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The Distribution of Dorsal Column Nuclear Efferents to the Basilar Pontine Gray in Normal and Neonatally Brain Damaged Rats

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THE DISTRIBUTION OF DORSAL COLUMN NUCLEAR EFFERENTS TO THE BASILAR PONTINE GRAY IN NORMAL AND NEONATALLY BRAIN DAMAGED RATS

By

Ross Kosinski

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DEDICATION

In memory of my loving mother.

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VITA

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INTRODUCTION

Ascending somatosensory pathways generally relay peripheral information in precise topographic patterns throughout all levels of the neuraxis (Brodal, 1981). Such a topographic progression of sensory inputs provides an anatomical substrate for the precise recognition of peripheral stimuli (Barr, 1979). For example, somatosensory information from tactile receptors ascends the dorsal white columns of the spinal cord somatotopically positioned within the fasciculus cuneatus and gracilis to terminate at caudal medullary levels within nuclei of the same name (Odutola, 1977). These dorsal column nuclei, cuneatus and gracilis, thus receive a somatotopic arrangement of primary afferent fibers from the fore- and hindlimb, respectively. The topographic arrangement of the representative receptive fields of these peripherally located tactile receptors is maintained via medial lemniscal projections to the contralateral somatosensory thalamus and subsequently the primary sensory cortical area through ipsilateral thalamocortical projects (see Dykes, 1982 for reviews).

Other well-established distribution patterns of DCN efferents include connections with the spinal cord (Gray et al., 1981; inferior olive and tectum (see Berkley et al., 1980), cerebellum (Grant, 1962; Feldman and Kruger, 1980), posterior thalamus (Lund and Webster, 1967;

Bold et al., in press) and zona incerta (Lund and Webster, 1967). Although previous studies have described DCN projections to the contralateral basilar pontine gray (PG) in several species including the rat (Lund and Webster; 1967), none has provided a precise description of their topographic distribution.

In view of the precise somatotopic distribution of the various DCN projections, and because electrophysiological studies have demonstrated tactile inputs from the fore- and hindlimb to be topographically organized in the pontine gray (Ruegg et al, 1977) and cerebellar hemispheres (Shambes et al., 1978), the first study of this dissertation examined whether DCN projections may distribute somatotopically within the PG. The normal topography of cuneo- and graciloportine projections was determined by using double anterograde tracing techniques within individual animals. Furthermore, because the DCN can be anatomically segregated into rostral and caudal subdivisions according to the physiological properties of neurons in these areas (see Dykes et al., 1982 for reviews), the exact origin of DCN projections was examined to determine whether these projections could be mediating tactile information to the cerebellar hemisphere via the PG. The injection of the retrograde tracer, horseradish peroxidase (HRP), into the DCN recipient zones of the PG was employed for this purpose. Evidence was provided for a somatotopic distribution of DCN-pontine originating from neurons located within regions of these nuclei that have been associated with the processing of highly specific tactile inputs.

Pontine afferents from the fore- and hindlimb sensorimotor cortices have been extensively investigated in the rat (Mihailoff et al., 1978; Kartje-Tillotson et al., 1981; Wiesendanger and Wiesendanger, 1982b) and were found to distribute somatotopically within the ipsilateral PG such that forelimb afferents are located mostly rostral to hindlimb afferents. Furthermore, electrophysiological studies have revealed a convergence within the PG of sensory cortical input and peripheral tactile input from corresponding regions of the animal's body surface (Ruegg et al., 1977) suggesting a possible anatomical convergence of ascending DCN inputs to the PG with descending sensory cortical projections. In order to determine whether such an anatomical overlap exists, the second study examined DCN-pontine projections in relation to fore- and hindlimb sensorimotor projections. Again, double anterograde tracing techniques were employed in individual animals to facilitate this analysis. Precise and partially overlapping pontine afferent patterns were revealed.

The analysis of DCN and sensorimotor cortical projections to the PG provided the anatomical framework for further studies concerning the possible remodelling of these projections in response to PG deafferentation by unilateral sensorimotor cortical ablations in the newborn. Such remodeling, commonly referred to as neural plasticity, can be very striking, particularly after neonatal lesions which often result in the redirection of axonal projections. Several studies have indicated that the formation of aberrant connections following neonatal

lesions is not a random process but instead may be a specific response to reinnervates neurons deprived of their normal input (Cunningham and Speas, 1975; Mustari and Lund, 1976; Finlay et al., 1979; Frost and Schneider, 1979). For example, disruption of pontine efferents by unilateral sensorimotor cortical lesions in newborn rats results in the formation of aberrant contralateral corticopontine fibers originating from the spared hemisphere. These aberrant fibers sprout across the midline to distribute to areas deprived of their normal cortical afferents by the neonatal cortical lesions (Leong, 1976a; Castro and Mihailoff, 1983). This remodeling, which has also been observed ultrastructurally to form synaptic contacts (Mihailoff and Castro, 1981), occurs after sensorimotor but not after occipital cortical lesions. This apparent specificity, expressed according to the location of the cortical lesion, has been further defined by recent studies demonstrating a topographic distribution of aberrant crossed fibers which corresponds to the normal ipsilateral distribution pattern (Kartje-Tillotson et al., 1981).

In addition to experimental paradigms based on deafferentation lesions, neuroplasticity has been commonly found after lesions which destroy target cells (see Lund, 1978; Cotman, 1978; Flohr and Precht, 1981 for reviews). In these cases, pathways which normally innervate the missing target cells alternatively projected to other areas. For example, neonatal hemicerebellectomy results in a considerable neuronal loss in the contralateral pontine gray; as a result, sensorimotor corticopontine projections, which normally innervate the missing cells,

formed aberrant crossed projections to the intact pontine gray (Leong, 1977; 1980; Castro and Mihailoff, 1983). These aberrant fibers were apparently able to successfully compete with the normal afferent inputs to the intact pontine gray.

In view of the corticopontine remodeling observed after cortical (deafferentation) and cerebellar (target removal) lesions, the third study was undertaken in search of remodeling of DCN afferents to the pontine gray using the same lesion conditions. The double anterograde tracing technique was used as in the previous studies to determine whether such DCN efferent remodeling occurred in a topographic fashion.

BACKGROUND

Peripheral stimulation of cutaneous inputs from one side of the rat's body surface will evoke electrical potentials in the contralateral primary (Welker, 1971; 1976) and secondary somatosensory cortices (Welker and Sinha, 1972) as well as in the ipsilateral cerebellum (Shambes et al., 1978). Although numerous pathways may be involved in this transmission, those mediated through the gracile and cuneate nuclei, commonly referred to as the dorsal column nuclei (DCN), to the somatosensory thalamus and basilar pontine gray (PG) are of particular importance to this dissertation.

Dorsal column nuclei - afferents, cytology and efferents

Afferent information coursing to higher levels of the neuraxis ascends within the dorsal funiculus of the spinal cord via long branches of dorsal root ganglion cells as the fasciculus gracilis or cuneatus. Fasciculus gracilis is primarily composed of fibers corresponding to the lower half of the animal's body and fasciculus cuneatus the upper half (Hand, 1966; Basbaum and Hand, 1973; Odutola, 1977; Ganchrow and Bernstein, 1981). Somatotopically, fibers from the tail and hindlimb are located most medially within fasciculus gracilis and fibers from the lower portion of the trunk are located laterally.

With respect to fasciculus cuneatus, fibers coursing from the forelimb appear most lateral while upper trunk fibers are located medially. This same topography is retained as these fasciculi terminate within the DCN, i.e., nuclei gracilis (hindlimb) and cuneatus (forelimb). Fibers transmitting cutaneous information with small receptive fields preferentially terminate throughout the more central regions of the DCN (caudal to the level of the obex), particularly in its dorsal aspects. Fibers transmitting cutaneous information with large receptive field properties and those associated with deeper, proprioceptive information terminate at more rostral, caudal and ventral regions of these brainstem nuclei (for reviews, see Dykes et al., 1982). The DCN thereby possess a specificity in relation to the physiological properties seen throughout their rostro-caudal axis.

Corresponding cytoarchitectonic studies in the monkey (Biedenbach, 1972), cat (Kuypers and Teurk, 1964; Hand, 1966; Keller and Hand, 1970; Ellis and Rustioni, 1981), and raccoon (Johnson et al., 1968) have demonstrated that the central regions of the DCN are characterized by large, rounded neurons with a dense plexus of short dendrites organized such that they appear as zones of cellular nests. The rostral, caudal and ventral regions of the DCN consist of small and large multiform neurons with long unbranched dendrites that are somewhat less organized into reticular zones. Although these physiological and cytoarchitectural patterns are recognized more readily in primates, cats and raccoons, similar patterns have been described for rodents (Basbaum and Hand, 1973; Odultola, 1977; Ganchrow

and Bernstein, 1981).

The efferent (i.e., medial lemniscal) connections of the DCN correspond to cytoarchitectural subdividions. For example, in the rat, cat and monkey, lemniscal fibers originating from the cell nest zone were found to distribute preferentially within the ventral posterior lateral nucleus (VPL) of the thalamus (Lund and Webster, 1967; Feldman and Kruger, 1980; Berkley, 1980). In comparison, the more rostral, ventral and caudal reticular zones of the DCN project to the medial portion of the posterior nuclear complex (POm) and the zona incerta (ZI) as well as additional thalamic regions including the magnocellular portion of the medial geniculate nucleus. In the above mentioned studies, gracilo- and cuneothalamic projections were observed to terminate somatotopically within the contralateral VPL such that the hindlimb (nucleus gracilis) is displayed caudal, lateral and dorsal to the forelimb (nucleus cuneatus) representation (Lund and Webster, 1967; Feldman and Kruger, 1980). Similar distribution patterns were identified within the contralateral POm and ZI of rats (Lund and Webster, 1967). Correspondingly, neurons within VPL respond to cutaneous inputs with small receptive field properties, while POm and ZI respond to polysensory inputs (Emmers, 1965; Davidson, 1965; Dykes et al., 1982).

In addition to the ZI and POm, other structures receiving afferents from the reticular regions of the DCN include the cerebellum, (Grant, 1962; Rinvik and Walberg, 1975; Cheek et al., 1975; Somana and Walberg, 1980; Feldman and Kruger, 1980; Gray et al., 1981), tectum and

inferior olivary complex (Groenewegen et al., 1975; Berkley, 1975; Berkley and Hand, 1976; Robards et al., 1976; Blomqvist et al., 1978; Robards, 1979; Edwards et al., 1979; Berkley et al., 1890), spinal cord (Burton and Loewy, 1976; 1977; Crutcher et al., 1978; Gray et al, 1981; Bromberg et al., 1981) and reticular formation (Salibi et al., 1980). Additionally, DCN projections to the PG have been briefly reported in the monkey and cat (Nauta and Kuypers, 1958), rat (Lund and Webster, 1967), hedgehog (Jane and Schroeder, 1971) and tree shrew (Schroeder and Jane, 1971). More recent studies reveal gracilo- and cuneopontine projections to distribute somatotopically within the contralateral caudal half of the PG in rats (Kosinski et al., 1982; Swenson et al., 1982; Swenson et al., in press). These latter studies found cuneopontine fibers located mostly rostral to graciloпontine fibers, but the origin of these fibers with respect to the cell nest or reticular zones of the DCN was not determined.

Thalamocortical and pontocerebellar projections

The VPL and POm nuclei of the thalamus and the PG further the transmission of sensory impulses to the cerebral and cerebellar cortices via ipsilateral thalamocortical projections and predominately contralateral pontocerebellar projections, respectively. The primary and secondary somatosensory cortices received ipsilateral thalamocortical projections from VPL in all species studies (Guillery et al., 1969; Hand and Morrison, 1970; Jones and Leavitt, 1973; Ralston and Sharp, 1973; Saporta and Kruger, 1977; Herkenham, 1979; Donoghue et

al., 1979; Spreafico et al., 1981; Lin and Chapin, 1981). Likewise, POm also projects to the ipsilateral primary and secondary sensory cortices (Herkenham, 1980; Spreafico et al., 1981; Lin and Chapin, 1981). In an autoradiographic investigation of thalamocortical projections in the rat (Herkenham, 1980), VPL distributed predominately throughout layer IV and the deeper portion of layer III of the first and second somatosensory cortices with sparse labeling observed in layer VI as well. The projections from POm differed regarding the primary and secondary areas; the projections to the primary area distributed throughout layers I and Va, whereas layers I and IV were labeled within the secondary area. The cortical connections of the ZI are less clear although they are thought to project to the primary and secondary sensory cortices in the rat (Wise and Jones, 1977) as well as the primary and secondary motor cortical areas (Bold and Neafsey, 1982).

Pontocerebellar projections from the caudal half of the PG, i.e., the recipient zone of DCN projections (Kosinski et al., 1982; Swenson et al., in press), traverse the middle cerebellar peduncle and distribute predominately throughout the contralateral, but also the ipsilateral, crus II and paramedian lobules (Mihailoff et al., 1981a, Mihailoff, in press) as well as the posterior vermal lobule VIII (Azizi et al., 1981) of the cerebellum. This divergence is at least partially attributed to axonal branching (Kawamura and Hashikawa, 1981; Mihailoff, in press). For example, using double retrograde fluorescent labeling methods, several neurons within the mid to caudal regions of

the PG were found to project bilaterally to either the same (homotopic) or different (heterotopic) regions of each cerebellar hemisphere (Mihailoff, in press). This double labeling of neurons was especially evident in the medial pontine nuclear region which has been previously suggested to contain bilaterally projecting cells (Castro and Mihailoff, 1983).

Cerebral and cerebellar topography

Electrophysiological studies coupled with cytoarchitectural techniques have provided detailed topographical analyses of the cerebral (Welker, 1981; 1976; Welker and Sinha, 1972; Hall and Lindholm, 1974; Donoghue and Wise, 1983) and cerebellar (Shambes et al., 1978; Joseph et al., 1978; Bower et al., 1981) cortices in the rat. Electrophysiologically, the rodent primary somatosensory and motor hindlimb cortical areas overlap almost completely comprising a single primary sensorimotor hindlimb representation (Hall and Linkholm, 1974; Donaghue and Wise, 1983). In comparison, the primary somatosensory and motor forelimb cortical areas only partially overlap (Hall and Lindholm, 1974; Donoghue and Wise, 1983). As found in other species, these primary cortical areas are located within the frontal and parietal cortices; however, in rodents their locations are typically referred to in relation to the bregma point of the skull with forelimb areas being rostral and hindlimb areas more caudal. The secondary somatosensory forelimb and hindlimb cortical areas can also be electrophysiologically identified on the lateral surface of each

hemisphere just above the rhinal sulcus (Welker and Sinha, 1972).

Somatosensory and motor cortical regions can also be distinguished cytoarchitecturally. The primary forelimb sensory cortical region is characterized by a dense granule cell layer IV. Somewhat medial and rostral to this sensory region is the primary forelimb motor cortical area which is characterized by a minimal layer IV and a more prominent layer V consisting of large pyramidal cells. The primary motor cortex has been further divided into medial and lateral portions according to cytoarchitectonics (Donoghue and Wise, 1983). The overlapping primary sensorimotor hindlimb cortical region reveals cytoarchitectural similarities between both the primary forelimb sensory and motor cortices possessing a dense granule layer IV and prominent layer V consisting of smaller pyramidal cells than found in the forelimb motor cortex (Welker, 1971; Donoghue et el., 1979). The secondary sensory cortical area exhibits a much less dense granule cell layer IV and is relatively uniform in appearance compared to the primary sensory area (Welker and Sinha, 1972).

Electrophysiological recording studies of cerebellar cortical activity evoked by peripheral cutaneous stimulation in rats revealed a "mosaic" or "fractured" type of cutaneous representation within the various cerebellar lobules (Shambes et al., 1978, Bower et al., 1981). For example, stimulation of facial skin areas evoked responses in patches across the ipsilateral paramedian obule (Shambes et al., 1978; Bower et al., 1981). In comparing peripheral and sensory cortical inputs to the cerebellar hemispheres, Bower et al. (1981) demonstrated

a highly organized topographic relationship in the paramedian lobule between inputs elicited from the contralateral sensory face regions of the cerebral cortex and from the corresponding ipsilateral side of the animal's face. Furthermore, the areas of the cerebral cortex being stimulated and the responsive region of the cerebellar cortex possessed the same receptive fields. Similarly, other electrophysiological studies have demonstrated that pontine neurons responsive to stimulation of the ipsilateral sensory forelimb areas of the cerebral cortex are also responsive to peripheral stimulation of the contralateral forelimb (Ruegg and Wiesendanger, 1975; Ruegg et al., 1977). Although this convergence within the PG was thought to occur through a transcortical circuit, it is possible that the convergence may occur at the level of the PG (Bower et al., 1981). In support of this hypothesis, recent anatomical studies demonstrate DCN projections to the contralateral PG (Swenson et al., in press) in areas which partially overlap with somatosensory corticopontine projections (Kosinski et al., 1982).

Corticothalamic projections

Several investigations have utilized anterograde and retrograde tracing techniques to determine the precise distribution of corticothalamic projections in various mammalian species including the rat (Wise, 1975; Jacobson and Trojanowski, 1975), cat (Jones and Powell, 1968; Romangnano and Maciewicz, 1975) and monkey (Jones and Powell, 1969; Friedman and Jones, 1981). Rodent corticothalamic

projections from the primary and secondary sensorimotor cortices were found to originate primarily from pyramidal cells located throughout layer IV and partially from cells in layer V (Wise, 1975; Jacobson and Trojanowski, 1975; Wise and Jones, 1977). The anterograde analysis of these projections has only been described in reference to the primary sensory cortical face and sensorimotor cortical hindlimb areas (Wise and Jones, 1977). Corticothalamic fibers from these primary sensory cortical regions terminate within the ipsilateral thalamus where they form dense connections within the ventral posterolateral (VPL) and ventral posteromedial (VPM) nuclear regions while less dense projections were observed within POm, ZI and the intralaminar nuclei. The sensory cortical face and hindlimb regions project somatotopically within the (VPM) and (VPL), respectively (Wise and Jones, 1977). In comparison, the topographic distribution observed within POm from these same cortical regions appeared reversed to that seen in the ventrobasal complex. Afferents from the facial somatosensory cortex distributed within the ventrolateral portion of POm while those from the hindlimb sensory cortex distributed within the dorsomedial portion (Wise and Jones, 1977). The precise distribution patterns of these sensory cortical projections to ZI or the intralaminar nuclei are less clear.

Pontine gray - cytology and afferents

The PG has been studied morphologically in a variety of species including the opossum (King et al., 1968; Mihailoff and King, 1975; Mihailoff, 1978a,b), rabbit and cat (Brodal and Jansen, 1946), rat

(Mihailoff et al., 1981b; Mihailoff and McArdle, 1981), monkey (Sunderland, 1940; Nyby and Jansen, 1951; Cooper and Fix, 1976; Cooper and Beal, 1978) and man (Olszewski and Baxter, 1954). These extensive investigations have described four principle pontine nuclear regions identified as medial, ventral, lateral and interpeduncular in reference to their position relative to the cerebral peduncle. These nuclear regions are further subdivided into subnuclei consisting of intrapeduncular, median, dorsomedian and dorsolateral nuclei. The various subdivisions of the PG however, do not possess any cytologically distinct boundaries or particular characteristics and therefore are only used for descriptive purposes (Mihailoff et al., 1981b).

Studies using Nissl and Golgi stains have further revealed complex dendritic and axonal arborizations within the pontine nuclei of the opossum (Mihailoff and King, 1975), rat (Mihailoff et al., 1981b), monkey (Cooper and Fox, 1976) and man (Ramon y Cajal, 1909). These studies have described two general varieties of pontine neurons, one having properties of a projection neuron and the other having properties of an interneuron, although there is some uncertainty concerning this latter type. In addition, many pontine neurons exhibit dendritic systems which extend into more than one afferent projection area thereby providing these cells with the ability to sample a variety of afferent inputs (Mihailoff et al., 1981b).

The laminar and topographic origin of corticopontine fibers has been investigated utilizing the retrograde HRP technique in the rat

(Wise and Jones, 1977; Wiesendanger and Wiesendanger, 1982a), cat (Kawamura and Chiba, 1979; Albus et al., 1981) and monkey (Jones and Wise, 1977). Following injections of HRP in the PG, labeled neurons are found within layer V and are predominately located within the somatosensory, motor and visual cortices. Less dense retrograde labeling has been associated with the cingulate, auditory and insular cortices as well.

Using anterograde tracing techniques in several species, sensorimotor corticopontine fibers were found to descend via the cerebral peduncle and terminate within the ipsilateral PG with only a slight contralateral projection. Each area of the sensorimotor cortex, i.e. primary forelimb and hindlimb cortical areas, appears to project throughout the rostrocaudal extent of the PG in discontinuous longitudinal columns or patches of terminal fibers as described for the opossum (Martin and King, 1968), rat (Mihailoff et al., 1978; Kartje-Tillotson et al., 1981; Wiesendanger and Wiesendanger, 1982b, Kosinski et al, 1982), cat (Brodal, 1968) and monkey (Dhanarajan et al., 1977, Brodal, 1978). Analysis of efferents from discrete areas of sensorimotor cortex revealed divergent, somatotopic projections to multiple regions of the PG (for reviews see Brodal, 1982). Combining two anterograde tracing techniques within a single rat has demonstrated a virtually non-overlapping topographic distribution of fore- and hindlimb corticopontine fibers within the various pontine areas receiving cortical inputs (Kartje-Tillotson et al, 1981). In preliminary studies (Kosinski et al., 1982), which have been expanded

upon in this dissertation, double anterograde tracing methods were also used to demonstrate the overlapping and non-overlapping nature of corticopontine fibers in relation to PG afferents from the DCN. These findings (fully presented below in Chapter 2) support physiological investigations demonstrating a convergence of inputs to the same pontine neurons following forelimb sensory cortex and forelimb tactile stimulation (Ruegg and Wiesendanger, 1975; Ruegg et al., 1977). These latter studies indicate that the PG may play a more integrative role rather than merely being a relay for the transmission of afferent inputs to the overlying cerebellum.

Plasticity Overview

The newborn developing nervous system demonstrates a remarkable ability to form anomalous connections in response to lesions incurred during the neonatal period (see Cotman, 1978; Lund, 1978; Tsukahara, 1981; Flohr and Precht, 1981 for reviews). The formation of these anomalous connections, referred to as neuronal remodeling or plasticity, has been well documented throughout all levels of the neuraxis for various species. Although the functional significance of plasticity is not clearly established, several studies have correlated recovery of lost behavioral tasks with the formation of anomalous connections suggesting that these connections might provide the anatomical basis for the observed behavioral recovery (Hicks and D'Amato, 1970; 1975; Schneider and Jhaveri, 1974; Goldberger and Murray, 1978; Laurence and Stein, 1978; Finlay et al, 1979; Goldberg,

1981).

Two types of experimental procedures are commonly employed in studies of neural plasticity: 1) the deafferentation of neuronal areas by lesions partially disrupting their afferent inputs thus providing available synaptic space within these regions and 2) the removal or ablation of a neuronal target which deprives growing axons of their normal synaptic contacts. In studies using deafferentation lesions in newborn animals, axons which normally innervate regions adjacent to the deafferented areas as well as other more remote fiber systems may "sprout", i.e., increase their terminal distribution, to innervate the neurons deprived of their normal afferent input. Likewise, in studies based on target removal lesions, axons deprived of their normal areas of innervation may increase their synaptic contacts with other targets that they may or may not normally innervate. This anomalous growth of axons in response to the neonatal removal of their neuronal target has been described in reference to an axon's ability to conserve or maintain a specific quantity of its terminal arborizations (for reviews see Devor and Schneider, 1975). Although these concepts of neuronal plasticity were originally interpreted regarding the remodeling of visual (Schneider, 1970; 1973) and motor pathways (Hicks and D'Amato, 1970) more recent studies have related them to neuonal remodeling in numerous systems including olfactory, auditory, limbic, and somatosensory (see Cotman, 1978; Lund, 1978; Tsukahara, 1981, Flohr and Precht, 1981; Finger and Stein, 1982 for reviews).

Cortical efferent plasticity

Neonatally induced lesions of one cerebral hemisphere result in the formation of anomalous corticofugal projections from the intact, unablated hemisphere which in general distribute to areas deafferented by the cortical lesion. These areas include the spinal cord (Hicks and D'Amato, 1970; Leong and Lund, 1973; Castro, 1975), DCN and caudal medullary reticular formation (Hicks and D'Amato, 1970; Leong, 1976a), as well as the trigeminal complex (Hicks and D'Amato, 1970), tectum (Lund and Lund, 1971; Leong and Lund, 1973; Leong, 1976a,b), red nucleus (Nah and Leong, 1976a,b; Sonnier et al., 1982), pretectum (Leong, 1976a), thalamus (Donoghue and Wells, 1977; Neuman et al., 1982), striatum (Coldman, 1978) and pontine gray (see below).

Corticopontine fibers, primarily an ipsilateral system, demonstrate an increased crossed distribution after unilateral sensorimotor cortical ablations in neonatal rats (Leong and Lund, 1973; Leong, 1976a,b; Mihailoff and Castro, 1981; Kartie-Tillotson et al., 1981; Castro and Mihailoff, 1983). These aberrant projections, which appear to represent an expansion of a small, contralateral component rather than a persistence of fibers which are normally more profuse during early developmental periods (Nah et al., 1980), distribute within areas deprived of inputs by the neonatal cortical ablation. Further work using double anterograde tracing techniques to map efferents from forelimb motor and hindlimb sensorimotor cortex demonstrated that the aberrant, crossed corticopontine fibers

distribute in a somatotopic pattern similar to the normal ipsilateral distribution pattern (Kartje-Tillotson et al., 1981). These studies indicate that plasticity, at least under these conditions, is not a random process resulting in the formation of meaningless connections and therefore support the concept that remodeling may contribute to functional recovery.

As an example of plasticity after target removal, an increase in crossed corticopontine fibers is also observed after neonatal hemicerebellar lesions (Leong, 1977; Castro and Mihailoff, 1983). These lesions result in considerable cell loss in the contralateral PG and cause cortical efferent fibers, which normally innervate the missing neurons, to cross and innervate pontine neurons on the intact side. These aberrant fibers, which apparently are successful in competing with normal pontine afferents, are found to be more dense when deafferenting cortical lesions are combined with hemicerebellectomy in newborn rats (Castro and Mihailoff, 1983). Such results indicate that neonatal deafferentation apparently providing synaptic space) or target removal (allowing for the tendency of axons to conserve their terminal arborizations) may act independently in influencing the remodeling of corticopontine projections observed after each lesion approach. Furthermore, these processes may be additive when combined within the same neuronal system (Castro and Mihailoff, 1983).

Plasticity of ascending systems

Using electrophysiological techniques, reorganizations in cutaneous receptive field maps have been described following lesions of the contralateral nucleus gracilis in adult rats (Wall and Egger, 1971). After such lesions, there is an initial loss of responsiveness in the representative hindlimb region of VPL to stimulations of the contralateral fore- or hindlimb. However, after three to seven days, these previously unresponsive cells begin to respond to stimulations of the hand and arm demonstrating a concomitant expansion of the foreleg representation into the hindlimb region of the VPL thalamus. This latent shift in responsiveness was suggested to reflect the sprouting of intact cuneothalamic terminals into neuronal regions previously occupied by nucleus gracilis terminals (Wall and Egger, 1971).

Electron microscopic examination of DCN projections following partial lesions of both nucleus gracilis and cuneatus in the adult rat (Tripp and Wells, 1978) revealed terminal degeneration within VPL to approximately sixty percent of control levels by 2-6 days postlesion. However, at fifty days postlesion, the medial lemniscal terminals were found to have not only returned to control levels, but also appeared relatively normal indicating the formation of new lemniscal inputs (Tripp and Wells, 1978). These results support electrophysiological data (Wee'71) indicating that cuneothalamic projections remodel into denervated regions of the VPL nucleus of the thalamus following DCN ablations in the adult rat.

Although neuronal plasticity is generally accepted to be more extensive following lesions in the neonatal period (see Cotman, 1978;

Tsukahara, 1981; Flohr and Precht, 1981), only one study has examined projections from the DCN after neonatal lesions (Frost, 1981). In an attempt to redirect ascending lemniscal fibers to the dorsolateral geniculate nucleus (DLG), sensorimotor cortical lesions were combined with enucleation in newborn hamsters (Frost, 1981). The cortical lesions produced market atrophy in VPL, a major terminal zone of ML fibers, and enucleation caused a massive deafferentation in the DLG. By this design, ML fibers were provided alternative sites within the DLG in the face of lesions which destroyed their normal targets within VPL, but remodelling into the DLG was not observed. Because medial lemniscal fibers appear well developed at parturition in the hamster, Frost (1981) suggested that these fibers may have lost their ability to conserve their axonal arborizations by forming anomalous connections. Alternatively, the ascending medial lemniscal fibers may have increased their terminal aborizations within nonthalamic loci that were neonatally deprived of afferents by the cortical lesions. This would require the analysis of nonthalamic DCN efferent projections following the same neonatal cortical lesions.

In addition to the remodelling of DCN efferent projections, previous studies have demonstrated remodeling of cerebellar afferent (Castro and Hazlett, 1979) and efferent connections following neonatal hemicerebellar lesions (Lim and Leong, 1975; Castro, 1975; Leong, 1977; Kawaguchi et al., 1979a,b; Fujito et al., 1980). Normally, spinocerebellar fibers enter the ipsilateral cerebellum and decussate within the cerebellar white matter to distribute bilaterally as mossy

fibers. As described for rats, neonatal hemicerebellar lesions destroy the neuronal target of the normal contralateral branches of spinocerebellar fibers causing the ipsilateral fibers within the spared cerebellar hemisphere to increase these terminal distribution (Castro and Hazlett, 1979). This ipsilateral increase in terminal distribution may have been augmented by the additional affect of deafferentation on cerebellar granule cells by the interruption of normal contralateral spinocerebellar fibers that course through the cerebellum in route to the spared cerebellar hemisphere.

Neonatal hemicerebellar lesions also result in the formation of aberrant cerebellar projections to the ipsilateral red nucleus and ventrolateral nucleus of the thalamus in rats (Lim and Leong, 1975; Castro, 1975; Leong, 1977) and kittens (Kawaguchi et al., 1979a; Fujito et al., 1980). This formation of aberrant cerebellar fibers was also demonstrated electrophysiologically in kittens (Kawaguchi et al., 1979b). The formation of bilateral cerebellar efferents after neonatal removal of one cerebellum is similar to the observed bilaterality of cortical efferent projections following unilateral cortical or cerebellar ablations in newborn animals (described above in the section on cortical efferent plasticity).

Origin and Distribution of Dorsal Column Nuclear Projections to the Basilar Pontine Gray in Rats

ABSTRACT

While a few studies have reported dorsal column nuclear projections to the pontine gray, none have provided a detailed examination of these pathways. In the present study, the distribution patterns of pontine afferents from the gracile and cuneate (dorsal column) nuclei were studied by combining two anterograde axonal tracing methods, the Fink-Heimer technique and autoradiography within individual animals. The retrograde horseradish peroxidase technique was also employed to determine the precise origin of these fibers. Results revealed somatotopic dorsal column nuclear projections within the caudal half of the contralateral pontine gray. Specifically, gracilo- and cuneopontine fibers distributed predominantly within two pontine nuclear regions, one in the medial pontine nucleus and another overlapping the ventral and lateral nuclei. Within these regions, fibers from nucleus cuneatus distributed rostrally to gracilis afferents. Following unilateral injections of horseradish peroxidase into the caudal pontine gray, retrogradely labeled neurons were found in the contralateral dorsal column nuclei at levels primarily caudal to the obex.

Consideration of these afferents in relation to previous reports concerning the distribution of corticopontine pathways and the topology of pontocerebellar projections suggests an integration within the pontine gray of neuronal activity comprising ascending and descending somatosensory systems which is relayed to the cerebellar cortex.

INTRODUCTION

In spite of numerous investigations of cerebral cortical (Mihailoff et al., 1978; Kartje-Tillotson et al., 1981; Wiesendanger and Wiesendanger, 1982b), cerebellar (Watt and Mihailoff, 1983), tectal (Burne et al., 1981) and spinal (Swenson et al., in press) inputs to the basilar pontine gray (PG), few studies have described ascending brainstem inputs, particularly from the dorsal column nuclei (DCN), to this large nuclear complex. Collaterals of medial lemniscal fibers distributing within the medial portion of the PG were first described in cats as "collaterales sensitives' by Ramon y Cajal (1909). Using degeneration staining methods following lesions of the DCN in cats, Walberg and Brodal (1953) reported bilateral inputs within rostral peduncular regions of the PG. Subsequent studies, also using degeneration staining methods after DCN lesions, have only briefly reported contralateral gracilo- and cuneopontine fibers in the rat (Lund and Webster, 1967) and in the cat and monkey (Nauta and Kuypers, 1958). Furthermore, these studies provide no information concerning the pattern of input. Similarly, PG afferents from the DCN of the tree shrew (Schroeder and Jane, 1971) and hedgehog (Jane and Schroeder, 1971) were merely described as "small" and "minimal" to the medial portion of the contralateral PG. Several other studies of DCN

projections in various species (Matzke, 1951; Bowsher, 1958; Hazlett et al., 1972; Berkley and Hand, 1978) make no mention regarding PG afferents from the DCN.

In view of the paucity of literature as well as the reported discrepancies regarding PG afferents from the DCN, the present study was initiated to investigate the origin, course and somatotopic distribution of DCN projections to the PG in rats. Subsequent studies examined these pontine afferents in relation to motor and somatosensory corticopontine projection patterns (Kosinski et al., 1982).

MATERIALS AND METHODS

Adult Long-Evans, blackhooded rats were used in this study. Two anterograde tracing techniques were combined in eight individual animals: 1) the Fink-Heimer stain for impregnation of degenerating axons and 2) autoradiography based on the neuronal uptake and axonal transport of injected tritiated leucine. These techniques were combined in order to determine the course and distribution pattern of the DCN projections as well as their relationship to one another.

Prior to surgery, animals were anesthetized with ketamine hydrochloride (100 mg/kg i.m., with supplemental doses as needed) and placed in a sterotaxic apparatus. The atlanto-occipital membrane was reflected to reveal the underlying DCN. Using a glass tipped (30-50 um diameter) 1 ul Hamilton syringe, 0.01 - 0.05 ul of tritiated leucine (50 uCi/ul) was injected into nucleus cuneatus and the adjacent nucleus gracilis was aspirated with a finely drawn glass pipette. Injection and lesion sites were typically made at levels just caudal to the obex.

After a seven day survival period, animals were sacrificed with an overdose of sodium pentobarbitol and perfused transcardially with physiological saline followed by 10% buffered formalin. Brains were removed and frozen sections were serially cut at 40 um in either the transverse or sagital plane. Every fourth section was used for the

Fink-Heimer technique and two sets of adjacent sections were processed autoradiographically (Cowan and Cuneod, 1975). The autoradiographic material was coated with NTB-2 emulsion and after 4 and 12 week exposure periods, developed with Kodak D-19 and counterstained with cresyl violet.

In order to determine the cells of origin of the DCN projections to the PG, 0.02-0.04 ul of horseradish peroxidase (HRP) conjugated to wheat germ agglutinin and dissolved in saline was sterotaxically injected into the caudal half of the PG in four animals. The HRP was delivered using a 1 ul Hamilton syringe with a micropipette (30-50 um diameter) cemented to its tip. In order to minimize possible uptake of the enzymatic tracer by the dorsally situated medial lemniscal fibers, a ventral surgical approach was used. Because of this approach, the trachea was intubated and then retracted along with the esophagus. The omohyoid muscle on one side was reflected as were the underlying superior laryngeal nerve and superior thyroid artery; the longissimus capitus muscles were also retracted revealing the underlying basal occiput. An opening in this bone was made with a dental drill; the dura was reflected and the micropipette inserted to a depth of 0.1-0.2 mm. Animals were sacrificed 1-2 days postoperatively and perfused transcardially with physiological saline followed by a 1% paraformaldehyde/1.25% gluteraldehyde solution and then by a 10% buffered sucrose solution. Brains were then removed and frozen sections cut serially at 40um in either the transverse or sagital plane. Sections were reacted with tetramethyl benzadine (TMB)

according to the protocol of Mesulam (1978). Every other section was couterstained with 1% pyronin Y for cytoarchitectural identification.

The distribution of degenerated or autoradiographically labeled axons within the PG and retrogradely labeled HRP-positive cells within the DCN was plotted using a camera lucida drawing tube. Terminology for the various pontine nuclear regions, as depicted in Figure 1a. was derived from a previous cytoarchitectural study of the PG (Mihailoff et al., 1981b).

RESULTS

Anterograde analysis

Alternate sections processed by the Fink-Heimer and autoradiographic methods revealed each lesion/injection site to be positioned just below the level of the obex (Figure 1b). Although coarse degenerative fibers from nucleus gracilis coursed through the medial aspect of nucleus cuneatus, the lesions of nucleus gracilis were consistently found not to encroach upon the more laterally and ventrally located cuneate nucleus. Fine degeneration observed within nucleus cuneatus but not emanating from it, is attributed to damage of the overlying fasciculus by the injection pipette. Additionally, the coincidental analysis of the topographic distribution of DCN projections to the ventral posterolateral (VPL) nucleus of the thalamus provided an internal control for the accurate placement of the lesion/injections. In agreement with previous reports (Lund and Webster, 1967; Feldman and Krugar, 1980), projections from nucleus gracilis were localized dorsolaterally and caudally to cuneatus projections within the contralateral VPL. Furthermore, densities of fiber bundles coursing through the medial lemniscus as well as distribution patterns within VPL appeared comparable for both tracing techniques.

The distribution of degenerative graciloPontine and radiolabeled cuneopontine fibers which coursed through the medial lemniscus to the contralateral PG is presented in Figure 2. At the most caudal pontine levels, graciloPontine fibers departed ventrally at right angles to the medial lemniscus, and cuneopontine fibers departed in the same manner at a slightly more rostral level. These pathways then passed medially, laterally and directly through the crus cerebri to distribute within the PG, and both demonstrated two major areas of termination within the caudal half of the PG -- one in the medial pontine nucleus (with some extension into the ventral nucleus) and the other in an area overlapping the ventral and lateral pontine nuclei (Figure 2).

While both pathways terminated in similar areas, they also demonstrated a distinctive somatotopy. Degenerative graciloPontine projections occupied the extreme medial, ventral and caudal portions of the medial pontine nucleus while somewhat more densely labeled cuneopontine fibers distributed slightly more rostrally, dorsally and laterally (Figures 2 and 3). Within the overlapping region of the lateral and ventral pontine nuclei, cuneatus projections distributed mostly rostral to those from nucleus gracilis (Figures 2 and 3). Although the contralateral dorsal peduncular nuclear region also appeared to receive DCN afferents, accurate analysis was made difficult by the proximity of heavily degenerative or radiolabeled medial lemniscal fibers. No projections were found rostral to mid PG levels from either DCN.

Retrograde analysis

Histological inspection demonstrated that the HRP injections were confined within the caudal PG in three of four animals (Figures 4 and 5); no damage of the medial lemniscus was found in these three animals. Although some HRP spread across the midline, reactive neurons, which were predominantly round to oval in shape and of rather uniform size, were only found contralaterally within the DCN. Within nucleus gracilis, the densest labeling was observed within its lateral and medial portions at levels ranging from the obex to approximately 600um caudally (Figures 4 and 5). An occasional reactive neuron could also be found at slightly more caudal levels. In comparison, a seemingly larger number of labeled cells were found throughout nucleus cuneatus except for its most rostral and caudal extent. The highest density of reactive neurons was primarily located dorsomedially in the cuneate nucleus at levels comparable to the position of labeled cells in nucleus gracilis (Figures 4 and 5).

DISCUSSION

The present description of DCN projections to the caudo-medial portion of the contralateral PG supports and extends previous findings obtained using the cat and monkey (Nauta and Kuypers, 1958), rat (Lund and Webster, 1967), tree shrew (Schroeder and Jane, 1971) and hedgehog (Jane and Schroeder, 1971). Additional new evidence is provided for DCN projections to an area overlapping the ventral and lateral pontine nuclear regions. Also, these pathways were found to distribute somatotopically, a feature which is characteristic of other pontine afferents.

The somatotopic pattern of DCN projections to the PG is reminiscent of afferents from primary sensorimotor cortex. Corticopontine projections from the fore- and hindlimb areas of the rat primary sensorimotor cortex distribute in discontinuous, longitudinal columns throughout the PG with hindlimb cortical afferents located mostly caudal to forelimb cortical afferents (Mihailoff et al., 1978; Kartje-Tillotson et al., 1981; Wiesendanger and Wiesendanger, 1982b). Similar to graciloponting projections, hindlimb corticopontine projections are described to be marginated along the ventral surface of the caudal PG whereas the forelimb projections are located in a slightly more dorsal and rostral position, much like the present description of cuneopontine fibers. Comparing DCN afferents to the PG

to previous reports of corticopontine afferents suggests a partial overlap in the distribution patterns of descending hindlimb corticopontine projections and ascending graciloportine projections, but overlap between forelimb corticopontine afferents and cuneopontine afferents is less apparent. Further experiments specifically designed to determine the extent of overlap between corticopontine and DCN pontine afferents within the caudal PG are presented in the next chapter of this dissertation.

Anatomical studies of the origin of pontocerebellar fibers in rats have demonstrated substantial retrograde labeling of neurons throughout the caudal half of the contralateral PG after HRP injections in various cerebellar lobules (Burne et al., 1978a; Azizi et al., 1981; Mihailoff et al., 1981a; Mihailoff in press). Comparison of these data to our findings indicates that afferent information to the PG from the DCN is relayed via pontocerebellar fibers to the vermal lobule VIII and the paramedian lobule. Specifically, pontine afferents from nucleus gracilis distribute among neurons projecting to the vermal lobule VIII (see Azizi et al., 1981) and the paramedian lobule (Mihailoff et al., 1981a) whereas cuneopontine projections partially correspond with the origins of pontocerebellar fibers to the paramedian lobule (see Mihailoff et al., 1981a). Corresponding electrophysiological studies demonstrate that peripherally evoked tactile inputs from the fore- and hindlimb are topographically organized within the ipsilateral paramedian lobule of rats (Shambes et al., 1978). These excitatory receptive fields were found to be similar to those recorded from the

cerebral primary sensory cortical area after the application of the same stimulus. Although direct DCN efferents to the cerebellum were suggested to mediate this cutaneous input to the paramedian lobule (Shambes et al., 1978), the apparent overlap within the PG of recipient zones of DCN afferents and the origin of pontocerebellar fibers to the paramedian lobule provides an alternative anatomical substrate.

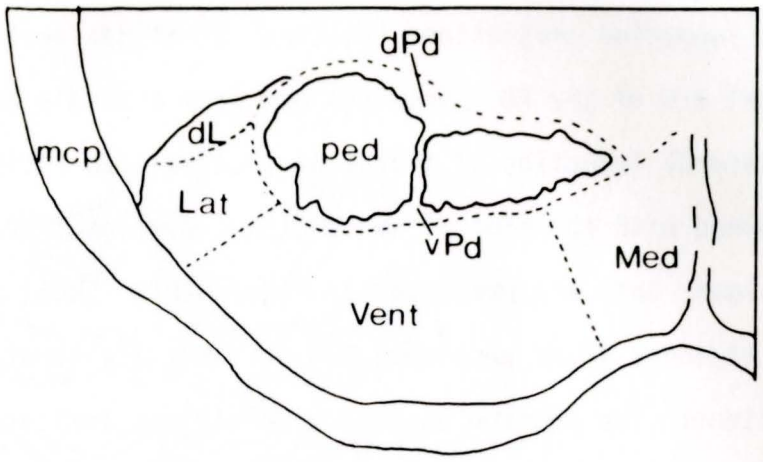
In contrast to the location of DCN neurons labeled after HRP injections into the cerebellar hemisphere of rats, i.e. rostral to the obex (Feldman and Kruger, 1980), both nucleus gracilis and cuneatus demonstrated round to oval reactive neurons at levels candal to the obex following injections of HRP into the contralateral caudal PG, as described in the present study. A greater number of similar, round to oval cells were identified within this same region caudal to the obex following HRP injections into the contralateral VPL nucleus of the rodent thalamus (Tan and Lieberman, 1978; Feldman and Kruger, 1980). The location of these neurons labeled after either PG or thalamic injections corresponds to the feline cell nest region of the DCN which is characterized by cells responding to cutaneous inputs with a high degree of place and modality specificly (Dykes et al., 1982). This data supports the possibility that the DCN relay somatosensory input to the cerebellum via the PG. In addition, further double labeling studies are needed to determine whether DCN pontine projections are actually collaterals of medial lemniscal fibers destined for the VPL nucleus of the thalamus.

The results obtained from the present study suggest that DCN

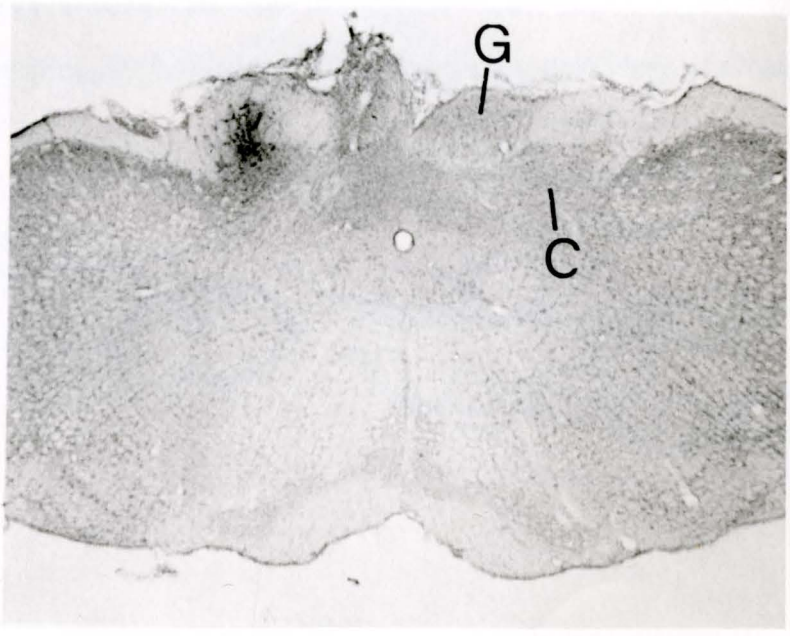
efferents to the PG relay somatosensory information, characterized by a high degree of place and modality specificity, from the fore- and hindlimb to the contralateral caudal half of the PG. This information may then be integrated with descending sensorimotor corticopontine inputs as indicated by previous electrophysiological studies which demonstrate that some pontine neurons respond to fore- and hindlimb sensory cortical stimulation as well as to tactile stimulation of the corresponding extremity (Ruegg and Wiesendanger, 1975; Ruegg et al., 1977). This integration is further examined anatomically by the experiments presented in the next chapter.

Fig. 1.a. Diagramatic illustration of the principal nuclei of the basilar pontine gray as modified from Mihailoff et al., (1981b). dPD,dorsal peduncular nucleus; vPd,ventral peduncular nucleus; dL, dorsalateral nucleus; Med,medial nucleus; Lat,lateral nucleus; Vent,ventral nucleus; mop,middle cerebellar peduncular; ped,cerebral peduncle.

Fig. 1.b. Low power photomicrograph of a representative cross section through the medulla of an animal having sustained a lesion of the left nucleus gracilis and an injection of the left nucleus cuneatus. Camera lucida drawings of the lesion/injection are depicted in Figure 2. Cresyl violet stain. Abbreviations: C,nucleus cuneatus; G,nucleus gracilis.



a



b

Fig. 2. Camera lucida drawings depicting the distribution pattern of radiolabeled cuneopontine (sections A-H) and degenerative graciloponti̇ne projections (sections A'-H') as seen throughout the caudal 4/5 of the PG. Drawings are from a single animal following a unilateral injection of tritiated leucine into nucleus cuneatus combined with ablation of the adjacent nucleus gracilis (depicted in the lower left and presented in Figure 1b). Sections A-A', B-B', etc. are adjacent 40 um sections; 200 um intervals separate the paired sections. The pontine nuclear subdivisions indicated in level D correspond to Figure 1a. A similar format is used in subsequent figures. Rectangles labeled A, B and C correspond to photographs presented in figure 3. Abbreviations: MCP,middle cerebellar peduncle; ML,medial lemniscus; ped,cerebral peduncle; PG,pontine gray.

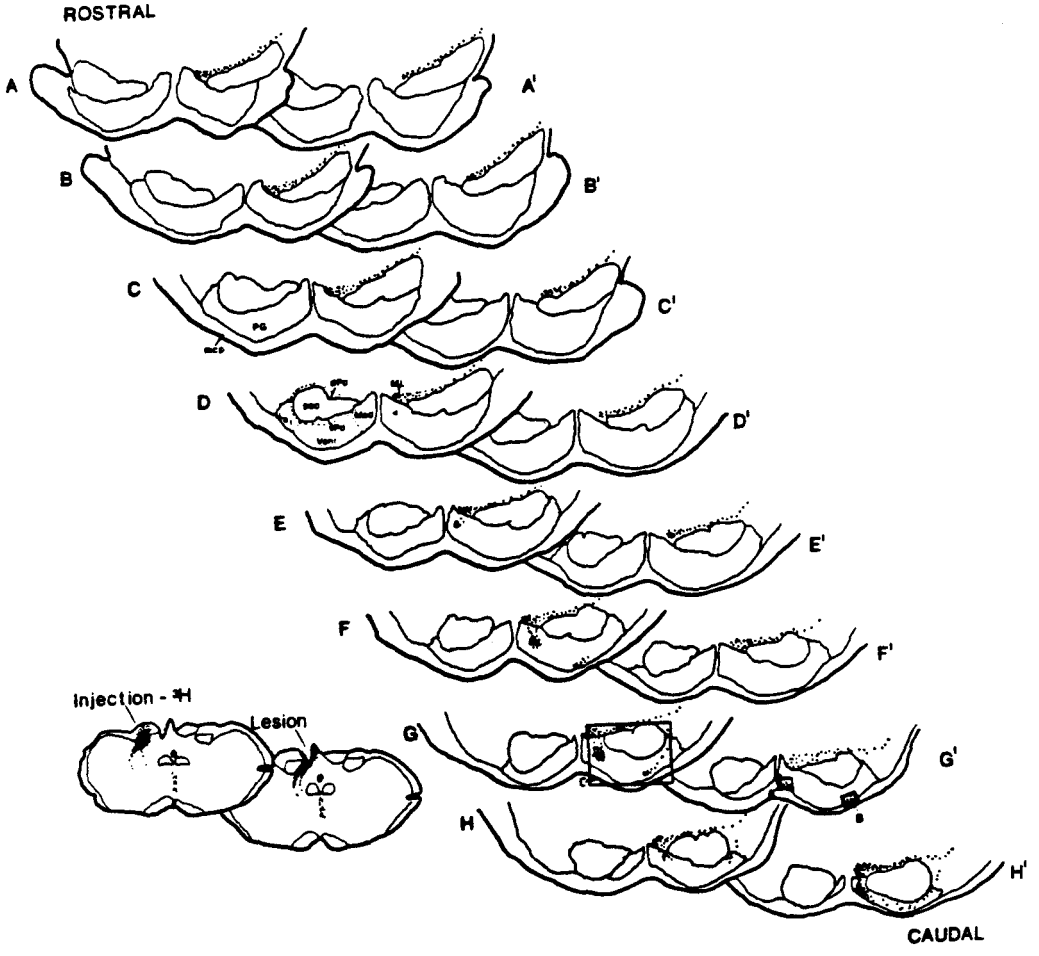

ROSTRAL
A
A'
B
B'
C
C'
D
D'
E
E'
F
F'
Injection - *H
Lesion
G
G'
H
H'
CAUDAL

Fig. 3. Photomicrographs corresponding to boxes labeled A-C in Figure 2. A. Medial pontine nucleus, F-H stain, 384x. B. Ventral pontine nucleus, F-H stain, 384x. C. Medial-Lateral pontine nuclei. Autoradiography (darkfield). 60.5x. Abbreviations: ML,medial lemniscus; ped,cerebral peduncle.

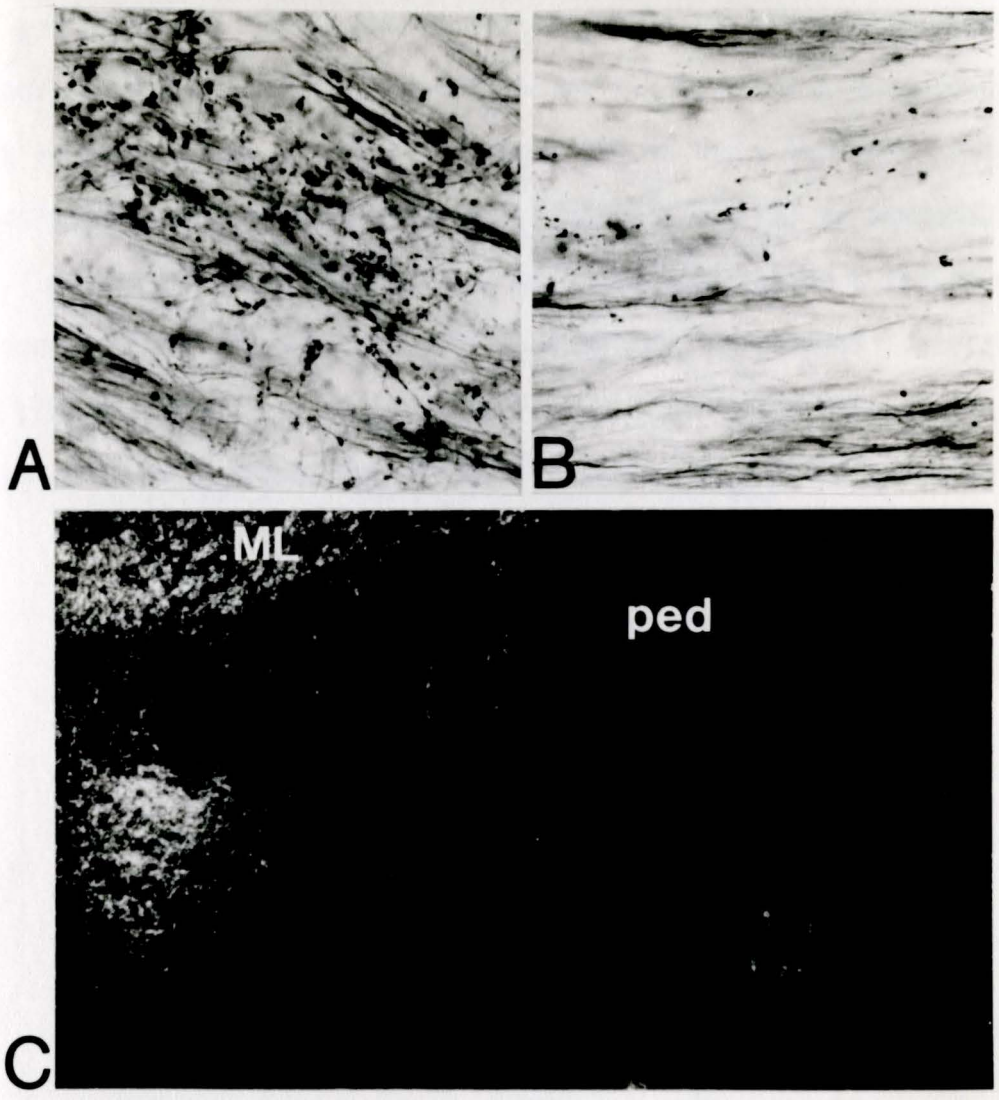

A
B
ML
ped
C

Fig 4. Sequence of camera lucida drawings at 160 micron intervals through the middle 1/3 of the dorsal column nuclei demonstrating the distribution pattern of retrogradely labeled cuneo- and graciloпontine neurons observed after an HRP injection into the caudal pontine gray as illustrated by the top drawing. The labeling within each section represents a composite taken from that section and 3 adjoining sections. Rectangles labeled A, B and C correspond to photographs in figure 5. Abbreviations: C,nucleus cuneatus; cc,central canal; C,nucleus gracilis; ped,cerebral peduncle; PG,pontine gray; o,obex.

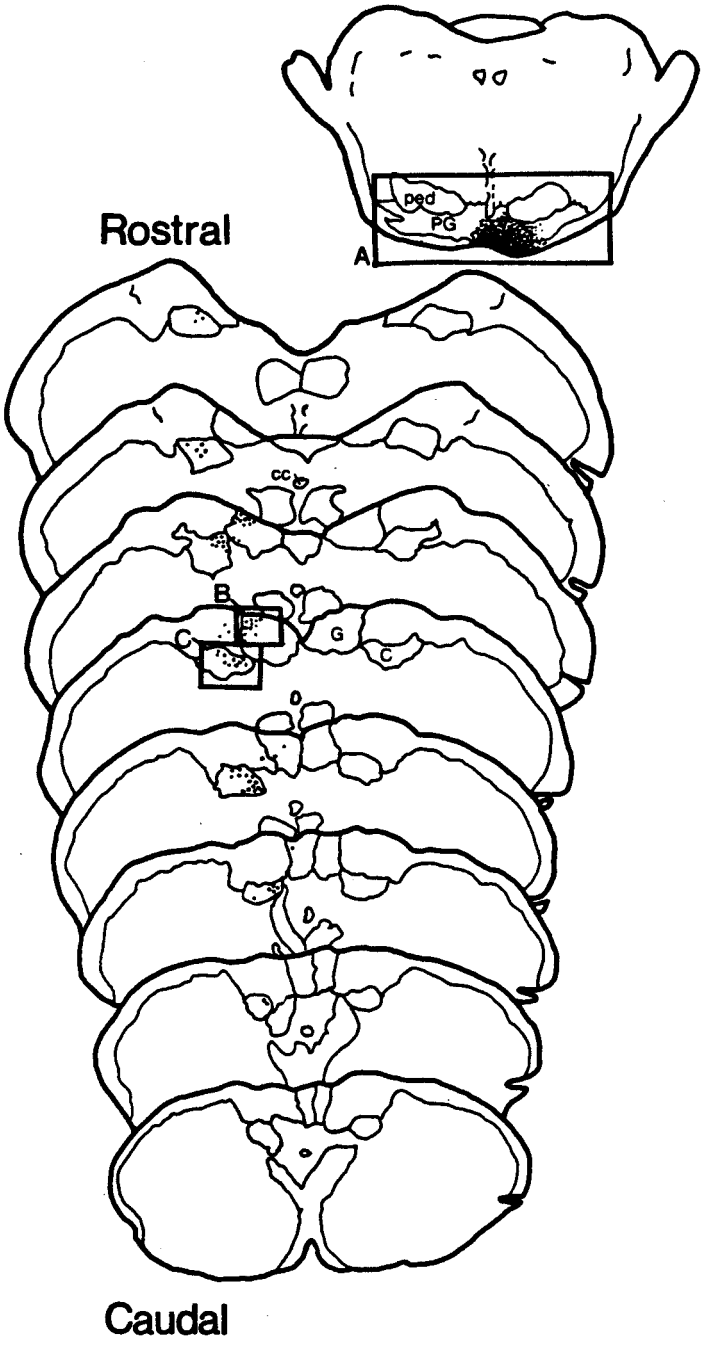
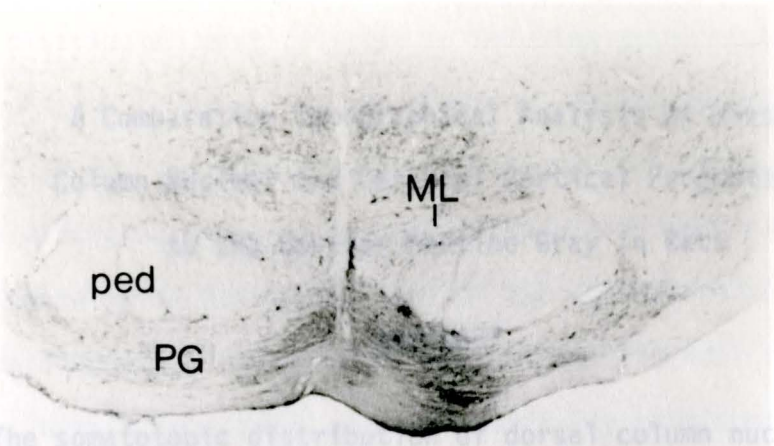

Rostral
ped
PG
A
cc
B
C
G
C
D
D
D
Caudal

Fig. 5. Photomicrographs corresponding to boxes labeled A-C in Figure 4. A. Caudal PG injection site of horseradish peroxidase, pyronin-Y stain, 24x. B. Nucleus gracilis darkfield-unstained, 153.6x. C. Nucleus cuneatus, darkfield-unstained, 153.6x. Abbreviations: ML,medial lemniscus; ped,cerebral peduncle; PG,pontine gray.



A

B

C

A Comparative Topographical Analysis of Dorsal Column Nuclear and Cerebral Cortical Projections to the Basilar Pontine Gray in Rats

ABSTRACT

The somatotopic distribution of dorsal column nuclear projections within the basilar pontine gray (as described in the preceding chapter) was re-examined in relation to the massive corticopontine projection system emanating most heavily from motor and somatosensory cortex. The distribution patterns of these two systems were compared combining autoradiographic and degeneration axonal tracing methods within individual animals.

Stereotaxic injections of tritiated leucine (50uCl/ul) and lesions by aspiration were made in animals under ketamine hydrochloride anesthesia. The injections (0.1-0.3ul) were centered in either the right forelimb sensory, motor or the hindlimb sensorimotor cortical areas as determined by intracortical microstimulation and multiunit recording techniques while the left dorsal column nucleus cuneatus or gracilis was aspirated.

The separate sensory and motor forelimb corticopontine fibers terminated in alternating zones or patches within discrete regions of the ipsilateral pontine gray. The hindlimb sensorimotor corticopontine

fibers distributed mostly caudal to forelimb projections, and at far caudal pontine levels, hindlimb afferents merged forming a dense band of label across the entire extent of the pontine gray. Similarly, pontine afferents from the dorsal column nuclei terminated somatotopically in the caudal half of the contralateral pontine gray with graciloпontine fibers distributing mostly caudal to cuneopontine fibers. Within individual animals, partially overlapping terminations were seen from nucleus cuneatus and the forelimb sensory cortical areas as well as from nucleus gracilis and the hindlimb sensorimotor cortical area. No overlap existed in the pontine terminations from nucleus cuneatus and the forelimb motor cortical area.

INTRODUCTION

Previous anatomical studies in the rat demonstrated somatotopic projections from the hindlimb sensorimotor cortex (Mihailoff et al., 1978; Karje-Tillotson et al., 1981; Wiesendanger and Wiesendanger, 1982b) as well as from the forelimb sensory (Wise and Jones, 1977; Mihailoff et al., 1978) and motor cortical areas (Kartje-Tillotson et al., 1981; Wiesendanger and Wiesendanger; 1982b) to the ipsilateral pontine gray (PG). Pontine afferents from the dorsal column nuclei (DCN), gracilis and cuneatus, were also found to project somatotopically within the caudal half of the contralateral PG in rats (Swenson et al., in press). The distribution patterns of afferents from the DCN were described as "reminiscent" of descending corticopontine projections from the fore- and hindlimb regions of the rat sensorimotor cortex (Experiment 1).

Electrophysiological recording studies of cerebellar cortex combining cerebral cortical and peripheral cutaneous stimulation revealed an overlapping topographical relationship within the cerebellar granule layers between activity elicited through stimulation of the contralateral primary sensory cortical area and the corresponding ipsilateral region of the animal's body surface (Bower et al., 1981). Bower et al. (1981) have suggested that the electrophysiological overlap of cutaneous and cortical inputs within

the cerebellar cortex may be organized at the level of the pontine gray (PG). This view concurs with previous studies demonstrating that various pontine neurons are responsive to both stimulation of the ipsilateral fore- and hindlimb area of the somatosensory cortex and the corresponding contralateral limb in cats (Ruegg and Wiesendanger, 1975) and monkeys (Ruegg et al., 1977).

The present anatomical study was undertaken to analyze the distribution of gracilo- and cuneopontine fibers in relation to corticopontine projections from the electrophysiologically defined sensorimotor hindlimb as well as sensory and motor forelimb cortical areas in the rat. Two anterograde tracing techniques, autoradiography and the degeneration staining method of Fink and Heimer (1967), were used simultaneously in individual animals in order to clearly define the possible convergence of descending cortical and ascending DCN inputs within the PG.

METHOD

Twenty male, Long-Evans, blackhooded rats ranging from two to six months of age were used in this study. Prior to surgery, animals were anesthetized with ketamine hydrochloride (100 mg/Kg i.m., with supplemental doses as needed) and placed in a sterotaxic apparatus. The cisterna magna was drained to prevent cortical swelling and the atlanto-occipital membrane reflected to reveal the underlying DCN. A craniotomy was then performed with care taken not to tear the underlying dura in order to minimize the possibility of cortical damage.

Intracortical multiunit recordings and microstimulation techniques were used to define the primary cortical forelimb and hindlimb areas. A glass insulated tungsten wire electrode (tip exposure of approximately 100 um) was inserted through the dura perpendicular to the cortical surface with a micromanipulator. Approximately 30 electrode penetrations per animal were made at 0.5 mm intervals. Each penetration was used first for recording and then for stimulating procedures. Evoked unit cluster responses to peripheral manipulations (stroking or tapping of the extremities and moving of joints) were recorded at a depth of 0.5 mm or less from the cortical surface. Sensory cortical areas were defined as those areas responding only to peripheral manipulations of the limbs. The electrode was then

lowered to a depth of 1.7 mm and an electrical stimulus applied at currents ranging from 5-100 uamps (stimulus parameters consisted of a 300 msec train of 0.25 msec pulses at 350 Hz). Threshold currents, specified as the lowest currents necessary to elicit a particular movement, were noted along with the evoked peripheral responses for each electrode penetration. Motor cortical areas were defined as those areas demonstrating evoked peripheral movements upon cortical stimulation of 100 uamps or less and which failed to demonstrate evoked potentials upon peripheral manipulations. Overlapping sensorimotor cortical areas, particularly characteristic of hindlimb cortex, were designated as those coextensive areas responsive to peripheral manipulations and able to produce peripheral movements upon cortical stimulations of currents up to 100 uamps.

Using a glass tipped (50 to 75 um diameter) 1 ul Hamilton syringe, 1-2 injections of 0.1-0.3 ul of tritiated leucine (50 uCl/ul) were centered in either the forelimb motor (n=6), forelimb sensory (n=6) or hindlimb sensorimotor cortical area (n=6). Following the cortical injections, either contralateral DCN, cuneatus corresponding to forelimb or gracilis to hindlimb, was aspirated using a finely drawn glass pipette. Three injection/lesion groups of animals were used: a) forelimb motor cortical injection/cuneatus lesion, b) forelimb sensory cortical injection/cuneatus lesion and c) hindlimb sensorimotor cortical injection/gracilis lesion. To control for axonal degeneration as a result of cortical mapping and injection procedures, the Fink-Heimer method was used on two animals that received cortical injections

without subsequent DCN lesions.

Seven days after the injection/lesion procedure animals were sacrificed with an overdose of sodium pentobarbital and perfused trascardially with physiological saline followed by 10% buffered formalin. Brains were removed and placed in a 30% sucrose and 10% buffered formalin solution until they sank. Frozen serial sections were then cut at 40 um in the transverse plane. Every fourth section was used for the Fink-Heimer technique and two sets of adjacent sections were processed according to routine autoradiographic protocol (Cowan and Cuneod, 1975). In the autoradiographic procedure, slides were dipped in Kodak NTB-2 emulsion, stored for 3 or 12 week exposure periods, developed with D-19 and counterstained with cresyl violet.

The distribution of pontine afferents was plotted using a camera lucida drawing attachment. The terminology of the pontine nuclear regions, as depicted in Figure 1, was derived from recent work on the cytoarchitecture of the rodent pontine gray (Mihailoff et al., 1981b.).

RESULTS

Although a slight amount of degeneration was observed in the crus cerebri in animals sustaining cortical injections of tritiated leucine without concomittant ablations of either DCN, virtually no degeneration argyrophilia was seen in the PG. Accordingly, the observed distribution of axonal degeneration within the PG after DCN lesions was not confounded by the degeneration of corticopontine projections resulting from electrophysiological analyses and tritiated leucine injections of the cerebral cortex. Also, the distribution of thalamic afferents observed after DCN ablations and after cortical injections corresponds to previous reports (Lund and Webster, 1967; Price and Webster, 1972; Wise and Jones, 1977; Feldman and Kruger, 1980) thus confirming the accurate placement of the lesions and injections used in this study.

Forelimb primary motor cortex injection/nucleus cuneatus lesion

Labeled corticopontine projections from the forelimb primary motor cortical area (Figure 2 sections A'-H') descended ipsilaterally in the medial third of the crus cerebri and distributed in five longitudinal zones or patches within the ipsilateral PG. At rostral levels, dense labeling was observed centrally within the ventral pontine nuclear region (Figure 2, section A' and B'). Less dense

labeling extended from this rostral zone to distribute along the ventral surface at mid pontine levels (Figure 2, section B'). Three small, but dense patches of terminal fibers were located at mid to caudal regions of the PG -- one within the dorsal peduncular nucleus, while the other two distributed in the ventral and medial pontine subdivisions (Figure 2, sections D'-C' and Figure 3C). A fine band of labeled fibers connected these latter regions along the ventral surface of the PG (Figure 2, section F'). Additionally, light areas of termination were located contralaterally along the midline raphe at these same mid to caudal PG levels. The most caudal and rostral regions of the PG revealed no terminal labeling form the forelimb primary motor cortical area.

Degenerative pontine afferents from a nucleus cuneatus ablation within the same animal (Figure 2, sections A-H) were found to course with medial lemniscal fibers to the side contralateral to the lesion. At mid to caudal pontine levels, these fibers turned ventrally and passed medial and lateral to the crus cerebri to distribute primarily within two regions of the PG. The densest region of termination was centered within the medial pontine nucleus (with some extension into the ventral nucleus) while the other region was located along the ventral surface of an area overlapping the ventral and lateral pontine nuclei (Figure 2, section E-H and Figures 3A and 3B). Neither of the projections extended into the most caudal levels of the PG. Although apparent projections were observed within the dorsal peduncular nucleus, their analysis was made difficult due to the proximity of

heavily degenerative medial lemniscal fibers. No additional projections were identified in rostral to mid pontine levels.

Pontine afferents from nucleus cuneatus did not appear to overlap with afferents from forelimb primary motor cortex (Figure 2F-F') but rather appeared to terminate in adjacent regions of the PG.

Forelimb primary sensory cortex injection/nucleus cuneatus lesion

Corticopontine fibers traced from the forelimb primary sensory cortical area descended ipsilaterally in the middle third of the crus cerebri and were found to terminate in five longitudinal zones throughout much of the ipsilateral PG (see Figure 4, sections A'-H'). Although heavily labeled fibers were located at rostral regions similar to those described for the motor cortical area, they were less dense and appeared to be closer to the crus cerebri (Figure 4, sections A' and B'). However, sparse labeling was observed to deviate ventrally from this rostral terminal zone (Figure 4, sections B' and C') as similarly found for afferents from the forelimb motor cortical area. At mid PG levels, light labeling was observed within the dorsal peduncular nucleus (Figure 4, sections D' and E') while two zones of dense terminations were seen further caudally. One zone was located laterally, along the ventral surface of an area overlapping the ventral and lateral pontine nuclei and appeared to be continuous with lightly labeled zones located rostrally (Figure 4, sections C'-F' and Figure 5C). A second zone appeared confined within more central regions of the medial pontine nucleus (Figure 4, sections D'-F' and Figure 5C).

No labeling was found at the most caudal extent of the PG or on the contralateral side.

Figures 4 and 5 also demonstrate degenerative pontine afferents after an ablation of nucleus cuneatus in the same animal used to map forelimb sensory corticopontine fibers. The observed afferent pattern is comparable to the description of cuneopontine fibers corresponding to Figures 2 and 3 and therefore is not repeated.

The topography of cuneopontine fibers resembled more closely afferents from forelimb sensory cortex in comparison to inputs from forelimb motor cortex. This is particularly evident in distributions observed within the central portions of the medial pontine nucleus and in the zone bridging the ventral and lateral pontine nuclei (compare Figures 2 and 4, sections D'-D and F'-F). Within the medial pontine nucleus, the density of both inputs appeared comparable with cuneatus fibers terminating predominately dorsal to, but partially overlapping, forelimb sensory corticopontine fibers (compare sections F and F' in Figure 4). While overlap within the ventral-lateral pontine nuclei appeared more complete than in the medial pontine region, the cuneopontine projections were comparatively less dense (compare Figures 4, sections E-E' and 5C). The zones receiving overlapping projections from forelimb sensory cortex and nucleus cuneatus formed alternating paired patches or zones with projections from forelimb motor cortex (compare Figure 2 with Figure 4). Throughout caudal levels of the PG, a medial to lateral distribution of these alternating motor and sensory patches were found across the transverse plane of the PG. At rostral

levels of the PG, where no cuneopontine projections were found, forelimb sensory cortical afferents were located dorsal to forelimb motor cortical afferent fibers.

Hindlimb primary sensorimotor cortex injection/nucleus gracilis lesion

Hindlimb sensorimotor corticopontine fibers were observed coursing ipsilaterally in the lateral third of the crus cerebri to distribute in five longitudinal zones within the ipsilateral PG (see Figure 6, sections A'-H'). As similarly described for the forelimb cortical projections, a dense zone of label was located just ventral to the crus cerebri at rostral levels (Figure 6, section B') which was slightly more caudal than described for forelimb corticopontine afferents. At mid-pontine levels, light labeling was found to be marginated along the midline raphe in the medial pontine nucleus (Figure 6, section E'). Further caudally, this midline labeling merged with additional, densely labeled fibers in the ventral and lateral pontine nuclei. Collectively, these fibers formed a transverse band of labeling along the entire ventral aspect of the caudal PG (Figure 6, sections F' and G' and Figure 7C). In contrast to the forelimb corticopontine projections, dense terminal labeling was seen throughout the furthest caudal regions of the PG, particularly within the ventral pontine nucleus (Figure 6, section H'). At these caudal levels, additional labeling was observed in the dorsal peduncular nucleus as well as in the ventral aspect of the contralateral ventral pontine nucleus (Figure 6, sections C' and H').

In the same animal, degenerative graciloPontine fibers coursed with medial lemniscal fibers enroute to the contralateral PG where they departed ventrally, passing medial and lateral to the crus cerebri to enter two major zones within the caudal fifth of the PG. At caudal levels, the heaviest graciloPontine fibers distributed in the central regions of the medial pontine nucleus (extending into the ventral pontine v=nucleus) while at a slightly more rostral level, they were found marginated along the ventral aspect of this pontine region (Figure 6, sections G-H and Figure 7A). Less dense labeling was located ventrally within a zone bridging the lateral and ventral pontine nuclei at these same caudal levels (Figure 6, sections G and H and Figure 7B).

Similar to the observed overlap between cuneoPontine and forelimb corticoPontine projections, graciloPontine fibers were found to partially overlap pontine afferents from the hindlimb sensorimotor cortical area (compare sections G'- H' with G-H of Figure 6). This overlap is most noticeable along the ventral portion of the medial and ventral-lateral pontine nuclei.

The alternating distribution patterns corresponding to sensory and motor forelimb corticoPontine fibers could not be examined in reference to hindlimb corticoPontine fibers since separate sensory and motor hindlimb cortices are not distinguishable in the rat (Hall and Lindholm, 1974; Donoghue et al., 1979; Donoghue and Wise, 1983).

DISCUSSION

Ascending somatosensory inputs from the DCN distributed somatotopically in the caudal PG. These cuneo- and graciloPontine projections were found to partially overlap with projections from forelimb sensory and hindlimb sensorimotor cortical areas, respectively. The anatomical imbrication of these descending and ascending pontine afferents has not been previously described in any species. However, electrophysiological studies have demonstrated pontine neurons to respond to peripheral stimulation of the fore- and hindlimb as well as to sensory, but not motor, cortical stimulation from corresponding fore- and hindlimb areas (Ruegg and Wiesendanger, 1975; Ruegg et al., 1977). The observed overlap of DCN and sensory cortical projections in the PG provides the anatomical substrate for this physiological convergence.

Regarding the transmission of sensory information to the cerebellar cortex, peripherally evoked fore- and hindlimb tactile inputs have been shown to distribute topographically in a "mosiac" or patchlike fashion across the longitudinal extent of the ipsilateral paramedian lobule in rats (Shambes et al., 1978). While these outaneous inputs were suggested to be mediated through direct DCN cerebellar connections (Shambes et al., 1978). However, the patchlike topography of fore- and hindlimb representations within the paramedian

lobule may reflect the anatomical organization of the DCN efferents to the PG. These indirect cerebellar projections may provide an additional source of cutaneous input to the paramedian lobule (Swenson et al, in press). Supportive anatomical studies on the origin of pontocerebellar projections after injections of horseradish peroxidase (HRP) into the paramedian lobule of rats (Mihailoff et al., 1978) revealed retrogradely labeled pontine neurons in caudal regions of the PG which appear to correspond to regions receiving DCN (Swenson et al., in press) as well as cortical afferents. The PG of rats may possess a similar organization of cutaneous inputs as previously described electrophysiologically for the cat (Ruegg and Wiesendanger, 1975) and monkey (Ruegg et al., 1977) and which receives anatomical support from the present study. Furthermore, in the rat, Bower et al., (1981) demonstrated tactile inputs from peripheral and primary sensory cortical areas, representing the same cuntaneous receptive fields, to overlap in patchlike regions of the cerebellar cortex suggesting such topographical relationships may be organized at the level of the PG.

Also observed in the present study, the corticopontine projections from electrophysiologically defined forelimb sensory and forelimb motor cortical areas appeared to project primarily in adjacent, non-overlapping pontine regions. A similar topographic configuration of forelimb sensory and motor corticopontine fibers was described for the cat (Brodal, 1968). In this study, two separate longitudinal columns, each containing adjacent sensory and motor

cortical pontine projections, were located medial and lateral to the crus cerebri. However, the internal organization within the columns appears reversed to our findings in rats, i.e., a dorsal-ventral somatotopy was observed at rostral PG levels and a medial-lateral somatotopy seen throughout the caudal half of the PG. Nonetheless, such a paired organization of corticopontine projections from the forelimb sensory and forelimb motor cortices (as well as from nucleus cuneatus) may reflect a similar arrangement of the origin of pontocerebellar fibers which have been shown to be organized in paired columns of cellular aggregates extending in the rostral-caudal plane in both cats (Hoddevik, 1975) and rats (Burne et al., 1978b).

In summary, the present study compared corticopontine projections from electrophysiologically defined sensory and motor forelimb as well as sensorimotor hindlimb cortical areas in relation to pontine afferents from the corresponding contralateral DCN. The results obtained confirm and extend previous anatomical findings and support physiological studies which demonstrated a convergence within the PG of peripherally evoked outaneous inputs with sensory, but not motor, cortical inputs (Ruegg and Wiesendanger, 1975; Ruegg et al., 1977). These anatomical and physiological data combined with studies on the organization of pontocerebellar fibers suggest an integration within the PG of information conveyed by cortical and DCN afferents which is then relayed in a precise topographic fashion to the granule layers of the cerebellar hemisphere.

Fig. 1. Diagramatic illustration of the principal nuclei of the basilar pontine gray as modified from Mihailoff et al., (1981b). Abbreviations: dPd,dorsal peduncular nucleus; vPd,ventral peduncular nucleus; d:, dorsolateral nucleus; Med,medial nucleus; Lat,Lateral nucleus; Vent,ventral nucleus; mop,middle cerebellar peduncular; ped,cerebral peduncle.

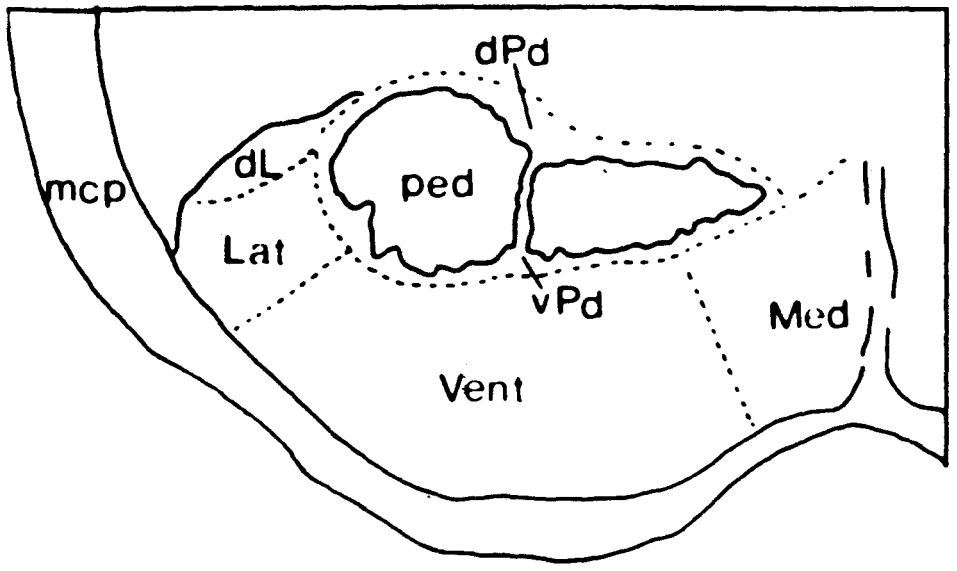

dPd
dL
mcp
ped
Lat
vPd
Med
Vent

Fig. 2. Camera lucida drawings depicting the distribution pattern of degenerative cuneopontine (sections A-H) and radiolabeled forelimb motor corticopontine projections (sections A'-H') as seen throughout the caudal 4/5 of the PG. Drawings are from a single animal following a unilateral ablation of nucleus cuneatus (depicted in lower left) and an injection of tritiated leucine into the forelimb motor cortical region (depicted upper right). Sections A-A', B-B', etc. are adjacent 40 um sections; 200 um intervals separate the paired sections. The pontine nuclear subdivisions indicated in level D correspond to Figure 1. A similar format is used in subsequent figures. Rectangles labelled A,B and C correspond to photographs in figure 3. Abbreviations: C,nucleus cuneatus; G,nucleus gracilis; mcp,middle cerebellar peduncle; ML,medial lemniscus; ped,cerebral peduncle; PG,pontine gray; XII,hypoglossal nucleus.

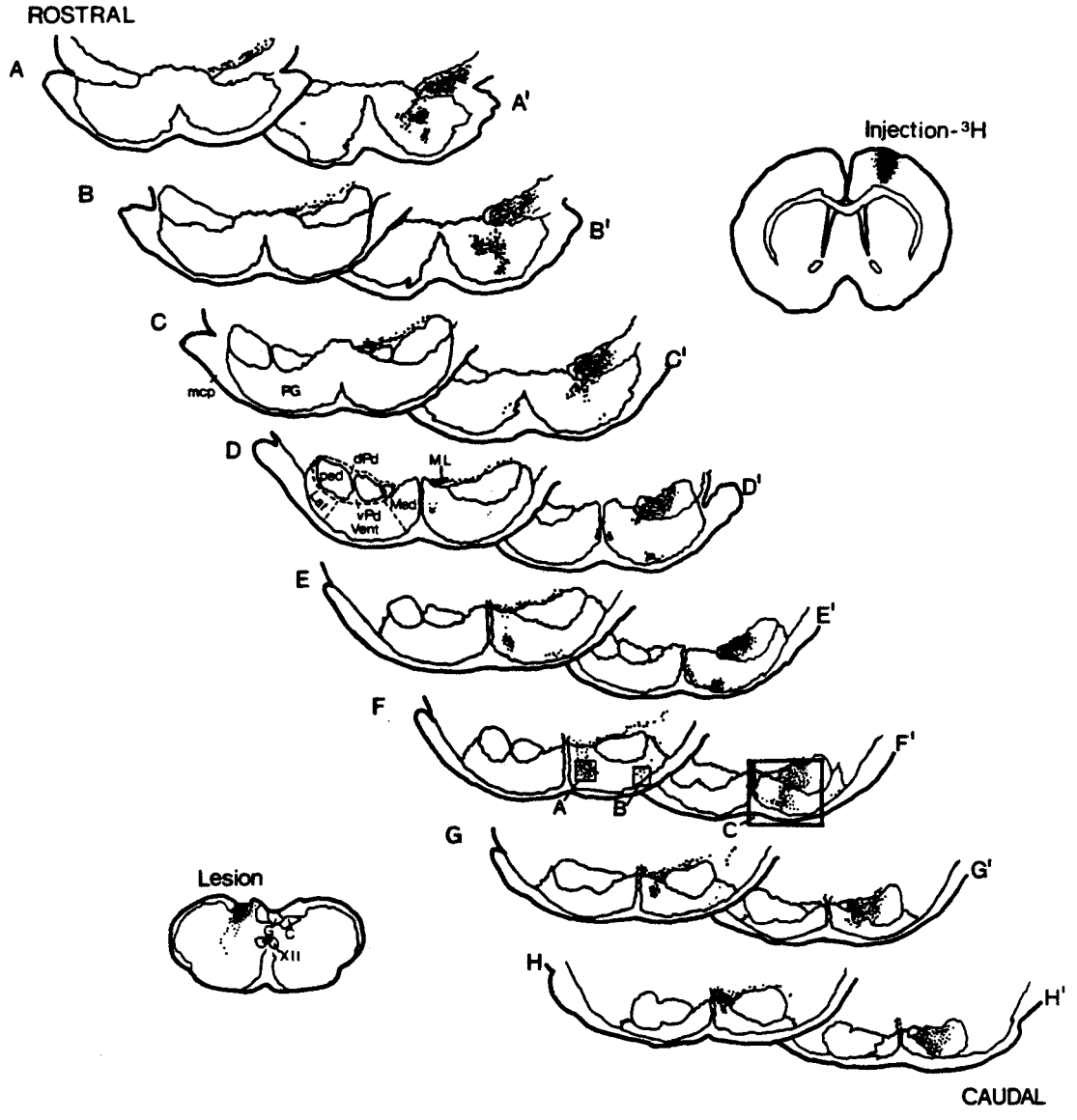

ROSTRAL
A
A'
B
B'
C
C'
mcp
PG
D
D'
dPd
ML
lped
Med
vPd
Vent
E
E'
F
F'
A
B
C
G
G'
H
H'
Lesion
XII
Injection-³H
CAUDAL

Fig. 3. Photomicrographs corresponding to boxes labeled A-C in Figure 2. A. Medial pontine nucleus, F-H stain, 384x. B. Ventral-Lateral pontine nuclei, F-H stain, 384x. C. Medial and Ventral pontine nuclei, Autoradiography (darkfield), arrow denotes midline, 60.5x.

Abbreviations: ped,cerebral peduncle.

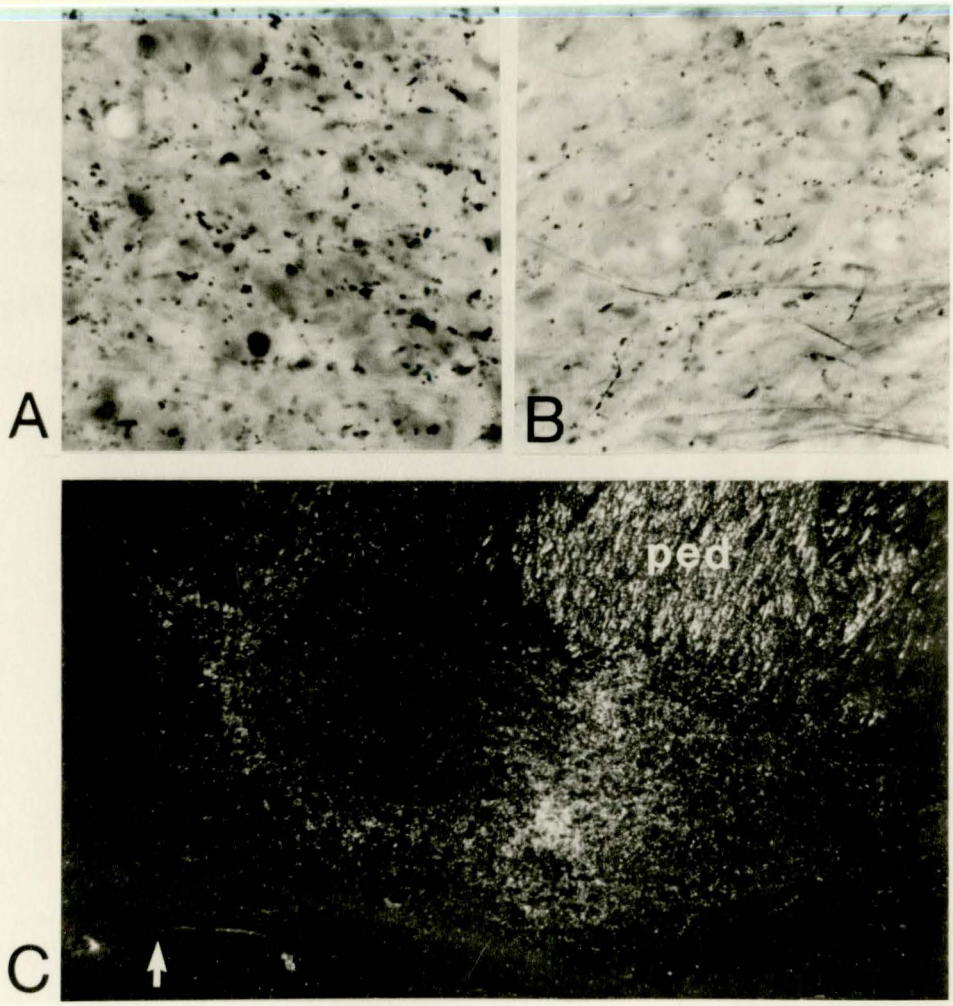

A
B
ped
C

Fig. 4. Camera lucida drawings depicting the distribution pattern of degenerative cuneopontine (sections A-H) and radiolabeled forelimb sensory corticopontine projections (sections A'-H') as seen throughout the caudal 4/5 of the PG. Drawings are from a single animal following a unilateral ablation of nucleus cuneatus (depicted in lower left) and an injection of tritiated leucine into the forelimb sensory cortical region (depicted in upper right). Sections A-A', B'-B', etc. are adjacent 40 um sections; 200 um intervals separate the paired sections. The pontine nuclear subdivisions indicated in level D correspond to Figure 1. Rectangles A, B and C correspond to photographs in figure 5. Abbreviations: C,nucleus cuneatus; G, nucleus gracilis; mcp,middle cerebellar peduncle; ML,medial lemniscus; peg,cerebral peduncle; PG,pontine gray; XII,hypoglossal nucleus.

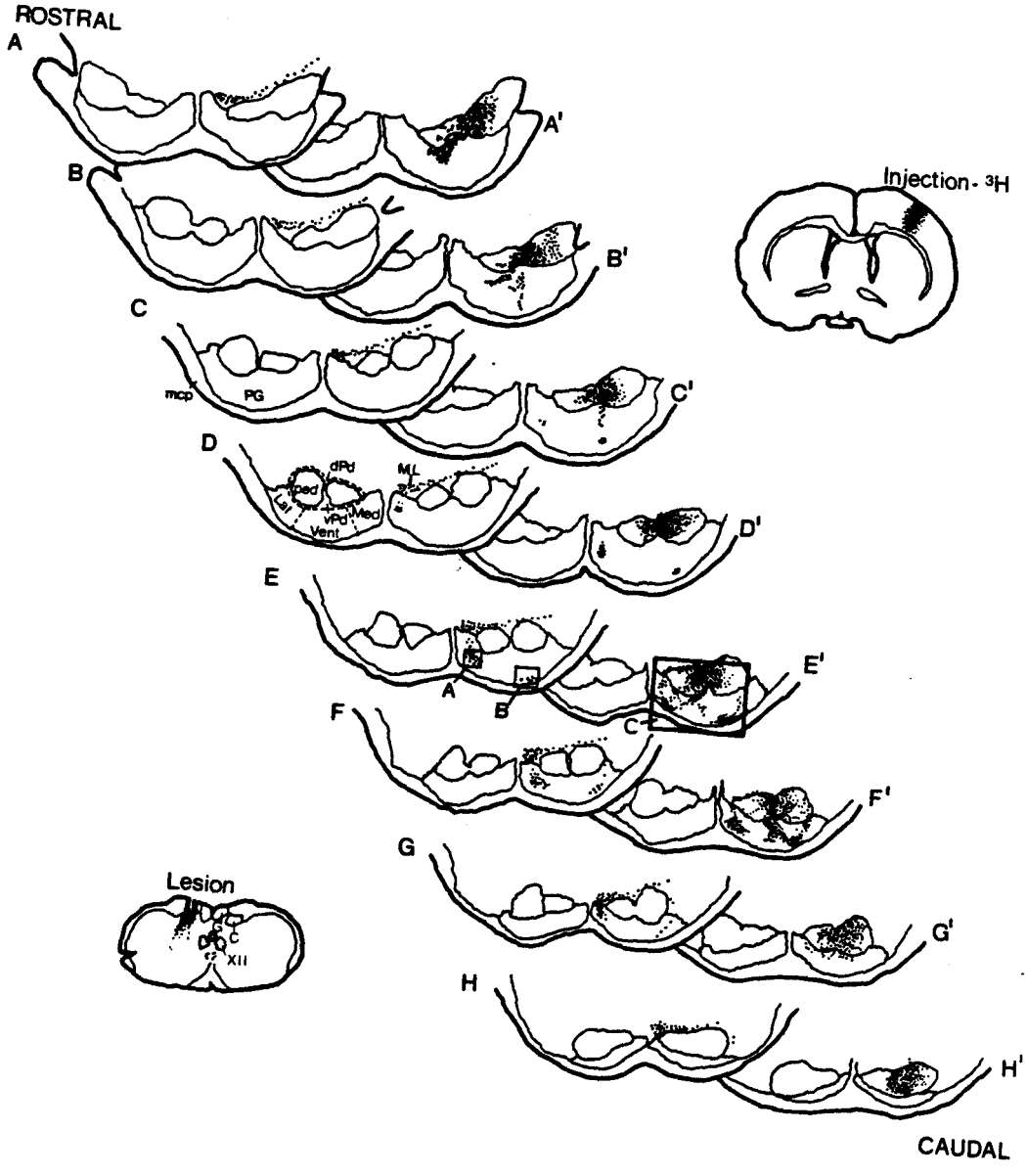

ROSTRAL
A
A'
B
B'
C
C'
mcp
PG
D
dPd
ped
ML
vPd
Med
Vent
D'
E
E'
A
B
C
F
F'
G
G'
H
H'
CAUDAL
Injection - 3H
Lesion
XII

Fig. 5. Photomicrographs corresponding to boxes labeled A-C in Figure 4. A. Medial pontine nucleus, F-H stain, 384x. B. Ventral pontine nucleus, F-H stain, 384x. C. Medial and ventral pontine nuclei, Autoradiography (darkfield); arrow denotes midline, 60.5x. Abbreviations: ped,cerebral peduncle.

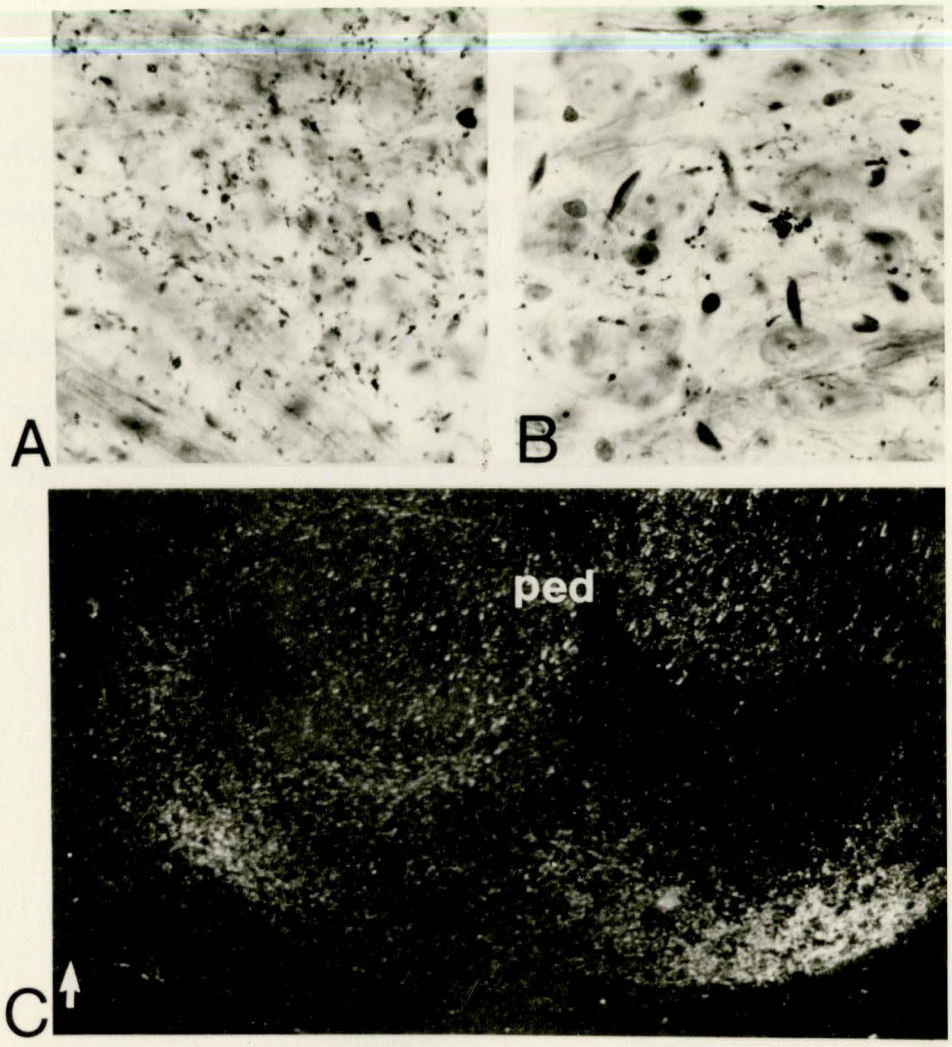

A
B
ped
C

Fig. 6. Camera lucida drawings depicting the distribution pattern of degenerative graciloPontine (sections A-H) and radiolabeled hindlimb sensorimotor corticopontine projections (sections A'-H') as seen throughout the caudal 4/5 of the PG. Drawings are from a single animal following a unilateral ablation of nucleus gracilis (depicted in lower left) and an injection of tritiated leucine into the hindlimb sensorimotor cortical region (depicted upper right). Sections A-A', B-B', etc. are adjacent 40 um sections; 200 um intervals separate the paired sections. The pontine nuclear subdivisions indicated in level D correspond to Figure 1. Rectangles A, B and correspond to photographs in figure 7. Abbreviations: C,nucleus cuneatus; G,nucleus gracilis; mcp,middle cerebellar peduncle; ML,medial lemniscus; ped,cerebral peduncle; PG,pontine gray; XII,hypoglossal nucleus.

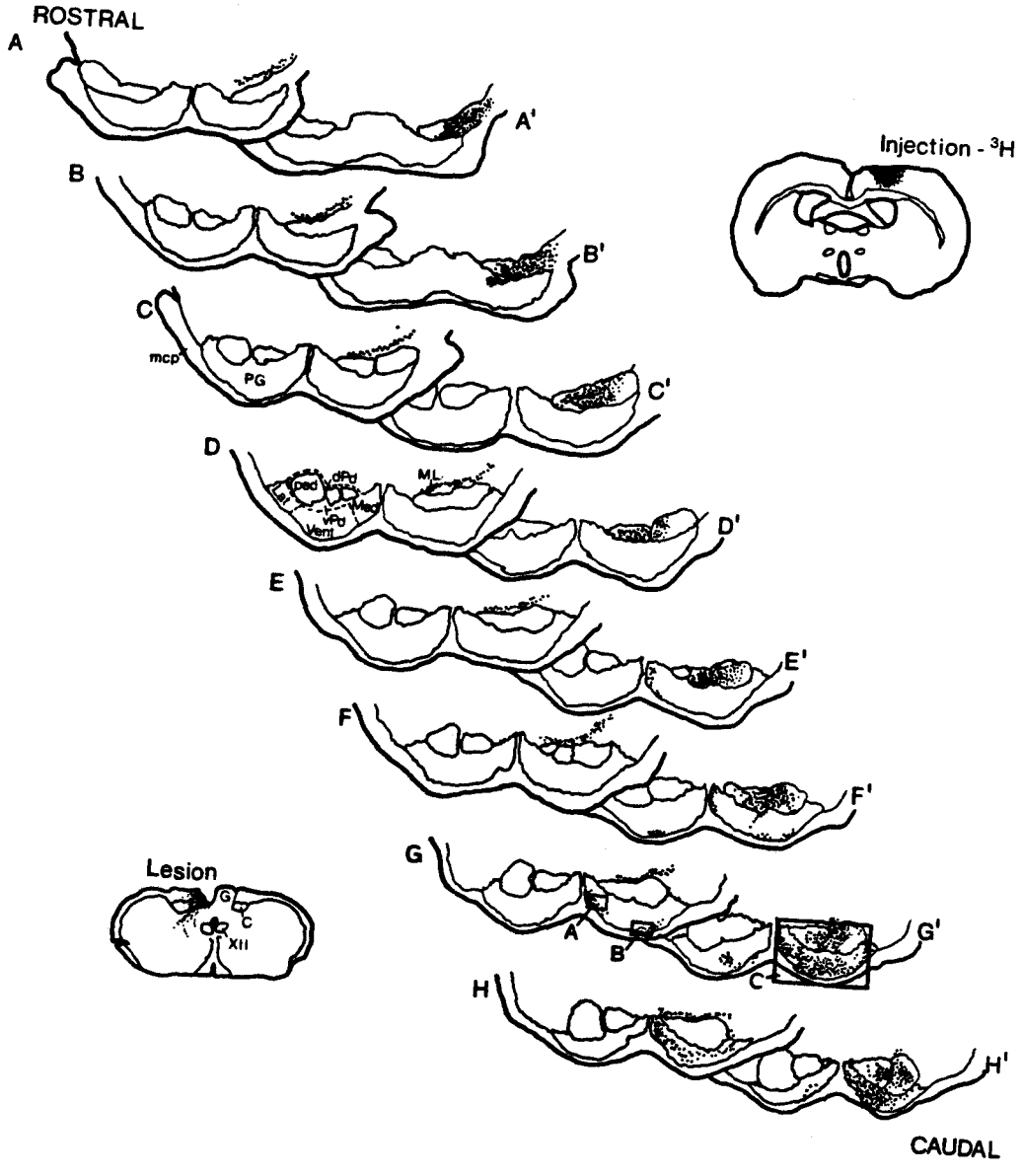
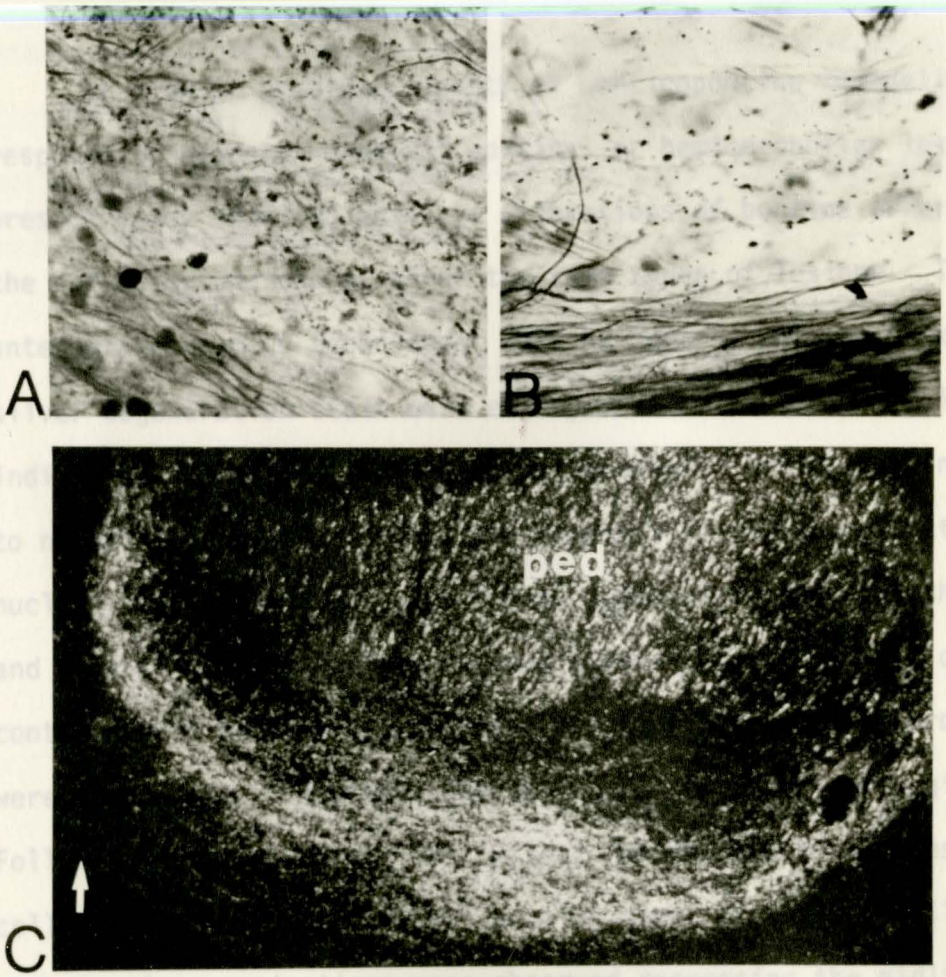

ROSTRAL
A
A'
B
B'
C
mcp
PG
C'
D
dPd
ML
Vem
D'
E
E'
F
F'
G
A
B
C
G'
H
H'
CAUDAL
Injection - ^{3}H
Lesion
G
C
XII

Fig. 7. Photomicrographs corresponding to boxes labeled A-C in Figure 6. A. Medial pontine nucleus, F-H stain, 384x. B. Ventral pontine nucleus, F-H stain, 384x. C. Medial and ventral pontine nuclei, Autoradiography (darkfield), arrow denotes midline, 60.5x. Abbreviations: ped,cerebral peduncle.


A
B
ped
C

Remodeling of Dorsal Column Nuclear Efferents after Cortical or Cerebellar Lesions in Newborn Rats

ABSTRACT

In view of previous reports of corticopontine remodeling in response to neonatal cerebral cortical or hemicerebellar lesions, the present study examined possible alterations of pontine afferents from the dorsal column nuclei after the same types of lesions. Two anterograde tracing techniques, autoradiography and the Fink-Heimer silver degeneration stain were employed simultaneously within individual animals to facilitate a topographic analysis. In response to neonatal cortical lesions, the terminal distribution patterns of nucleus gracilis and cuneatus fibers appeared expanded in the medial and lateral pontine nuclear regions within the caudal half of the contralateral pontine gray. Also, aberrant cuneopontine projections were found rostrally within the ventral pontine nuclear region. Following neonatal cerebellar lesions, which results in considerable cell loss in the contralateral pontine gray, aberrant gracilo-, but not cuneopontine, projections were observed recrossing the midline to terminate within the ventromedial portion of the intact ipsilateral pons.

Because neonatal sensorimotor cortical ablations produce

extensive neuronal loss within the somatosensory thalamus, thus depriving medial lemniscal fibers of their primary target of termination, the possibility of medial lemniscal remodeling to the opposite intact thalamus was examined. However, no aberrant medial lemniscal projections recrossing to the intact ipsilateral somatosensory thalamus were found. These results were further corroborated by the analysis of evoked cortical responses after peripheral stimulation which correlated with the normal laterality of medial lemniscal projections.

INTRODUCTION

Efferents from the dorsal column nuclei (DCN), cuneatus and gracilis, were recently demonstrated to distribute somatotopically within the caudal half of the contralateral pontine gray (PG) in rats (Kosinski et al., 1982; Swenson et al., in press and as described in Experiment 1). These pontine afferents were found primarily within two pontine regions, one in the medial subdivision and another overlapping the lateral and ventral subdivisions. Within these pontine regions, graciloPontine fibers were found mostly caudal to cuneopontine fibers. In additional studies, these somatotopic projections were examined in relation to the somatotopic distribution of fore- and hindlimb sensorimotor corticopontine projections (Kosinski et al., 1982; and as described in Experiment 2). Within individual animals, contralateral cuneo- and graciloPontine projections were seen to partially overlap with ipsilateral forelimb sensory and hindlimb sensorimotor corticopontine projections, respectively.

The present study examined whether DCN projections to the PG would form aberrant connections after neonatal cortical lesions which deafferented the pontine gray (PG). The use of two anterograde tracing techniques, autoradiography and the Fink-Heimer degeneration staining method facilitated the topographic analysis of DCN efferents. In

addition to PG deafferentation, the same cortical lesions also cause substantial cell loss within the somatosensory thalamus (Kolb et al., 1983), the primary target of medial lemniscal fibers originating from the DCN (Lund and Webster, 1967). Thus the possible remodeling of DCN efferents in the absence of their target cells in the thalamus was examined in addition to the possibility of remodeling after PG deafferentation. The plasticity of pathways mediating somatosensory information to the cerebral cortex was also studied electrophysiologically by recording the laterality of evoked cortical potentials after peripheral stimulation of each side of the body surface in adult animals that had sustained unilateral, neonatal cortical lesions.

Similar to the cell loss in the thalamus after cortical lesions, considerable cell loss is found in the PG contralateral to hemicerebellar lesions. Accordingly, the possible remodeling of DCN efferents in response to PG target removal was also examined.

The results obtained demonstrated aberrant DCN projections after PG deafferentation or target removal, including an anomalous recrossing of fibers, but no anomalous recrossing of medial lemniscal fibers was observed in relation to thalamic cell loss.

MATERIALS AND METHODS

Long-Evans, blackhooded rats were used in this study. Using hypothermic anesthesia, newborn pups (1-2 days of age) received right sensorimotor cortical (RSMC) or left hemicerebellar (LCb) ablations by aspiration with finely drawn glass pipettes. Non-lesion control animals (1-2 pups per litter) were also anesthetized by hypothermia and had their tails snipped for identification. Following surgery, the pups were returned to their mothers until weaning. A total of thirty-five animals were used in three experimental and two control groups as indicated in Table I.

TABLE I

Group/Animals	Neonatal Lesions	Adult Lesion/Injection	Intracortical Multiunit Recording
(1) / 6	none (control)	LNG / LNC	
(2) / 11	RSMC	LNG / LNC	
(3) / 6	LCb	LNG / LNC	
(4) / 6	none (control)		yes
(5) / 6	RSMC		yes

At 2-6 months of age, these same animals were anesthetized with ketamine hydrochloride (100 mg/Kg i.m., with supplemental doses as needed) and placed in a stereotaxic apparatus. In groups 1-3, the atlanto-occipital membrane was reflected to reveal the underlying dorsal column nuclei. The left nucleus gracilis (LNG) was then aspirated with a finely drawn glass pipette and the adjacent nucleus cuneatus (LNC) inject with 0.01-0.05 ul of tritiated leucine (50 uCi/ul) using a glass tipped (30-50 um tip diameter) 1 ul Hamilton syringe. Lesions and injections were typically centered at levels just caudal to the obex (as described in Experiment 1).

After a seven day survival period, animals were sacrificed with an overdose of sodium pentobarbitol and perfused transcardially with physiological saline followed by a 10% buffered formalin solution. Brains were removed and frozen sections were cut serially at 40 um in either the transverse or sagittal plane. Every fourth section was processed according to the Fink-Heimer technique in order to examine degenerating axons from the nucleus gracilis and two sets of adjacent sections were processed autoradiographically (Cowan and Cuneod, 1975) in order to examine radiolabeled nucleus cuneatus efferents. The autoradiographic material was coated with NTB-2 emulsion and after 4 and 12 week exposure periods, developed and counterstained with cresyl violet. The distribution of DCN efferents was plotted using a camera lucida drawing attachment. The terminology for the various pontine nuclear regions, as depicted in Figure 1a, was derived from a previous cytoarchitectural study of PG neurons (Mihailoff et al., 1981b).

(Additional references were derived from the atlas of Paxinos and Watson 1982).

In groups 4 and 5, the cisterna magna was drained to prevent cortical swelling and a craniotomy performed. In order to define the primary sensory forelimb area, a glass insulated tungsten wire electrode (tip exposure of 70-100 um) was stereotaxically inserted through the dura perpendicular to the cortical surface. Unit cluster responses evoked by peripheral manipulations of the forelimbs (stroking or tapping of the limbs and moving of joints) were recorded at a depth of 0.5 mm or less from the cortical surface and noted for laterality. Animals were then sacrified with an overdose of sodium pentobarbitol and perfused transcardially with physiological saline followed by a 10% buffered formalin solution. Brains were removed and frozen sections were cut serially at 40 um in the transverse plane. Sections were then stained with cresyl violet for the cytoarchitectural identification of the electrode tracks.

RESULTS

Each of the subtitles listed below corresponds to the animal groups as presented in Table I.

Control Group 1: none-LNG lesions/LNC injection

Following the lesion/injection of the gracile and cuneate nuclei, alternatively processed sections revealed degenerative and radiolabeled medial lemniscal fibers coursing to both the contralateral PG and thalamus. As described in Experiment 1, gracilo- and cuneopontine fibers terminated somatotopically in predominately two areas within the caudal half of the contralateral PG -- one in the medial pontine nuclear region (extending into the ventral nucleus) and the other in an area overlapping the ventral and lateral pontine nuclear regions (Figures 2 and 3). Within each nuclear region, cuneopontine fibers were located rostral to graciloпontine fibers. Additionally, within the medial pontine nuclear region, terminations from nucleus cuneatus were located dorsolaterally to terminations from nucleus gracilis. Both dorsal column nuclei also projected densely to the contralateral ventral posterolateral (VPL) thalamic nucleus as well as to the posterior nuclear complex (PO) and the zona incerta (ZI). Within the thalamic nuclei, the projections from cuneatus distributed slightly more rostral and ventromedial than those from gracilis projections.

These thalamic patterns conform to previous investigations (Lund and Webster, 1967; Feldman and Kruger, 1980).

Group 2: RSMC-LNG lesions/LNC injections

The smallest and largest extent of the neonatal cortical lesions as seen in the adult animal is illustrated in Figure 1b. A representative cross-section through an area of a cortical lesion is presented in Figure C. While portions of the basal ganglia and hippocampus were unintentially ablated, the thalamus did not appear to be damaged at surgery. However, due to the cortical lesion, the ipsilateral thalamus in the adult animal revealed a marked atrophy compared to the intact, contralateral thalamus (Figure 1c). In addition, the crus cerebri ipsilateral to the cortical ablation was considerably reduced except for a small lateral portion as seen at caudal pontine levels (Figure 1d).

Due to the atrophy of the crus cerebri after cortical lesions, the precise distribution of DCN projections observed within the caudal half of the PG was difficult to analyze because the various pontine nuclear regions are normally determined by their position relative to the crus cerebri. However, as reported for Group 1 animals, DCN projections were found to terminate primarily within two regions of the contralateral PG -- one being in the medial subdivision and the other in an area overlapping the lateral and ventral subdivisions (compare Figure 2 and 4). In comparison to control animals, a slight, but apparent increase in the density and extent of terminal labeling in

each of these pontine regions was observed as well as a further extension of the medial labeling into the ventral pontine region (compare Figures 3A-C with 5A-C). As compared to controls, gracilo- and cuneopontine projections appeared to extend more rostrally within both of these pontine nuclear regions (compare Figure 2, sections D and G' with Figure 4, sections C and F') Additionally, aberrant projections from nucleus cuneatus were observed at even further rostral levels of the contralateral PG just ventral to the medial lemniscus apparently terminating within the ventral pontine nucleus (Figure 4, section A and Figure 5D).

At the thalamic levels, medial lemniscal fibers appeared reduced in comparison to control animals (Group 1). The paucity of termiantions made it difficult to observe the distribution pattern which was further confounded by the high degree of thalamic atrophy and distortion. However, no medial lemniscal fibers were found recrossing the midline to innervate the intact thalamus.

Group 3: LCb-LNG lesions/LNC injections

Following neonatal hemicerebellectomy, the contralateral PG showed considerable cellular atrophy throughout its rostrocaudal extent as presented in Figure 6. A representative cross section taken at caudal levels of the PG is presented in Figure 7A. However, in spite of this atrophy, the distribution pattern of DCN projections to the contralateral PG appeared similar, although reduced, to that observed in control animals (compare Figures 6 and 7 with Figures 2 and 3). The

rostral to caudal topographic distribution of cuneo- and graciloponti ne projections was preserved in the medial and overlapping ventral-lateral pontine nuclear region as was the dorsal to ventral somatotopy of the projections in the medial pontine region. However, unlike controls, a modest number of fibers emanating from nucleus gracilis were found crossing the midline within caudal levels of the PG (Figure 6, sections F', G' and Figure 7A). Although the midline raphe was skewed toward the atrophied side of the PG, these aberrant graciloponti ne fibers, which have now re-crossed the midline were clearly seen to distribute, ipsilateral to their origin, within the ventral and medial portion of the medial pontine nucleus. No aberrant ipsilateral projections were observed from the cuneate nucleus.

Control Group 4: none- Intracortical Multiunit Recording

In normal control animals, evoked responses were recorded within the primary forelimb sensory cortical area by manipulation of the contralateral forelimb; no responses were obtained with manipulation of the ipsilateral limb. These patterns which correspond to the laterality of major somatosensory pathways, concurs with previous electrophysiological investigations in the rat (Welker, 1971; 1976; Hall and Lindholm, 1974; Donoghue and Wise, 1983).

Group 5: RSMC- Intracortical Multiunity Recording

In adult animals that sustained unilateral sensorimotor cortical lesions at 1-2 days of age, evoked responses observed within the intact

primary forelimb sensory cortical area were equivalent to controls. Responses were observed after manipulation of the contralateral limb but none were observed corresponding to manipulation of the ipsilateral forelimb. Furthermore, no apparent differences in the cortical topography of peripherally evoked forelimb responses were observed.

DISCUSSION

DCN projections in response to neonatal RSMC lesions - Pontine deafferentation

Neonatal lesions of the right sensorimotor cortex, which disrupts afferents primarily to the ipsilateral PG, resulted in an incresae in PG afferents from the left DCN. While gracilo- and cuneopontine fibers retained their somatotopic appearance, i.e., cuneatus fibers located mostly rostral to gracilis fibers, their terminal areas of distribution were slightly enlarged in comparison to control animals. Additionally, cuneopontine fibers formed aberrant connections within the ventral pontine nucleus at rostral portions of the contralateral PG.

Cuneo- and graciloпontine fibers are normally found to partially overlap with primary forelimb sensory and hindlimb, sensorimotor corticopontine projections (Kosinski et al., 1982; as well as in Experiment 2) within the caudal half of the PG. Furthermore, while DCN pontine projections do not normally distribute at rostral pontine levels (Kosinski et al., 1982; Swenson et al., in prss; see Experiments 1 and 2), this region has been shown to receive dense terminations from the ipsilateral forelimb sensory and motor cortical areas (Mihailoff et al., 1978; Kartje-Tillotson et al., 1981; Kosinski et al., 1982; as also described in Experiment 2).

Evidence in support of the aforementioned results comes from

studies which suggest that neonatal cortical lesions deprive pontine neurons of their normal corticopontine input thereby providing an availability of postsynaptic sites on pontine neurons normally receiving cortical projections (Leong, 1976b; Mihailoff and Castro, 1981). The formation of anomalous contralateral corticopontine fibers seems to result, at least partially, from the sprouting of normally existing crossed corticopontine fibers which have expanded to fill those pontine areas deprived of their normal cortical inputs (Mihailoff and Castro, 1981). Additionally, these anomalous projections distribute in a fashion similar to the topographic distribution of normally crossed corticopontine fibers, i.e., forelimb cortical areas represented more rostrally within the PG than those from hindlimb cortical areas (Kartje-Tillotson et al., 1981). However, normally crossed corticopontine fibers have not been described in relation to the rostral half of the contralateral PG and thus there is not an apparent anatomical substrate for the reinnervation of rostrally located contralateral pontine neurons (Kartje-Tillotson, personal communication). Since there appears to be only a slight amount of remodeling depicted at rostral levels after neonatal cortical lesions (Kartje-Tillotson, 1981; Castro and Mihailoff, 1783), the cuneopontine remodeling observed at rostral regions of the PG (after the same lesion paradigm described for corticopontine remodeling) may reflect this failure of crossed corticopontine fibers to sprout rostrally. Although corticopontine projections normally form synapses on the distal portions of PG dendrites (Mihailoff et al., 1981c), the precise

location of the synaptic terminals of DCN pontine projections has not been described. The slight expansion of DCN pontine fibers seen within the more caudal levels of the PG after neonatal cortical lesions may be attribted to terminal fibers sprouting into neonatally vacated corticopontine synaptic sites located more distally on pontine neurons. Similarly, the aberrant cuneopontine projections distributing within the rostral portion of the PG may also be occupying corticopontine synaptic sites, i.e., from forelimb sensory or motor cortical areas, left vacant as a result of the neonatal ablation.

In contrast to gracilo- and cuneopontine fibers, no aberrant DCN-thalamic projections were observed following neonatal cortical lesions. Correspondingly, no electrophysiological evidence for such remodeling was detected within the intact sensorimotor cortex upon peripheral stimulation of the ipsilateral extremities. This lack of medial lemniscal remodeling may be attributed to the consistent sparing of the second sensory cortical area (SII of Welker and Sinha, 1972) in animals that sustained neonatal RSMC lesions. The second sensory cortical area receives peripheral information corresponding to the contralateral half of the body surface (Welker and Sinha, 1972) and receives ipsilateral projections from somatosensory thalamic regions such as VPL (Saporta and Kruger, 1977; Donoghue et al., 1979; Herkenham, 1979; Lin and Chapin, 1981). In view of these data, medial lemniscal fibers to the atrophied thalamus may have formed synaptic contacts with thalamic neurons that were sustained by their connections with the intact second sensory cortical area which appeared spared

after cortical lesions. The presence of such connections might thereby preclude the formation of anomalous lemniscal fibers recrossing to innervate the intact thalamus.

In related studies using hamsters, medical lemniscal projections similarly failed to form anomalous connections after sensorimotor cortical lesions even when other thalamic nuclei, such as the DLG, were theoretically made "available" by additional lesions which deafferented these other nuclei (Frost 1981). From these data, Forst (1981) suggests that non-lemniscal fibers may have sprouted to reinnervate deafferented thalamic areas and thereby prevented lemniscal remodelling. Frost further suggests that lemniscal fibers may have compensated for the decrease in their thalamic arborizations by sprouting within nonthalamic sites that were deprived of cortical input by the neonatal lesions. Such "compensatory sprouting", which was originally proposed by Schnieder (1973) in reference to the remodeling of visual connections in hampsters, is supported by our observation. Possibly the observed increase of DCN projections to the contralateral PG after neonatal cortical lesions may have reduced the ability of DCN projections to sprout within the thalamus. As a further test of this hypothesis, additional studies are needed to determine if DCN projections to the PG are indeed collaterals of medial lemniscal fibers (as was suggested above in Experiment 1 - Discussion).

DCN projections in response to neonatal LCb lesions - Pontine target removal

Following neonatal cerebellar lesions, DCN projections were found to distribute within the caudal half of the contralateral atrophied PG. However, compared to control animals, these projections appeared less dense in both the medial and overlapping ventral to lateral pontine regions. At caudal pontine levels, a small, but obvious, aberrant bundle of graciloportine fibers crossed the midline to distribute within the comparable, intact ipsilateral medial pontine nuclear region, but similar aberrant cuneopontien fibers were not identified. However, some pontine neurons normally project to both cerebellar hemispheres (Rosina et al., 1980; Mihailoff, in press) and many of these bilaterally projecting neurons are located in medial pontine regions which appear to correspond to the location of cuneopontine terminations i.e., within the medial pontine region at mid to caudal PG levels. Therefore, the normal ipsilateral component of these pontocerebellar projections may sustain these pontine neurons following neonatal cerebellar lesions and provide an anatomical substrate for the remodeling of somatosensory input from the foreleg to the intact cerebellar hemisphere. Since there appear to be only scarce ipsilateral pontocerebellar connections in more caudal regions of the medial pontine nuclei, i.e., gracilis recipient areas of the PG, the resulting pontine atrophy in this region may have induced graciloportine fibers to sprout across the midline in attempts to conserve the total amount of their terminal arborizations. This double-crossing of gracilis afferents may represent the redirection of ascending sensory inputs from the hindleg to the intact ipsilateral PG.

The remodeling of sensorimotor corticopontine projections has also been described in response to neonatal cerebellar lesions in the rat (Leong, 1977; 1978; Castro and Mihailoff, 1983). Corticopontine fibers which normally terminate on distal dendrites of pontine neurons (Mihailoff et al., 1981c) were found to expand to intermediate dendritic locations within the ipsilateral atrophied PG (Leong, 1980) presumably reinnervating cerebellopontine synapses left vacant by the neonatal hemicerebellar ablation (Leong, 1980). The expansion of these cortical fibers within the atrophied PG may have additionally competed with DCN pontine projections for their terminal space possibly explaining the observed attenuation of the latter projections.

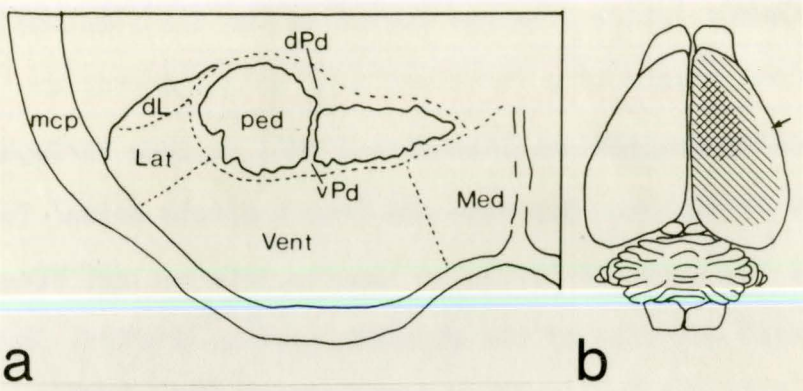
In summary, the present study provided evidence for the remodeling of ascending somatosensory inputs to the PG following unilateral sensorimotor and hemicerebellar lesions in newborn rats. Gracilo- and cuneopontine projections appeared to expand their terminal fibers possibly reinnervating corticopontine synaptic sites left vacant by neonatal cortical ablations, but no evidence was provided for the remodeling of DCN-thalamic fibers following the same neonatal lesions. In response to the cerebellar lesions, aberrant gracilo-, but not cuneopontine, projections were observed crossing the midline to distribute within the ipsilateral PG in regions comparable to normal graciloPontine terminations.

Fig. 1.a. Diagramatic illustration of the principal nuclei of the basilar pontine gray as modified from Mihailoff et al., (1981b). Abbreviations: dPd,dorsal peduncular nucleus; vPd,ventral peduncular nucleus; dL,dorsolateral nucleus; Med,medial nucleus; Lat,lateral nucleus; Vent,ventral nucleus; mcp,middle cerebellar peduncular; ped,cerebral peduncle.

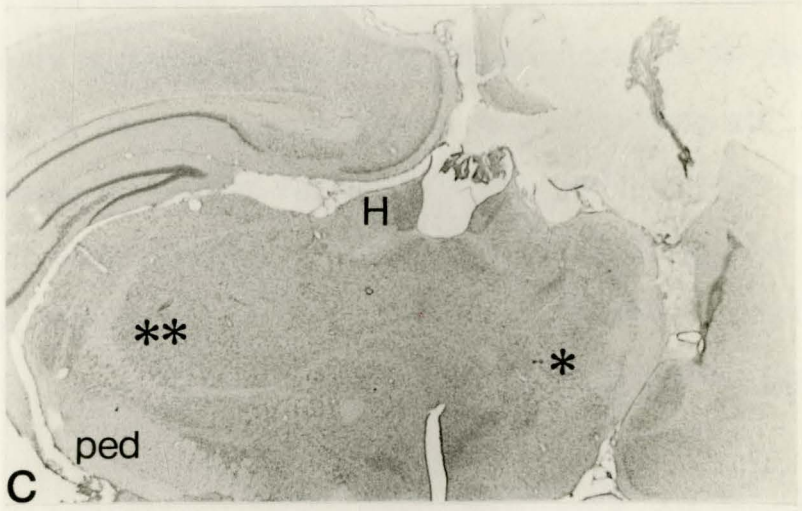
Fig. 1.b. Diagram of the dorsal view of a rat brain depicting the largest (slanted lines) and smallest (cross hatching) extent of the sensorimotor cortical lesions performed neonatally. The cortical damage for all other animals was intermediate between these two extremes, at least encompassing the area removed in the smallest lesion. Note that the far lateral portion of the cortex (arrow) was spared even after the largest lesion.

Fig. 1.c. Low power photomicrograph of a representative cross section through the level of the ventral posterolateral thalamic region of an animal having sustained a RSMC lesion neonatally. Note the extent of the cortical ablation as well as the intact contralateral (**) and atrophied ipsilateral (*) ventral posterolateral thalamic nucleus. Thionin stain. Abbreviation: H,habenula; ped,cerebral peduncle.

Fig. 1.d. Low power photomicrograph of a representative cross section through the caudal PG of an animal having sustained a similar neonatal RSMC lesion. Note the greatly atrophied cerebral peduncle (arrow) which is ipsilateral to the neonatal cortical lesion. Abbreviations: ML,medial lemniscus; ped, cerebral peduncle.



a b



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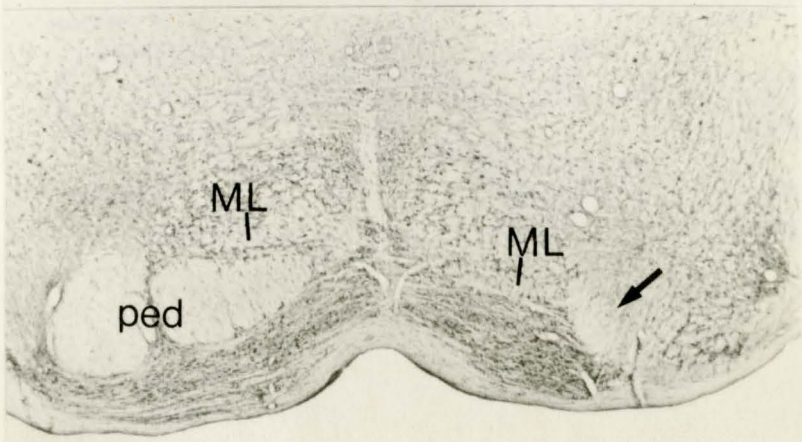
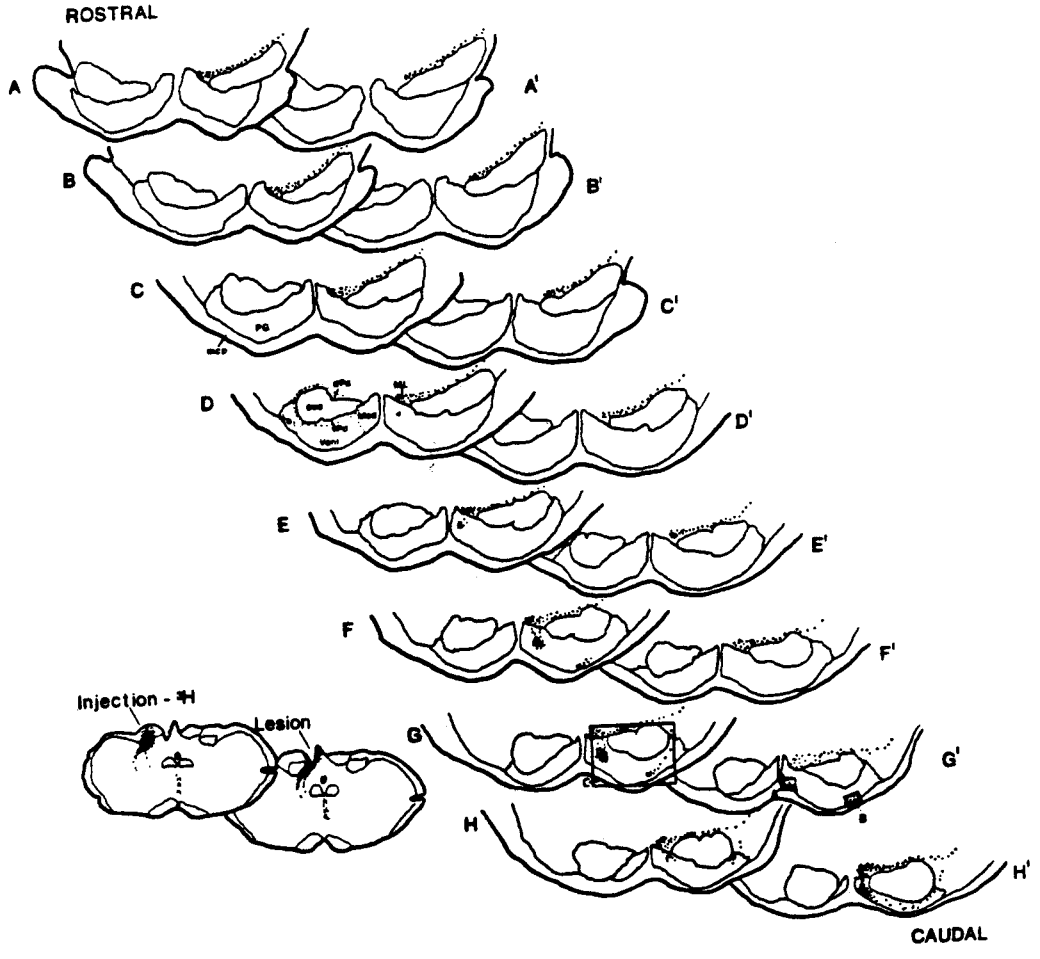


d

Fig. 2. Camera lucida drawings depicting the distribution pattern of radiolabeled cuneopontine (sections A-H) and degenerative graciloпontine projections (sections A'-H') as seen throughout the caudal 4/5 of the PG. Drawings are from a single animal following a unilateral injection of tritiated leucine into nucleus cuneatus combined with ablation of the adjacent nucleus gracilis (depicted in the lower left and presented in Figure 1b). Sections A-A', B-B', etc. are adjacent 40 um sections; 200 um intervals separate the paired sections. The pontine nuclear subdividions indicated in level D correspond to Figure 1a. A similar format is used in subsequent figures. Rectangles A, B and C correspond to photgraphs in figure 3. Abbreviations: mcp,middle cerebellar peduncle; ML,medial lemniscus; ped,cerebral peduncle; PG,pontine gray.


ROSTRAL
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CAUDAL

Fig. 3. Photomicrographs corresponding to boxes labeled A-C in Figure 2. A. Medial pontine nucleus, F-H stain, 384x. B. Ventral pontine nucleus, F-H stain, 384x. C. Medial Lateral pontine nuclei. Autoradiography (darkfield), 60.5x. Abbreviations: ML,medial lemniscus: ped,cerebral peduncle.

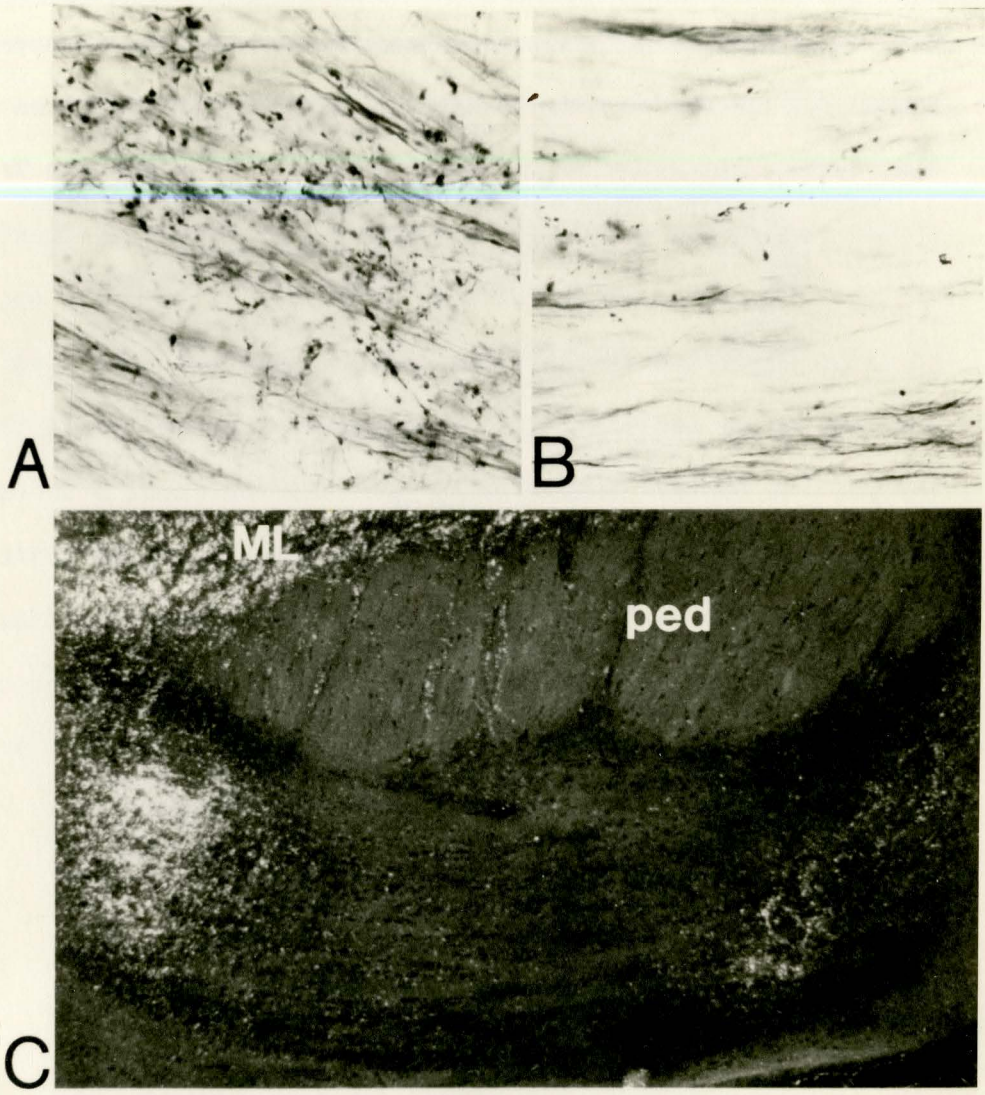
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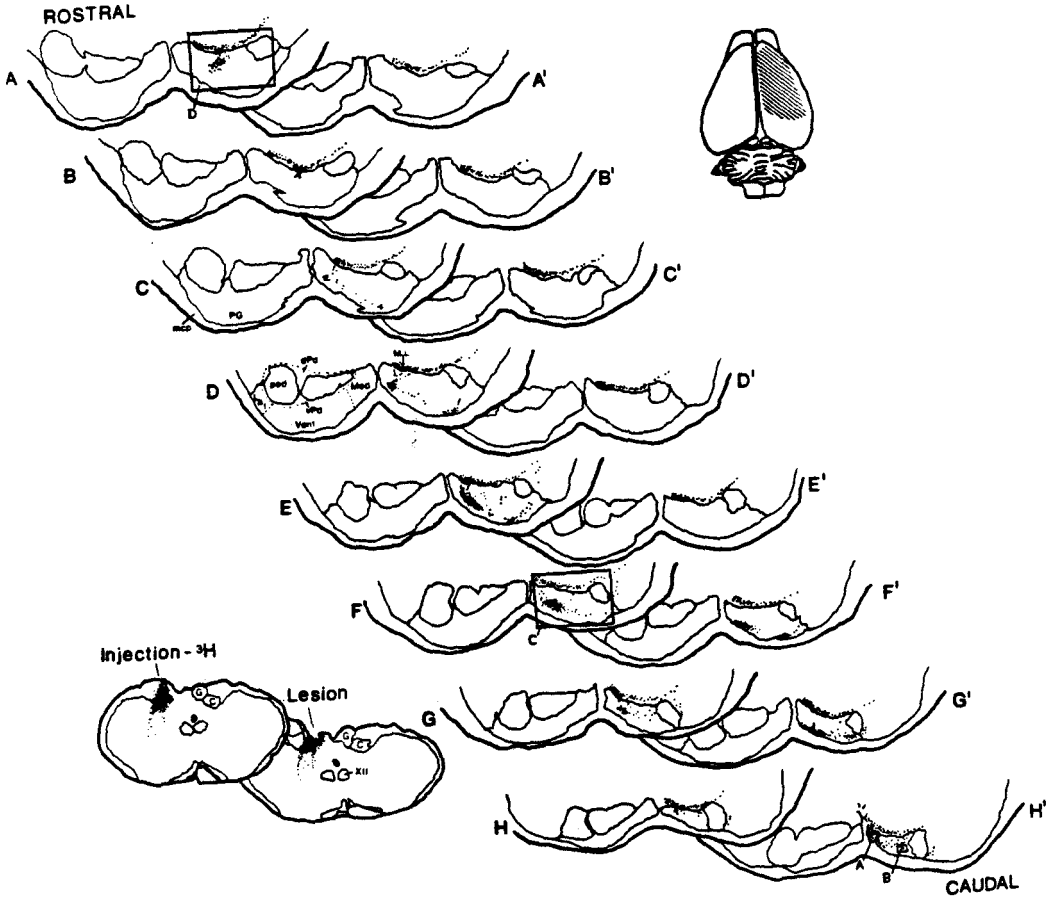
Fig. 4. Camera lucida drawings depicting the distribution pattern of radiolabeled cuneopontine (sections A-H) and depgenerative graciloponţine projections (sections A'-H') as seen throughout the caudal 4/5 of the PG from an adult animal having sustained a neonatal RSMC lesion (depicted in the upper right). Drawings are from a single animal sustaining a unilateral injection of tritiated leucine into nucleus cuneatus combined with ablation of the adjacent nucleus gracilis (depicted in the lower left). Sections A-A', B-B', etc. are adjacent 40 um sections; 200 um intervals separate the paired sections. The pontine nuclear subdivisions indicated in level D correspond to Figure 1a. Rectangles A, B and C correspond to photgraphics in figure 5. Abbreviations: C,nucleus cuneatus; G,nucleus gracilis; G,nucleus gracilis; mcp,middle cerebellar peduncle; ML,medial lemniscus; ped,cerebral peduncle; PG,pontine gray; XII,hypoglossal nucleus.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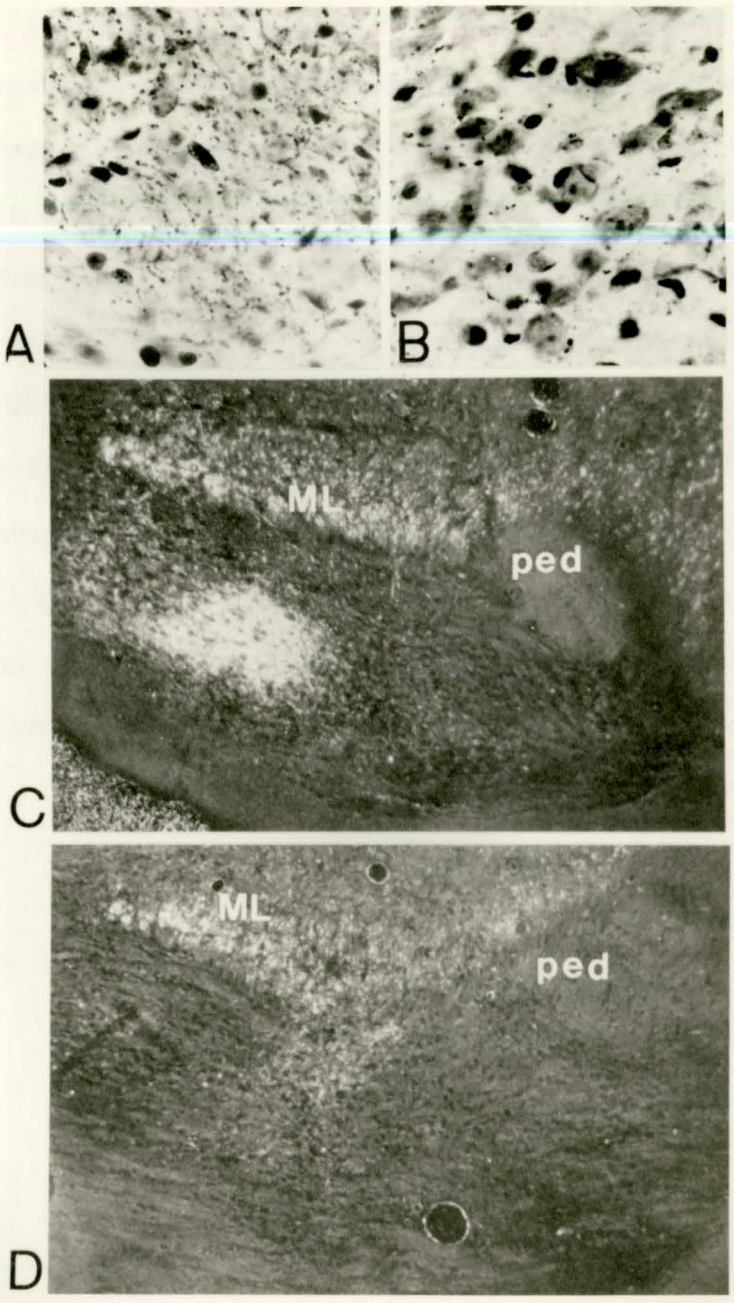
Lesion
CAUDAL

Fig. 5. Photomicrographs corresponding to boxes labeled A-D in Figure 2. A Medial pontine nucleus, F-H stain, 384x. B. Ventral-Lateral pontine nucleus, Autoradiography (darkfield), 60.5x. D. Ventral pontine nucleus, Autoradiography (darkfield), 60.5x Abbreviations: ML,medial lemniscus; ped,cerebral peduncle.


A
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C
ML
ped
D

Fig. 6. Camera lucida drawings depicting the distribution pattern of radiolabeled cuneopontine (sections A-H) and degenerative graciloпontine projections (sections A'-H') as seen throughout the caudal 4/5 of the PG from an animal having sustained a neonatal LCb lesion (depicted in the upper right). Drawings are from a single animal following a unilateral injection of tritiated leucine into nucleus cuneatus combined with an ablation of the adjacent nucleus gracilis (depicted in the lower left). Sections A-A', B-B', etc. are adjacent 40 um sections; 200 um intervals separate the paired sections. The pontine nuclear subdivisions indicated in level D correspond to Figure 1a. Rectangles A, B and C correspond to photgraphs in figure 7. Abbreviations: C,nucleus cuneatus; C, nucleus gracilis; mcp,middle cerebellar peduncle; ML,medial lemniscus; ped,cerebral peduncle; PG,pontine gray; XII,hypoglossal nucleus.

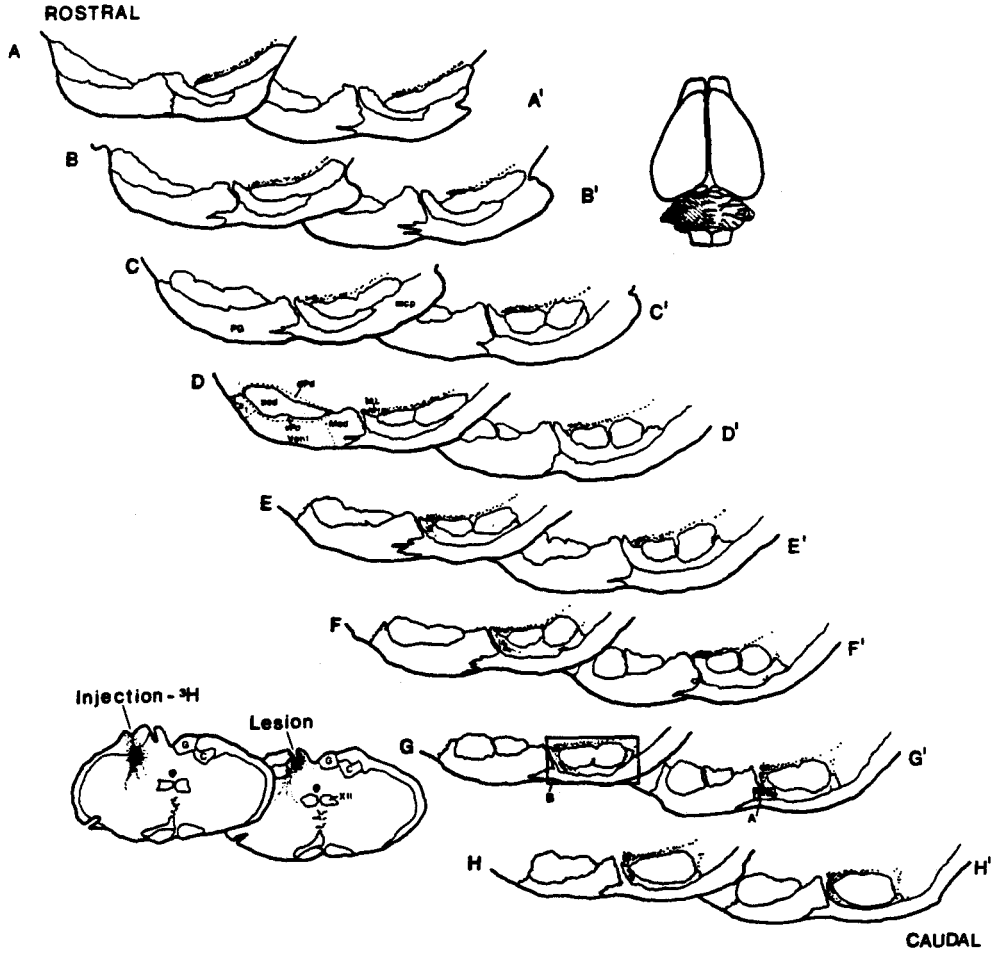
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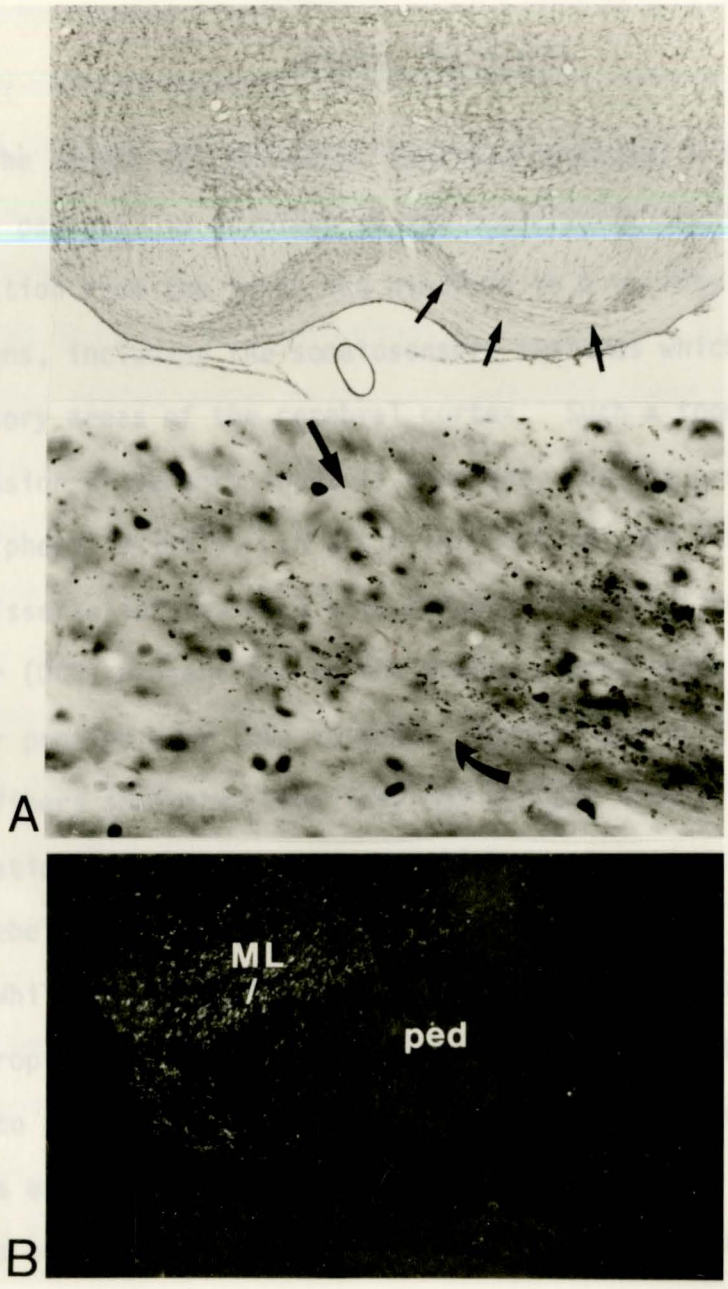
Fig. 7A. Low power photomicrograph of a representative section through the caudal PG of an animal having sustained a neonatal LCb lesion. Note the atrophied PG contralateral to the neonatal cerebellar lesion as indicated by the arrows.

Fig. 7B. Photomicrographs corresponding to boxes labeled A-B in Figure 4. A. Medial pontine nuclear regions, F-H stain, 384x. Note the aberrant graciloPontine fibers crossing the midline into the intact, ipsilateral PG. Arrows indicate skewed midline. B. Medial-Lateral PG, Autoradiography (darkfield), 60.5x. Abbreviations: ML,medial lemniscus; ped,cerebral peduncle.


A
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GENERAL DISCUSSION

The dorsal column-medial lemniscal pathway, a major ascending sensory pathway, is involved in the topographic transmission of tactile information from the fore- and hindlimb to a variety of brainstem locations, including the somatosensory thalamus which relays this input to sensory areas of the cerebral cortex. Such a topographic progression of sensory input is necessary for the precise recognition of peripheral stimuli. In a similar fashion, the first experiment of this dissertation revealed a topographic distribution of dorsal column nuclear (DCN) projections to the contralateral caudal half of the basilar pontine gray (PG) in rats. Further analysis of the origin of these fibers indicated that they may mediate highly specific, tactile information from the extremities which is then transmitted from the PG to cerebellar levels.

While the cerebellum was once thought to be concerned primarily with proptioceptive functions, in the last few decades it has also been shown to receive topographically arranged tactile inputs from various regions of the body surface including the fore- and hindlimbs (see Shambes et al., 1978). Electrophysiological studies have demonstrated a precise arrangement of evoked tactile inputs from the fore- and hindlimb of one side of the body to form "mosaic" or "fractured" arrangements in discrete zones or patches within the granule layers of

the ipsilateral paramedian lobule (Shambes et al., 1978). While this tactile cerebellar input has been suggested to be mediated through a transcortical loop, i.e., DCN - somatosensory thalamus - primary sensory cortical areas - pontine gray - cerebellum (Jansen and Brodal, 1954; Jansen, 1957; Oscarsson, 1970; Eccles, 1973; Ruegg and Wiesendanger, 1975; Ruegg et al., 1977), recent studies by Shambes et al (1978) indicate that peripherally evoked tactile inputs reach the cerebellar cortex prior to the cerebral cortex and therefore via a separate pathway. In support of these electrophysiological findings, the results from the first experiment of this dissertation suggest that DCN - pontine projections are at least contributing to the transmission of this tactile cerebellar input.

Electrophysiological studies have further revealed that primary sensory cortical stimulation as well as peripherally evoked tactile inputs activate different but intermingled populations of granule cells in both cats (Allen et al., 1974) and rats (Bower et al., 1981). Since fore- and hindlimb sensorimotor corticopontine projections distribute throughout the ipsilateral PG in a patch-like appearance resembling the tactile arrangement of peripheral inputs recorded in the cerebellum (Shambes et al., 1978), the intermingling or converging of tactile sensory inputs within the cerebellar cortex has been postulated to be influenced through a similar convergence of sensory inputs located at pontine levels (Bower et al., 1981). In comparing the distribution of cuneo- and graciloponting projections described in the first study with previous investigations of the distribution of fore- and hindlimb

sensorimotor corticopontine projections (Mihailoff et al., 1978; Kartje-Tillotson et al., 1981; Wiesendanger and Wiesendanger, 1982b), such an overlap seemed plausible for caudal levels of the PG. Consequently, information about the spatial location of peripheral stimuli appear to reach tactile regions of the cerebellum via both ipsilateral primary sensorimotor corticopontine projections (Bower et al., 1981) and contralateral DCN projections.

The second study of this dissertation investigated whether an anatomical convergence exists between pontine afferents from primary fore- and hindlimb sensorimotor cortical areas and the corresonding DCN, cuneatus and gracilis. Indeed, corticopontine afferents from the ipsilateral forelimb sensory and hindlimb sensorimotor cortical areas were found to partially overlap with contralateral cuneo- and graciloпontine projections within the caudal half of the PG. Although the functional implications of these patch-like converging sensory inputs are at best speculative, electrophysiological studies indicate that such converging sensory inputs have at least a "collaborative" effect on pontine neurons which subsequently influence the corresponding patches of granule cells within the various cerebellar lobules (Bower et al., 1981). These latter affects have in addition, been shown to directly modify the response properties of an immediately overlying patch-like arrangement of Purkinje cells (Bower and Woolston, 1983). Exactly how this information affects cerebellar function has not been explored, however it has been suggested to provide appropriate spatiotemporal cues for processing sensory information necessary for

"accomplishing orderly tactuomotor behaviors" (Shambes et al., 1978).

The precise arrangement of ascending DCN and descending sensory cortical pathways within the PG not only provides for unique studies concerning the relationships of cerebellar afferents with cerebellar functions, it also provides a basis for additional studies concerned with the interactions of these afferents in response to neonatal brain damage. In particular, neonatal sensorimotor cortical lesions of one hemisphere deprive ipsilateral pontine neurons of their cortical inputs and thus presumably providing avaialable synaptic space for other PG afferents, e.g., sensorimotor coritical and DCN projections. In comparison, neonatal hemicerebellar lesions result in a marked cellular atrophy within the contralateral PG which thereby deprives both ipsilateral corticopontine and contralateral DCN projections as well as other PG afferents of their target cells.

While corticopontine projections have been extensively investigated following the neonatal cortical or cerebellar lesions (Leong and Lund, 1973; Leong, 1976a,b; 1977; 1980; Mihailoff and Castro, 1981; Kartje-Tillotson, 1981; Castro and Mihailoff, 1983), DCN projections to the PG have not been investigated following either lesion. The third study of this dissertation, therefore, examined these ascending somatosensory pontine afferents in response to similar neonatal cortical and cerebellar lesions and attempted to compare the remodeling of these projections with the previously described remodeling of corticopontine projections.

Following either neonatal lesion, the remodeling of DCN fibers

appeared to reflect the normal imbrication of pontine afferents from the DCN and sensorimotor cortex (as described in the second study). For example, DCN-pontine projections were primarily found to expand their normal terminal distributions following neonatal cortical lesions suggesting that these DCN projections sprouted to occupy corticopontine synaptic sites left vacant by the neonatal cortical lesions. In contrast, DCN-pontine projections were primarily found to be attenuated following hemicerebellar lesions possibly reflecting the atrophy of pontine neurons as well as a concomittant expansion of ipsilateral sensorimotor corticopontine projections (Leang, 1980). The results concerning DCN projections following either neonatal lesion, in comparison to previous studies describing the remodeling of sensorimotor corticopontine projections indicates that the distribution of these modified pontine afferents may retain a proximity in relation to their terminal fibers as described for normal animals in the second experiment of this dissertation.

While many studies have linked the growth of aberrant projections following neonatal lesions with recovery of function (Hicks and D'Amato, 1970; Schneider, 1973; 1979; Castro, 1977; Neuman et al., 1982), others have correlated such aberrant growth with maladaptive or detrimental behavior (Schneider, 1979). Although the present dissertation did not attempt to provide functional correlation concerning the observed remodeling of DCN projections after neonatal brain damage, it does reveal that ascending projections establish closely related connections with descending sensorimotor corticopontine

projections and that these projections retain a certain anatomical congruency in response to neonatal brain damage, suggesting such anomalous pathways may be of functional significance to the animal. Further studies utilizing both anatomical and electrophysiological techniques are intended to extend the results obtained in the three studies of this dissertation in order to compare the interactions of ascending and descending pathways in both normal and neonatally brain damaged animals. The basilar pontine system, which in recent years has been found to receive input from practically all levels of the neuraxis, provides a good model for such examination.

BIBLIOGRAPHY

Albus, K., Donate-Oliver, F., Sanides, D. and Fries, W. The distribution of pontine projection cells in visual and association cortex of the cat: An experimental study with HRP, J. Comp. Neurol., 201 (1981) 175-189.

Allen, G.I., G.B. and Ohno, T., Somatotopically organized inputs from fore- and hindlimb areas of sensorimotor cortex to cerebellar Purkyne cells, Explo. Brain Res., 20 (1974) 255-272.

Azizi, S.A., Mihailoff, G.A., Burne, R.A. and Woodward, D.J. The pontocerebellar system in the rat: An HRF study. I. Posterior vermis, J. Comp. Neurol., 197 (1981) 543-558.

Barr, M.L., The Human Nervous System. An Anatomical Viewpoint, Harper and Row, Maryland, 1979.

Basbaum, A.I. and Hand P.J., Projections of cervicothalamic dorsal roots to the cuneate nucleus of the rat, with observations on cellular "bricks", J. Comp. Neurol., 148 (1973) 347-360.

Biedenbach, M.A., Cell density and regional distribution of cell types in the cuneate nucleus of the rhesus monkey, Brain Res., 45 (1972) 1-14.

Berkley, K.J., Different targets of different neurons in nucleus gracilis of the cat, J. Comp. Neurol., 163 (1975) 285-304.

Berkley, K.J., Spatial relationships between the terminations of somatic sensory and motor pathways in the rostral brainstem of cats and monkeys. I. Ascending somatic sensory inputs to lateral diencephalon, J. Comp. Neurol., 193 (1980) 283-317.

Berkley, K.J., Blomqvist, A., Pelt, A. and Flink, R., Differences in the collateralization of neuronal projections from the dorsal column nuclei and lateral cervical nucleus to the thalamus and tectum in the cat: an anatomical study using two different double-labeling techniques, Brain Res., 220 (1980) 273-290.

Berkley, K.J. and Hand, P.J., Projections to the inferior olive from the gracile, cuneate and trigeminal nucleu in the cat, Anat. Rec. 184 (1976) 359.

Berkley, K.J. and Hand, P.J., Efferent projections of the gracile nucleus in the cat, Brain Res., 153 (1978) 263-283.

Blomqvist, A., Flink, R., Bowsher, D., Griph, S. and Westman, J., Tectal and thalamic projections of dorsal column and lateral cervical nuclei: A quantitative study in the cat, Brain Res., 141 (1978) 335-341.

Bold, E.L., Kosinski R.J. and Neafsey, E.J., The topography of dorsal column nuclear projections to sensorimotor cortex through the thalamic posterior nuclear complex in rats, Neurosci. Abstr., in press.

Bold, E.L., and Neafsey, E.J., A comparison of the thalamic afferents to the rostral and caudal forelimb regions of the rat motor cortex, Neurosci. Abst., 8 (1982) 542.

Bower, J.M., Beerman, D.H., Gibson, J.M., Shambes, G.M. and Welker, W., Principles of organization of a cerebro-cerebellar circuit: Micromapping the projections from cerebral (SI) to cerebellar (granule cell layer) tactile areas of rats, Brain Behav. Evol., 18 (1981) 1-18.

Bower, J.M., and Woolston, D.C., Congruence of spatial organization of tactile projections to granule cell and Purkinje cell layers of cerebellar hemispheres of the albino rat: Vertical organization of cerebellar cortex, J. Neurophys., 49 (1983) 745-766.

Bowsher, D., Projection of the gracile and cuneate nuclei in Macaca mulatta: An experimental degeneration study, J. Comp. Neurol., 110 (1958) 135-155.

Brodal, A. and Jansen, J., The ponto-cerebellar projection in the rabbit and cat, Experimental investigations, J. Comp. Neurol., 84 (1946) 31-118.

Brodal, A., Neurological Anatomy In Relation to Clinical Medicine, Oxford University Press, New York, 1981.

Brodal, P., The corticopontine projections in the cat. I. Demonstration of a somatotopically organized projection from the primary sensorimotor cortex, Expl. Brain Res., 5 (1968) 210-234.

Brodal, P., The corticopontine projections in the rhesus monkey. Origin and principles of organization, Brain, 101 (1978) 251-283.

Brodal, P., The cerebropontocerebellar pathways: Salient features of its organization. In: S.L. Palay and V. Chan-Palay (Eds.), The Cerebellum New Vistas, Springer-Verlag, Berlin-Heidelberg, 1982, 108-133.

Bromberg, M.B., Burnham, J.A. and Towe, A.L., Doubly projecting neurons of the dorsal column nuclei, Neurosci. Lett., 25 (1981) 215-220.

Burne, R.A., Azizi, S.A., Mihailoff, G.A. and Woodward, D.J., The tectopontine projection in the rat with comments on visual pathways to the basilar pons, J. Comp. Neurol., 202 (1981) 287-307.

Burne, R.A., Briksson, M.A., Saint-Cyr, J.A. and Woodward, D.J., The organization of the pontine projection to lateral cerebellar areas in the rat: Dual zones in the pons, Brain Res., 139 (1978a) 340-347.

Burne, R.A., Mihailoff, G.A., and Woodward, D.J., Visual corticopontine input to the paraflocculus: A combined autoradiographic and HRP study, Brain Res., 143 (1978b) 139-146.

Burton, H. and Loewy, A.D., Descending projections from medullary somatosensory relay nuclei, Neurosci. Abstr., 2 (1976) 904.

Burton, H., and Loewy, A.D., Projections to the spinal cord from medullary somatosensory relay nuclei, J. Comp. Neurol., 173 (1977) 773-792.

Castro, A.J., Ipsilateral corticospinal projections after large cerebral lesions in neonatal rats, Expl. Neurol., 46 (1975) 1-8.

Castro, A.J., Limb preference after large lesions of the cerebral hemisphere in neonatal rats, Physio. Behav., 18 (1977) 605-608.

Castro, A.J. and Hazlett, J.C., Spinocerebellar sprouting in response to neonatal hemicerebellectomy, Anat. Rec., 193 (1979) 501-502.

Castro, A.J. and Mihailoff, G.A., Corticopontine remodelling after cortical and/or cerebellar lesions in newborn rats, J. Comp. Neurol., in press.

Cheek, M.D., Rustioni A., and Trevino, D.L., Dorsal column nuclei projections to the cerebellar cortex in cats as revelaed by the use of the retrograde transport of horseradish perioxidase, J. Comp. Neurol., 164 (1975) 31-46.

Cooper, M.H. and Beal, J.A., The neurons and the synaptic endings in the primate basilar pontine nuclei, J. Comp. Neurol., 180 (1978) 17-42.

Cooper, M.H. and Fox, C.A., The basilar pontine gray in the adult monkey (Macaca mulatta): A golgi study, J. Comp. Neurol., 168 (1976) 145-174.

Cotman, C.W., Neuronal Plasticity, Raven Press, New York, 1978.

Cotman, C.W., Lewis, E.R. and Hand, D., The critical afferent theory: A mechanism to account for septohippocampal development and plasticity. In H. Flohr and W. Precht (Eds.), Lesion-Induced Neuronal Plasticity in Sensorimotor Systems, Springer-Verlag, New York, 1981, 13-26.

Cowan, W.M. and Cuneod, M. (Eds.), The Use of Axonal Transport for Studies of Neuronal Connectivity, Elsevier, Amsterdam, 1975.

Crain, B.J. and Hall, W.C., The normal organization of the lateral posterior nucleus in the golden hamster and its reorganization after neonatal superior colliculus lesions, Behav. Brain Res., 3 (1981) 223-238.

Crutcher, K.A., Humbertson, A.O. and Martin, G.F., The origin of brain stem-spinal pathways in the North American opossum (Didelphis Virginiana), J. Comp. Neurol., 179 (1978) 169-194.

Cunningham, T.J. and Speas, G., Inversion of anomalous uncrossed projections along the mediolateral axis of the superior colliculus: Implications for retinocollicular specificity, Brain Res., 88 (1975) 73-79.

Davidson, N., The projection of afferent pathways on the thalamus of the rat, J. Comp. Neurol, 124 (1965) 377-390.

Devor, M. and Schneider, G.F., Neuroanatomical plasticity: The principle of conservation of total axonal arborization. In: F. Vital-Durand and M. Jeannerod (Eds.), Aspects of Neural Plasticity, Institute National de la Sante et de la Recherche Medicale, Paris, 1975, 191-202.

Dhanarajan, P., Ruegg, D.G. and Wiesendanger, M., An anatomical investigation of the corticopontine projection in the primate (Saimiri sciureus). The projection from motor and somatosensory areas, Neurosci., 2 (1977) 913-922.

Donoghue, J.P., Kerman, K.L. and Ebner, F.F., Evidence for two organizational plans within the somatic sensory-motor cortex of the rat, J. Comp. Neurol., 183 (1979) 647-664.

Donoghue, J.P. and Wells, J., Synaptic rearrangement in the ventrobasal complex of the mouse following partial cortical deafferentation, Brain Res., 125 (1977) 351-355.

Donoghue, J.P. and Wise, S.P., The motor cortex of the rat: Cytoarchitecture and microstimulation mapping, J. Comp. Neurol., 212 (1983) 76-88.

Dykes, R.W., Parallel processing of cutaneous information in the somatosensory system of the cat, Le Journal Canadien Des Sciences Neurologiques, 9 (1982) 9-19.

Dykes, R.W., Rasmusson, D.D., Sretavan, D. and Rehman, N.E., Submodality segregation and receptive-field suquences in cuneate, gracile and external cuneate nuclei of the cat, J. Neurophys., 47 (1982) 389-416.

Eccles, J.C., A re-evaluation of cerebellar function in man. In: Desmedt (Ed.), New Developments in Electromyography and Clinical Neurophysiology, Volume 3, Karger, Basel, 1973, 209-224.

Edwards, D.B., Ginsburgh, C.L., Kenkel, C.K. and Stein, B.E., Sources of subcortical projections to the superior colliculus in the cat, J. Comp. Neurol., 184 (1979) 309-329.

Ellis, L.C. Jr. and Rustioni, A., A correlative HRP, Golgi, and EM study of the intrinsic organization of the feline dorsal column nuclei, J. Comp. Neurol., 197 (1981) 341-367.

Emmers, R., Organization of the first and second somesthetic regions (SI and SII) in the rat thalamus, J. Comp. Neurol., 124 (1965) 215-227.

Feldman, S.G. and Kruger, L., An axonal transport study of the ascending projection of medial lemniscal neurons in the rat. J. Comp. Neurol., 192 (1980) 427-454.

Finger, S. and Stein, D.G., Brain Damage and Recovery: Research and Clinical Perspectives, Academic Press, New York, 1983.

Fink, R.P. and Heimer, L., Two methods for selective silver impregnation of degenerating axons and their synaptic endings in the central nervous system, Brain res., 4 (1967) 369-374.

Finlay, E.I., Wilson, K.G. and Schneider, G.E., Anomalous ipsilateral retinotectal projections in Syrian hamsters with early lesions: Topography and functional capacity, J. Comp. Neurol., 183 (1979) 721-740.

Flohr, H. and Precht, W., Lesion-Induced Neuronal Plasticity in Sensorimotor Systems, Springer-Verlag, New York, 1981.

Friedman, D.P. and Jones, E.G., Thalamic input to areas 3a and 2 in monkeys, J. Neurophys., 45 (1981) 59-85.

Frost, D.O., Orderly anomalous retinal projections to the medial geniculate, ventrobasal, and lateral posterior nuclei of the hamster, J. Comp. Neurol., 203 (1981) 227-256.

Frost, D.O., and Schneider, G.E., Plasticity of retinofugal projections after partial lesions of the retina in newborn Syrian hamsters, J. Comp. Neurol., 185 (1979) 517-568.

Fujito, Y., Tsukahara, N. and Yoshida, M., Synaptic plasticity in chronically hemicerebellectomized or hemispherectomized kittens, Neurosci, Lett., 4: Suppl. (1980) 42.

Ganchrow, D. and Bernstein, J.J., Projections of caudal fascioulus gracilis to nucleus gracilis and other medullary structures, and Clarke's nucleus in the rat, Brain Res., 205 (1981) 383-390.

Goldberg, M.E., The role sprouting might play during the recovery of motor function In: H. Flohr and W. Precht (Eds.), Lesion-Induced Neuronal Plasticity in Sensorimotor Systems, Springer-Verlag, New York, 1981, 130-140.

Goldberg, M.E. and Murray, M., Recovery of movement and axonal sprouting may obey some of the same laws. In: C.W. Cotman (Ed.), Neuronal Plasticity, Raven Press, New York, 1978, 73-97.

Goldman, P.S., Neuronal plasticity in primate telencephalon: Anomalous projections induced by prenatal removal of frontal cortex, Science, 202 (198=78) 768-770.

Grant, G., Projection of the external cuneate nucleus onto the cerebellum in the cat: An experimental study using silver methods, Expl. 5 (1962) 179-195.

Gray, T.S., Hazlett, J.C. and Martin, G.F., Organization of projections from the gracile, medial cuneate and lateral cuneate nuclei in the North American opossum. Horseradish peroxidase study of the cells projecting to the cerebellum, thalamus and spinal cord, Brain Behav. Evol., 18 (1981) 140-156.

Groenewegen, H.J., Doesten, A.J.P. and Voogel, J., The dorsal column nuclear projections to the nucleus ventralis psoterior lateralis thalami and the inferior olive in the cat; An autoradiographic study, J. Comp. Neurol., 162 (1975) 505-518.

Guillery, R.W. Adrian, H.O., Woolsey, C.N. and Rose, J.E., Activation of somatosensory areas I and II of cat's cerebral cortex by focal stimulation of the ventrobasal complex. In: D.F. Purpura and M.D. Yahr (Eds.), The Thalamus, Columbia University Press, New York, 1966, 197-206.

Hall, R.D. and Lindholm, E.P., Organization of motor and somatosensory neocortex in the albino rat, Brain Res., 66 (1974) 23-38.

Hand, F., Lumbosacral dorsal root terminations in the nucleus gracilis of the cat, J. Comp. Neurol., 126 (1966) 137-156.

Hand, P.J. and Morrison, A.R., Thalamocortical projections from the ventrobasal complex to somatic sensory areas I and II, Expl. Neurol., 26 (1970) 291-308.

Hazlett, J.C., Dom, R. and Martin, G.F., Spino-bulbar, spino-thalamic and medial lemniscal connections in the American opossum, Didelphis marsupialis virginia, J. Comp. Neurol., 146 (1972) 95-118.

Herkenham, M., The afferent and efferent connections of the ventromedial thalamic nucleus in the rat, J. Comp. Neurol., 183 (1979) 487-518.

Herkenham, M., Laminar organization of thalamic projections to the rat neocortex, Science, 207 (1980) 532-535.

Hicks, S.P. and D'Amato, C.J., Motor-sensory and visual behavior after hemispherectomy in newborn and mature rats, Expl. Neurol., 29 (1970) 416-438.

Hicks, S.P. and D'Amato, C.J., Motor-sensory cortex corticospinal system and developing locomotion and placing in rats, Amer. J. of Anat., 143 (1975) 1-42.

Hoddevik, G.H., The pontocerebellar projections to the paramedian lobule in the cat: An experimental study with the use of horseradish peroxidase as a tracer, Brain Res., 95 (1975) 291-307.

Jacobson, S. and Trojanowski, J.Q., Corticothalamic neurons and thalamocortical terminal fields: An investigation in rat using horseradish peroxidase and autoradiography, Brain Res., 85 (1975) 385-401.

Jane, J.A. and Schroeder, D.M., A comparison of dorsal column nuclei and spinal afferents in the European hedgehog (Erinaceus europeous), Expl. Neuol., 30 (1971) 1-17.

Jansen, J., Afferent impulses to the cerebellar hemispheres from the cerebral cortex and certain subcortical nuclei: An electro-anatomical study in the cat, Acta. Physicl. Scand., 41: suppl. 143, (1957) 1-99.

Jansen, J. and Brodal, A., Aspects of Cerebellar Anatomy, Tanum, Oslo, 1954.

Johnson, J.I., Welker, W.I. and Pubols, B.H., Somatotopic organization of raccon dorsal column nuclei, J. Comp. Neurol., 132 (1968) 1-44.

Jones, E.G. and Leavitt, R.Y., Demonstration of thalamo-cortical connectivity in the cat somato-sensory system by retrograde axonal transport of horseradish perioxidase, Brain Res., 63 (1973) 414-418.

Jones, E.G. and Powell, T.P.S., The projection of the somatic sensory cortex upon the thalamus in the cat, Brain Res., 10 (1968) 369-391.

Jones, E.G. and Powell, T.P.S., The cortical projection of the ventroposterior nucleus of the thalamus in the cat, Brain Res., 13 (1969) 298-318.

Jones, E.G., Wise, S.P. and Coulter, J.D., Differential thalamic relationships of sensory-motor and parietal cortical fields in monkeys, J. Comp. Neurol., 183 (1979) 833-882.

Joseph, J.W., Shambes, G.M., Gibson, J.M. and Welker, W.I., Tactile projections to granule cells in caudal vermis of the rat's cerebellum, Brain Behav. Evol., 15 (1978) 141-149.

Kartje-Tillotson, G., Neafsey, E.J. and Castro, A.J., The specificity of corticopontine plasticity, Anat. Rec., 199 (1981) 133A.

Kawaguchi, S., Yamamolo, T., Samejima, A., Itoh, K., and Mizuno, N., Morphological evidence for axonal sprouting of cerebellothalamic neurons in kittens after neonatal hemicerebellectomy, Expl. Brain Res., 35 (1979a) 511-518.

Kawaguchi, S., Yamamoto, T., Samejeima, A., Electrophysiological evidence for axonal sprouting of cerebellothalamic neurons in kittens after neonatal hemicerebellectomy, Exp. Brain Res., 36 (1979b) 21-39.

Kawamura, K. and Chiba, M., Cortical neurons projecting to the pontine nuclei in the cat. An experimental study with the horseradish perioxidase technique, Exp. Brain Res. 35 (1979) 269-285.

Kawamura, K. and Hashikawa, T., Projections from the pontine nuclei proper and recticular tegmental nuclei onto the cerebellar cortex in the cat. An autoradiographic study, J. Comp. Neurol. 201 (1981) 395-413.

Keller, J.H. and Hand, P.J., Dorsal root projections to nucleus cuneatus of the cat, Brain Res., 20 (1970) 1-17.

King, J.S., Martin G.F., and Biggert, T., The basilar pontine gray of the opossum. I. Morphology, J. Comp. Neurol., 133 (1968) 439-446.

Kolb, B., Sutherland, R.J. and Whishaw, I.Q., Abnormalities in cortical and subcortical morphology after neonatal neocrotical lesions in rats, Expl. Neurol., 79 (1983) 223-244.

Kosinski, R.J., Kartje-Tillotson, G. and Castro, A.J., Topographic analysis of dorsal column nuclear and motor cortical projections to the basilar pontine gray in rats, Neurosci. Abstr., 8 (1982) 446.

Kuypers, H.G.J.M. and Tuerk, J.D., The distribution of cortical fibers within the nuclei cuneatus and gracilis in the cat, J. Anat. Long., 98 (1964) 143-162.

Laurence, S. and Stein, D.G., Recovery after brain damage and the concept of localization of function. In: S. Finger (Ed.), Recovery From Brain Damage, Flenum Press, New York, 1978, 369-407.

Leong, S.K., A qualitative electron microscopic investigation of the anomalous corticofugal projections following neonatal lesions in the albino rat, Brain Res., 107 (1976a) 1-8.

Leong, S.K., An experimental study of the corticofugal system following cerebral lesions in the albino rat, Expl. Brain Res., 26 (1976b) 235-247.

Leong, S.K., Sprouting of the corticopontine fibers after neonatal cerebellar lesion in the albino rat, Brain Res., 123 (1977) 164-169.

Leong, S.K., Effects of deaffenting cerebellar or cerebral inputs to the pontine and red nuclei in the albino rat, Brain Res., 155 (1978) 357-361.

Leong, S.K., A qualitative electron microscopic study of the corticopontine projections after neonatal cerebellar hemispherectomy, Brain Res., 194 (1980) 299-310.

Leong, S.K. and Lund, R.D., Anomalous bilateral corticofugal pathways in albino rats after neonatal lesions, Brain Res., 62 (1973) 218-221.

Lim, K.H., Leong, S.K., Aberrant bilateral projections from the dentate and interposed nuclei in albino rats after neonatal lesions, Brain Res., 96 (1975) 306-309.

Lin, C.S. and Chapin, J.K., Some connections of SI and SII in the rat, Neurosci. Abstr., (1981) 396.

Liu, C-N. and Chambers, W.W., Intraspinal sprouting of dorsal root axons, Arch. Neurol. Psych., 79 (1958) 46-61.

Lund, R.D., Development and Plasticity of the Brain, Oxford Univ. Press, New York, 1978.

Lund, R.D. and Lund, J.S., Synaptic adjustments after deafferentation of the superior colliculus of the rat, Science, 171 (1971) 804-806.

Lund, R.D. and Webster, K.E., Thalamic afferents from the dorsal column nuclei; An experimental anatomical study in the rat, J. Comp. Neurol., 130 (1967) 301-312.

Martin, G.F. and King, J.S., The basilar pontine gray of the opossum (Didelphis virginiana). II. Experimental determination of neocrotical input, J. Comp. Neurol., 133 (1968) 447-462.

Matzke, H.A., Course of fibers arising from nucleus gracilis and cuneatus of cat, J. Comp. Neurol., 94 (1951) 439-452.

Mesulam, M.M., Tetramethyl benzidine for horseradish perioxidase neurohistochemistry: A non-carcinogenic blue reaction product with superior sensitivity for visualizing neural afferents and efferents, J. Histochem. and Cytochem., 26 (1978) 106-117.

Mihailoff, G.A., Principle neurons of the basilar pons as the source of a recurrent collateral system, Brain Res. Bull., 3 (1978a) 319-332.

Mihailoff, G.A. Anatomic evidence suggestive of dendrodendritic synapses in the opossum basilar pons, Brain Res. Bull., 3 (1978b) 333-340.

Mihailoff, G.A., Intra- and interhemispheric collateral branching in the rat pontocerebellar system, a flourescence double-label study, J. Comp. Neurol., in press.

Mihailoff, G.A., Burne, R.A., Azizi, S.A., Norell, G. and Woodward, D.J., The pontocerebellar system in the rat: An HRP study. II. Hemispheral components, J. Comp. Neurol., 197 (1981a) 559-577.

Mihailoff, G.A., Burne, R.A. and Woodward, D.J., Projections of the sensorimotor cortex to the basilar pontine nuclei in the rat: An autoradiographic study, Brain Res., 145 (1978) 347-354.

Mihailoff, G.A. and Castro, A.J., Autoradiographic and electron microscopic degeneration evidence for axonal sprouting in the rat corticopontine system, Neurosci. Lett., 21 (1981) 267-273.

Mihailoff, G.A. and King, J.S., The basilar pontine gray of the oppossum: A correlated light and electron microscopic analysis, J. Comp. Neurol., 159 (1975) 521-552.

Mihailoff, G.A. and McArdle, G.B., The cytoarchitecture, cytology, and synaptic organization of the basilar pontine nuclei in the rat. II. Electron microscopic studies, J. Comp. Neurol., 195 (1981) 203-219.

Mihailoff, G.A., McArdle, C.B. and Adams, C.E., The cytoarchitecture, cytology, and synaptic organization of the basilar pontine nuclei in the rat. I. Nissl and Golgi studies, J. Comp. Neurol., 195 (1981b) 181-201.

Mihailoff, G.A., Watt, G.B. and Burne, R.A., Evidence suggesting that both the corticopontine and cerebellopontine systems are each composed of two separate neuronal populations. An electron microscopic and horseradish peroxidase study in the rat, J. Comp. Neurol., 195 (1981c) 221-242.

Mustari, M.J. and Lund, R.D., An aberrant crossed visual corticotectal pathway in albino rats, Brain Res., 112 (1976) 37-44.

Nah, S.H. and Leong, S.K., Bilateral corticofugal projections to the red nucleus after neonatal lesions in the albino rat, Brain Res., 107 (1976a) 433-436.

Nah, S.H. and Leong, S.K., An ultrastructural study of the anomalous corticorubral projection following neonatal lesions in the albino rat, Brain Res., 111 (1976b) 162-166.

Nah, S.H., Ong, L.S. and Leong, S.K., Is sprouting the result of a persistent neonatal connection, Neurosci. Lett., 19 (1980) 39-44.

Nauta, W.J.H. and Kuypers, H.G.J.M., Some ascending pathways in the brain stem reticular formation In: H.H. Jasper (Ed.), Recticular Formation of the Brain, Little, Brown, Boston, 1958, 3-39.

Neumann, S., Pritzel, M. and Huston, J.P., Plasticity of cortico-thalamic projections and functional recovery in the unilateral detelencephalized infant rat, Behav. Brain Res., 4 (1982) 377-388.

Nyby, O. and Jansen, J., An experimental investigation of the corticopontie projection in Macaca mulatta, Skr. Norske Vidensk.-Akad., I. Mat.-nat. Kl. No. 3 (1951) 1-47.

Odutola, A.E., Patterns and fields of dorsal column fiber treminals in the cuneo-gracile nuclei of the rat, Expl. Neurol. 54 (1977) 112-120.

Olszewski, J. and Baxter, D., Cytoarchitecture of the Human Brainstem, J.B. Lippincott Co., Philadelphia, 1954.

Oscarsson, O., Functional organization of spino-cerebellar paths. In: Iggo (Ed.), Handbook of Sensory Physiology, Vol. 2: Somatosensory System, Springer, Berlin, 1970.

Paxinos, G. and Watson, C., The Rat Brain: In sterotaxic coordinates, Academic Press, New York, 1982.

Price, T.R. and Webster, K.E., The corticothalamic projections from the primary somatosensory cortex of the rat, Brain Res., 44 (1972) 636-640.

Ralston, H.J. and Sharp, P.V., The identification of thalamocrotical relay cell in the adult cat by means of retrograde axonal transport of horseradish perioxidase, Brain Res., 62 (1973) 273-278.

Ramon y Cajal, S., Histologie du System de L'Homme et des Vertebres, Vol. I, Institute Ramon y Cajal, Madrid, 1909, 892-908.

Rinvik, E. and Walberg, F., Studies on the cerebellar projections from the main and external cuneate nuclei in the cat by means of retrograde axonal transport of horseradish perioxidase, Brain Res., 95 (1975) 371-381.

Robards, M.J., Somatic neurons in the brainstem and neocortex projecting to the external nucleus of the inferior colliculus: An anatomical study in the opossum, J. Comp. Neurol., 184 (1979) 547-566.

Robards, M.J., Watkins, D.W. III and Masterton, R.B., An anatomical study of some somesthetic afferents to the intercollicular terminal zone of the midbrain of the opossum, J. Comp. Neurol., 170 (1976) 499-524.

Romagnano, M.A. and Maciewicz, R.J., Peroxidase labeling of motor cortex neurons projecting to the ventrolateral nucleus in the cat, Brain Res., 83 (1975) 469-473.

Rosina, A., Privini, L., Bentivoglio, M. and Kuypers, H.G.J.M., Ponto-neocerebellar axonal branching as revealed by double flourescent retrograde labeling technique, Brain Res., 195 (1980) 461-466.

Ruegg, D.G., Seguin, J.J. and Wiesendanger, M., Effects of electrical stimulation of somatosensory and motor areas of the cerebral cortex on neurones of the pontine nuclei in squirral monkeys, Neurosci., 2 (1977) 923-927.

Ruegg, D.G. and Wiesendanger, M., Corticofugal effects from sensorimotor area I and somatosensory area II on neurones of the pontine nuclei in the cat, J. Physiol., Lond., 247 (1975) 745-757.

Salibi, N.A., Saade, N.E., Bonna, N.R. and Jabbur, S.J., Dorsal column input into the reticular formation. Nature, 288 (1980) 481-483.

Saporta, S. and Kruger, L., The organization of thalamocortical relay neurons in the rat ventrobasal complex studies by the retrograde transport of horseradish perioxidase, J. Comp. Neurol., 174 (1977) 187-208.

Schneider, G.E., Mechanisms of functional recovery following lesions of visual cortex or superior colliculus in neonate and adult hamsters, Brain Behav. and Evol., 3 (1970) 295-323.

Schneider, G.E., Early lesions of superior colliculus: Factors affecting the formation of abnormal retinal projections, Brain Behav. and Evol., 8 (1973) 73-109.

Schneider, G.E., Is it really better to have your brain lesion early? A revision of the "Kennard Principle", Neuropsych., 17 (1979) 557-583.

Schneider, G.E. and Jhaveri, S.R., Neuroanatomical correlates of spared or altered function after brain lesions in the newborn hamster. In: D.C. Stein, J.J. Rosen and N. Butters (Eds.), Plasticity and Recovery of Function in the Central Nervous System, Academic Press, New York, 1974, 65-109.

Schroeder, D.M. and Jane, J.A., Projections of dorsal column nuclei and spinal cord to brainstem and thalamus in the tree shrew, Typia glis, J. Comp. Neurol., 142 (1971) 309-350.

Shambes, G.M., Gibson, J.M. and Welker, W., Fractured somatotopy in granule cell tactile of rat cerebellar hemispheres revealed by micromapping, Brain Behav. Evol., 15 (1978) 94-140.

Somana, R. and Walberg, F., A re-examination of the cerebellar projections from the gracile, main and external cuneate nuclei in the cat, Brain Res., 186 (1980) 33-42.

Sonnier, B.J., Villablanca, J.R., Olmstead, C., McAllister, J.P., and Gomez, F., Autoradiographic tracing of a crossed cortico-rubral pathway in cats with neonatal ablation of one cerebral hemisphere, Neurosci. Abstr. 8 (1982) 746.

Spreafico, R., Hayes, N.L. and Rustioni, A., Thalamic projections to the primary and secondary somatosensory cortices in cat: Single and double retrograde tracer studies, J. Comp. Neurol., 203 (1981) 67-90.

Steward, O., Reinnervation of dentate gyrus by homologous afferents following entrohinal cortical leions in adult rats, Science, 194 (1976) 426-428.

Sunderland, S., The projection of the cerebral cortex on the pons and cerebellum in the macaque monkey, J. Anat., London, 74 (1940) 201-226.

Swenson, R.S., Kosinski, R.J. and Castro, A.J., Analysis of caudal brainstem and spinal cord projections to the pontine gray in rats. Neurosci. Abstr., 8 (1982) 446.

Swenson, R.S., Kosinski, R.J. and Castrol, A.J., Topography of spinal, dorsal column nuclear and spinal trigeminal projections to the pontine gray in rats, J. Comp. Neurol., in press.

Tan, C.K. and Lieberman, A.R., Identification of thalamic projection cells in rat cuneate nucleus: A light and electron microscopic study using horseradish peroxidase, Neurosci. Lett., 10 (1978) 19-22.

Tripp, L.N. and Weels, J. Formation of new synaptic terminals in the somatosensory thalamus of the rat after lesions of the dorsal column nuclei. Brain Res., 155 (1978) 362-367.

Tsukarhara, N., Synaptic plasticity in the mammalian central nervous system, Neurosci., 4 (1981) 351-379.

Wall, F.D. and Egger, M.D., Formation of new connections in adult rat brains after partial deafferentation, Nature, 232 (1971) 542-545.

Walberg, F. and Brodal, A., Spinopontine fibers in the cat: An experimental study, J. Comp. Neurol., 99 (1953) 251-288.

Watt, C.B. and Mihailoff, G.A., The cerebellopontine system in the rat. I. Autoradiographic studies, J. Comp. Neurol., 215 (1983) 312-330.

Welker, C., Microelectrode delineation of fine grain somatotopic organization of SMI cerebral neocortex in albino rat, Brain Res., 26 (1971) 259-275.

Welker, C., Receptive fields of barrels in the somatosensory neocortex of the rat, J. Comp. Neurol., 166 (1976) 173-190.

Welker, C., and Sinha, M.M., Somatotopic organization of SmII cerebral neocortex in albino rat, Brain Res., 37 (1972) 132-136.

Wiesendanger, R. and Wiesendanger, M., The corticopontine system in the rat. I. Mapping of corticopontine neurons, J. Comp. Neurol., 208 (1982a) 215-226.

Wiesendanger, R. and Wiesendanger, M., The corticopontine system in the rat. II. The projection pattern, J. Comp. Neurol., 208 (1982b) 227-238.

Wise, S.P., The laminar organization of certain afferent and effernt fiber systems in the rat somatosensory cortex, Brain Res., 90 (1975) 139-142.

Wise, S.P. and Jones, E.G., Cells of origin and terminal distribution of descending projections of the rat somatic sensory cortex, J. Comp. Neurol., 175 (1977) 129-158.

APPROVAL SHEET

The dissertation submitted by Ross J. Kosinski has been read and approved by the following committee:

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The final copies have been examined by the director of the dissertation and the signature which appears below varifies the fact that any necessary changes have been incorporated and that the dissertation is now given final approval by the committee with reference to content and form.

The dissertation is therefore accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

November 28, 1983
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