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CORRELATED LIGHT AND ELECTRON MICROSCOPIC
ANALYSIS.

The Ohio State University, Ph.D., 1974
Anatomy

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THE BASILAR PONTINE GRAY OF THE OPOSSUM:
A CORRELATED LIGHT AND ELECTRON MICROSCOPIC ANALYSIS

DISSERTATION

Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

By

Gregory A. Mihailoff, B.S., M.Sc.

* * * * *

The Ohio State University
1974

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Finally, to my mother and late father, I shall ever be indebted for instilling in me the desire to attain such a goal and to my wife and her parents for their support, sacrifice and patience, I will be forever grateful.

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INTRODUCTION

In the present investigation, an attempt has been made to analyze the cytoarchitecture and the synaptic organization of the basilar pontine gray by comparing information obtained from both the light microscope and the transmission electron microscope. This type of correlated light and electron microscopic approach has been employed previously in the study of other cell stations (King et al., '71, '72, '73; Bowman and King, '73) involved in precerebellar circuitry, although there are relatively few studies of any type dealing with the pontine nuclei. The earliest descriptive accounts of the pontine gray utilizing the Golgi technique were provided by Vincenzi (1885), Held (1893) and Pusateri (1896). However, the work of Ramon Y Cajal ('09) contains the most extensive description of this region using primarily the brains of newborn mice and reveals the presence of two basic types of nerve cells; projection neurons whose axons form the bundles of pontocerebellar fibers comprising the brachium pontis and neurons described as mossy

cells which exhibit a locally ramifying axon. The more recent literature includes several depictions of the nuclear aggregations within the pontine gray as observed in Nissl stained preparations (Voris and Hoerr, '32; Sunderland, '40; Brodal and Jansen, '46; Olszewski and Baxter, '54; Abdel-Kader, '68; King et al, '68). Each of these accounts emphasizes the presence of neuronal aggregates dorsal, ventral, lateral and medial to the cerebral peduncle as a general feature of the basilar pons and in some instances certain smaller subdivisions are reported. A previous ultrastructural study of the pontine gray (Hollander et al, '69) includes a description of the normal fine structure of the cat basilar pons and provides evidence that corticopontine axons terminate preferentially on small dendritic profiles. With regard to afferent connectivity, accounts detailing the wide variety of axonal systems providing synaptic end-structures in the pontine gray include: the pretectum (Graybiel, '74), the ventral lateral geniculate nucleus (Rosenquist and Edwards, personal communication to Dr. George Martin), the superior colliculus (Papex and Freeman, '30; Martin, '69), the dorsal column nuclei (Nauta and Kuypers, '58; Lund and Webster, '67; Jane and Schroeder, '71; Schroeder and Jane, '71), the cerebral cortex (Sunderland, '40; Abdel-Kader, '68; Martin and King, '68; Brodal, '68a,b, '71 a,b, '72 a,b,c;

Harting and Martin, '70; Martin et al., '71) and the cerebellar nuclei (Brodal et al., '72; Yuen et al., '74). The above series of papers by P. Brodal detailing the corticopontine system in the cat are particularly significant and reveal a rather precise longitudinal organization of corticopontine terminals within the pontine gray which is not reflected in its normal cytoarchitecture.

In light of the diversity of input to the basilar pontine gray, the data compiled in this study of its normal fine structure will serve as a control for experimental electron microscopic investigations designed to further clarify its synaptic organization. It is hoped that such anatomical data will stimulate further electrophysiological study of the pontine nuclei and thereby contribute to the understanding of its functional role in the transfer of information from various cortical and subcortical centers to the cerebellum.

MATERIALS AND METHODS

The basilar pontine gray of the American opossum (Didelphis marsupialis virginiana) was serially sectioned in the transverse plane and stained for Nissl substance (Windle, '43; Fernstrom, '58). Nuclear aggregations as well as cytological features of individual basilar pontine neurons were determined from these preparations. Four different modifications of the Golgi method were utilized to reveal the morphology of neurons in various regions of the pontine nuclei. Brains of young opossums weighing less than 1 kg were processed by either the method of Valverde ('70) or Porter and Davenport ('49). Adult opossum brains were impregnated by the method of Fox et al ('51) or the Kopsch technique (as modified in the laboratory of Dr. Raymond Guillery, personal communication to Dr. James King). Material from the Kopsch impregnations was used most extensively in this study. These brains were fixed by either formaldehyde or glutaraldehyde-paraformaldehyde perfusion, chromated in 3.5% potassium dichromate and impregnated in 0.75% silver nitrate. Drawings

of individual neurons impregnated by the Golgi method were made with a 54X or 100X oil immersion objective using a Leitz microprojection apparatus.

For electron microscopic study, all brains of both control and experimental animals were fixed by intracardiac perfusion. Anesthesia was achieved by intravascular administration of sodium pentobarbitol (36 mg/kg body weight), a tracheotomy was performed and the opossum artificially ventilated by a small animal respirator. A thorocotomy was then performed, the heart exposed and the parietal pericardium dissected away. Sodium heparin (0.65 mg/kg body weight) and a vasodilator, sodium nitrite (0.09 mg/kg body weight) were introduced directly into the left ventricle. After five minutes, the cardiovascular system was thoroughly flushed with 200-300 cm³ of normal saline (which contained 0.5 gm/100 cm³ procaine hydrochloride in several animals as a substitute for the sodium nitrite) and fixed with 400 cm³ of a 1% glutaraldehyde, 2% paraformaldehyde solution followed by 700 cm³ of a 2% glutaraldehyde, 4% paraformaldehyde solution. The fixative was prepared with 0.2N sodium cacodylate buffer and the final solution adjusted to a pH of 7.2. After approximately 1100 cm³ of perfusate was passed through the animal over a period of 15 to 20 minutes, the brain was removed and immersed in the more concentrated fixative for 1 hour. The entire basilar pons was then excised

from the brain stem and divided into right and left halves. With the aid of a dissecting microscope, small blocks of tissue were removed from areas medial, ventral and lateral to the longitudinally oriented cerebral peduncle in each half of the basilar pons as illustrated in Fig. 1. The tissue was then washed in 0.2N sodium cacodylate buffer, post-fixed in 1% osmium tetroxide, block stained in saturated uranyl acetate, dehydrated in ethyl alcohols and embedded in Maraglas in such a manner that subsequent thin and semi-thin sections would always be cut in the transverse plane. The location of each block of tissue within the basilar pons was verified in toluidine blue stained semi-thin sections by noting the position of the cerebral peduncle and the ventral surface of the brain stem. The blocks were then trimmed and thin sectioned in the transverse plane on a Reichert OM-U2 ultramicrotome. The thin sections were picked up on copper grids, stained with lead citrate and observed with either an RCA EMU-3F or a Phillips EM300 electron microscope.

In the experimental electron microscopic portion of this investigation, adult opossums were subjected to either cortical or cerebellar ablations. The cortical cases included unilateral decortications as well as smaller lesions restricted to striate and peristriate regions. One animal was subjected to a dual

Fig. 1. A photomicrograph of a transverse section of the basilar pons illustrating the manner in which blocks of tissue were excised from areas medial (M), ventral (V) and lateral (L) to the cerebral peduncle (PED).

Fig. 2. Two line drawings illustrating the location of lesions involving motor-sensory (a) and visual (b) cortex (reader's left) and the brachium conjunctivum and deep cerebellar nuclei (reader's right).

lesion involving the forelimb and face portion of the motor-sensory amalgum in one hemisphere and the visual cortex in the opposite hemisphere (Fig. 2, line drawing to the reader's left). Cerebellar lesions involved hemicerebellectomy or unilateral transection of the superior cerebellar peduncle. Fig. 2 (line drawing to the reader's right) illustrates a cerebellar lesion which slightly impinges on the dorsal aspect of the brain stem, but more importantly interrupts the brachium conjunctivum perhaps sparing a small number of its most ventral fascicles. In addition, a large portion of the deep cerebellar nuclei are involved in the lesion. In all experimental animals, both halves of the pontine gray were examined with the side contralateral to the lesion serving as a control in cortical cases while the side ipsilateral to the lesion served as a control in brains with cerebellar lesions. Survival times in the cortical lesions included 2, 3, 4, 5, 6, 8 and 10 days (10 animals) while those for cerebellar cases were 3, 4, 5, and 6 days (8 animals).

OBSERVATIONS

Nissl Preparations

Nuclear aggregations within the basilar pontine gray.

In rostral transverse sections, cells tend to aggregate adjacent to the cerebral peduncle as it becomes incorporated into the basilar pons and in doing so form the ventral portion of a peripeduncular nucleus (Fig. 3, line arrows). Further caudally the ventral part of the peripeduncular nucleus diminishes in size while concomitantly the dorsal portion is formed by cells lying dorsal to the cerebral peduncle some of which may actually be situated among the fibers of the medial lemniscus (Fig. 5, line arrows). In rostral sections, the ventral region of the pontine gray is characterized by large numbers of transversely oriented fibers although near the extreme ventral surface there are two obvious aggregations of cells, one situated ventromedially (Fig. 3, open block arrows), the other ventrolaterally (Fig. 3, solid block arrows). These two cell groups appear to form longitudinally oriented columns extending through the rostral one-third of the pontine gray and their caudal extent is

indicated in Fig. 4 (open and solid block arrows). The ventral region in the caudal two-thirds of the basilar pons is free of any conspicuous subdivisions. Medial and lateral nuclei of the pontine gray are formed rostrally as expansions of the peripeduncular nucleus (Fig. 4, arrows marked M and L), and are present throughout the rostrocaudal extent of the basilar pons. A median nucleus (Fig. 5, curved arrows), a subdivision of the medial nucleus, is apparent in mid portions of the pontine gray as a small cluster of cells adjacent to the midline and, in caudal sections, a dorsolateral subdivision of the lateral nucleus can be identified (Fig. 5, open block arrows). The dorsal region of the caudal one-half of the basilar pons is characterized by a supralemniscal extension of the pontine nuclei. This supralemniscal process is first observed as an intensely staining cluster of cells adjacent to the medial lemniscus (Fig. 6, solid block arrows) which then expands dorsally and laterally to insinuate itself between the medial lemniscus and the overlying pontine reticular formation (Fig. 7, solid block arrows). The neurons comprising the extreme lateral part of the supralemniscal process are continuous with a dorso-lateral expansion of the peripeduncular nucleus and the entire complex of cells appears to enclose the medial lemniscus.

The reticulotegmental nucleus. Depending on the plane of section, the reticulotegmental nucleus is first observed just beyond the mid rostrocaudal extent of the basilar pons as a small cluster of cells adjacent to the midline and dorsal to the supra-lemniscal process (Fig. 7, open block arrow). Further caudally, a few cells may extend laterally (Fig. 8, solid block arrow) but the main portion of the nucleus expands dorsally adjacent to the midline (Fig. 8, open block arrow), and is present well beyond the caudal limit of the basilar pontine gray.

Individual neurons. Basilar pontine neurons range in size from 9 to 35 μ and demonstrate morphologic features compatible with those described in an earlier study of the pontine gray (King et al, '68). The two extremes of the size spectrum are illustrated by cells in the medial nucleus (Fig. 9) and examples of neurons of an intermediate size are illustrated in the lateral (Fig. 10) and ventral (Fig. 11) nuclei. Although one given cell size may be observed more frequently in one nucleus than in another, there is no strict localization within the subdivisions of the pontine gray on the basis of perikaryal dimension.

Golgi Preparations

Examples of typical basilar pontine neurons are illustrated in Fig. 12. These nerve cells are judged to be projection neurons because only the initial segments of their axons have impregnated well (Fig. 12, open block arrows). The

neuron in the lower right of Figure 12 is enlarged in Figure 13 to illustrate its axon and the characteristic irregular shaped protrusions which typically issue from the dendrites. These appendages may arise individually, in a series of three or four giving the dendritic surface a serrated appearance or two such dendritic protrusions may be arranged so as to form a claw-like structure. This feature is indicated by the solid block arrow on the neuron in Figure 13 and at higher magnification in the inset of the same figure. Dendritic claws have been observed on all portions of the dendritic expanse, but are more numerous proximally. Although the above dendritic appendages appear to be a general feature of pontine projection neurons there is some regional variation within the pontine gray. A variety of projection neuron, observed most frequently near the ventral extreme of the pontine gray (Fig. 14) is characterized by stalked appendages arising from some, but not all of its dendrites (Fig. 14, inset). The bulbous terminals of these appendages vary considerably in shape and range from approximately 1 to 3 μ in diameter. In addition, it is apparent in transverse sections that the dendrites of these neurons prefer a dorso-ventral or medio-lateral orientation in contrast to the more random or radially disposed dendrites on the previously illustrated cells (Fig. 12). However, there is no hint of any longitudinal dendritic organization in horizontally sectioned material.

For the most part, nerve cells located in the medial and lateral nuclei exhibit a radial dendritic pattern (Fig. 12, upper right) with proximal dendrites ranging in size from 5 to 8 μ in their greatest transverse dimension and tapering to 0.5 to 1.5 μ at their distal extremity. The majority of the dendrites of peripeduncular neurons are oriented in such a way that they conform to the shape of the cerebral peduncle and course adjacent to its surface (Fig. 12, lower left). However, in some instances these nerve cells give rise to dendrites which penetrate into the confines of the peduncle or extend out of the peripeduncular zone into the surrounding ventral nucleus (Fig. 12, upper left). The axons of the above pontine neurons in most instances arise from the somata although a few apparently issue from proximal dendrites. Axons generally cannot be traced for distances greater than 20 to 40 μ since at that point they apparently acquire a myelin sheath or pass out of the plane of section. No collaterals have been observed arising from this initial impregnated portion of the axon. However, the possibility remains that collaterals may be given off after these axons have aggregated at the ventral surface of the basilar pons as they form the middle cerebellar peduncle. In several impregnations, a number of fine, beaded processes have been observed issuing from the dense fiber plexus at the ventral surface of the pons and coursing into the pontine gray. One cannot be sure, however,

that these thin processes are not simply pontine afferents that are coincidentally located in that position.

Neurons at the smaller end of the size spectrum (9-18 μ) have been observed to give rise to one or several axon-like processes (Fig. 15, solid block arrows). The axon-like process on the left (Fig. 15, A, solid block arrow) issues from a conical elevation on a proximal dendrite and courses laterally then ventrally as it gradually diminishes in diameter and becomes beaded (Fig. 15, A, small open block arrows). The approximate total length of this process is 375 μ , but only its distal 150 μ is extremely thin and beaded. Other small neurons (only partially illustrated) demonstrate similar processes (Fig. 15, B, lower right, solid block arrows) and in addition, give rise to thin, beaded processes (Fig. 15, B, large open block arrow) which have been referred to as a "string of beads" (Rafols and Valverde, '73). The latter processes range from 10 to 30 μ in length and exhibit several small dilatations or swellings along their course and/or at their termination. Axons also originate from the soma (Fig. 15, C, lower center) and give rise to collaterals which, as in this example, can be followed for some distance as it becomes attenuated and beaded. Three or four long and relatively unbranched main dendritic trunks arise from the cell bodies of these neurons. Dendritic claws are not present and stalked appendages are infrequently observed.

Electron Microscopic Preparations

The fine structure of the basilar pontine gray will be described in the following sequence: characteristic neuronal profiles, basic types of presynaptic profiles, and results of cortical and cerebellar lesions. The pontine gray appears to be morphologically similar in all areas sampled, however, no quantitative data has been assembled to more accurately assess possible regional differences.

Neuronal Profiles. Based on Golgi impregnations, it has been demonstrated that some neurons at the smaller end of the size spectrum (measuring 18μ or less in their greatest dimension) give rise to a locally ramifying axon or axon-like process. Cells of a comparable size have been observed in control electron microscopic material and an example is illustrated in Fig. 16. These small neuronal profiles have been observed in all areas of the pontine gray and are characterized by a thin rim of cytoplasm containing numerous polysomes and only very infrequent stacks of granular endoplasmic reticulum. In addition, in most instances, these cells exhibit an extensive network of smooth endoplasmic reticulum arranged in a circumnuclear pattern. The perikaryal surface of these profiles for the most part is

invested in glial lamellae and only occasionally is an axosomatic contact observed (Fig. 16, open block arrow). For comparison, a typical larger neuronal profile likely representing a projection neuron is illustrated at the same magnification in Fig. 17. These larger profiles exhibit a much greater profusion of cytoplasmic organelles including distinct aggregates of granular endoplasmic reticulum and appear to give rise to radially oriented dendrites when the plane of section passes through the nucleolus. In addition, the perikaryon of these neurons occasionally gives rise to small spine-like appendages. Axosomatic synapses are more frequently observed on these profiles (which may simply be a reflection of the greater incidence of larger neuronal profiles), but remain rare in terms of absolute numbers.

Presynaptic Profiles. Careful examination of synaptic end-structures in the basilar pons reveals the presence of four basic types of presynaptic profiles. Large presynaptic profiles range in size from 2 to 8μ (Figs. 18 and 19) and contain round vesicles (average diameter, $450\text{ \AA}$) as well as a central core of mitochondria. Such profiles contact neuronal somata (Fig. 16, open block arrow) and the shafts of various sized dendrites in an asymmetrical manner. Characteristically,

they also form multiple asymmetric contacts with several small postsynaptic profiles (Fig. 19, asterisks). The origin of these small profiles is seen to better advantage in Fig. 18 which illustrates a large axon terminal apparently sectioned in the transverse plane. The solid block arrow indicates a stalk originating from a transversely sectioned proximal dendrite and in turn giving rise to at least two and possibly three small branches (Fig. 18, asterisks) whose orientation is suggestive of the claw-like structures previously illustrated on the dendrites of projection neurons (Fig. 13, inset) or the multilobed appendages demonstrated in the inset (Fig. 18). Careful study of numerous examples of similar synaptic complexes indicates that the small peripheral profiles may originate from different dendrites, however, whether or not these dendrites represent more than one neuron could not be ascertained. In several instances in our control material, large presynaptic profiles could be distinguished by the fact that they contained either a very small number of synaptic vesicles or conversely were densely packed with vesicles. Further observation and quantitation is necessary in order to identify the source of these profiles and to evaluate the possibility of sub-populations of large axon terminals.

The remaining three types of axon terminals are described

as small profiles since they consistently measure less than 2μ in their greatest dimension. The category of small bouton illustrated in Fig. 20 (open block arrow) occasionally contains a few microtubules and a dense core vesicle in addition to round synaptic vesicles ranging from $270 \text{ \AA}$ to $420 \text{ \AA}$ in diameter and averaging $380 \text{ \AA}$. These axon terminals form asymmetric synaptic junctions primarily with small diameter (1.5μ or less) dendrites and less frequently with somata and more proximal dendritic profiles (Fig. 18, open block arrow). In several instances a bouton of this type has been observed to contact two neighboring dendritic profiles simultaneously (Fig. 24, open block arrow). Conversely, examples of a single transversely sectioned small diameter dendrite receiving more than one bouton are rare. A second type of small bouton is similar in size (Fig. 20, solid block arrow) and also forms asymmetric synaptic contacts with small diameter dendritic profiles. However, the spheroidal vesicles contained within these profiles are of a larger size, ranging from $440 \text{ \AA}$ to $640 \text{ \AA}$ and averaging $500 \text{ \AA}$. This type of bouton appears to be the least numerous presynaptic profile observed in the pontine gray. The third category of small bouton, as illustrated in Fig. 21, contains flattened or elliptical vesicles and forms symmetrical synaptic junctions with somata and proximal dendrites, but most frequently with small dendritic profiles. Quite often, in fortunate sections, this type of bouton is observed to be of the en passage character. Also in

numerous instances a flat vesicle bouton contacts a dendritic profile which in the same plane of section receives a round vesicle bouton that may be directly apposed to the flat vesicle terminal.

Experimental Electron Microscopic Preparations

Cerebellar Cases. The superior cerebellar peduncle was unilaterally transected at a level just caudal to the inferior colliculus. The lesion in each case involved portions of the deep cerebellar nuclei as well as axons emanating from these cerebellar zones destined to form the contralateral descending limb of the brachium conjunctivum. Regions of the pontine gray contralateral to the lesion previously described as cerebellar terminal zones at the light microscopic level (Yuen et al., '74) were examined for ultrastructural evidence of degenerating axon terminals. Specifically, portions of the medial, lateral and ventral nuclei were sampled (see Materials and Methods, Fig. 1). At survival times of 3 and 4 days, a larger type of axon terminal (2 to 4.2 μ) containing round vesicles and forming asymmetric synaptic contacts was observed to undergo a filamentous degenerative reaction (Fig. 25). Typically, the degenerating terminals were in synaptic contact with proximal, intermediate and distal dendritic shafts, as well as the characteristic claw-like postsynaptic profiles (Fig. 25, asterisks) described

in the control material. By day 6, many degenerating terminals were dark, shrunken and enveloped by glial processes.

Cortical Cases. In a second series of experimental animals either the entire neocortex was ablated or lesions were restricted to specific functional areas as described by Lende ('63a, b). Examination of the pontine gray ipsilateral to the lesion in decorticate specimens indicates that at survival times as early as 2 days both electron dense (Fig. 22) as well as filamentous (Fig. 23) degenerating axon terminals were present. Moreover, both types of degenerating profiles are demonstrable at all survival times up to and including 10 days. Boutons undergoing the electron dense reaction observed at an early stage of degeneration (Fig. 22), when compared with normal terminals (Fig. 20) appear to contain small round vesicles and form asymmetric synaptic junctions. Filamentous degenerating terminals (Fig. 23, solid block arrows) when compared with a normal bouton (Fig. 24, open block arrow) also form asymmetric junctions and exhibit small round vesicles. Of those dark degenerating terminals in which the size of the postsynaptic element could be accurately determined (sectioned in the transverse plane), 18 were found to be in contact with spine-like profiles or dendrites measuring less than one and one-half micra in diameter (compare postsynaptic dendrites (Dd) in Figures 20 and 22) while only 2 examples contacted dendrites

measuring larger than three micra. These data suggest that the electron dense terminals have a propensity for smaller diameter dendrites. Examination of 33 filamentous terminals contacting postsynaptic profiles sectioned in the transverse plane reveals that 17 contact small diameter dendrites (1.5μ or less) whereas 16 were opposed to larger diameter dendritic profiles (3μ or more) suggesting that more filamentous endings have a predelection for the proximal portions of the dendritic tree. In noting the size of both types of degenerating profiles, it was observed that the largest electron dense terminal in an early stage of degeneration (day 2) prior to any significant shrinkage, measured 1.4μ while the largest filamentous endings measured 2.8μ . Indeed, although there was considerable variation and overlap in the size of both types of degenerating profiles, the filamentous endings tended to be larger than those in the early stage of the dark reaction.

The above data could be interpreted to suggest that two types of cortical axons may synapse on basilar pontine neurons. Thus smaller cortical lesions were accomplished and in the two animals subjected to lesions involving only striate and peristriate cortex (5 days survival) only filamentous degenerating terminals were observed. In a third animal a lesion (5 days survival) was placed within the forelimb and face portion of the motor-sensory amalgum in one hemisphere and in the striate and peristriate regions in the opposite hemisphere. In the

pontine gray ipsilateral to the motor-sensory lesion numerous dark degenerating terminals were present and only an occasional profile demonstrated a filamentous reaction. In contrast, on the side ipsilateral to the visual area lesion all degenerating terminals were of a filamentous nature.

DISCUSSION

Nissl Preparations

In a previous cytoarchitectural study of the opossum basilar pons (King et al., '68) the pontine gray was divided into four zones based on their relationship to the cerebral peduncle and named medial, lateral, ventral and dorsal nuclei. A subsequent report detailing the corticopontine and cerebello-pontine projection systems in this species (Yuen et al., '74) clearly demarcated separate peripeduncular and medial cell groups. Neurons forming the peripeduncular nucleus were recognized in the earlier report by King et al ('68), but were included as part of the four main nuclei. The present investigation confirms the results of the above studies and in Nissl preparations documents additional details such as: (1) the two longitudinal cell columns in the ventral nucleus; (2) a dorso-lateral subdivision of the lateral nucleus similar to that seen in the cat, and (3) a supramelemniscal extension as described for the human by Olszewski and Baxter ('54). In addition, the reticulotegmental nucleus can be clearly distinguished from the dorsal regions of the pontine gray.

In comparison to other mammals, the four major nuclei described in the opossum are comparable to those described in the pontine gray of the cat and rabbit (Brodal and Jansen, '46), rabbit (Abdel-Kader, '68), macaque monkey (Sunderland, '40) and human (Olszewski and Baxter, '54).

The longitudinal cell columns described in the ventral nucleus appear to correspond to the two zones of degeneration in that region as described by Yuen et al ('74) following cortical ablations. Similar longitudinal columns of corticopontine degeneration were first described by Brodal ('68) in the cat but were located primarily in areas medial and lateral to the cerebral peduncle.

The neurons located dorsal to the medial lemniscus have been considered part of the reticulotegmental nucleus in the cat (Brodal and Brodal, '71). However, based on the discontinuity between the cells located near the surface of the medial lemniscus and those situated more dorsally adjacent to the midline (Fig. 7) these neurons have been divided into a supra-lemniscal portion of the basilar pons and the reticulotegmental nucleus in the present description.

Golgi Preparations

Three types of projection neurons were differentiated by Ramon Y Cajal ('09) on the basis of extent and orientation of

their dendritic tree and a single type of intrinsic neuron was illustrated. Cajal did not detail types of dendritic protrusions or appendages, but simply states that spines were observed on the projection neurons and they are obvious in his illustrations. The intrinsic neuron was described as a mossy cell since all surfaces of the cell including the soma were profusely covered with spines while the locally ramifying axon in many instances took origin from a dendrite. The adult opossum basilar pons exhibits neurons which are similar to those described by Cajal in that two basic types of neurons are present, projection neurons and an interneuron. However, the present investigation indicates that basilar pontine projection neurons differ somewhat with regard to their dendritic morphology. The most numerous variety is characterized by irregularly shaped dendritic protrusions and claws. A lesser number of cells, most frequently apparent near the ventral extreme of the basilar pons, display varying sizes and numbers of stalked dendritic appendages. Most projection neurons exhibit a radial dendritic pattern although those in peripeduncular regions often assumed a more polar orientation. Functional implications attached to the varying dendritic morphology of projection neurons are admittedly tenuous due to the vagaries of the Golgi technique. As evidence to this fact, the literature contains a number of hypotheses with regard to the function of dendritic spines or appendages. Those on Purkinje

cell dendrites may act summarily as a current injection mechanism (Chan-Palay, '70) in which the clustering of spines is particularly significant and affords one axon terminal the opportunity of contacting a large number of spines. Discharge of that terminal then would readily allow the summation of potentials in each of the spines thereby apparently increasing the efficiency of the incoming signal. The clustering of small spine-like protrusions about large axon terminals described in control electron microscopic material in the present report would seem to lend itself to such a functional interpretation. Other investigators (Schiebel and Schiebel, '68) have suggested that dendritic protrusions may serve to parcel out or isolate different afferent systems reaching the area in question. The latter interpretation suggests a valid functional implication for dendritic protrusions in the pontine gray.

Projection cell axons have not been satisfactorily impregnated in our material even in double or triple impregnation procedures; therefore, in most instances only the initial and presumed non-myelinated portion of the axon can be identified. Although no collaterals have been observed to issue from this impregnated portion of the axon thin, beaded fibers have been traced from the plexus of axons forming the middle cerebellar peduncle at the ventral surface of the pons suggesting their possible origin as collaterals of projection axons. Cajal does

not illustrate collaterals arising from any of the various types of projection neurons, but in all other respects our observations regarding projection neurons are consistent with those in the newborn mouse.

The intrinsic neuron described in the opossum exhibits few dendritic spines or protrusions and characteristically gives origin to more than one axon or axon-like process that usually arises from a dendrite and becomes thin and beaded in the vicinity of the parent cell. The major difference between this cell and the interneuron described by Cajal is the fact that in the newborn mouse the somatic and dendritic surfaces of the intrinsic cell display a tremendous number of spines, a feature which may only reflect its neonatal character. The basilar pontine intrinsic neuron bears some features comparable to the interneurons described in the dorsal lateral geniculate nucleus of the cat (Famiglietti and Peters, '72), rat (Wong-Riley, '72) and mouse (Rafols and Valverde, '73) in that they exhibit locally ramifying axons or axon-like processes of somatic or dendritic origin as well as the thin beaded structures referred to as long stalked appendages by Famiglietti and Peters ('72) or "strings of beads" by Rafols and Valverde ('73). Thalamic interneurons, however, demonstrate a much more profuse system of dendritic appendages some of which are actually presynaptic end-bulbs. A more favorable comparison might be made with the interneuron demonstrated

in the lateral cerebellar nucleus of the rat (V. Chan-Palay, '73a) which exhibits few dendritic spines and gives rise to an axon arborizing in the immediate vicinity of the cell. Such axons most frequently originate from dendrites.

Normal Fine Structure

The only previous ultrastructural account of the basilar pons was provided by Hollander et al ('69) utilizing the cat.

These investigators described three basic types of pre-synaptic profiles which included large and small categories of boutons containing round synaptic vesicles and a third variety of terminal exhibiting flattened vesicles. No attempt was made in that report to characterize the types of neuronal profiles observed in the pontine gray and further no correlation of the fine structure revealed in the study was made with previously reported light microscopic data. In the present investigation, two types of neuronal profiles were observed in control electron microscopic material and distinguished on the basis of perikaryal dimension and certain cytoplasmic features. One type, due to its small size, the relative paucity of granular endoplasmic reticulum and its extensive Golgi complex is very likely the electron microscopic correlate of the small cells described as interneurons in our Golgi impregnated material. The fine structure of these small neuronal profiles is similar to that of the intrinsic neurons of

the lateral cerebellar nucleus of the rat as illustrated by V. Chan-Palay ('73b). The larger neuronal profile illustrated in the present report was suggested to be an example of a basilar pontine projection neuron due to the fact that it gave rise to dendrites in a radial fashion and exhibited cytoplasmic features consonant with descriptions of projection neurons in other areas of the central nervous system (Peters et al., '70). All types of presynaptic profiles, with the exception of those terminals containing the large round synaptic vesicles were observed to contact the somata of both types of basilar pontine neurons. The absolute numbers of axosomatic synapses, however, was quite low in all regions of the pontine gray, an observation also made by Hollander et al ('69) in the cat.

With regard to presynaptic profiles in the opossum basilar pons, four categories of axon terminals were described, three of which were similar to those illustrated in the cat (Hollander et al., '69). Based on size the four types of profiles were placed in two categories, those ranging in size from 2 to 8 μ and those always measuring less than 2 μ in their greatest dimension. The smaller category of presynaptic profiles actually included three of the four basic types. The first type was characterized by its content of small round or spherical vesicles (380 Å) and asymmetric synaptic junctions formed primarily, but not exclusively with small diameter dendrites. A second type of axon terminal also

forming asymmetric contacts with small diameter dendrites was distinguished by the fact that it contained larger round 500 Å synaptic vesicles. This type of profile was not described in the cat and was relatively rare in the present study. The third category of small axon terminal was observed to contain flattened or elliptical vesicles and to contact in a symmetrical junction a wide variety of postsynaptic profiles but again primarily small dendritic profiles.

An inhibitory function for flat vesicle terminals was first proposed by Uchizono ('65). In a study directed toward characterization of the electrophysiological properties of basilar pontine neurons (Sasaki et al., '70) IPSPs were revealed in both unitary and intracellular recording following antidromic activation of basilar pontine neurons produced by stimuli delivered to the brachium pontis or a cerebellar folium. A similar inhibition was also observed following stimulation of cerebral cortical gray matter. However, a subsequent report by Allen et al ('71) indicated that stimulation of the internal capsule coupled with ablation of cortical gray matter abolishes the inhibition of pontine neurons suggesting that the inhibition following cortical gray stimulation was, in effect, due to disfacilitation of corticopontine fibers produced by an intracortical inhibitory mechanism. A similar interpretation may explain the inhibition in pontine neurons produced by cerebellar

folial stimulation (Sasaki et al., '70). That is, current spread may activate Purkinje cells which then inhibit the deep cerebellar nuclei thereby disfacilitating pontine neurons by removing the excitatory input of the deep nuclei. On the other hand, the disfacilitation theory cannot explain the inhibition following stimulation of the brachium pontis as reported by Sasaki et al ('70). In this situation, a pontocerebellar axon is stimulated antidromically as are its collaterals which are postulated to activate an inhibitory interneuron which in turn impinges on neighboring basilar pontine neurons. The characteristics of the resultant IPSPs indicates that the inhibition occurs on distal dendritic segments of pontine neurons and correlates with findings in the present investigation which demonstrate that the majority of flat vesicle terminals contact small diameter dendritic profiles. It is therefore suggested that the flat vesicle profiles may represent the synaptic end-structures of an inhibitory interneuron.

Larger axon terminals in the pontine gray contain round vesicles ($450 \text{ \AA}$) and form asymmetric junctions with large and a lesser number of small dendritic shafts. Several small postsynaptic profiles which arise as dendritic protrusions and frequently form claw-like structures similar to those illustrated on projection neurons in Golgi material are typically seen in a circumferential manner surrounding the larger synaptic profiles.

Several fortunate sections indicate, but do not demonstrate unequivocally, that the small postsynaptic profiles contacted by a single large axon terminal may have arisen from at least two different dendrites. The possibility of subgroups within the population of large axon terminals is suggested by the number of vesicles contained in these profiles, some exhibiting fewer numbers of synaptic vesicles while others are densely packed with vesicles. The use of vesicle packing density as the only criterion in differentiating types of axon terminals is rather tenuous in the light of increasing evidence suggesting that the number of vesicles in a given terminal is a function of the physiological state of that terminal at the time of fixation (Heuser and Reese, '73). The presence in the pontine gray of synaptic profiles similar to mossy fiber collateral terminals as described in the rat lateral cerebellar nucleus (Chan-Palay, '73c) suggests the possible existence of collaterals arising from pontocerebellar axons. Evidence in support of collateralization is found in the aforementioned study concerned with the electrophysiological properties of basilar pontine neurons (Sasaki et al., '70). Single stimuli delivered to the brachium pontis in some instances or to a cerebellar folium in others produced repetitive firing of neurons in the pontine gray as indicated by field potential analysis and unitary recording. Latency and neuron response characteristics, according to Sasaki, suggest that collaterals

of pontocerebellar axons synapse directly on and fascilitate neighboring basilar pontine cells.

Experimental Fine Structure

Cerebellar Lesions. A projection from the deep cerebellar nuclei to the pontine gray by way of the descending limb of the brachium conjunctivum has only recently been documented at the light microscopic level by A. Brodal et al ('73) in the cat and Yuen et al ('74) in the opossum. This feedback circuit had been demonstrated previously by physiological methods by Tsukahara and Bando ('70) who found that stimulation of the interpositus complex in the cat elicited monosynaptic EPSPs in basilar pontine neurons. In an attempt to identify axon terminals of the descending brachium conjunctivum, a number of animals were subjected to either ablations of the deep cerebellar nuclei or transection of the brachium conjunctivum just caudal to the inferior colliculus and proximal to its decussation. Large axon terminals in the pontine gray contralateral to the lesion were observed to undergo filamentous degeneration at early survival times (3 and 4 days) followed by a transition to electron dense degeneration at later times (6 days). However, in an effectively complete transection of the superior cerebellar peduncle (a very small number of axons may have been spared) numerous large presynaptic profiles remained free of degeneration. It is suggested therefore that at least some, but not

all large boutons arise from axons of the descending brachium conjunctivum which themselves originate from the deep cerebellar nuclei, particularly the interpositus anterior and dentate nuclei. The identifying features of cerebellar nuclear axon terminals in both control and experimental material in the basilar pons are strikingly similar to those reported in similar studies of the cerebellar nuclear afferents to the red nucleus (King et al., '71, '72). That is to say in the red nucleus, large axon terminals with round vesicles and forming asymmetric contacts on small dendritic appendages, undergo early filamentous and later electron dense degeneration following stereotaxic lesions of the deep cerebellar nuclei. The question of subpopulations of large boutons in the basilar pons unfortunately must go unanswered for the present although the data gathered clearly warrants continued investigation for possible additional sources of large terminals.

Cortical Lesions. Following unilateral decortication, both electron dense and filamentous degenerating boutons are observed in the ipsilateral pontine gray at survival times as early as 2 days. At 6 days survival, the majority of electron dense terminals are shrunken, crenated and surrounded by glial processes although numerous filamentous boutons are still evident. Even at 10 days survival, both types of degenerating profiles are observed, however, the number of filamentous terminals appears to

have markedly decreased. The degenerating profiles in all cases form asymmetric synaptic junctions, contain round vesicles and usually measure less than 1.8μ although some of the filamentous endings are as large as 2.8μ . The dark degenerating boutons typically contact small (1.5μ or less) diameter dendrites, a fact also demonstrated by Hollander et al ('69) in the cat. The distribution of the filamentous terminals differed somewhat in that they appeared to terminate in almost equal numbers on large (3.0μ or greater), as well as small diameter dendrites.

Previous accounts describing cortical axon terminals in various areas of the central nervous system have indicated that these boutons display the electron dense type of degeneration which is not preceded by a filamentous reaction (Szentagothai et al., '66; Lund, '69; King et al., '72; Valverde, '73). However, in the squirrel monkey (Mathers, '72), both filamentous and dark degenerating terminals have been observed in the pulvinar at early survival times (3 days) following lesions involving peristriate and posterior temporal cortices. Campos-Ortega and Hayhow ('73) in the inferior pulvinar of the rhesus monkey describe filamentous degenerating boutons and only infrequently a dark degenerating terminal following occipital lobotomy. The above evidence and that of the present study suggests that two populations of axons may undergo degeneration subsequent to the ablation of certain cortical areas. In order

to further assess this problem in the opossum two animals received lesions restricted to striate and peristriate cortex and it was found that only filamentous degenerating terminals were present in the pontine gray at 5 days survival. Because the above cortex does not contribute any fibers to the cortico-spinal system in the opossum (Martin and Fisher, '68), the filamentous axon terminals may be part of a direct corticopontine axon system. This possible interpretation was further investigated by placing a dual lesion in an animal in which forelimb and face motor-sensory cortex was ablated in one hemisphere and a large visual area lesion placed in the opposite hemisphere. In this way not only would the survival times be the same for the two lesions but variables related to fixation and tissue handling could be controlled. Only filamentous boutons were observed in the pontine gray (5 days survival) ipsilateral to the visual area lesion whereas primarily electron dense degenerating terminals were present on the side of the motor-sensory lesion with only a very infrequent filamentous terminal. Such data may be interpreted as evidence to suggest that two populations of cortical neurons send axons to the opossum basilar pons.

Support for the above interpretation is also available from physiological studies. Allen et al ('69) have demonstrated

in the cat that, based on conduction velocities, two different cortical systems reach the basilar pons, one system conducting at a mean velocity of 34.0m/sec the other at 10.7m/sec. Since conduction velocity is directly proportional to fiber diameter it is possible, but by no means certain, that one might expect to find two populations of degenerating axon terminals in the pontine gray or at least a rather broad range in size of synaptic profiles. Indeed, this observation has been made in the present study when it was pointed out that at early survival times, presumably prior to significant shrinkage of the bouton, dark terminals tend to be smaller than the majority of filamentous degenerating boutons. Consequently, based on their small size, their nearly exclusive contact with small diameter dendrites and their apparent origin from areas of cortex projecting on the spinal cord it is felt that the dark degenerating boutons represent the terminals of collaterals of axons continuing beyond the basilar pons. Certainly, the suggestion of right angle collaterals is seen in sagittal sections of brains previously subjected to motor-sensory cortical lesions and processed by the Fink-Heimer technique (G. F. Martin, personal communication). The filamentous boutons, because of their somewhat larger size, their more numerous contacts with larger diameter dendrites and their origin from cortical regions which in the opossum do not give rise to corticospinal fibers are apparently formed by the axon terminals of a direct corticopontine fiber system.

ABBREVIATIONS

BP	=	brachium pontis
Dd	=	dendrites
L	=	lateral nucleus
M	=	medial nucleus
ML	=	medial lemniscus
PED	=	cerebral peduncle

PLATE I

- Fig. 3 A photomicrograph of a representative transverse section in the rostral one-third of the basilar pons. The ventral peripeduncular nucleus is indicated by the arrows adjacent to the cerebral peduncle (PED). A ventromedial (open block arrows) and a ventrolateral (solid block arrows) cell column is present at this level. Nissl stain (2.5X objective).
- Fig. 4 A photomicrograph of a representative transverse section at the mid-portion of the basilar pons. Medial (open block arrow, M) and lateral (open block arrow, L) are indicated as are the caudal extents of the ventromedial (open block arrow) and ventrolateral (solid block arrow) longitudinal cell columns. Nissl stain (2.5X objective).
- Fig. 5 A photomicrograph of a representative transverse section in the caudal one-third of the basilar pons. The straight arrows indicate the dorsal portion of the peripeduncular nucleus adjacent to the cerebral peduncle (PED). A subdivision of the lateral nucleus, the dorsolateral nucleus

is indicated by open block arrows while the median nucleus (curved arrows) is formed adjacent to the midline. Nissl stain (2.5X objective).

- Fig. 6 A photomicrograph of a transverse section at the mid-portion of the basilar pons illustrating an intensely staining cluster of cells (solid block arrows) located dorsal and medial to the medial lemniscus (ML). Nissl stain (2.5X objective).
- Fig. 7 A photomicrograph of a transverse section slightly caudal to that in Figure 6. The dorsomedial extension of cells has expanded at this level to lie dorsal to the medial lemniscus (ML) forming the supralemniscal (solid block arrows) portion of the basilar pons. The rostral tip of the reticulotegmental nucleus is indicated by an open block arrow. Nissl stain (2.5X objective).
- Fig. 8 A photomicrograph of a transverse section at the caudal extreme of the basilar pons. A few cells of the reticulotegmental nucleus extend laterally (solid block arrow) while on the opposite side of the midline a small cluster of cells representing

the rostral portion of the reticulotegmental nucleus are indicated by the open block arrow. Nissl stain (2.5X objective).

Fig. 9 A high power photomicrograph of nerve cells in the medial nucleus that represent the two extremes of the size spectrum of basilar pontine neurons. Nissl stain (54X oil immersion objective).

Fig. 10 A high power photomicrograph of nerve cells in the lateral nucleus illustrating examples in the intermediate portion of the size spectrum of basilar pontine neurons. Nissl stain (54X oil immersion objective).

Fig. 11 A high power photomicrograph of cells in the ventral nucleus illustrating examples in the intermediate portion of the size spectrum of basilar pontine neurons. Nissl stain (54X oil immersion objective).

PLATE II

Fig. 12 A composite drawing of four basilar pontine projection neurons to illustrate variations in the typical dendritic pattern. The neuron in the upper right is located in the lateral nucleus while that in the lower right is from the medial nucleus. On the left are examples of nerve cells in peripeduncular regions. The axon of each cell is indicated by an open block arrow. Golgi-Kopsch preparation.

PLATE III

Fig. 13 A higher magnification microprojection drawing of the basilar pontine nerve cell illustrated in the lower right of Figure 12. The line drawing in the lower right indicates the location of the neuron in reference to the cerebral peduncle (PED) and brachium pontis (BP). The open block arrow illustrates the axon whereas the solid block arrow indicates a claw-like dendritic appendage. Golgi-Kopsch preparation.

INSET

A high power photomicrograph of the dendritic claw illustrated in Figure 13. Golgi-Kopsch preparation. (54X oil immersion objective).

PLATE IV

Fig. 14

A microprojection drawing of two basilar pontine neurons located, as indicated in the orientation drawing in the lower right, ventromedial to the cerebral peduncle (PED) and brachium pontis (BP). These neurons are characterized by stalked appendages (solid block arrow) and the dorso-ventral and mediolateral orientation of their primary dendrites. Golgi-Kopsch preparation.

INSET

A high power photomicrograph of the stalked appendage indicated in Figure 14 by the solid block arrow. Golgi-Kopsch preparation. (54X oil immersion objective).

PLATE V

Fig. 15

A microprojection drawing of an interneuron located ventral to the cerebral peduncle (PED) and brachium pontis (BP). The solid block arrows on the centrally illustrated nerve cell (A) indicate axon-like processes which become attenuated and beaded (small open block arrows). At the lower right (B) is a partially illustrated cell giving origin to two axon-like processes (solid block arrows) which also give rise to en passage varicosities (small open block arrows). A thin, beaded process (large open block arrow) originates from a secondary dendrite. The cell in the lower center (C) exhibits an axon of somatic origin which gives off a collateral (solid block arrow) that becomes beaded (small open block arrows). Golgi-Kopsch preparation.

PLATE VI

Fig. 16

A profile of the somata of a small basilar pontine neuron. Cytoplasmic features include scattered polysomes and an extensive circum-nuclear Golgi complex. The open block arrow indicates a large presynaptic profile forming an axosomatic synapse. (electron micrograph).

Fig. 17

A profile of the somata of a large basilar pontine neuron reproduced at the same magnification as the neuron in Figure 16. When compared with the smaller profile, in addition to the obvious size difference, this larger cell exhibits a much greater profusion of cytoplasmic organelles and gives rise to four dendrites (Dd) in a radial manner. (electron micrograph).

PLATE VII

Fig. 18

An illustration of a transversely sectioned proximal dendrite (Dd) giving rise to a protrusion (solid block arrow) which may correspond to the dendritic claw indicated in Figure 13, inset, or part of a multi-lobed appendage as demonstrated in the inset of this figure. The small profiles (asterisks) are contacted by a transversely sectioned large bouton. The shaft of the proximal dendrite receives a small bouton (open block arrow) containing round vesicles as well as several microtubules. (electron micrograph).

INSET

A high power photomicrograph of a multi-lobed appendage observed on a projection neuron dendrite. Golgi-Kopsch preparation. (54X oil immersion objective).

Fig. 19

An illustration of a longitudinally sectioned large axon terminal. These presynaptic profiles exhibit the smaller sized round vesicles and

characteristically form multiple asymmetric
contacts with small postsynaptic profiles
(asterisks). (electron micrograph).

PLATE VIII

- Fig. 20 An illustration of two different types of the smaller category of axon terminals observed in control material. The open block arrow indicates a type of bouton characterized by small, round synaptic vesicles. A second type of small bouton (solid block arrow) is distinguished by its larger spheroidal synaptic vesicles. Both axon terminals contact a small diameter dendrite (Dd) and display asymmetrical junctions. (electron micrograph).
- Fig. 21 An illustration of a small type of axon terminal containing flattened or elliptical vesicles and displaying a distinct symmetrical synaptic junction. (electron micrograph).
- Fig. 22 An illustration of a small electron dense degenerating axon terminal subsequent to a unilateral decortication (2 day survival). The profile contains round synaptic vesicles and forms an asymmetric contact with a small dendritic profile (Dd) in a manner similar to the normal terminal

(open block arrow) in Figure 20. (electron micrograph).

PLATE IX

- Fig. 23 An illustration of two filamentous degenerating axon terminals (solid block arrows) subsequent to a unilateral decortication (4 day survival). Compare these degenerating endings with the normal axon terminal indicated by the open block arrow in Figure 24. (electron micrograph).
- Fig. 24 An illustration of a smaller type of presynaptic profile (open block arrow) in control material containing small round vesicles and forming asymmetric synaptic contacts with small diameter dendrites (Dd). (electron micrograph).
- Fig. 25 An illustration of a large filamentous degenerating terminal (4 day survival) subsequent to transection of the superior cerebellar peduncle which contacts a dendritic shaft (Dd) as well as two small postsynaptic profiles (asterisks). (electron micrograph).

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