UCSF
UC San Francisco Electronic Theses and Dissertations

Title
The cytoarchitecture and connectivity of the midbrain periaqueductal grey in the monkey

Permalink
https://escholarship.org/uc/item/55r7z0zf

Author
Mantyh, Patrick William

Publication Date
1981

Peer reviewed|Thesis/dissertation

eScholarship.org Powered by the California Digital Library
University of California

THE CYTOARCHITECTURE AND CONNECTIVITY OF THE MIDBRAIN PERIAQUEDUCTAL GREY IN THE MONKEY

by

PATRICK WILLIAM MANTYH

DISSERTATION

Submitted in partial satisfaction of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

ANATOMY

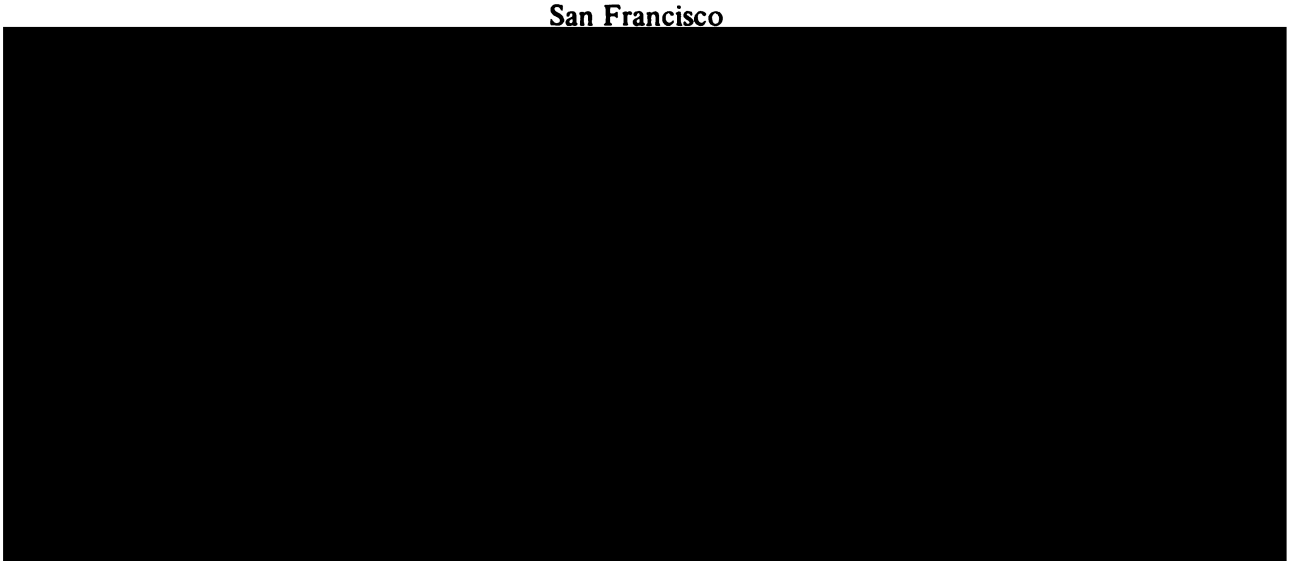
in the

GRADUATE DIVISION

of the

UNIVERSITY OF CALIFORNIA

San Francisco



Date University Librarian

Degree Conferred: APR 5 1981

Dedicated to my family and friends

THE CYTOARCHITECTURE AND CONNECTIVITY OF THE MIDBRAIN PERIAQUEDUCTAL GREY IN THE MONKEY

Table of Contents: Outline

Abstract

In a comprehensive analysis of the periaqueductal grey (PAG), Nissl, Weil and Golgi stained sections through the midbrain were examined in 15 rat, 8 cat, 8 macaque, and 12 squirrel monkey cases.

Nissl and Weil stained material did not reveal any cyto- or myelo-architectural borders within the PAG, except for an apparent cell poor region immediately surrounding the cerebral aqueduct. No significant difference in cell size or shape was found within various regions of the PAG in any of the animals examined.

Golgi impregnated material revealed the PAG to be composed of a heterogenous group of cells which do not appear to be regionally segregated according to size, shape, or dendritic arborization. Most cells observed had extensive dendritic arborizations in the coronal plane with lesser, but still extensive arborizations in the sagittal plane. The cell somas ranged in size from 10-35mm and had shapes ranging from small spherical to large pyramidal. Also noticeable in the Golgi sections was a dense plexus composed of both neuronal processes and glia in the border immediately surrounding the aqueduct. The lateral border, or annulus, of the PAG, formed chiefly by the mesencephalic root of V seen in Nissl or Weil material, is not readily seen in Golgi impregnated material. The stratum profundum of the superior colliculus and the adjacent ventrolateral tegmentum appeared similar in cytoarchitecture to the PAG. Both PAG neurons and neurons from these surrounding areas have dendritic arbors that interdigitate with each other without respect to borders defined by Nissl cytoarchitecture or more apparent myeloarchitecture features. Some PAG cell dendrites extended beyond the stratum profundum into the

stratum intermedium of the superior colliculus. No striking differences in the organization of the PAG, or its constitutent cell morphology was observed between rat, cat, macaque, or squirrel monkey.

The PAG in the animals examined appears to be composed of a population of cells with a wide range of soma size and shape that have extensive dendritic arbors, which often extend into adjacent parts of the tectum or tegmentum. Therefore, cytoarchitectural features of the PAG do not reveal apparent anatomical correlates to the variety of functional subdivisions suggested by physiological studies.

The afferent and efferent connections of periaqueductal grey in the monkey were also investigated in this study by stereotoxically placing combined horseradish peroxidase (HRP) and tritiated amino acid (in monkeys) injections ranging from .01-.1ml in various regions of the periaqueductal grey (PAG) of 10 squirrel monkeys, 4 cats and 15 rats. After a 2-4 day survival time the animals were perfused and alternate frozen sections processed for either HRP histochemistry (Mesulam '78) or autoradiography (Cowan et. al., '72).

From this study it appears the PAG receives wide ranging afferents from the rostralmost pole of the frontal cortex to the sacral spinal cord. HRP positive cells were found in the following areas: Frontal granular cortex; Amygdala; central and basal lateral nuclei; Substantia innominata; Hypothalamus; ventromedial, supraoptic, suprachiasmatic, dorsomedial, lateral, periventricular, posterior, periarcuate; Zona incerta; Mesencephalon: superior colliculus cuneiformis, PAG; Pons: locus coeruleus; Medulla: parvo-, gigantocellular reticular, raphe magnus and raphe pallidus, spinal trigeminal nuclei; Spinal cord. Of all these areas, the hypothalamus and zona incerta have the heaviest projection to the PAG.

Efferent projections were observed in the following nuclei after tritiated amino acid injections into the PAG: Hypothalamus: ventromedial, supraoptic, suprachismatic, dorsomedial, lateral, periventricular, posterior, supramamillary, preoptic area; Dorsal thalamus: centromedian, parafascicularis, medialis pars medialis, paraventricular thalami, centralis medialis, reuniens, rhomboidalis, Ventral tegmental area of Tsai, Zona incerta, Mesencephalon: cuneiformis, PAG, superior colliculus; Pons: raphe magnus, raphe pallidus, locus coeruleus; Medulla: parvo- and giganto-cellular reticular formation, and the nucleus ambiguous. Efferent and afferent connections of the PAG show a significant degree of overlap. This reciprocity is especially evident in the hypothalamic and brainstem areas.

Afferent and efferent connections remain quiet constant from one animal to another even though the area of the PAG injected varied. These findings, along with Nissl, Weil, and Golgi analysis of the PAG do not support the division of the PAG into distinct cell groups, each with its own cytoarchitecture and connectivity.

The implications of these findings with regard to this region of the brain being a visceral, nociceptive and cognitive integrator are discussed.

Abbreviations

III, Oculomotor complex

IV, Trochlear nerve nucleus

V, Tract of mesencephalic trigeminal nucleus

VI, Abducens nucleus

VII, Facial nucleus

XII, Hypoglossal nucleus

AlO, AlO area

Ab, Ambiguous nucleus

AD, Anterodorsal nucleus of the thalamus

ALH, Anterior lateral hypothalamic area

AM, Anteromedial nucleus of the thalamus

AMV, Anterior medullary velum

APL, Lateral preoptic area

APM, Medial preoptic area

AQ, Cerebral aqueduct

Arc, Arcuate nucleus of the hypothalamus

ARG, Autoradiograph

AV, Anteroventral nucleus of the thalamus

BAc, Basal amygdala

BC, Brachium conjunctivum

BL, Basolateral nucleus of the amygdala

BM, Basomedial nucleus of the amygdala

C, Cervical cord

Cd, Caudate nucleus

Ce, Central nucleus of the amygadala

Cel, Central lateral nucleus of the thalamus

Cem, Central medial nucleus of the thalamus

CG, Central grey

ChO, Chiasm of the optic tract

CL, Claustrum

CM, Centromedian nucleus of the thalamus

Cs, Central superior nucleus

CSL, Central superior lateral nucleus of the thalamus

Cun, Cuneiformis nucleus

DG, Dorsal tegmental nucleus of Gudden

DH, Dorsal nucleus of the hypothalamus

DV, Dorsal nucleus of the vagus

FF, Fields of Forel

Fx, Fornix

G, Gracile nucleus

GP, Globus pallidus

H, Field H

HRP, Horseradish peroxidase

IC, Internal capsule

IP, Interpeduncular nucleus

L, Lumbar cord

LA, Lateral nucleus of the amygdala

Lc, Locus coeruleus

LD, Lateral geniculate nucleus

Lim, Limitans nucleus of the thalamus

LP, Lateral posterior nucleus of the thalamus

MD, Mediodorsal nucleus of the thalamus

MesRF, Mesencephalic reticular formation

MTT, Mamillothalamic tract

Mv, Mesencephalic nucleus of the trigeminal nerve

NRM, Nucleus raphe magnus

NSv, Nucleus of the spinal trigeminal tract

NTS, Nucleus of the solitary tract

Olp, Principal olivary nucleus

OT, Optic tract

Pa, Paraventricular nucleus of the thalamus

PAG, Periaqueductal grey

PaH, Paraventricular nucleus of the hypothalamus

Pbl, Parabrachial nucleus, lateral part

Pc, Paracentralis nucleus of the thalamus

Pf, Parafascicular nucleus of the thalamus

Pgl, Paragigantocellular nucleus

PHA, Posterior hypothalamic area

Po, Pontine reticular formation, pars oralis

PuM, Pulvinar nucleus, medial part

Put, Putamen

PVB, Periventricular bundle

Py, Pyramid

R, Reticular nucleus of the thalamus

Reu, Reunion nucleus of the thalamus

Rm, Raphe magnus

RN, Red nucleus

Ro, Raphe obscuris

S, Sacral cord

SC, Superior colliculus

SGV, Substantia grisea ventralis

SI, Substantia innominata

SNc, Substantia nigra pars compacta

SNd, Substantia nigra pars diffusa

SO, Superior olivary nucleus

SqM, Squirrel monkey

SU, Supramamillary nucleus

T, Thoracic cord

V3, Third ventricle

Ves, Vestibular nucleus

Vm, Ventromedial nucleus of the hypothalamus

VPI, Ventral posterior inferior nucleus of the thalamus

VPL, Ventral posterior lateral nucleus of the thalamus

VPM, Ventral posterior medial nucleus of the thalamus

ZI, Zona incerta

CHAPTER 1: NISSL, WEIL, AND GOLGI ANALYSIS OF THE PERIAQUEDUCTAL GREY IN THE RAT, CAT AND MONKEY, CYTOARCHITECTURE OF A PAIN MODULATORY CENTER

INTRODUCTION

The periaqueductal grey (PAG), is composed of a group of densely packed cells which surround the cerebral aqueduct in the midbrain. Positioned between the brainstem and the diencephalon the PAG is in a unique position to sample and/or influence a wide variety of both ascending sensory systems and descending modulating sensory pathways.

A great deal of research interest has been focused on the PAG in recent years because of its role in pain modulation. The PAG has been identified as the most effective site in the brain for the production of stimulation produced analgesia (SPA) (Mayer and Liebeskind, 1974). The key role of this mescencephalic central grey region in the production of the SPA was originally discovered by Reynolds in 1969. In this paper he reported that the analgesia elicted upon PAG stimulation was so efficatious that a laparotomy could be performed on an otherwise unanesthetized rat. During this period of SPA it was reported that the rat appeared to be receptive to all modalities of sensation except that of nociception. This phenomena of SPA in the rat was confirmed by Mayer and Price (1972). This selective blocking of nociceptive modalities during SPA has since been successfully applied to humans with intractable pain (Hosobuchi et. al., 1977). In these patients, as in animal models, the modification of sensory input has been reported to be limited to the nociceptive modalities while other sensory modalities seem relatively unaffected.

It has been hypothesized that this phenomena of SPA utilizes part of the same system by which exogenous opiates and endogenous opiate-like compounds exert their analgesic effects (Basbaum and Fields, 1978). This proposal is supported by several lines of

evidence as reviewed by Basbaum and Fields (1978). The most effective sites for SPA and opiate administration for analgesia overlap considerably in the PAG (Mayer and Liebeskind, 1974). The PAG is also moderately rich in opiate receptors (Pert et. al., 1976) and endogenous opiate-like compounds (Simantov et. al., 1976). Finally and perhaps most significantly, it has been shown that naloxone a specific opiate antagonist partially blocks SPA (Adams, 1976; Akil et. al., 1976). It is hypothesized that once the endogenous opiate-like compounds have been released in the PAG during SPA, these opiates activate PAG neurons which project to medullary serotonergic and noradrenergic neurons. These medullary neurons in turn project to the spinal cord where they produce an inhibition of nociceptive input. Thus, when the PAG is properly stimulated it is able to profoundly inhibit nociceptive input.

The older literature documents a wide spectrum of physiological effects that can be elicited by PAG stimulation. These effects include bladder distention (Skultety, 1959), gastric motility (Skultety, 1963), vocalization (Kanai and Wang, 1962), strong emotional reflexes (Hunsperger, 1956), and inhibition of oxytocin release (Aulsebrook and Holland, 1969). Thus while many current investigators are interested solely in the SPA generated upon excitation of the PAG, it is important to bear in mind that PAG stimulation is also probably producing other physiological actions besides analgesia. Such physiological data suggest that the PAG is a functionally diverse structure which is involved in a multiplicity of actions during its normal functioning. Biochemical analysis has also confirmed that the PAG is a heterogeneous structure. Serotonin

(Palkovits et. al., 1974), noradrenaline (Versteeg et. al., 1976), acetylcholine (Palkovits and Jacobowits, 1976), and dopamine (Versteeg et. al, 1976) are found in moderate to high concentrations in the PAG. Opiate-like putative neurotransmitters such as β-endorphin (Dupont et. al., 1980), and enkephalin (Simantov et. al., 1977; Hokfelt et. al., 1977) have also been shown to be present in moderate to high concentrations in the PAG. These latter findings are hypothesized to partially underlie the analgesic action of SPA by the release of these endogenous opiate-like compounds upon stimulation of the PAG. More recent work has suggested an even greater number of putative neurotransmitters to be present in the PAG in moderate to high concentrations when compared to surrounding areas of the brain. Such putative neurotransmitters as, bombesin (Pert et. al., 1980), GABA (Fahn, 1976), substance P (Hokfelt et. al., 1977), histamine (Taylor et al, 1972) and angiotensin II (Fuxe et. al., 1976), have been shown to be present in the PAG. Such a concentrated collection of putative neurotransmitters in one area is rather uncommon in most areas of the brain and spinal cord. Such findings again suggest the heterogenity and wide spectrum of biochemical and physiological actions of the PAG.

Although the physiology and biochemistry of the PAG is beginning to be better understood, the anatomy of the structure is still ill defined. The purpose of the present study is to describe the normal cytoarchitecture and myeloarchitecture of the PAG and to look for possible morphological correlates to the wide range of physiological and biochemical functions which are influenced by this unique midbrain periaqueductal structure. It is believed that with a more realistic and detailed anatomical knowledge of the PAG, more detailed

physiological and biochemical studies can be undertaken, which should in turn lead to more accurate answers to how the PAG functions. Also important in this study is to note what, if any, are the morphological differences between the various species examined here. In previously published physiological and biochemical studies on the PAG the most frequently used species were the rat, cat, monkey, and human. Results obtained from one species are often difficult to correlate with other species since the basic form of the structure is not known to be similar from one species to the next. Therefore this study will descirbe the basic cytoarchitecture of the PAG across three experimental species and in subsequent companion studies the afferent and efferent connections of the PAG will be examined in these same species.

MATERIALS AND METHODS

The Nissl, Weil, and Golgi analyses reported in this study were conducted on the brains of 15 rats, 8 cats, 8 macaque and 12 squirrel monkeys. All animals used in this study were adults.

For all the Nissl, Weil, Golgi, and HRP histochemistry material, the animals were anesthetized with pentobarbitol. After being anesthetized to a point where no nociceptive or corneal reflexes were present, the animals were perfused transcardially. First a phosphate buffered (pH 7.4) heparinized saline solution (37°) was infused through the vascular system to clear it of most of the circulating blood. After the saline solution flowing out of the right atrium was free of noticeable red blood cells a second solution of buffered fixative was perfused through the vasculature. This buffered fixative

(pH 7.4) contained 2.0% gluteraldehyde, 1.0% paraformaldehyde, and 5% sucrose. This solution was allowed to flow through the vasculature for 15-30 minutes. After this time a third solution of 5% buffered sucrose was perfused through the vasculature to eliminate any excess fixative. Upon completion of the perfusion the brain was carefully removed and allowed to sit in a 30% buffered (pH 7.4) sucrose solution for 2-5 days. After the above steps the processed brains were well fixed and in appropriate condition to begin the individual histological protocols listed below.

Nissl

The fixed cryoprotected brains were cut on a freezing microtome at 50μm in either the coronal or sagittal plane with each third section being saved for the Nissl stain. The cut sections were stored in phosphate buffer (pH 7.4) and subsequently mounted in serial order onto gelatinized slides and allowed to air dry overnight. These slides were then hydrated and placed in .25% cresylecht violet solution (pH 5.3) and stained for 5-15 minutes and then differentiated and dehydrated through a series of graded alcohols. After being dehydrated in an absolute alcohol solution the slides were passed through several changes of xylene and coverslipped with Permount as the mounting medium. Sections were then studied and photographed using an Olympus BH microscope and a Nikon M-35S camera at magnifications from 2-100X.

Weil

The Weil stained material was cut, sectioned and studied in the same way as the Nissls. The staining procedure used was the same as that given by Emmers and Akert (1963).

Golgi

For Golgi impregnation, a modified Golgi Kopsh protocol consistently gave excellent results. The fixed brains were cut into 3mm slices and placed on a rotator in a solution of 1 part 40% formaldehyde plus 4 parts 3.5% $K_2Cr_2O_7$ for a total of 30 hours. The slices were then placed into a solution of 3.5% $K_2Cr_2O_7$ for one week. Without washing the tissue was placed in several changes of .75% $AgNO_3$ for one week intervals and stored in the dark. After this period the tissue was sampled to see if proper impregnation of PAG neurons had taken place. If unsatisfactory the tissue was cycled through the entire procedure again. If satisfactory impregnation had occurred the tissue was then taken out and placed in 70% alcohol for one hour. The tissue was then cut in a coronal or sagittal plane on a Vibratome at 90-100μm. Serial sections were collected in 95% alcohol.

Loose sections were then passed through several changes of absolute alcohol and finally placed in xylene for 5-60 minutes to clear the tissue and remove excess silver deposits. After the xylene clearing, the tissue was mounted on gelatinized slides and coverslipped with permount as the mounting medium. For graphic reconstruction of individual cells an Olympus BH with an attached drawing tube was used. Sections were observed and drawn at 10X, 20X, 40X magnifications. For drawing purposes outlines of each animal's

PAG was drawn to scale using a wall projector. Individual cells were then drawn at an appropriate magnification onto this PAG scale outline using a drawing tube. Thus on a 3' by 3' piece of drawing paper the entire PAG could be reconstructed for analysis with all well impregnated cells drawn onto the PAG in their proper position. Such a finished drawing gave not only the detailed structure of well impregnated PAG neurons but also the topographical position and orientation each cell had within the PAG.

Horseradish Peroxidase Histochemistry

To examine if the Golgi method had a tendency to impregnate only specific classes of PAG neurons we attempted to use a technique which would label PAG neurons irrespective of their neuronal type and compare this with the Golgi material. A 30% solution of horseradish peroxidase (HRP) Sigma type VI .2-1.0 µl was injected into the PAG of blocks of fixed cat brain tissue. After these injections the blocks were placed in a solution of phosphate buffered saline for 1-2 days to allow diffusion via capillary action to take place. After this time the tissue was cut in 90mm sections on a freezing microtome and reacted for HRP histochemistry with DAB-cobalt intensification. Neurons in the PAG which had been cut and exposed to the HRP had taken up the HRP. This HRP uptake probably occured by capillary action and had extensively filled the neuron, so that comparisons between these HRP filled cells and Golgi impregnated neurons could be made. The rational behind this protocol was that while the reasons for selective neuronal impregnation by the Golgi method remain unknown, the diffusion of the HRP injection should be unselective, for any cut

process is a very small tube presumably capable of capillary action. This protocol proved satisfactory for demonstrating that all types neurons labeled by this nonselective HRP method also was labeled in the Golgi impregnated material. Therefore it appears in the PAG that the Golgi method did not have a tendency to impregnated selective classes of cells.

RESULTS

Anatomical Boundaries of the PAG

The periaqueductal grey is a cell rich, myelin poor, alar plate region which surrounds the cerebral aqueduct. In this report the PAG is tentatively considered to be that area ventral and medial to the superior or inferior colliculi, medial to the annulus formed by the root of the trigeminus mesencephalic cells and their fibers, and that area dorsal to the oculomotor and trochlear nuclei. The caudal, iter region of the PAG is marked by the locus coeruleus and the rostral end of the PAG demarcated by the posterior commissure. The only cell group that is partially within these defined borders which will not be considered part of the PAG is the dorsal supratrochlear raphe nucleus. Although the dorsal lateral aspects of the dorsal raphe intermingle with the ventral aspect of the PAG, for the purposes of this description the dorsal raphe will be considered a separate entity in this paper because: it is composed of large cells with a distinctive fusiform shape, it has a set of connections with other areas of the brain that differ markedly from the PAG (Jouvet et al, 1976), and some of its neurons characteristically contain serotonin, a neurotransmitter which is not synthesized by any PAG neuron but is characteristically

synthesized by neurons in certain raphe nuclei.

Cytoarchitecture

The PAG is composed of a heterogeneous group of neurons which vary widely in their somatic shape, size, and dendritic arborization. Nissl stained material shows a gradual increase in both intensity of cellular staining and apparent packing density as one moves from the central part outwards to the periphery in the PAG (Figures 1-3). In Nissl stained material one also notes that the size of the neuronal somas vary between 10-35 microns. Occasionally one sees a very large 35-40mm oval cell with a prominent nucleus. These large oval cells are most probably displaced mesencephalic V cells. The borders of the PAG as viewed with the Nissl stain are quite well defined except for the caudal ventral region which merges with the dorsal raphe. However it is possible to cytoarchitecturally distinguish the two neuronal populations, for the dorsal raphe cells tend to stain darker and be larger than the surrounding PAG cells.

Golgi impregnated material produces a strikingly different view of the PAG as compared to Nissl stained material. With Golgi stained material (Figures 4-8) the PAG appears more complex and heterogeneous than is revealed with the Nissl stain. The somas of the PAG neurons appear more varied in shape, larger and better defined. More importantly in the central region of the PAG, which appeared to stain poorly with the Nissl stain, there appear large numbers of cells and a very dense plexus of small diameter fibers (Figure 9a). The degree to which PAG neurons have spines seemed directly related to how over-impregnated the neurons were. If the neurons were over-impregnated

one saw large numbers of spines on PAG neurons. However when PAG neurons were only well impregnated, so that the dendritic arbors were quite dense and could be traced over long distances, the spines were difficult to visualize. Therefore it appears some PAG neurons have spines but, because of difficulty and inconsistent staining of them, a quantitative estimate of their occurance on PAG neurons can not be made here.

The types of cells found to constitute the PAG showed a striking degree of variability when compared to nearby regions. In the same Golgi material the superficial layers of the superior colliculus, the inferior colliculus, and the medial genculate body all displayed the cell types similar to those as described by previous authors (Ramon y Cajal, 1909; Geniec and Morest, 1971; Morest, 1964). Because of the variability in PAG neruons when compared to the surrounding regions it was felt that to impose a strict classification of cell types on the PAG neurons would be more a subjective exercise in ignoring differences, rather than accurately reflecting the heterogenous nature of cells in the PAG. However, several broad classes of cells can be described in the PAG, under which a variety of subtypes may be grouped. The five major nonglial cell types found in the PAG will be described below.

The first cell type is the fusiform shaped neuron which is found in all regions of the PAG. These neurons tend to have long eleptical soma with one to several dendritic processes emerging from each end of the soma as pictured in figure 10a. The diameter of the fusiform soma ranges from 10-30 microns. While the fusiform neurons are found in all regions of the PAG, they are most prominent in the central region

of the PAG. This cell type is also evident in the most rostral regions of the PAG where the central grey is reduced to a slender tube underneath the posterior commissure as the cerebral aqueduct becomes the third ventricle. This fusiform cell type also can be found in other periventricular regions of the brain such as the nucleus periventricularis hypothalami. The orientation of these fusiform cells in the PAG changes as one moves from the center to the periphery. In the most central regions these cells can be found encircling the cerebral aqueduct, but as one moves more peripherally these fusiform cells become arranged in a more radial fashion. It appears these cells stain weakly with cresyl violet for although they are present in moderate to high numbers immediately surrounding the cerebral aqueduct as viewed with a Golgi stain (Figures 4-8), this same region appears to stain poorly when visualized with a cresyl violet stain (Figure 1-3). These fusiform cells appear to have very thin dendritic and axonal processes as viewed with the Golgi stain (Figure 9a). These axons are probably unmyelinated for the central region of the PAG, where these cells are present in large numbers, appears as a very poorly or unmyelinated region when viewed with a myelin stain (Figure 13-15).

A second type of cell found in the PAG is the multipolar neuron as shown in figure 9b and c. These neurons have soma diameters which range from 10-30μ and they are found in all areas in the PAG. These cells have somas that are usually in the shape of a typical multipolar neuron with the dendrites and axons coming off the corners of the cell body. These neurons can have extensive dendritic arborizations with a preferential spread in the coronal plane with lesser, but still

extensive arborizations in the sagittal plane. The arborizations often extend into the deeper layers of the superior colliculus or the surrounding tegmentum where they appear to interdigitate with tectal and tegmental cells.

A third neuronal type found in this region is the stellate cell. These polygonal neurons have a rather oval soma with 3 to 6 processes coming off the cell body at a variety of angles. While the other neuronal types found in the PAG have a wide variety of intermediate forms, the stellate cells seem subdividable into two subclasses. The first subclass is the microstellate cell with somas between 10-12mm. These cells can be found in all parts of the PAG and are characterized by their very small round somas and their extremely fine neuronal processes. These cells, while being the smallest cells in the PAG, still have extensive arbors which radiate out preferentially in the coronal plane. The second type of stellate neurons present in the PAG has a soma between 17-30mm in diameter (Figure 9d). These cells have distributions and patterns of arborization similar to the fusiform cells. As with the fusiform cells, these neurons have extensive dendritic arbors which often extend into the surrounding deep layers of the superior colliculus and the deep tegmentum.

The fourth neuronal type is the pyramidal-shaped cell which is found in all areas of the PAG (Figure 10). This cell type, which is the largest cell type in the PAG, has a soma diameter between 15-35μm. This cell type also has a distribution opposite that of the fusiform cell, that is the pyramidal-like cells, while present in all regions of the PAG, tend to be larger in both number and size as one nears the periphery. These pyramidal cells tend to have the most extensive

arborizations of any cells in the PAG as viewed in both the Golgi and HRP preparations. Their extensive dendrite appendages spread to every region of the PAG, and often extend far into the superior colliculus (Figure 10d) or the deep or intermediate tegmentum (Figure 10c). These pyramidal cells, along with the large fusiform and stellate cells appear very similar to the deep tectal and tegmental cells that interdigitate with these PAG neurons. Whether the similar form shown by these PAG, tectal and tegmental neurons denotes similar function is unknown.

The last cell type of note in the PAG is the ependymal cell shown in Figures 9b and 9c. These ependymal cells line the cerebral aqueduct and are quite difficult to impregnate, but once this is accomplished, these cells appear very similar to those described by Ramon y Cajal (1909), that line the central canal in the spinal cord. These primitive type cells have relatively small somas (15μm) and appear to have "feet" which they line the aqueduct with and have an eliptical cell body whose long axis is at right angles to the aqueduct it lines (Figure 9c). Unlike Cajal's spinal ependymal cells which had only one process extending peripherally away from the aqueduct, some of these epedymal cells occasionally have tufts of processes coming off the peripheral end of the soma (Figure 9b). This tuft is composed of 1-7 processes which are very thin and tend to travel peripherally for 20-50μm away from the aqueduct and then bend sideways and travel parallel to the aqueduct. When several of these ependymal cells are labeled in one section, they form a dense plexus of these fibers which are confined to the inner 1/3 of the circumaqueductal grey matter.

The HRP histochemistry which was performed on fixed cat PAG material gave very similar results to that which was obtained with the Golgi method (Figure 12). In this DAB reacted material, no new classes of cells were observed, compared to the Golgi material. The one cell type that was not labeled with this HRP histochemistry, but was seen in the Golgi material, was the ependymal cell. Although the HRP was injected into several sites where the ependymal processes were visualized to be present with the Golgi material, no ependymal soma were ever well labeled in the HRP material. The reason for this lack of ependymal filling is unknown. However, the neurons which did fill with the HRP have delicate dendritic and axonal arborizations that extended several millimeters beyond their somas. These neurons are very similar to the cells seen in well impregnated Golgi material. The intense neuronal filling accomplished by this HRP-fixed tissue method is shown in Figure 12c. This cell filling is reminiscent of that obtained with intracellular HRP microinjections. More importantly, this technique served to confirm that in our Golgi material, we were labeling all classes of cells that are present in the PAG and that the Golgi impregnated neurons accurately reflected the neuronal architecture of each cell type except the ependymal cell.

Myeloarchitecture

The fiber system of the PAG as revealed by Weil, Golgi and HRP histochemistry suggests a central→peripheral gradient with regards to the size of the neuronal fibers and their degree of axon myelinization. The Weil material, in which myelinated fibers are stained darkly, displays an increasing degree of myelinization as one

moves from the central to the periphery of the PAG (Figures 13-15). In Golgi and HRP material one also notices that the size of the fibers tends to be larger in more peripheral regions with a gradual decrease in caliber as one approaches the aqueduct. It should be noted, however, that there is a dense plexus of neuronal fibers near the aqueduct seen in Golgi sections (Figure 9a). These small diameter fibers can also be visualized with HRP histochemistry (Figure 12b). Thus, since one sees these fibers with either the Golgi or HRP technique but not with a myelin stain, one can conclude that there is a fiber system in the innermost aspect of the PAG that is composed chiefly of poorly or amyelinated fibers. As one moves from the caudal iter region of the PAG where the PAG has its greatest diameter, to more rostral regions where the diameter gradually shrinks, the degree of myelinizaton and the relative size of the neurons is dependent on the absolute distance one is from the center of the ventricle. Thus, as one moves rostral and the PAG narrows, the PAG does not shrink as a whole. Rather, its smaller celled, less myelinated inner region remains, while the slightly larger celled more heavily myelinated peripheral region disappears.

When one compares the PAG fiber systems, as revealed by each of the three techniques utilized here, a similar picture emerges. First, there is a gradual increase in the fiber diameter and degree of myelinization as one moves from the center → periphery in the PAG. This increase in both fiber diameter and myelinization correlates well with the gradual increase in neuronal size as one moves central → periphery. Second, there is a tendency in this fiber system to move from a circular, to a radial organization as one moves from the center

to the periphery in the PAG. This radial organization of the PAG fibers is so organized that the bundle of Schutz which is streaming through the PAG in a route parallel to the aqueduct, is at right angles to the processes of the PAG's neurons. Thirdly, the borders of the PAG which appear clearly demarcated with the Nissl stain, are not well defined in Golgi stains, owing to the spread of dendrites beyond the PAG borders and the presence of similar types of cells in structures adjacent to the PAG.

DISCUSSION

In undertaking this study we have addressed three major questions:

(1) What is the cellular morphology of the PAG, and in light of this data, is the PAG divisible into distinct cell groups which might be correlated with specific functions?

(2) Is the morphology of this structure similar in the various species of animals used in the investigation of the PAG?

(3) If there are not clearly delineated subnuclei in the PAG, are there any anatomical correlates to the variety of functions this structure is involved in?

Each of these questions will be discussed below, in view of the findings obtained in this study.

Neuronal Populations

In attempting to answer the question of whether the PAG is divisible into distinct subnuclei one must first define what the criteria for such a subdivision might be. Ramon-Moliner and Nauta

(1966) addressed this question and stated "A nucleus or center can be identified on the basis of any one of the variety of distinctive properties: specific functional effects following stimulation or lesion; histochemical properties; pigment content, specific afferent or efferent relationships; or simple evident cytological individuality." Morest (1965) also addressed this question in a paper using the Golgi technique and stated "Specific neuronal types are differentiated and mapped according to the number, size, distribution and branching pattern of the dendrites and the forms of the axons and appendages. A neuronal population, or nucleus, may then be identified as a spatial distribution of neurons of a specific morphological type." Although the above definitions are different in scope, they have in common the requirement that the nuclei have a distinctive difference, in either form or function, which distinguishes it from its neighbors.

Previous investigators have addressed the question of whether the PAG should be subdivided into distinct subnuclei. Ramon y Cajal (1909) in the young cat using the Golgi technique felt the region was not subdivisible on the basis of distinct cell types. Kolliker (1896) in the human also using the Golgi technique described the PAG as being composed of a wide variety of cell types which were arranged in a heterogeneous fashion. However, recent investigators Hamilton (1973 and 1974), using chiefly the Nissl stain or degeneration techniques in the cat, subdivided the PAG region into three subnuclei, each with its own distinctive cytoarchitecture and connectivity. In this subdivision schema, three subnuclei were described, a dorsal, medial, and lateral nucleus. The dorsal nucleus was described as being

composed of small and darkly staining cells, fusiform to spherical in shape and these cells projected only to the pretectal region and lateral habenula. The medial nucleus was described as being composed of small spindle shaped cells that stain darkly with cresyl violet and project to only the Fields of Forel and the ventral tegmental area. The lateral nucleus was described as being composed of neurons spherical or triangular in shape that stain lightly with Nissl techniques and which project to the paraventricular and parafasicular nucleus of the thalamus, and to the posterior hypothalamus.

More recent studies employing the Golgi techniques have examined the cells in the PAG in the cat and in the lateral region of the PAG in man. In both studies, a variety of cell types were observed; seven cell types were reported as being present in the PAG of the cat (Lui and Hamilton, 1980) and four in the lateral region of the PAG in man (Laemele, 1979). Both studies maintain that the PAG should be subdivided into three regions; medial, dorsal, and lateral, this being based on previous Nissl (Hamilton, 1973) and silver degeneration techniques (Hamilton, 1974). However, the original subdivision of the PAG was based on the observation in Nissl stained material that each of the three distinct regions had a homogeneous cell type. Furthermore in the companion studies of the afferent and efferent connections of the PAG (Mantyh, 1980) it was found that the previously proposed subdivisions of the PAG have the majority of their connections in common. Thus it seems inappropriate for recent investigators using the Golgi technique to maintain that such subdivisions are valid when their own results show a heterogeneous distribution of cell types within each nucleus of the PAG. Some of

the previous subdivisions that were not seen in this study could be due to species differences. However, across the range of animals examined here; rat, cat, macaque and squirrel monkey, no marked cytoarchitectural species differences were noted. Therefore it is concluded in this study that previous subdivisions of the PAG based on cytoarchitectural and connectivity grounds seem inappropriate.

The cytoarchitectural organization of the PAG suggested by this study is one in which there is a heterogeneous group of cells in all regions of the PAG. Thus, while any type of cell can be found in all regions of the PAG, the size of the cell soma gradually increases the further one moves away from the aqueduct. Therefore, in the rostral region of the PAG one tends to find smaller cells because the distance from the center of the aqueduct is less than in more caudal regions. This central → peripheral gradient is also apparent in the diameter of the neuronal processes and the degree of myelinization.

This study along with the companion hodological studies on the afferent and efferent connections of the PAG (Mantyh, 1980), suggest this mesencephalic central grey region is more heteregeneous and complex than has been previously described. This heterogeneity in the structure of the PAG is apparent on the cytological level examined in this study and is in good correlation with published physiological and biochemical studies of the PAG. The diversity of the cell morphology of the PAG as reported here is not to suggest an unsortable mass of cell types. Rather by appreciating the anatomical heterogeneity of this structure more detailed and realistic hypotheses and investigations can be conducted on the PAG. Hopefully with this diversity on the way in which the PAG is organized in mind, we believe

that the function of the PAG can be more fruitfully pursued with new and improved anatomical, physiological, and biochemical techniques.

Comparative Anatomy

The cytoarchitecture of the PAG in rat, cat, macaque and squirrel monkey did not vary in any striking or noticeable way when examined with the Nissl, Weil, and Golgi method. The same wide range of cells were present in all animals examined, as was the heterogeneous nature of these cell types distribution. The cell sizes in all species remained constant (from 10-35mm), while the number of cells comprising the PAG was at least in the rat and the most in the macaque. The one aspect which did vary was the per cent of mesencephalic cross section occupied by the PAG in a given stereotaxic coronal section. The rat had the largest ratio with the macaque having the smallest ratio. This declining per cent of area it occupied appears to be due to massive increases in the ascending lemniscal and cerebellar fiber systems and descending cortical projection fibers found in the cat and monkey.

The apparent change in the configuration of the PAG from circular tube in the rat (Figure 3) to an eleptical tube in the monkey (Figure 1) as seen in the coronal plane is not due to any appreciable change in the basic shape of the region but is caused by increased cephalic flexure that cat and monkey have compared to the rat. Such a change does not change the PAG or its relation to surrounding structures but simply changes its appearance in the stereotaxic planes. Thus, in this morphological study and in the companion study of the afferent and efferent connections of the PAG in the same species (Mantyh, 1980)

the PAG maintains a phylogenetic stability in its basic structure and its afferent and efferent connections.

For the study of PAG function rats are must often used in behavioral and biochemical studies, cats for the anatomy of the structure, and monkeys for a physiological model which is thought to most closely resemble humans. From this and companion studies it appears anatomically, at the light level, that the cytoarchitecture and connectivity of this structure is phylogenetically stable. However, this study can not answer whether this structure is stable when examined physiologically or biochemically. Certainly in all these animals SPA can be achieved, but whether this is accomplished by the same neurotransmitters or similar physiological mechanisms remains unclear.

Functional Correlates

One criterion that is commonly used (Olszewski and Baxter, 1954; Ramon-Moliner and Nauta, 1966) in subdividing a region such as the PAG is to see if specific regions can be correlated with specific functions. Stimulation experiments are commonly employed to uncover such localized functional units. Such stimulation experiments on the PAG document the diversity of functions associated with this central grey region. Stimulation of the PAG produced reactions such as; rage accompanied by intense vocalization (Kanai and Wang, 1962), changes in visceral motility (Skultety, 1963), inhibition of oxytocin release (Aulsebrook and Holland, 1969), strong emotional reflexes (Hunsperger, 1956), and analgesia (Reynolds , 1968). Although these responses can be repeatably obtained during PAG stimulation, the precise

localization of the stimulus within the PAG which produces these effects is unknown. As described in the present study, the PAG is composed of a very heterogeneous group of neurons which surround the cerebral aqueduct. Coursing through this central grey region are large numbers of poorly or unmyelinated nerve fibers which are rather loosely known as the dorsal bundle of Schutz (Schutz, 1891) or the periventricular bundle (Ranson, 1943). Upon stimulation of the PAG both the neurons and the nerve fibers en passage will be stimulated. Thus it is difficult to ascertain whether a stimulation response is due to the excitation of PAG neurons and/or the fibers of passage. A second difficulty in attempting to localize functional subnuclei in the PAG is that the site of the electrode is often not histologically identified and the extent of the current spread is difficult to gauge. These uncertainties in PAG stimulation experiments make the assignment of specific PAG functions to specific PAG subregions a difficult task. An example of this difficulty is the attempts of defining which one of Hamilton's (1973 and 1974) proposed subnuclei in the PAG is responsible for SPA. A wide variety of loci have been identified including; ventral rat (Mayer and Price, 1972), cat (Oliveras et al., 1974); lateral, rat (Reynolds, 1969), dorsal, rat (Prieto et al., 1979), monkey (Yezierski, et. al., 1980), and rostral periventricular bundle human (Hosobuchi, 1977), all of which have been reported to produce an effective analgesia. Therefore it appears that even when species differences are eliminated, and the stimulation and observational parameters are standarized, it would be difficult to assign any one of the previously proposed subnuclei, a specific and unique function associated with SPA. These physiological data again

suggest a more complex organizational schema of the PAG than has been previously proposed.

Biochemical analysis of the PAG has also provided an insight into the heterogeneity of this structure. Using radioimmunoassay, enzymatic isotope assay, and/or immunohistochemistry, a variety of neurotransmitters have been shown to be present within the PAG. Neurotransmitters such as acetylcholine (Palkovits et. al., 1976), dopamine (Versteeg et. al., 1976), histamine (Taylor et. al., 1972), serotonin (Palkovits et. al., 1974), and dopamine (Versteeg et. al., 1976) have all been shown to be present in moderate concentrations in the PAG. A wide variety of neuropeptides have also been localized in moderate concentrations in the PAG including; enkephalin (Hokfelt et. al., 1977), Substance P (Hokfelt et. al., 1977), bombesin (Pert et. al., 1980), GABA (Fahn, 1976), angiotensin II (Fuxe et. al., 1976), and β-lipotropin (Watson, 1977). Such a plethora of neurotransmitters and neuropeptides within the PAG is consistent with the heterogeneous anatomical and physiological nature of this mesencephalic structure. What is relevant to this discussion is the pattern of distribution of these neurotransmitters and neuropeptides within the PAG. If such neurotransmitters or neuropeptides underlie a specific function or a specific afferent connection with the PAG, one might expect these substances to be distributed within specific functional units, i.e., the previously proposed subnuclei. However, one does not see the proposed subnuclei patterns in the distribution of these substances. Rather one usually observes a mosaic-like distribution pattern within the PAG suggesting a more complex and heterogeneous organization of the PAG than the three proposed subnuclei. These biochemical results

seem consistent with the recent anatomical and physiological PAG data, all of which suggests this structure is more complex than has been previously described.

The present cytoarchitectural study combined with published immunohistochemical data suggest the PAG is composed of a variety of overlapping functional or morphological units. This is suggested by the intermingling of large numbers of cell types observed in this study and the overlapping distribution of the neurotransmitters which are present within the PAG. Such an organization would be consistent with the stimulation experiments where one stimulation area gives rise to several quite different responses. This could result from several distinct cell types each with a different function being situated in the same area and thus being stimulated simultaneously.

Therefore, this study along with the companion hodological studies on the PAG's connections suggest this mesencephalic central grey region is more heterogeneous and complex than has been previously described. This heterogeneity is apparent on the anatomical level examined here and is in good correlation with the published physiological and biochemical studies. Such diversity of cell morphology of the PAG as reported here is not to suggest an unsortable mass of cell types. Rather by appreciating the anatomical heterogeneity of this structure more detailed and realistic hypotheses and investigations can be done on the PAG. Hopefully with this diversity in mind the way in which the PAG is organized to function can be more fruitfully pursued by applying modern anatomical and biochemical techniques.

SUMMARY AND CONCLUSIONS

(1) The PAG is composed of a tightly packed group of neurons which show a slight increase in soma size, dendritic diameter, and degree of myelinization as one moves central → peripheral.

(2) The PAG has a wide variety of cell types including multipolar, fusiform, stellate and pyramidal neurons. Because each of these neuronal types is found in nearly all regions of the PAG, strict subdivisions, each with a homogeneous cell type, seem inappropriate.

(3) Clearly delineated subnuclei based on; soma size, dendritic arborizations, pigmentation, evidence cytological individuality or a unique set of afferent or efferent connections could not be discerned for the PAG in this or companion hodological studies (Mantyh, 1980).

(4) Regional subdivision within the PAG may exist based on physiological and biochemical properties but such subdivisions were not seen in the cytoarchitectural or histological studies conducted here.

(5) Therefore terms as dorsal, dorsal-lateral, ventromedial, and ventrolateral should be employed only in a topographical sense but not to denote distinct subnuclei each with its own unique cytoarchitecture and set of connections.

BIBLIOGRAPHY

Adams, J.E. 1976 Naloxone reversal of analgesia produced by brain stimulation in the human. Pain, 2:161-166.

Akil, H., D.J. Mayer, and J.C. Leibeskind 1976 Antagonism of stimulation-produced analgesia by naloxone, a narcotic antagonist. Science, 191:961-962.

Aulsebrook, L.H. and R.C. Holland 1969 Central inhibition of oxytocin release. Am. J. Physiol. 216:830-840.

Basbaum, A.I. and H.L. Fields 1978 Endogenous pain control mechanisms: Review and hypothesis. Annals of Neurology, 4:451-462.

Emmers, R. and K. Akert 1963 A stereotoxic atlas of the brain of the Squirrel monkey. p. XII, Univ. of Wisconsin Press.

Fahn, S. 1976 Regional distribtuion studies of GABA and other putative neurotransmitters and their enzymes. In GABA in nervous system function. Roberts, E., T. Chase, and D. Tower eds. Raven Press, New York.

Fuxe, K., D. Ganten, T. Hokfelt, and P. Bolme 1976 Immunohistochemical evidence for the existence of Augrotensin II containing nerve terminals in the brain and spinal cord in the rat. Neuroscience Letters, 2:229-234.

Geniec, P. and D.K. Morest 1971 Neuronal architecture of the human posterior colliculus. Acta Oto-Laryngologica, Supplement 295:1-33.

Hamilton, B.L. and F.M. Skultety 1970 Efferent connections of the periaqueductal grey matter in the cat. J. Comp. Neurol. 139:105-114.

Hamilton, B.L. 1973 Cytoarchitectural subdivisions of the periaqueductal grey matter in the cat. J. Comp. Neurol., 149:1-28.

Hamilton, B.L. 1974 Projections of the nuclei of the periaqueductal grey matter in the cat. J. Comp. Neurol., 152:45-58.

Hokfelt, T., A. Ljungdahl, L. Terenius, R. Elde, and G. Nilsson 1977 Immunohistochemical analysis of peptide pathways possibly related to pain and analgesia: Enkephalin and Substance P. Proc. Natl. Acad. Sci. USA, 74:3081-3085.

Hosobuchi, Y., J.E. Adams, and R. Linchitz 1977 Pain relief by electrical stimulation of the central grey matter in humans and its reversal by nalaoxone. Science 197:183-186.

Hunsperger, R.W. 1956 Role of substantia gresea centralis mesencephali in electrically induced rage reactions. In: Progress in Neurobiology. Vol. I J. Avierus Kappers ed. Elsevier, New York pp. 289-294.

Kanai, T. and S.C. Wang 1962 Localization of the central vocalization mechanism in the brainstem of the cat. Exp. Neurol., 6:426-434.

Kolliker, A. 1896 Handbuck der gewebelehre des Menschen, Verlag von Wilhelm Engelmann, Keipzig p. 335.

Krieger, M.S., L.A. Conrad, and D.W. Pfaff 1979 An autoradiographic study of the efferent connections of the ventromedial nucleus of the hypothalamus. J. Comp. Neur. 183:785-816.

Laemle, L.K. 1979 Neuronal populations of the human periaqueductal grey, nucleus lateralis. 186:93-108.

Lui, R.P.C. and B.L. Hamilton 1980 Neurons of the periaqueductal grey matter as revealed by Golgi study. J. Comp. Neurol. 189:403-418.

Mayer, D.J. and J.C. Liebeskind 1974 Pain reduction by focal electrical stimulation of the brain: An anatomical and behavioral analysis. Brain Research, 68:73-93.

Mantyh, P.W. 1980 The afferent and efferent connections of the periaqueductal grey in the monkey. Neurosci. Abstr. 17.6.

Morest, D.K. 1964 The neuronal architecture of the medial geniculate body of the cat. J. Anat. (Lond.) 98:611-630.

Morest, D.K. 1965 The laminar structure of the medial geniculate body of the cat. J. Anat. (Lond.) 99:143-160.

Oliveras, J.L., J.M. Besson, G.Guilbaud, and J.C. Liebeskind 1974 Behavioral and electrophysiological eivdence of pain inhibition from midbrain stimulation in the cat. Exp. Brain Res., 20:32-44.

Olszewski, J. and D. Baxter 1954 Cytoarchitecture of the human brain stem. J.P. Lippincott Company, Montreal, p. 8.

Palkovits, M., M. Brownstein, and J.M. Saavedra 1974 Serotonin content of the brain stem nuclei in the rat. Brain Research. 80:237-249.

Palkovits, M. and D.M. Jacobowitz 1976 Topographical atlas of catecholamine and acetyl cholinesterase-containing neurons in the rat brain. II Hindbrain (Mesencephalon and Rhomencephalon) J. Comp. Neurol.

Pert, C.B., M.J. Kihar, and S.H. Snyder 1976 Opiate receptor; autoradiographic localization in rat brain. Proc. Natl. Acad. Sci. USA 73:3729-3733.

Pert, A., T.W. Moody, C.B. Pert, L.A. Dewald, and J. Rivier 1980 Bombesin: receptor distribution in brain and effects on nociception and locomotor activity. Brain Res., 193:209-220.

Prieto, G.P., G.J. Giesler Jr., and J.T. Cannon 1979 Evidence for stie specificity in naloxone's antagonism of stimulation-produced analgesia in the rat. Neurosci. Abstr. 2090.

Ramon y Cajal, S. 1909 Histologie du Systeme Nerveus de l'homme et des vertebres. Volumes I and II. Maloine, Paris, Reprinted: Consejo Superior de Investigaciones Cientificas, 1952, Madrid.

Ramon-Moliner, E. and W.J.H. Nauta 1966 The Isodendritic core of the brainstem. J. Comp. Neurol., 126:311-336.

Reynolds, D.V. 1968 Surgery in the rat during electrical analgesia induced by focal brain stimulation. Science 164:444-445.

Simantov, R., M.J. Kuhar, G.W. Pasternak, et al. 1976 The regional distribution of a morphine-like factor enkephalin in monkey brain. Brain Research, 106:189-197.

Skultety, F.M. 1959 Relation of periaqueductal grey matter to stomach and bladder motility. Neurol. 9:190-197.

Skultety, F.M. 1963 Stimulation of the periaqueductal grey and hypothalamus. Arch. Neurol. 8:38-50.

Taylor, K., E. Gfeller, and S. Snyder 1972 Regional localization of histamine and histidine in the brian of rhesus monkey. Brain Research 41:171-179.

Versteeg, D., J. Van der Gugten, W. de Jong, and M. Paklovits 1976 Regional concentrations of noradrenaline and dopamine in rat brain. Brain Research 113:563-574.

Watson, S.J., J.D. Barchas, and C.H. Li 1977 Beta-lipotropin: localization of cells and axons in rat brain by immunocytochemistry. Proc. Natl. Acad. Sci. U.S.A. 74:5155-5158.

Yezierski, T., K. Wilcox, and W.D. Willis 1980 A pharmacological study of inhibition of primate spinothalamic tract cells from periaqueductal grey and nucleus raphe magnus. Neurosci. Abstr. 17.9.

Figure 1. Photomicrograph of Nissl-stained coronal section through the periaqueductal grey at the level of the superior colliculus; saimiri sciureus, (animal SM-7).

Monkey Nissl Stain Coronal Section

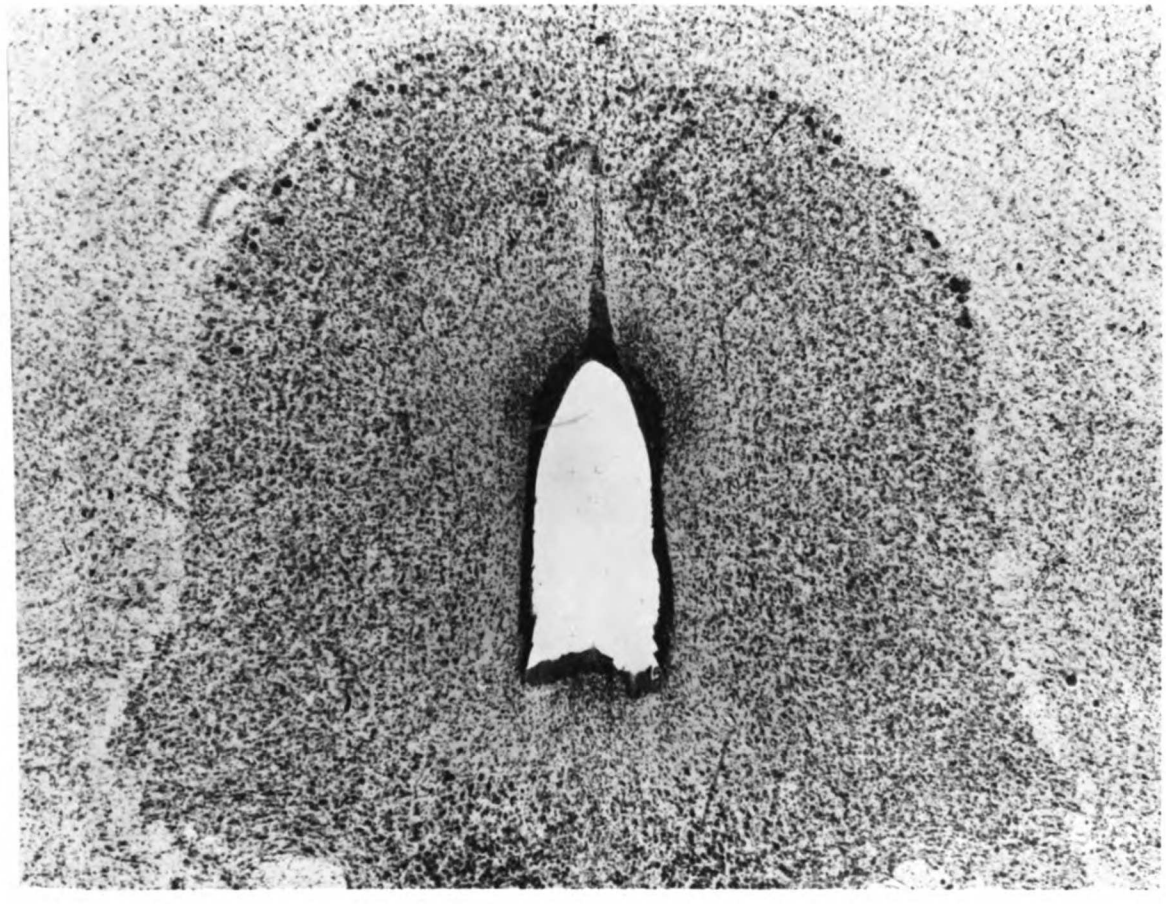


Figure 2. Photomicrograph of Nissl-stained coronal section through the periaqueductal grey at the level of the superior colliculus; cat (animal IA-3).

Cat Nissl Stain Coronal Section

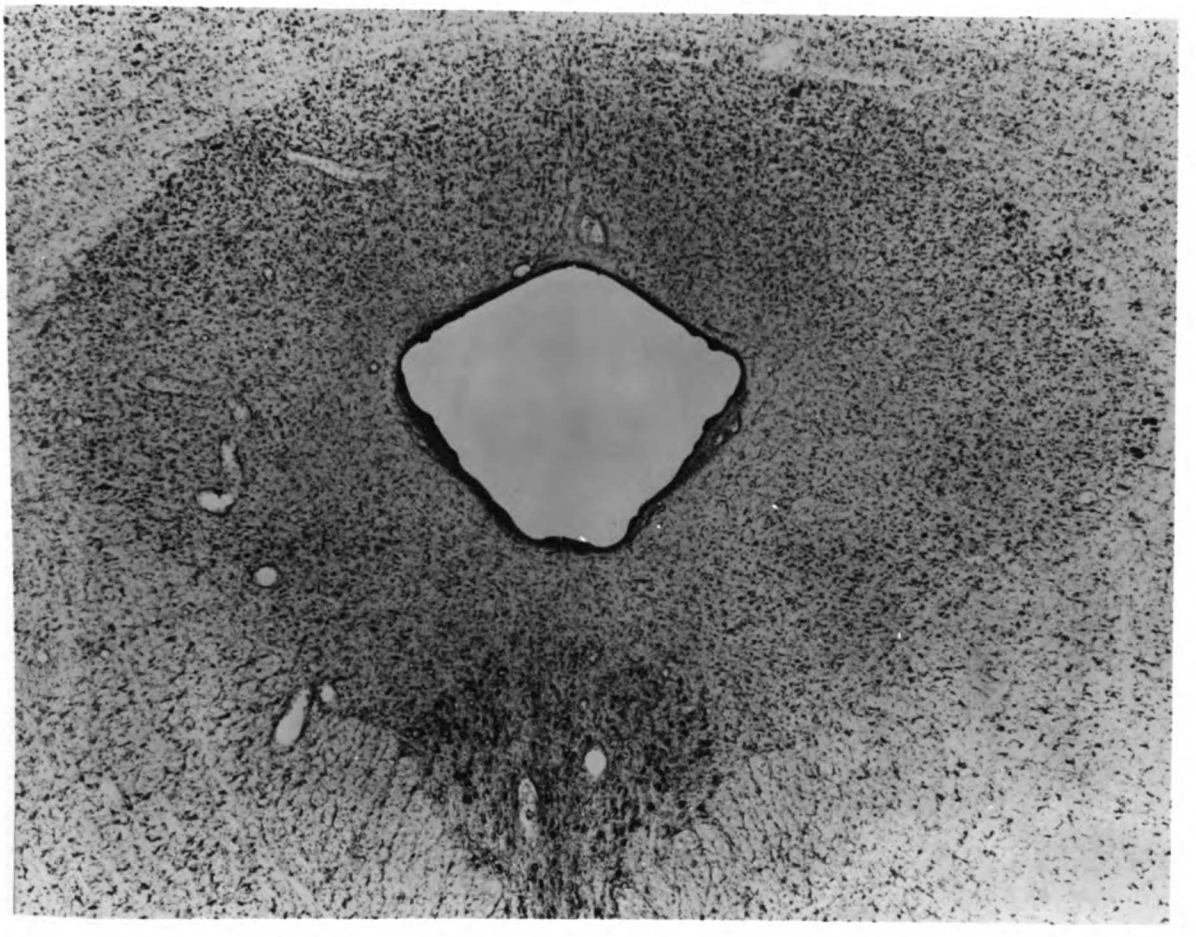


Figure 3. Photomicrograph of Nissl-stained coronal section through the periaqueductal grey at the level of the superior colliculus; rat (animal RCG-1).

Rat Nissl Stain Coronal Section

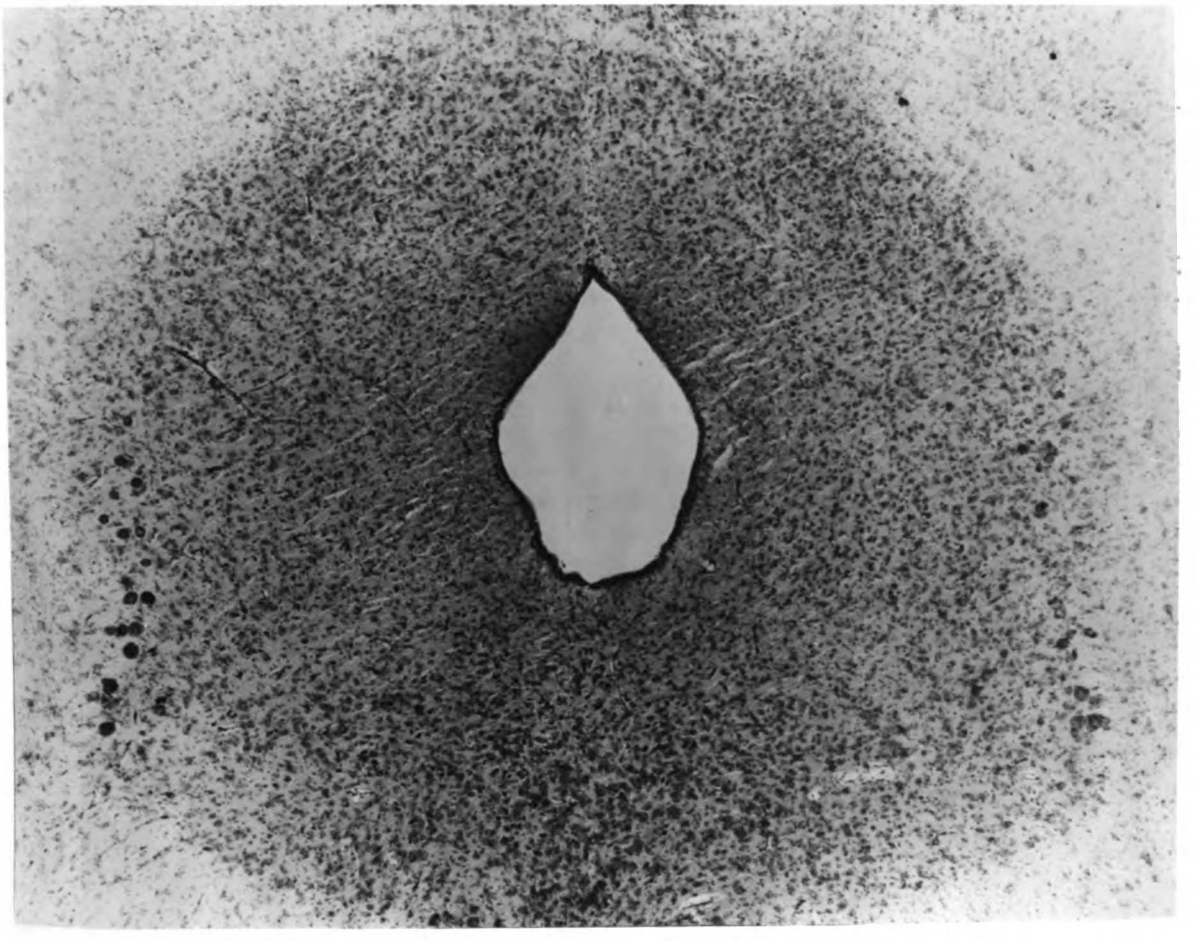


Figure 4. Composite drawing of neurons of the periaqueductal grey in coronal section made with the aid of a drawing tube. Section is at the intercollicular level. Golgi preparations; macaca mulatta; (animal M-6).

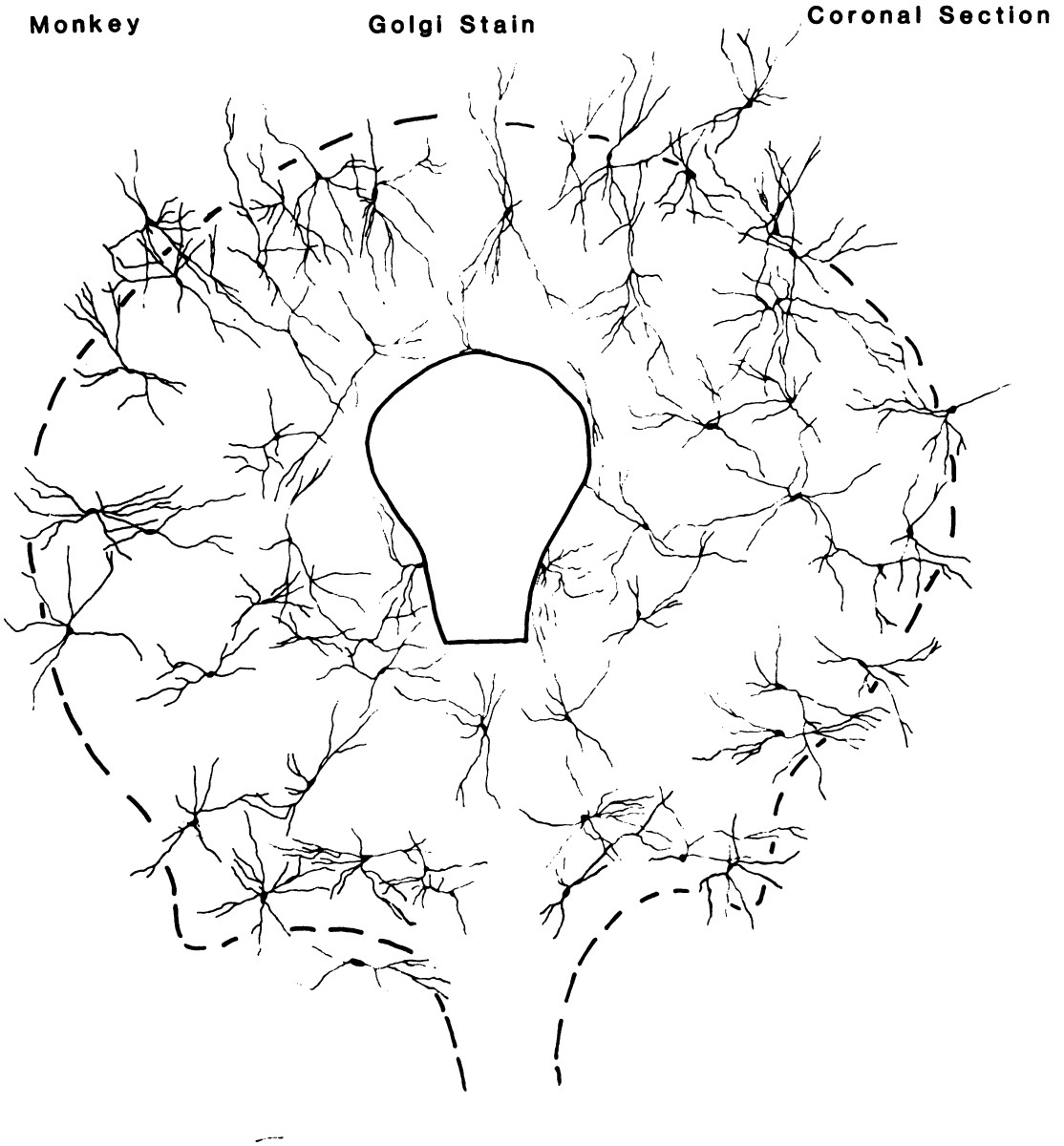

Monkey
Golgi Stain
Coronal Section

Figure 5. Composite drawing of neurons of the periaqueductal grey in coronal section made with the aid of drawing tube. Section is at the level of the caudal superior colliculus. Golgi preparation; macaca mulatta, (animal M-3).

Monkey Golgi Stain Coronal Section

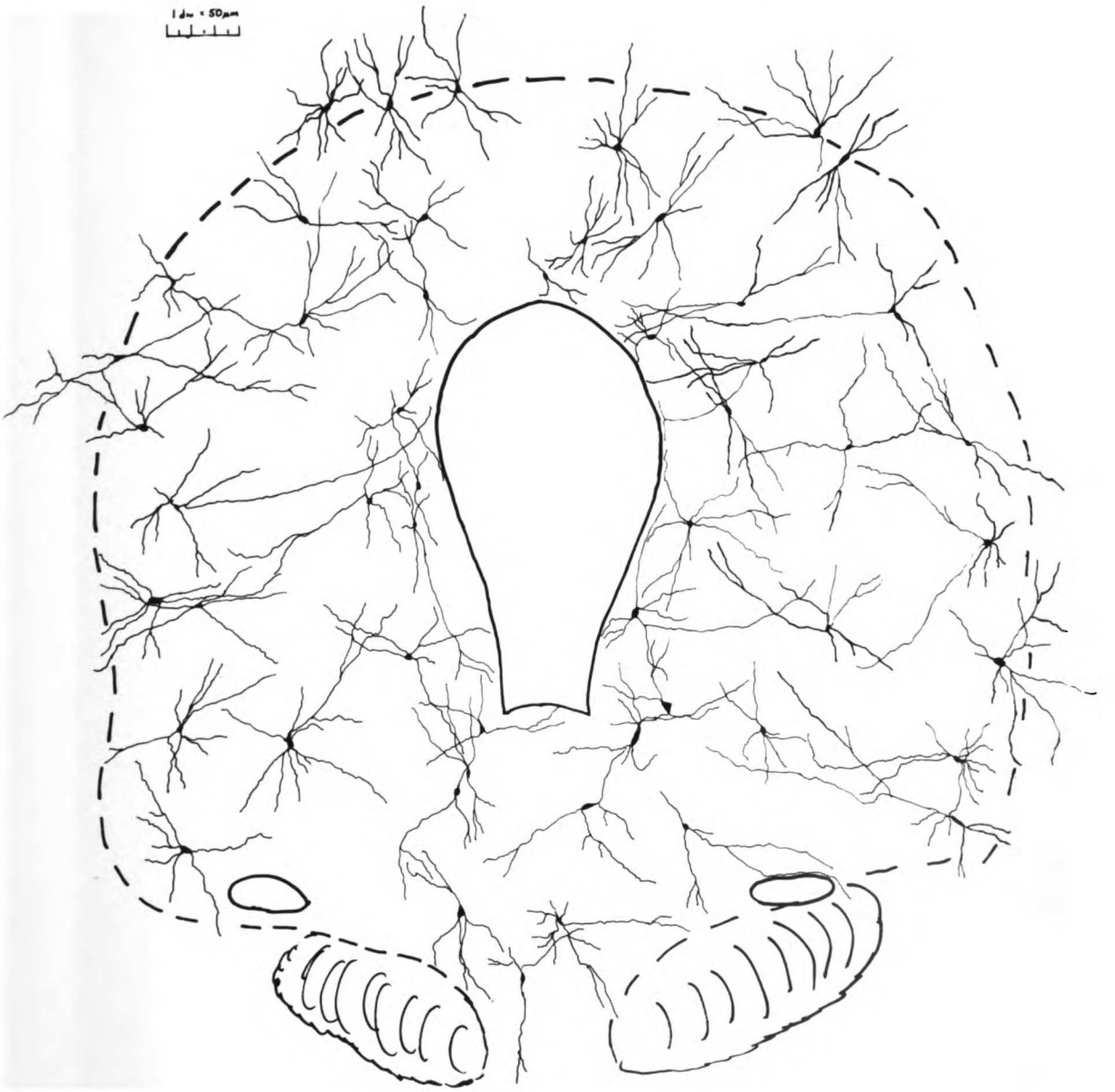


Figure 6. Composite drawing of neurons of the periaqueductal grey in coronal section made with the aid of a drawing tube. Section is at the intercollicular level. Golgi preparations; macaca mulatta; (animal M-2).

Monkey

Golgi Stain

Coronal Section

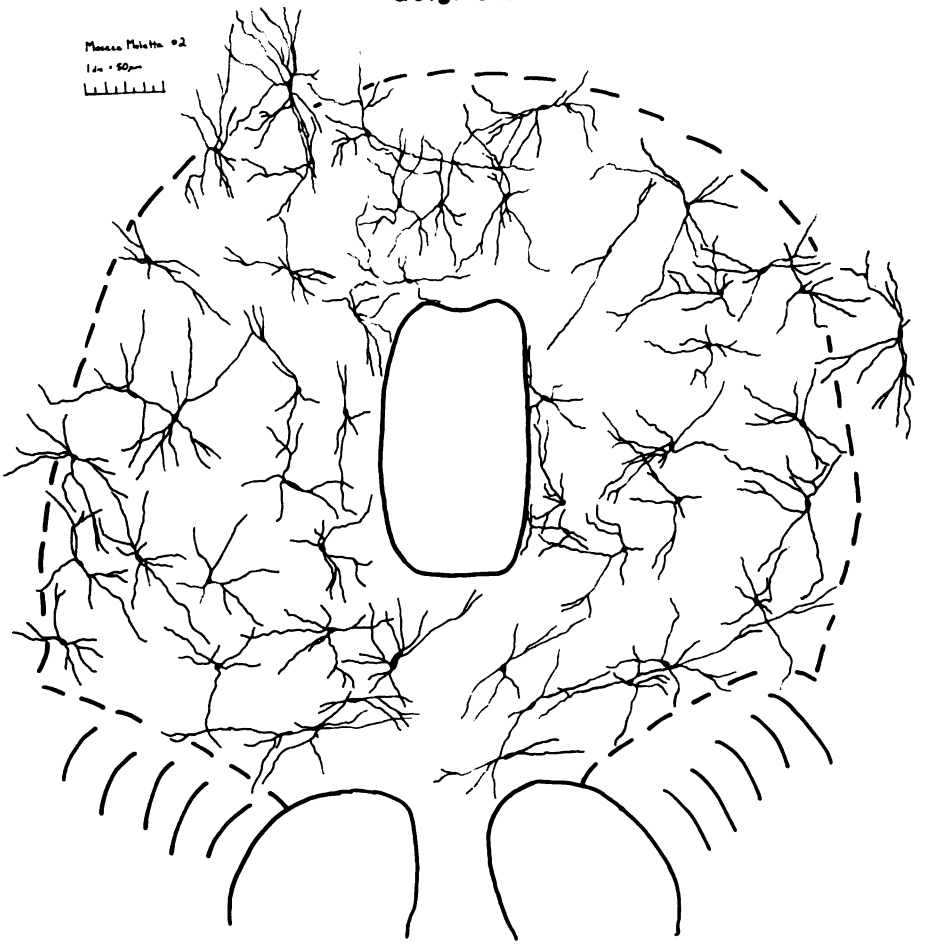


Figure 7. Composite drawing of neurons of the periaqueductal grey in coronal section made with the aid of a drawing tube. Section is at the intercollicular level. Golgi preparations; cat, (animal CG-3).

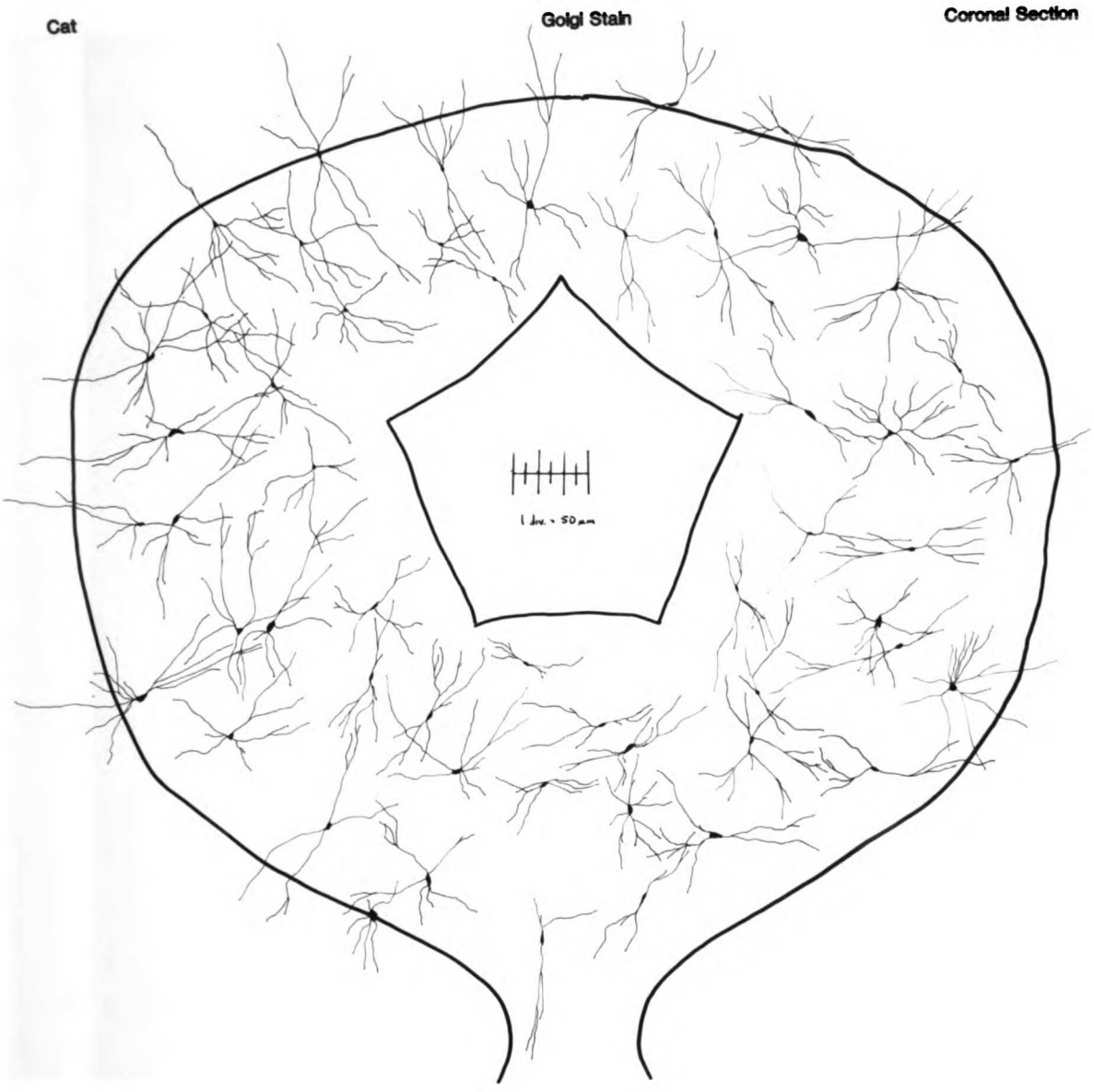

Cat
Golgi Stain
Coronal Section
1 div. = 50 μm

Figure 8. Composite drawing of neurons of the periaqueductal grey in coronal section made with the aid of a drawing tube. Section is at the intercollicular level; Golgi preparations. rat, (animal RPAG-7).

Rat Golgi Stain Coronal Section

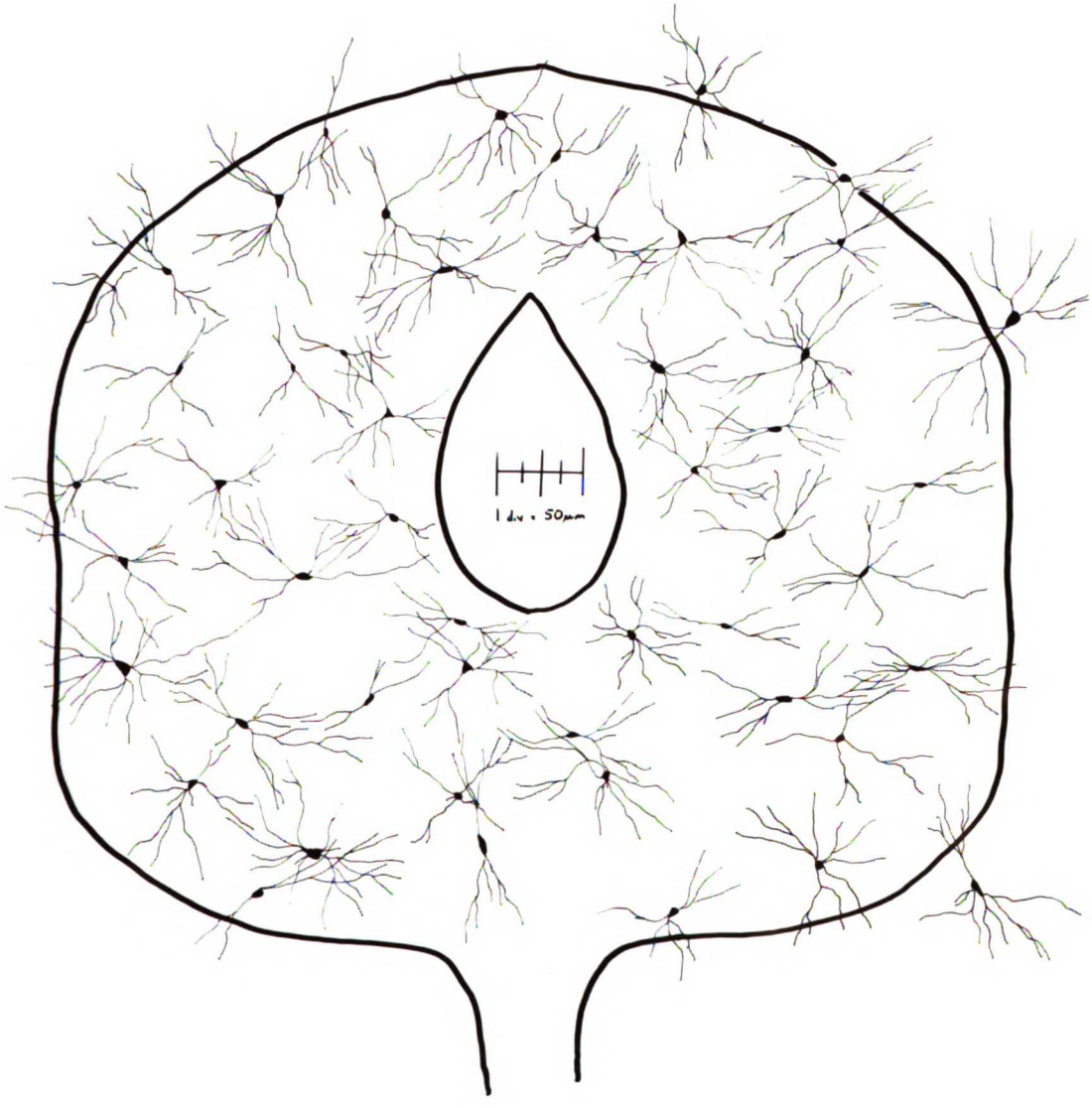


Figure 9. A. A photomicrograph showing the region of the dorsolateral periaqueductal grey. Note the dense fiber plexus immediately surrounding the cerebral aqueduct (AQ). Golgi preparation; saimiri sciureus (Scale = 100μ).

B. A photomicrograph showing the dorsal region of the periaqueductal grey. Note the tufts arising from the ependymal cell whose cell body immediately borders on the cerebral aqueduct (AQ). Golgi preparation; saimiri sciereus (Scale = 50μ).

C. A photomicrograph showing the midline dorsal region of the periaqueductal grey. Note the single fibers arising from the ependymal cell body which immediately borders on the ventral cerebral aqueduct. Golgi preparation; saimiri sciureus (Scale = 50μ).

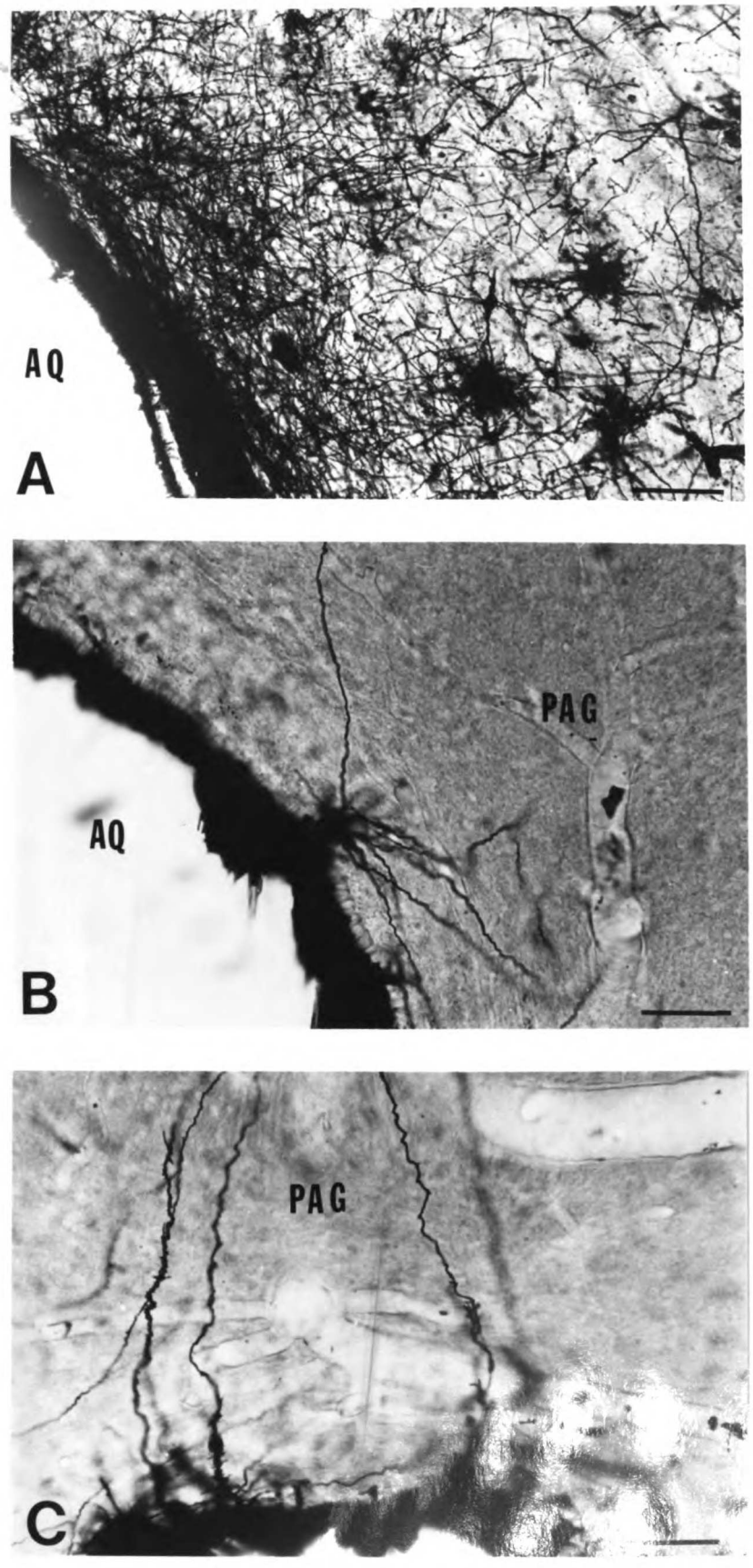

AQ
A
PAG
AQ
B
PAG
C

Figure 10.

A. Photomicrograph of a fusiform shaped neuron located in the ventromedial region of the periaqueductal grey. Golgi preparation; macaca mulatta (Scale = 50μm).

B. Photomicrograph of a multipolar shaped neuron located in the medial region of the periaqueductal grey. Golgi preparation; saimiri sciureus (Scale = 50μm).

C. Photomicrograph of a multipolar shaped neuron in the medial region of the periaqueductal grey. Golgi preparation; cat, (Scale = 50μm).

D. Photomicrograph of a stellate shaped neuron in the dorsolateral region of the periaqueductal grey. Golgi preparation; cat (Scale = 50μm).

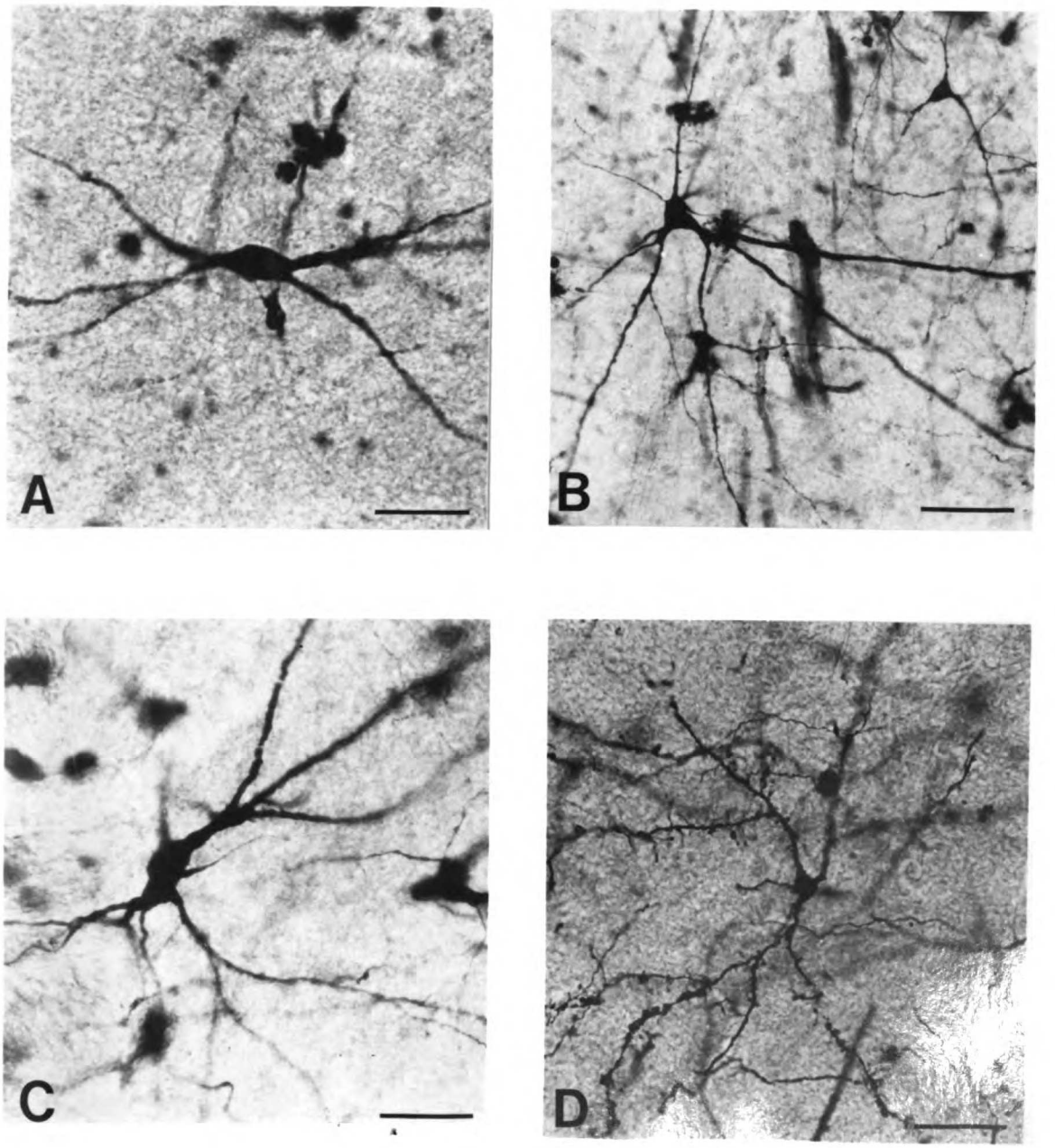

A
B
C
D

Figure 11.

A. Photomicrograph of a pyramidal shaped cell in the dorsal midline region of the periaqueductal grey. Golgi preparation; cat (Scale = 50μm).

B. Photomicrograph of a cluster of multipolar shaped neurons in the dorsal medial aspect of the periaqueductal grey. Golgi preparation; cat (Scale = 50μm).

C. Photomicrograph of a pyramidal shaped neuron located in the medial most region of the deep tectum. Note that the neurons' dendrites extend both ventrally into the periaqueductal grey and dorsally into the deep layers of the superior colliculus. Golgi preparation; macaca mulatta (Scale = 50μm).

D. Photomicrograph of a pyramidal shaped neuron located in the lateral most aspect of the periaqueductal grey. Note that the neuron sends processes to both the lighter region (PAG) and to the darker dorsal region (superior colliculus). Golgi preparation; macaca mulatta (Scale = 50μm).

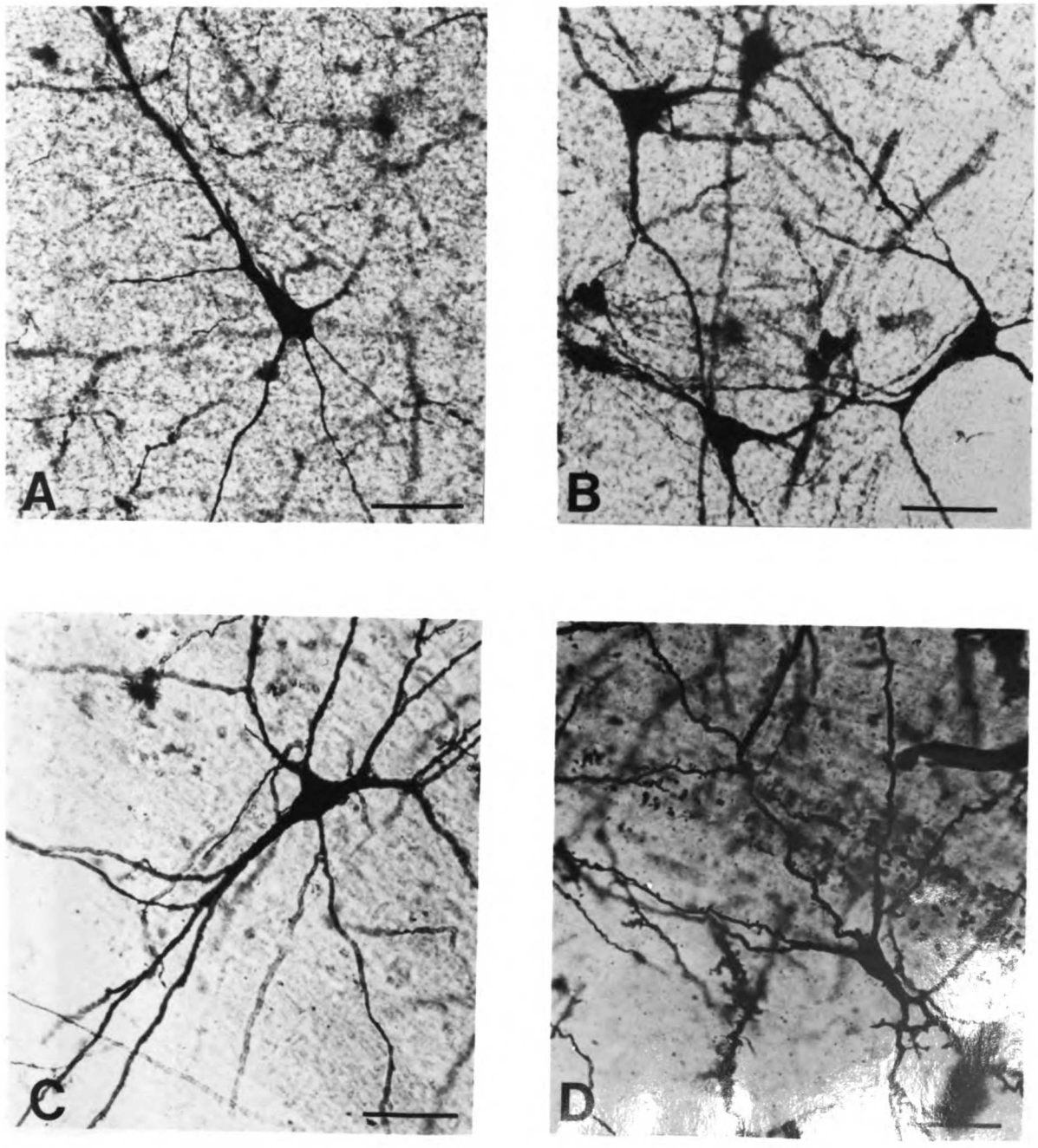

A
B
C
D

Figure 12.

A. Photomicrograph of a fusiform shaped neuron in the dorsolateral region of the periaqueductal grey; Fixed brain with horseradish peroxidase histochemistry preparation; cat (Scale = 50μm).

B. Photomicrograph of the ventromedial region of the periaqueductal grey showing the variety of neurons labeled. Fixed brain ith horseradish peroxidase histochemistry preparation; cat (Scale = 50μm).

C. Photomicrograph of a multipolar neuron in the ventral aspect of the periaqueductal grey. Fixed brain with horseradish peroxidase histochemistry preparation; cat (Scale = 50μm).

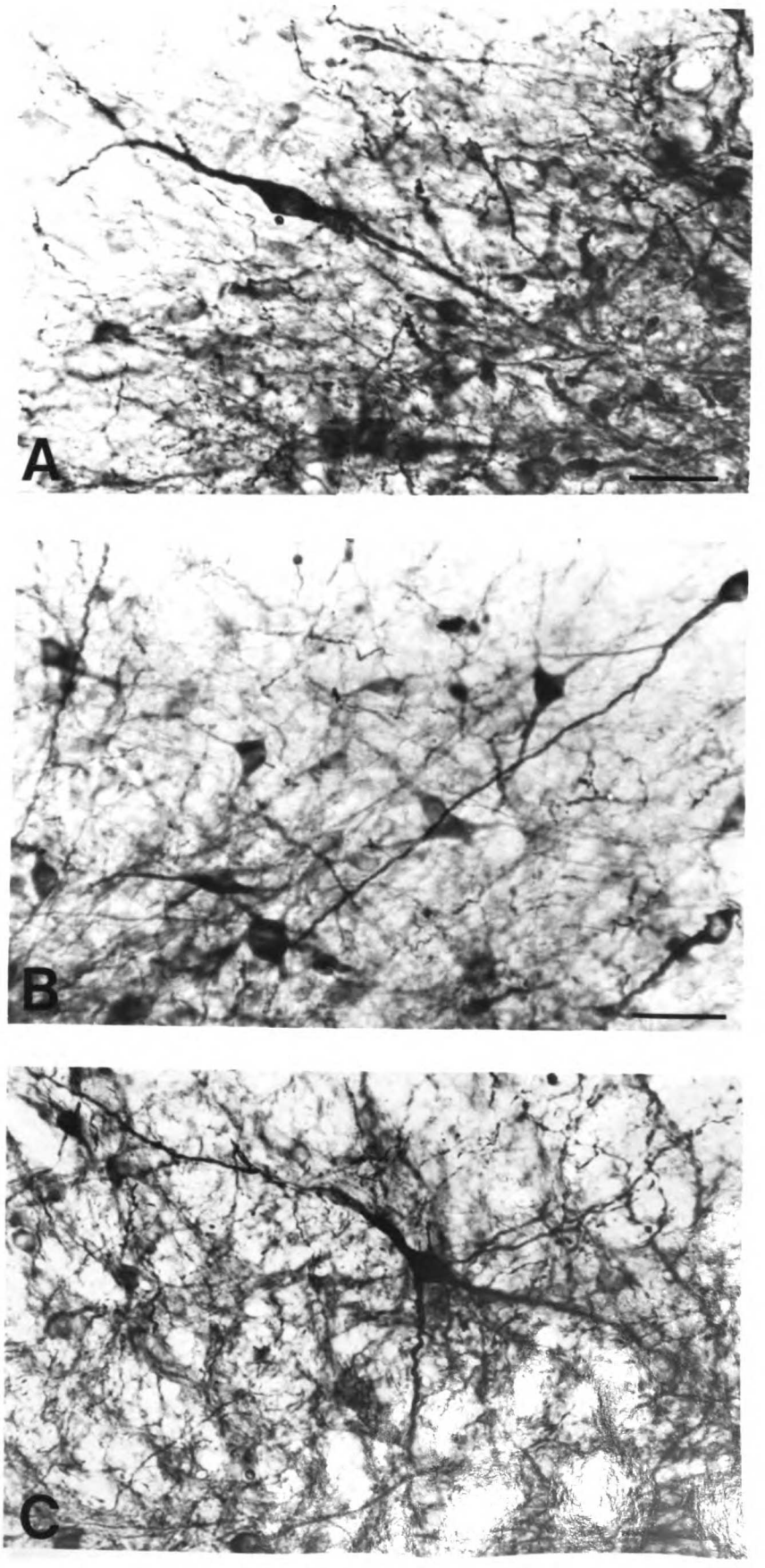

A
B
C

Figure 13. Photomicrograph of a Weil stained coronal section through the periaqueductal grey; macaca mulatta.

Monkey Weil Stain Coronal Sectior

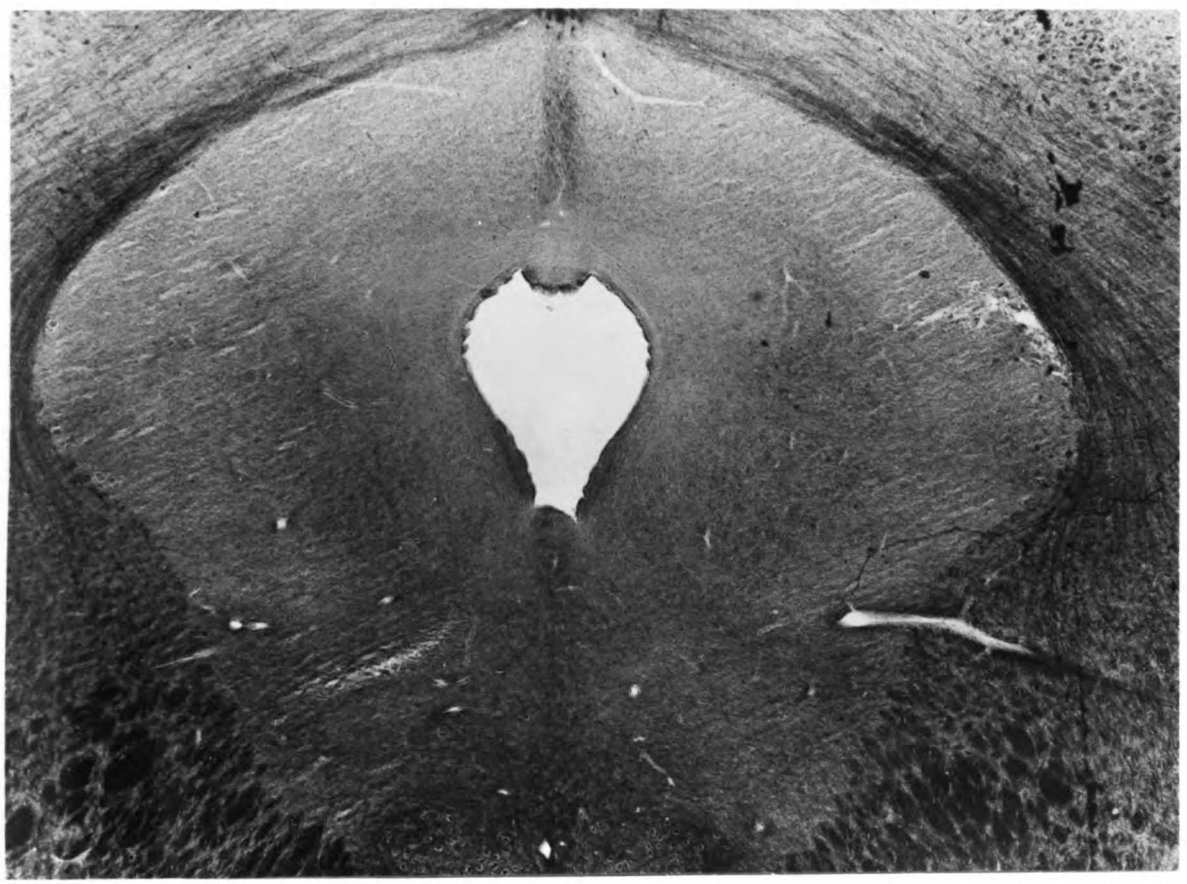


Figure 14. Photomicrograph of a Weil stained coronal section through the periaqueductal grey; cat.

Cat Weil Stain Coronal Section

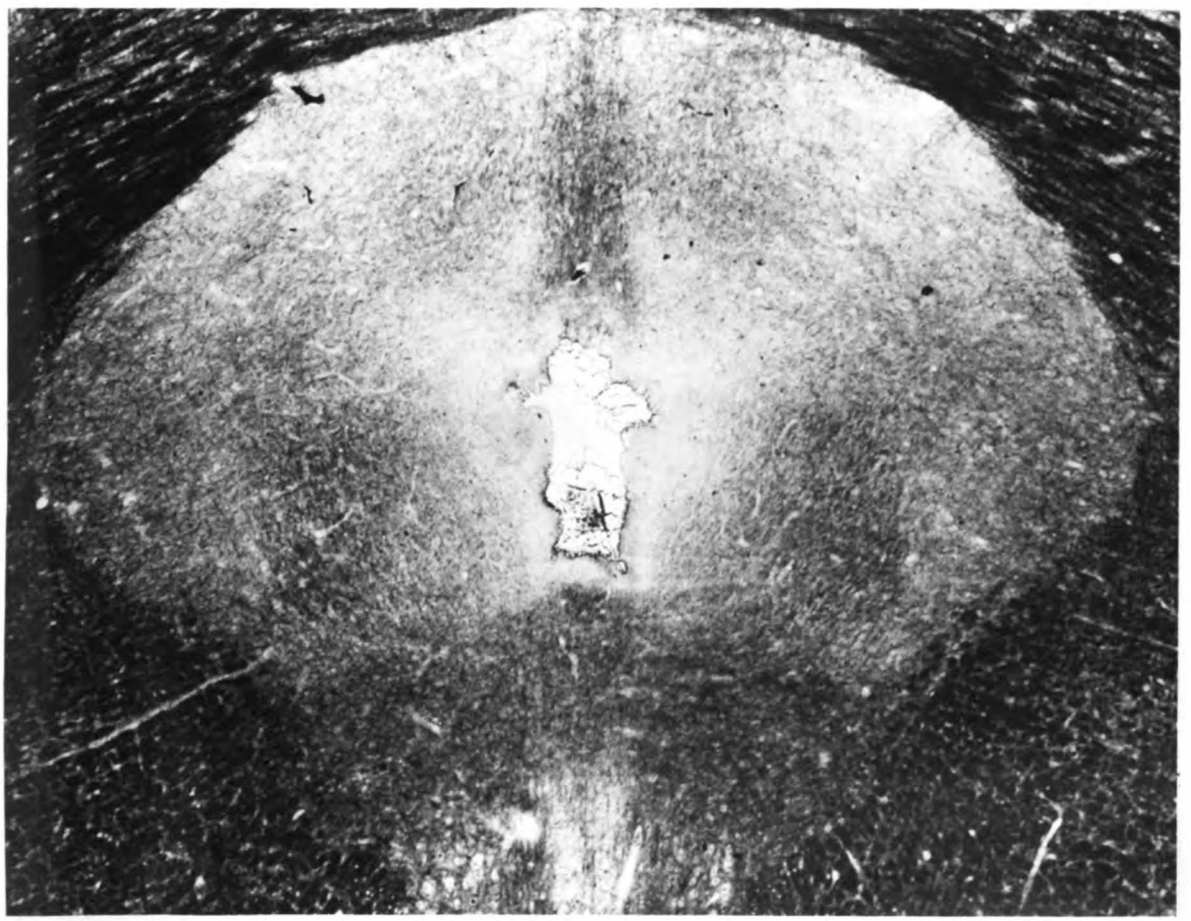
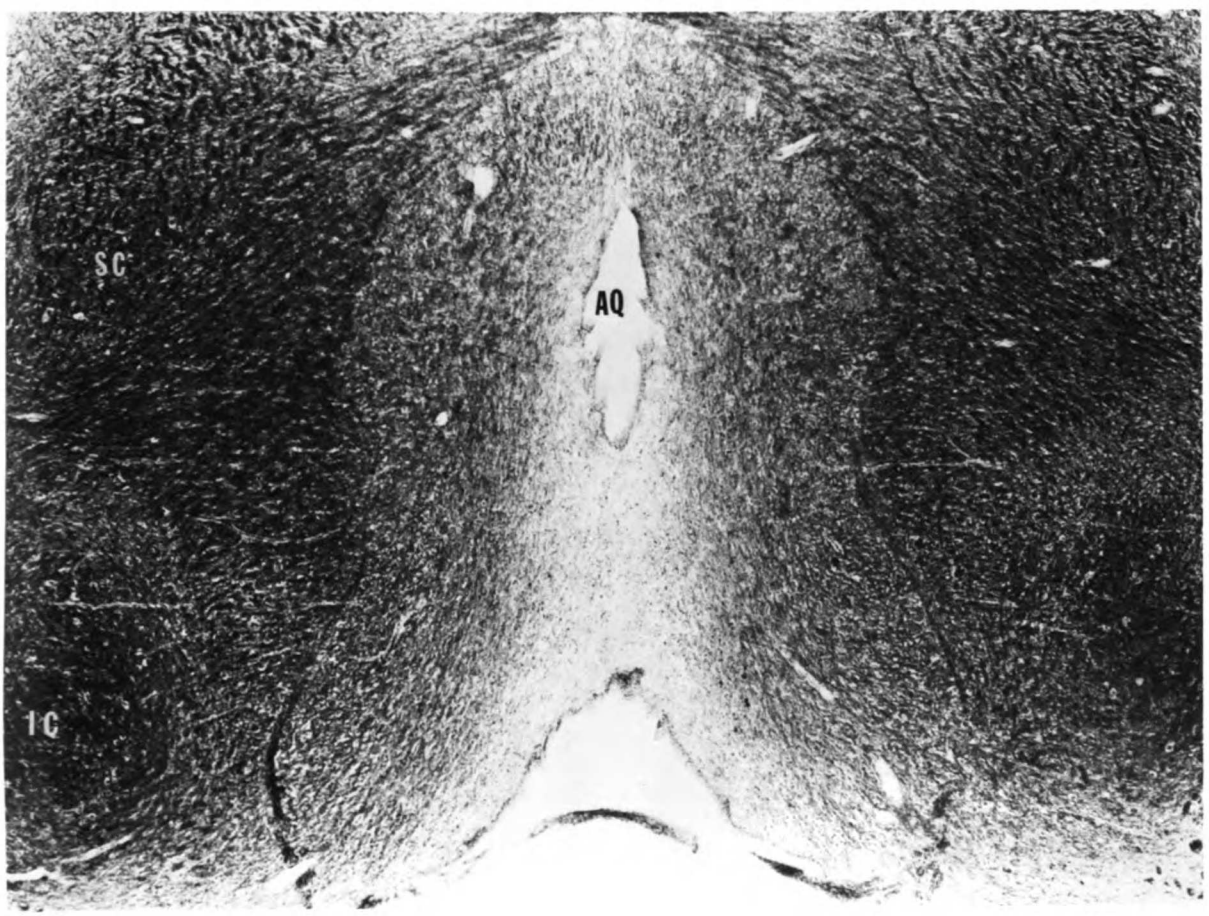


Figure 15. Photomicrograph of a Weil stained horizontal section through the periaqueductal grey; rat.

Rat Weil Stain Horizontal Section



CHAPTER 2: FOREBRAIN PROJECTIONS TO THE PERIAQUEDUCTAL GREY, THE DESCENDING AFFERENT CONNECTIONS OF A PAIN MODULATORY CENTER

INTRODUCTION

The periaqueductal grey (PAG) is a midline structure which surrounds the cerebral aqueduct from the level of the locus coeruleus to the posterior commissure. Physiologically this central grey region has been implicated in a wide variety of functions including, inhibition of oxytocin release (Aulsebrook and Holland, 1969), vocalization (Kanai and Wang, 1962), emotional rage (Hunsperger, 1956), lordosis reflex in sexual behavior (Sakuma and Pfaff, 1980), and analgesia (Reynolds, 1969). What these various responses have in common is unclear but they all do appear to have visceral or emotional overtones which might be considered functions of the limbic system. The involvement of this central grey region in the production of stimulation produced analgesia (SPA) has elicited much interest in this midbrain structure. The phenomenon of SPA was originally described in the rat (Reynolds, 1968) and it has been replicated in the cat (Oliveras et al, 1974), monkey (Goodman and Holcombe, 1975) and man (Hosobuchi et al, 1977). However SPA is not unique to the PAG, for there are other regions of the brain such as the caudate nucleus (Lineberry and Vierck, 1975) the internal capsule (Adams et al, 1974) and the septum (Gol, 1967) where appropriate stimulation also produces a pronounced SPA. The unique aspect of the SPA produced in the PAG is that it appears to work in a manner similar to centrally administered opiates. There is a variety of evidence in support of this view. The same regions that prove most efficatious in the production of SPA overlap with regions that produce analgesia upon intracerebral injection of morphine (Liebeskind and Mayer, 1974). Second, SPA appears to be cross tolerant to morphine administration in

the PAG (Mayer and Hayes, 1975). Lastly, SPA is partially reversed by the opiate antagonist nalaxone (Adams, 1976). Thus the PAG is unique among the regions in the brain which produce analgesia upon stimulation, in so far as a specific and identified pharmacological processes seems to partially underlie its action.

Previous neuroanatomical literature has suggested that this central grey region received inputs from a variety of rostral brain areas. These included widespread cortical areas (Kunzle, 1974), the hippocampus (Nauta, 1958), amygdala (Hopkins and Holstege, 1978) and the hypothalamus (Conrad and Pfaff, 1976; Saper et. al., 1976, 1979). For a brainstem region to receive such widely disparate input is considered unusual and in light of this extensive convergence of forebrain input Nauta (1958) coined the phrase "limbic midbrain area" to describe this central grey region.

The purpose of this study is to investigate the sources of afferent input to the midbrain PAG originating in the forebrain by means of modern anatomical techniques. Such an analysis should indicate which forebrain structures influence the PAG. That these rostral brain regions do play a role in influencing PAG function is evidenced by the fact that stimulation of the axonal fibers originating from these rostral regions and projecting to the PAG, elicits similar physiological responses as does stimulation of the PAG itself. Thus previous physiological data suggests these forebrain regions play a significant role in the control of PAG function. This study will therefore explore the anatomical substrate by which these rostral brain regions exert their descending control.

MATERIAL AND METHODS

Ten adult squirrel monkeys, four adult cats and 15 adult rats were used in this study. The animals were anesthetized with intraperitoneal sodium pentobarbital (Nembutal 20-40 mg/kg) and placed in a stereotaxic apparatus with an appropriate head holder. With aseptic procedures the skull was exposed and a small burr hole was made in the skull at the appropriate level. Through this hole a 25 gauge insulated needle with a specially beveled tip, or a glass electrode (tip diameter 10-30 μm), attached to a 1ml Hamilton syringe was lowered through the burr hole to a predetermined depth using the stereotaxic coordinates of Emmers and Akert (1963) for the monkey, Berman (1968) for the cat and Albe-Fessard et. al. (1971) for the rat. In some monkeys stimulation via the insulated needle was used to help define when the tip of the needle was in the PAG. Upon stimulation one could generally elicit eye movement (III or IV nerve) and/or vocalization (Jurgens and Pratt, 1979) when the tip was in the PAG. The injections in rats and cats consisted of .01-.05 μl of 30% horseradish peroxidase HRP (Sigma type VI) over a period of 1-2 hours. In the monkey a mixture of .01-.1 μl was injected which consisted of 30% HRP (Sigma type VI) and 5-10 μC of ^{3}H-leucine. This isotope mixture was prepared by dessicating the isotope to dryness and then reconstituting it in the 30% HRP solution. After this injection the wound was closed and the animals returned to their cages.

After a survival time of 1-3 days the animals were reanesthetized with sodium pentobarbital and perfused through the heart with a heparinized phosphate buffered saline solution (pH 7.4) followed by a mixture of 2.0% gluteraldehyde and 1.0% paraformaldehyde in a 0.1 M

phosphate buffer, pH 7.4. After perfusing with fixative the brain was left in place for 1 hour to allow the fixative to penetrate, after which a 10% sucrose solution in phosphate buffer pH 7.4 was perfused through the animal to eliminate any excess fixative. The brain and spinal cord were then removed, blocked and stored in 30% sucrose solution in phosphate buffer for 2 days to reduce ice crystal formation in the frozen sections. Sections were then cut in the coronal stereotaxic plane at 50 µm on a freezing microtome and in monkeys alternate 25 µm sections were cut for autoradiography. The 50 µm sections were then reacted for the presence of HRP using the tetramethylbenzidine (TMB) technique (Mesulum, 1978). After the peroxidase reaction was completed, the sections were mounted on gelatinized slides, counterstained, dehydrated, coverslipped and examined with both dark and bright field optics.

Injections sites were reconstructed from alternate serial sections through the midbrain. It was assumed that the area of dense staining approximated the zone of active uptake of horseradish peroxidase. Both the injection site and labelled cells were plotted onto projection drawings of serial sections. Each section in the reconstructed figures contains those cells (indicated as dots) recorded in one representative 50 µm section.

RESULTS

The results presented here will emphasize the observations from the primate material. The cat and rat material will be discussed only if significant differences were observed compared to the primate. These rat and cat experiments were undertaken in order to determine if

significant species differences occur in PAG connectivity. These experiments reveal that few discernable differences in the forebrain input to the PAG occur between the monkey, cat and rat.

The most difficult problem in HRP studies is determining the effective uptake region at the injection site. To overcome this problem we made very small injections (average of .025 µl 30% HRP), waited a long period after the injection before withdrawing the electrode to minimize leakage along the electrode track, and used the most sensitive chromagen available to visualize the extent of the spread of HRP. By using these techniques we feel we have minimized this difficulty.

The injection sites obtained in the primates used in this study are shown in Figure 16. As can be seen from the diagram a variety of discrete injections were made into various regions of the PAG. Although different regions of the PAG were injected in different animals or even in different species, no matter where HRP was injected into the PAG, a similar pattern of afferent connections were observed, with only minor exceptions. Because of the consistency of these findings we shall present a summary of our results here from a representative injection site found in experiment SM-2 (Fig. 16). This injection site was in the dorsal lateral area of the PAG and had a rostral to caudal spread of approximately 2.0 mm. No consistent differences were observed in cell labelling patterns between the rostral and caudal injection sites.

In the following presentation of the results we shall describe the regions in which HRP positive cells were consistently found beginning with the rostral-most areas and moving caudal through the diencephalon toward the PAG injection sites.

Cortical Regions

The frontal granular cortex exhibited a considerable number of HRP positive cells after PAG injections. These cells appeared chiefly in the dorsolateral cortex extending from the rostral pole of the frontal lobe back to the level of the anterior commissure. At this level labelled cells in the insular cortex also came into view (Figures 17 and 18). The labelled cells in the frontal cortex were pyramidal in nature and appeared to segregate into two classes, one class consisting of large cells 25 μm in diameter (Figure 19) and a second class of small cells 18 μm diameter (Figure 19). These cells appeared predominantly on the side ipsilateral to the injection with a small but consistent number being present in the contralateral cortex. These cells were present in a band confined to layer 5 of the cortex. (Fig. 19). Cells were also consistently seen in the insular cortex. These labelled insular cells were similar to those found in the frontal granular cortex and are pyramidal in shape and are also located in a band in layer 5. Injections into all areas of the PAG caused some labelling of these cells. These insular cells, however, appeared to project preferentially to the more peripheral regions of the PAG because larger numbers of cells were found in animals in which the injection sites were more peripherally located in the PAG. These frontal and insular cortical cells were also partially labelled following deep tectal and deep tegmental injections in which the PAG was unlabelled. Therefore it appears that this projection from cortex to midbrain is primarily to the periphery of the PAG and adjacent tectal and tegmental regions.

Amygdala

Ipsilateral amygdala cells were consistently labelled after injections into all regions of the PAG. These labelled cells had small round soma (15 μm in diameter). They were, however, few in number (3 labelled cells per 50 μm section) and were found to be located exclusively in the basal lateral and central amygdala nuclei (Figure 18).

Hypothalamus

The hypothalamus demonstrated by far the largest number of labelled cells of all the regions of the brain in which HRP positive cells were found after injections of HRP into the PAG. This labelling was not uniform within the hypothalamus but tended to be distributed into discrete regions and nuclei of the hypothalamus. Described below are the hypothalamic areas which contained retrogradely labelled cells.

Anterior Region

In the rostral-most areas of the hypothalamus large numbers of labelled cells could be consistently found in the medial preoptic nucleus, which merges with the caudal septum (Figure 20a). These labelled cells were present in a band at this level which swept into the lateral preoptic area and included a portion of the medial substantia innominata. As one moved caudal these labelled cells continued to be present and merged into the anterior hypothalamic nuclei. In the anterior hypothalamic nuclei the lateral part was more heavily labelled than the medial part which contained few labelled

cells. This band of labelled cells seemed to sweep from the most rostral areas of the medial and lateral preoptic region caudalward to include the lateral anterior nuclei and to some extent the lateral aspect of the medial anterior nuclei (Figure 20b). Few, if any, labelled cells were observed in the supraoptic, paraventricular or suprachiasmatic hypothalamic nuclei. Injections into different regions of the PAG did not show any appreciable variation in the numbers or distribution of labelled cells seen in the anterior hypothalamic region.

Medial Region

The medial region of the hypothalamus contained moderate to heavy labelling after HRP injections into any area of the PAG. The fusiform periventricular cells which line the third ventricle above the arcuate nucleus were the most consistently well labelled cells (Figures 20c and 21). HRP positive cells also appeared in the dorsal hypothalamic area just dorsal and lateral to the periventricular cells. These dorsal and lateral cells seemed to form lateral wings (Fig. 21) that attached to the periventricular cell system that extended so far lateral that they appear to merge with the labelled cells in the zona incerta. In other words all of the labelled cells in the periventricular nucleus, dorsal nucleus, and the zona incerta were to varying degrees fusiform in shape, and they appeared to run from medial to lateral in a contiguous fashion (Fig. 21).

The ventromedial nucleus of the hypothalamus, a well delineated nuclear lying group just lateral to the periventricular cells and dorsal to the arcuate nucleus, contained moderate numbers of labelled

stellate shaped cells which were approximately 20 μm in diameter and quite distinct from the more medial, fusiform periventricular cells. Unlike the band of labelled cells in the anterior or periventricular region of the hypothalamus the labelled cells in the ventromedial nucleus were well delineated and appeared as a distinct nuclear group. The arcuate nucleus that is situated immediately below the ventromedial nucleus had few labelled cells. This nuclear zone is a teardrop shaped periventricular nucleus adjacent to the ventral most aspect of the third ventricle and dorsal to the infundibulum. Its boundaries as described by Krieg (1932) in the rat can be clearly defined in the rat and primate but it is more difficult to discern in the cat. Although cells in the arcuate nucleus itself were not well labelled in any case, the rather ill defined region just above the arcuate zone proper and ventral to the ventromedial nucleus contained large numbers of well labelled cells. These labelled cells were variable in shape and extended somewhat lateral to the arcuate nucleus in both a rostral and caudal direction. This periarcuate labelled region did not seem confined to any of the classically described hypothalamic nuclear groups and it appears to be a seperate "periarcuate" nucleus. Other distinct nuclei in the medial hypothalamic region such as the tuberal or tuberomammillary nuclei contained few if any labelled neurons.

Lateral Region

The lateral region of the hypothalamus traversed by the medial forebrain bundle contained moderate numbers of well labelled neurons (Figure 20c). These cells were variable in shape including such forms

as stellate, fusiform and spherical. The number of labelled cells in this lateral region was never as great as the number of labelled cells observed in the medial or posterior hypothalamic regions but was comparable to in number the labelling seen in the anterior hypothalamic regions.

Posterior Region

In the posterior region of the hypothalamus heavy labelling of neurons was seen in the posterior hypothalamic nucleus which immediately borders the third ventricle (Figure 20c, 20d, 22a and 22b). This periventricular region had large numbers of labelled fusiform cells which seemed contiguous with the more rostral dorsomedial nucleus and the lateral zona incerta. In this region of the hypothalamus the distinct borders of nuclear groups are difficult to delineate and if such borders do in fact exist the cells labelled here did not respect them. In contrast to the large number of labelled cells observed in the posterior hypothalamic nuclei, the well delineated mammillary nuclei contained few if any labelled cells.

Zona Incerta

This nuclear zone, which is located lateral to the hypothalamus, dorsal to the subthalamic nucleus, ventral to the ventral basal complex and medial to the nucleus reticularis thalami, consistently contained large number of heavily labelled cells (Figure 20d and 20e). These labelled cells were fusiform in shape (Fig. 22c)and appeared to be organized into an upper and lower tier of cells. Some labelled cells were observed in both tiers after injections into any region of

the PAG (Figure 20d and 20e). As has been previously mentioned, these labelled cells were often contiguous with labelled cells in the posterior or dorsomedial hypothalamus. Few if any labelled cells were observed in the reticularis thalami although the lateral border of the zone incerta and the medial border of the reticularis thalami is difficult to define.

Mesencephalic Reticular Formation

This poorly defined region consistently sends a moderate to heavy projection to the ventrally adjacent PAG (Figure 20f). Labelled mesencephalic reticular formation (Mes RF) cells are heterogeneous in shape and range from small spherical to large pyramidal. Some of the neurons of this diffusely organized region that project to the PAG might include cells located just lateral to the exiting third nerve belonging to the AIO area. Whether these are the AIO neurons which contain dopamine is not known, although the position of these deeper Mes RF labelled neurons approximates with the AIO area.

DISCUSSION

Before discussing the anatomical and functional implications of this investigation it is important to realize the limitations and caution one should take in interpreting the results from such an HRP study. In all descriptions of the results obtained here we have used the word "projecting to" rather than "terminating in" the PAG. The reason for this is that HRP can can be taken up by damaged axons as well as axonal terminals (LaVail, 1975). In the PAG there is a large number of poorly myelinated axons traveling through this region which

are known collectively as the dorsal bundle of Schutz (Schutz, 1891). Introduction of an electrode into the PAG in order to inject HRP would probably disrupt some of these axons. Therefore, neurons in the brain which send axons through the PAG that do not terminate there have a possibility of being retrogradely labelled. The actual occurrence of the labelling of such injured "axons en passage" has been minimized in this study by using different angles of approach, fine injection needles, and by noting the consistency of a projection to the PAG in different animals.

Although the possibility of labelling axons of passage has been minimized in this study it nevertheless cannot be completely ruled out when using HRP as a tracer in a region such as the PAG. Therefore, in the discussion we shall site other investigations whenever possible which might serve to either confirm, or deny, whether apparent anatomical connections do occur or whether the labelled neurons are due to the uptake of HRP by axons en passage. It should be noted, however, that this HRP technique does give an accurate representation of those neuronal cells and fibers that would be stimulated if a stimulating electrode was used in this same position as our injecting needle. Stimulating electrodes are known to affect axons of passage, axonal terminals, and cell bodies alike. HRP similarly labels injured axons of passage and axonal terminals. Therefore a comparable population of cells and fibers will be affected by both techniques. Thus while the HRP technique does have its limitation in determining only areas of true axonal synapses, it nevertheless does accurately reflect which structures might be affected by stimulation electrodes placed in similar areas.

The frontal cortex projection to the PAG seen in this study has been reported in previous studies using the monkey (Goldman and Nauta, 1976; Kunzle and Akert, 1977). In these previous autoradiographic studies it has been reported that the caudal-most extent of the frontal cortex projection ended in the PAG (Goldman and Nauta, 1976). Since these fibers projected no further than the midbrain level, the labelled frontal cortex cells observed in the present investigation are most probably due to terminal uptake of HRP and not damaged fibers of passage. The functional significance of this cortex-PAG projection is unknown. It is of interest however that these same cortical regions have been reported (Freeman and Watts, 1946; Foltz and White, 1962) to be involved in the affective aspects of pain. Ablation of these cortical areas causes the patient to still recognize nociceptive input but the association of this nociception with pain is apparently lost. Whether the cortical-PAG connections shown to be present in this study are actually involved in the affective aspects of pain perception is unknown.

The connection of the amygdala with the PAG was previously reported by Hopkins and Holstege (1978) using autoradiographic techniques in the monkey. The cells of origin of this amygdala-PAG connection observed in the present HRP study were quite small in number and contained within the basolateral and central nuclei of the amygdala (Figure 18).

The hypothalamic projection to the PAG is by far the heaviest projections received by the PAG from any region of the brain. Previous autoradiographic studies in the rat (Conrad and Pfaff, 1976a, 1976b) and monkey (Saper et. al., 1976, 1979) have also suggested that

the PAG is the midbrain area where nearly all hypothalamic projections to the brainstem converge. In these autoradiographic studies the furtherest caudad that the massive hypothalamic projection could be traced was the midbrain PAG. Such results suggest these hypothalamic neurons labelled in the present study was caused chiefly by HRP pickup from axonal terminals and not damaged axons of passage. What is striking about the hypothalamic cells labelled here is the large number of nuclei, and neurons within these nuclei that were well labelled (Figure 20). Such a massive projection from a functionally diverse area of the brain such as the hypothalamus suggests that the functions of the PAG also are diverse and heterogeneous in nature. Of special note in this hypothalamic-PAG projection are those labeled neurons in the lateral hypothalamus and the periarcuate region. The lateral hypothalamus is often stated to be the main area for hypothalamic "inputs" while the medial hypothalamus is thought to be primarily concerned with hypothalamic "output" (Brooks and Kuizumi, 1974). In the present study, however, the lateral hypothalamus contributed a significant projection to the PAG. Therefore, the hypothesis that the lateral hypothalamus lacks long distance connections seems inappropriate in light of the present data.

The discovery of a heavy projection to the PAG from a periarcuate cell group and not the arcuate nucleus per se was somewhat surprising. Previous authors have identified the arcuate nucleus as being the sole source of β-endorphin fibers which projects to the PAG (Watson et. al., 1977). The release of β-endorphin from the arcuate terminals in the PAG during stimulation produced analgesia (SPA) is hypothesized to partially underlie the resulting analgesia (Hosobuchi et. al., 1979).

However, in the arcuate nucleus as defined by Krieg (1932), few labelled neurons were seen after injections into any region of the PAG. One finds only a diffuse background of endogenous staining in the arcuate nucleus even in the absence of any HRP injection into the PAG. However, cells in the surrounding periarcuate regions were heavily labelled after PAG injections. This discrepancy between the location of the previously described β-endorphin cell bodies and the labelled neurons observed in this study is probably due to an anatomical misnomer in identifying the region in the original immunohistochemical description of the β-endorphin cell bodies (Watson et. al., 1977). In examining the labelled β-lipotropin cell bodies shown in this report (Watson et. al., 1977) one notes that few β-endorphin positive cells are actually present in the arcuate nucleus as defined by Krieg (1932) but most are present in the dorsally adjacent periarcuate regions. In view of this discrepancy it would seem more appropriate to refer to the location of these β-endorphin cells as being present in the basal hypothalamus rather than as the infundibular related arcuate nucleus.

The zona incerta cells which have been shown to project to the PAG in this study have not been investigated autoradiographically, most probably due to the difficulty in injecting this region given its sheet like form. The zona incerta has been shown to receive projections from the dorsal column nuclei (Lund and Webster, 1967), principal sensory nucleus of the trigeminus (Smith, 1975) and possibly from the spinothalamic tract (Bovie, 1979). The function of this sheet like structure is unknown although it does have neurons similar in morphology and distribution to the laterally continuous nucleus

reticularis thalami. The projection of a topographically organized structure such as the zona incerta provides an anatomical substrate to the previously suggested possibility of a topographic organization of the PAG (Liebeskind and Mayer, 1971).

The mesencephalic reticular formation (MesRF) sends a heterogeneous projection to the PAG. Previous autoradiographic experiments have not been performed on this ill defined area so the resolution of the question of fibers of passage or true axonal terminations cannot be made at this time. However, scattered neurons in the ventral Mes RF region that are in about the same area as the AIO dopaminergic cell group were consistently labelled. Dopamine is known to be present in the PAG (Versteeg et. al., 1976). Since the number of dopamine containing nuclei is quite limited and neurons near the AIO group are found to project to the PAG, the possiblity exists that these deep Mes RF labelled cells are in reality AIO neurons which contribute the dopaminergic axons known to be present in the PAG.

Previous anatomical reports (Hamilton, 1973, 1974) have stated that the PAG can be divided into three subnuclei, dorsal, lateral and ventral group, each having its own distinctive cytoarchitecture and connectivity. In a previous report (Mantyh, 1980) we have noted that these regions of the PAG have more features of their cytoarchitecture in common than have they notable differences. The present report presents further evidence to support the contention that these hypothesized subnuclei do not each have a distinctive set of connections. Our data, however, suggest that most regions of the PAG share in common the majority of their sources of afferent connections. Therefore, on anatomical grounds it seems inappropriate to view the

various regions of the PAG as each being a distinct entity based on either cytoarchitectural (Mantyh, 1980) or, based upon the present findings, connectivity grounds.

The heterogenity of connections with the PAG made by forebrain structures is also remarkable. These diverse connectional origins include: cortical areas, the amygdala, subnuclei of the hypothalamus, the zona incerta, and the mesencephalic reticular formation. Such connections suggest the PAG has the anatomical substrates necessary for being involved in a wide variety of physiological functions. Such heterogeneity of function has already been suggested of the PAG in physiological studies following either stimulation (Kanai and Wang, 1962; Reynolds, 1969; Skutely, 1959, 1963) or ablation techniques (Skultety, 1966; Taylor and Farrell, 1962). What is germane to this discussion is the anatomical and physiological heterogeneity of this structure. Studies on the PAG which focus on only one of its many physiological functions are probably oversimplifying the complex nature of this central grey region. Therefore, when discussing the normal in vivo functioning of this region it is important to keep in mind that it is probably involved in a multitude of physiological mechanisms. In this respect it is unfortunate that in the current literature of human stimulation produced analgesia no thorough description of the global changes experienced by the patient during SPA is available. In such as a complex region such as the PAG it would indeed be remarkable if one could stimulate this region and obtain one and only one physiological effect, that of analgesia. In animal models in which data collection is much more difficult, given that the animal cannot verbally relate its physiological changes, a

multitude of physiological responses unrelated to analgesia have been commonly obtained during stimulation of the PAG. In such experimental animals, analgesia is also commonly obtained during stimulation and in fact the reason animals are used in these experiments is that they do approximate the human condition. In attempting to unravel how the PAG functions it would be of great use to obtain more complete descriptions of the patient's physiological responses during PAG stimulation. Simply focusing on the question of analgesia being obtained or not seems to oversimplify a key region of the brain and to thus lose the interesting and otherwise unavailable insights that only humans can provide into this complex region of the brain. Therefore a more complete description of the effects of SPA on patients including those changes observed in animal models such as, emotional mood, gastric motility, rage reactions, sexual function and changed perception to different types of sensory stimuli would be of great help in revealing what functions this central grey region plays in regulating normal human behavior.

SUMMARY

In summary the present investigation has stresses four points on the anatomy of the periaqueductal grey:

1) the PAG receives a variety of inputs from a diverse group of forebrain structures which include: the frontal granular cortex, the insular cortex, the amygdala, the hypothalamus, the zona incerta and the mesencephalic reticular formation.

2) The majority of these neurons project to the PAG via the periventricular bundle. Fibers of this system can be seen hugging the cerebral aqueduct at the level of the posterior commissure. It is apparently this bundle that the neurosurgeon stimulates during stimulation produced analgesia.

3) The different regions of the PAG have in common the majority of their afferent forebrain connections. Thus subdivision schemas based on each subnuclei of the PAG having its own distinctive cytoarchitecture or connectivity seems inappropriate.

4) The diversity of the rostral forebrain areas projecting to the PAG is in good correlation with its known heterogenity in its physiological functions. Such diversity in the known anatomy and physiology suggests that reports of stimulation of the PAG producing only analgesia might be overlooking other simultaneous physiological changes.

BIBLIOGRAPHY

Albe-Fessard, D., F. Stutinsky and S. Libouban 1971 Atlas Stereotaxic du diencephale du Rat Blanc, Centre National de la Recherche Scientifique, Paris.

Adams, J.E. 1976 Naloxone reversal of analgesia produced by brain stimulation in the human. Pain 2:161-166.

Adams, J.E., Y. Hosobuchi and H.L. Fields 1974 Stimulation of internal capsule for relief of chronic pain. J. Neurosurg. 41: 740-744.

Aulsebrook, L.H. and R.C. Holland 1969 Central inhibition of oxytonin release. Am. J. Physiol. 216:830-840.

Berman, A. 1968 The brain stem of the cat. Univ. of Wisconsin Press, Madison.

Beyer, D., F. Mena, P. Pacheco and M. Alcaray 1962 Blockage of lactation by brainstem lesions in the cat. Am. J. Physiol. 202: 465-468.

Bovie, J. 1979 An anatomical reinvestigation of the termination of the spinothalamic tract in the monkey. J. Comp. Neurol. 186:343-370.

Brooks, C.M. and K. Kuizumi 1974 In: The hypothalamus and control of integrative process in Medical Physiology (ed. V. Mountcastle). Mosby, St. Louis.

Conrad, L.C.A. and D.W. Pfaff 1976a Efferents from medial basal forebrain and hypothalamus in the rat. I. An autoradiographic study of the medial preoptic area. J. Comp. Neurol. 169:185-220.

Conrad, L.C.A. and D.W. Pfaff 1976b Efferents from medial basal forebrain and hypothalamus in the rat. II. An autoradiographic study of the anterior hypothalamus. J. Comp. Neurol. 169:221-262.

Emmers, R. and K. Akert 1963 A stereotaxic atlas of the brain of the squirrel monkey (saimiri sciureus). University of Wisconsin Press, Madison.

Felten, D.L., A.M. Laties and M.B. Carpenter 1974 Monoamine-containing cell bodies in the squirrel monkey brain. Am. J. Anat. 139:153-166.

Foltz, E.L. and L.E. White, Jr. 1962 Pain "relief" by frontal cingulumotomy. J. Neurosurg. 19:89-100.

Freeman, W. and J.W. Watts 1946 Pain of organic disease relieved by prefrontal lobotomy. Proc. roy. Soc. Med. 39:445-447.

Gol, A. 1967 Relief of pain by electrical stimulation of the septal area. J. Neurol. Sci. 5:115-120.

Goldman, P.S. and W.J.H. Nauta 1976 Autoradiographic demonstration of a projection from prefrontal association cortex to the superior colliculus. Brain Res. 116:145-149.

Goodman, S.J. and V. Holcombe 1975 Selective and prolonged analgesia in monkey resulting from brain stimulation. In: First World Congress on Pain, 264.

Hamilton, B.L. 1973 Cytoarchitectural subdivisions of the periaqueductal grey matter in the cat. J. Comp. Neurol. 149:1-28.

Hamilton, B.L. 1974 Projections of the nuclei of the periaqueductal grey matter in the cat. J. Comp. Neurol., 152:45-58.

Hosobuchi, Y., J.E. Adams and R. Linchitz 1977 Pain relief by electrical stimulation of the central gray matter in humans and its reversal by naloxone. Science 197:183-186.

Hosobuchi, Y., J. Rossier, F. Bloom, and R. Buillemin 1979 Stimulation of human periaqueductal grey for pain relief increases immunoreactive β-endorphin in ventricular fluid. Science 203:279-281.

Hopkins, D.A. and G. Holstege 1978 Amygdaloid projections to the mesencephalon, pons and medulla oblongata in the cat. Exp. Brain Res. 32:529-547.

Hunsperger, R.W. 1956 Role of substantia gresea centralis mesencephali in electrically induced rage reactions. In: Progress in Neurobiology.

Jurgens, U. and R. Pratt 1979 Role of the periaqueductal grey in vocal expression of emotion. Brain Res. 167:367-387.

Kanai, T. and S.C. Wang 1962 Localization of the central vocalization mechanism in the brainstem of the cat. Exp. Neurol. 6:426-434.

Krieg, W.J.S. 1932 The hypothalamus of the albino rat. J. Comp. Neurol. 55:19-89.

Kunzle, H. and K. Akert 1977 Efferent connections of cortical, Area 8 (frontal eye field) in Macca fascicularis. A reinvestigation using the autoradiographic technique. J. Comp. Neurol. 173:147-164.

LaVail, J.H. 1975 Retrograde cell degeneration and retrograde transport techniques. In: The Use of Axonal Transport for Studies of Neuronal Connectivity (eds. Cowan W.M. and Cuenod M.) pp/ 217-248. Elsevier, Amsterdam.

Liebeskind, J.C. and D.J. Mayer 1971 Somatosensory evoked responses in the mesencephalic central grey matter of the rat. Brain Res. 27:133-151.

Lineberry, C.G. and C.J. Vierck 1975 Attenuation of pain reactivity by caudete nucleus stimulation in monkeys. Brain Res. 98:110-134.

Lund, R.D. and K.E. Webster 1967 Thalamic afferents from the spinal cord and trigeminal nuclei. An experimental anatomical study in the rat. J. Comp. Neurol. 130:313-328.

Mantyh, P.W. 1980 Nissl, Weil and Golgi Analysis of the Periaqueductal Grey in the Rat, Cat and Monkey. Neurosci. Abstracts.

Mayer, D.J. and R. Hayes 1975 Stimulation-produced analgesia: development of tolerance and cross-tolerance to morphine. Science 188:941-943.

Mesulum, M.M. 1978 Tetramethyl-benzidine for horseradish peroxidase neurohistochemistry; a non-carcinogenic blue reaction product with superior sensitivity for visualizing neural afferents and efferents. J. Histochem. Cytochem. 26:106-117.

Nauta, W.J.H. 1958 Hippocampal projections and related neural pathways to the mid-brain in the cat. Brain 81:319-340.

Oliveras, J.L., J.M. Besson, G. Guilbaud et al 1974 Behavioral and electrophysiological evidence of pain inhibition from midbrain stimulation in the cat. Exp. Brain Res. 20:32-44.

Reynolds, D.V. 1968 Surgery in the rat during electrical analgesia induced by focal brain stimulation. Science 164:444-445.

Saper, C.B., L.W. Swanson and W.M. Cowan 1976 The efferent connections of the ventromedial nucleus of the hypothalamus of the rat. J. Comp. Neurol. 169:409-442.

Saper, C.B., L.W. Swanson and W.M. Cowan 1979 Some efferent connections of the rostral hypothalamus in the squirrel monkey (saimiri sciureus) and Cat. J. Comp. Neurol. 184:205-242.

Schutz, H. 1891 Anatomishe Untersuchungen uber den Faseruer lauf im zentralen Hohlengrau und den Nerven-faserschwund in demselben bli der progressiven Paralyse der Irren. Arch. Psychiat. 22:527-587.

Skultety, F.M. 1959 Relation of periaqueductal gray matter to stomach and bladder motility. Neurol. 9: 190-197.

Skultety, F.M. 1963 Stimulation of the periaqueductal grey and hypothalamus. Arch. Neurol. 8:38-50.

Skultety, F.M. 1966 Changes in caloric intake following brainstem lesions in cats. Arch. Neurol. 14:670-680.

Smith, R.L. 1975 Axonal projections and connections of the principal sensory trigeminal nucleus in the monkey. J. Comp. Neurol. 163:347-376.

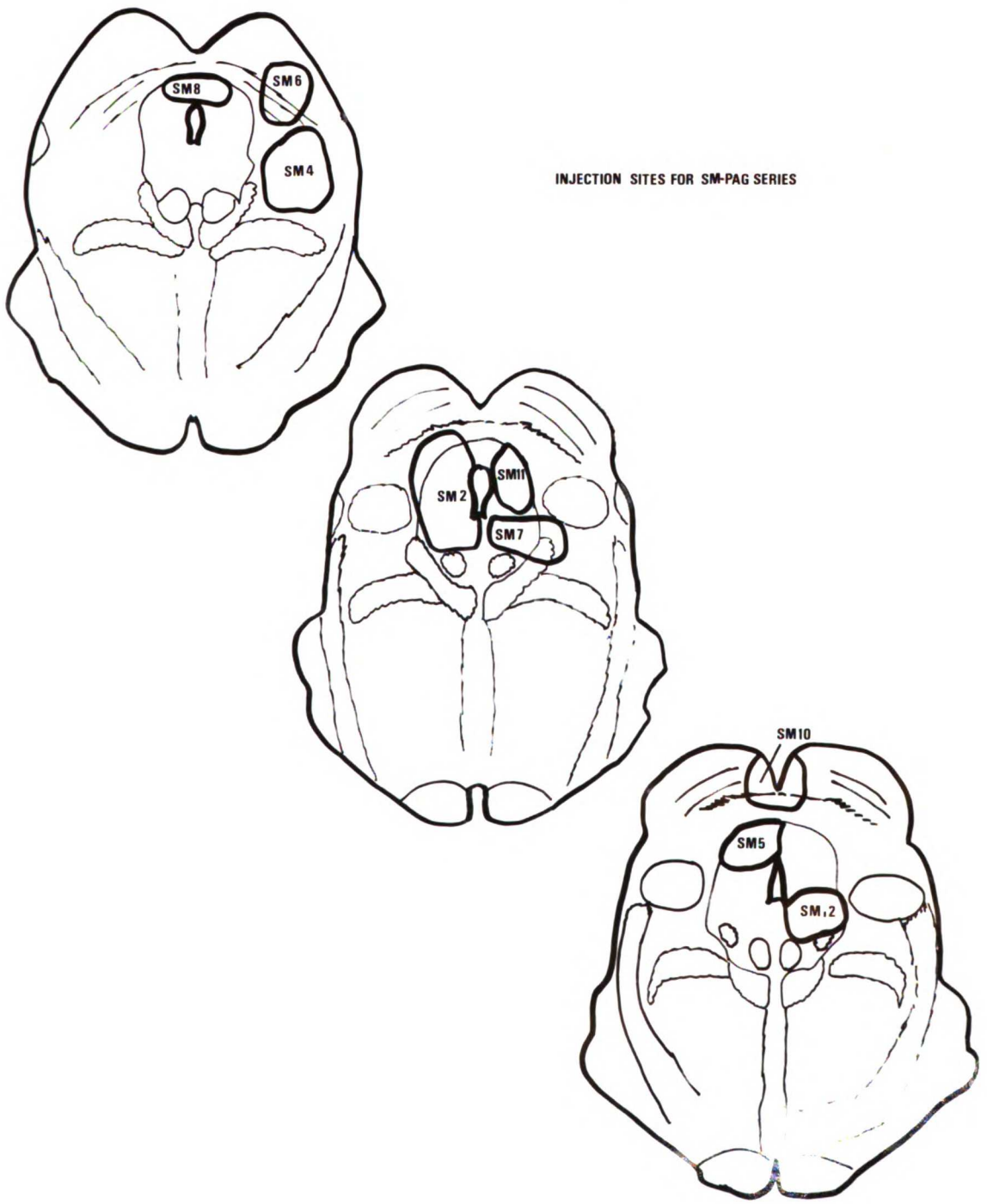
Sukuma, Y. and D.W. Pfaff 1980 Convergent effects of lordosis-relevant somatosensory and hypothalamic influences on central gray cells in the rat mesencephalon. Exp. Neurol. 70:269-281.

Taylor, A.N. and G. Farrell 1962 Effects of brainstem lesions on aldosterone and cortical secretion. Endocrin. 70:556-566.

Versteeg, D., J. Van der Gugten, W. de Jong and M. Palkovits 1976 Regional concentrations of noradrenaline and dopamine in rat brain. Brain Res. 113:563-574.

Watson, S.J., J.D. Barchas and C.H. Li 1977 b-Lipoprotropin: Localization of cells and axons in rat brain by immunohistochemistry. P.N.A.S. (USA) 74:5155-5158.

Figure 16. A schematic drawing of the squirrel monkey brain to show the extent of the various injection sites in and around the periaqueductal grey; saimiri sciureus.



INJECTION SITES FOR SM-PAG SERIES

Figure 17. A series of tracings illustrating the position of retrogradely filled HRP cells in the frontal granular cortex following an injection involving the entire right PAG. In this and all similar chartings of HRP material to follow the injection site is shown in stippling and the black dots indicate the positions in which retrogradely filled HRP neurons were located. All figures are arranged from rostral to caudal as if brain were cut in coronal section. saimiri sciureus, (experiment SM-2).


HRP-SqM
a
b
c
d
e
Cd
Put

Figure 18. A tracing illustrating the location of retrogradely labeled HRP cells at the level of the anterior commissure following an injection involving the entire right PAG; saimiri sciureus, (experiment SM-2).

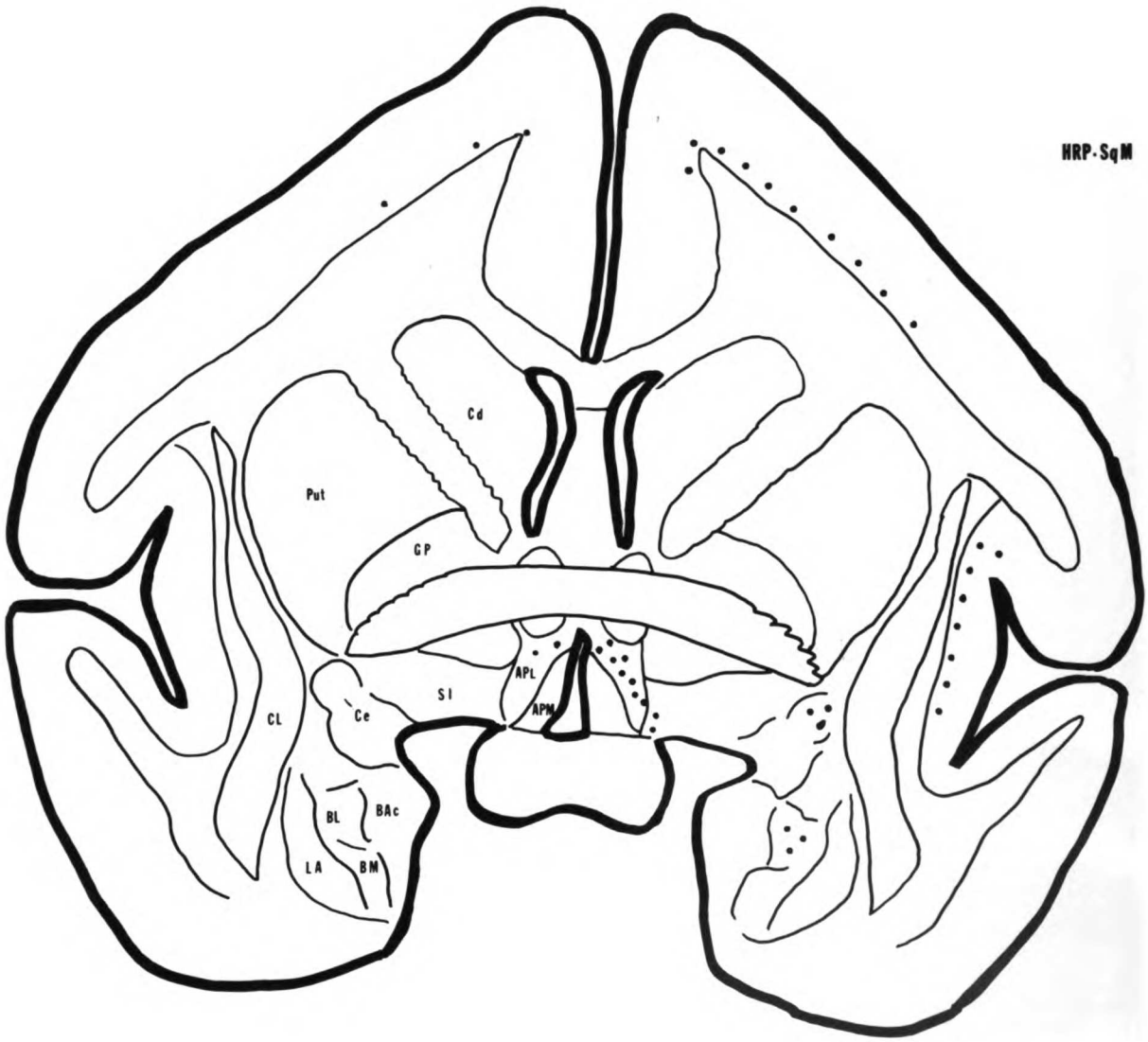
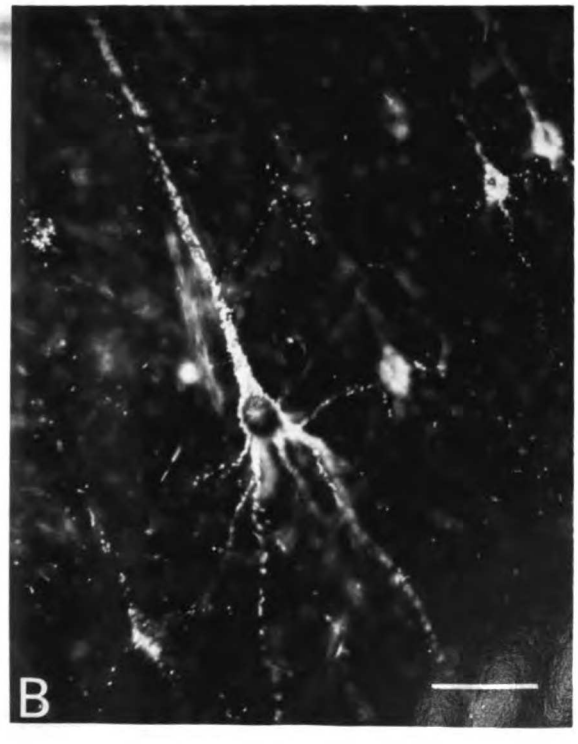

HRP-SqM
Cd
Put
GP
APL
APM
SI
Ce
CL
BL
BAc
LA
BM

Figure 19. A. A darkfield photomicrograph to show the distribution of HRP labeled neurons in layer V of the dorsomedial frontal cortex as seen in coronal section following an injection into the dorsal PAG; saimiri sciureus, (experiment SM-8). Scale = 50μm.

B. A darkfield photomicrograph to show the larger pyramidal neurons in layer V of the granular frontal cortex as seen in coronal section following an injection into the dorsal PAG; saimiri sciureus, (experiment SM-8). Scale = 50μm.

C. A darkfield photomicrograph to show the two classes of HRP filled neurons in layer V of the granular cortex in coronal section following an injection into the dorsolateral PAG; saimiri sciureus, (experiment SM-5). Scale = 50μm.


A


B


C

Figure 20. A series of tracings illustrating the position of retrogradely filled HRP neurons in the forebrain following an injection involving the entire right PAG; saimiri sciureus, (experiment SM-2).

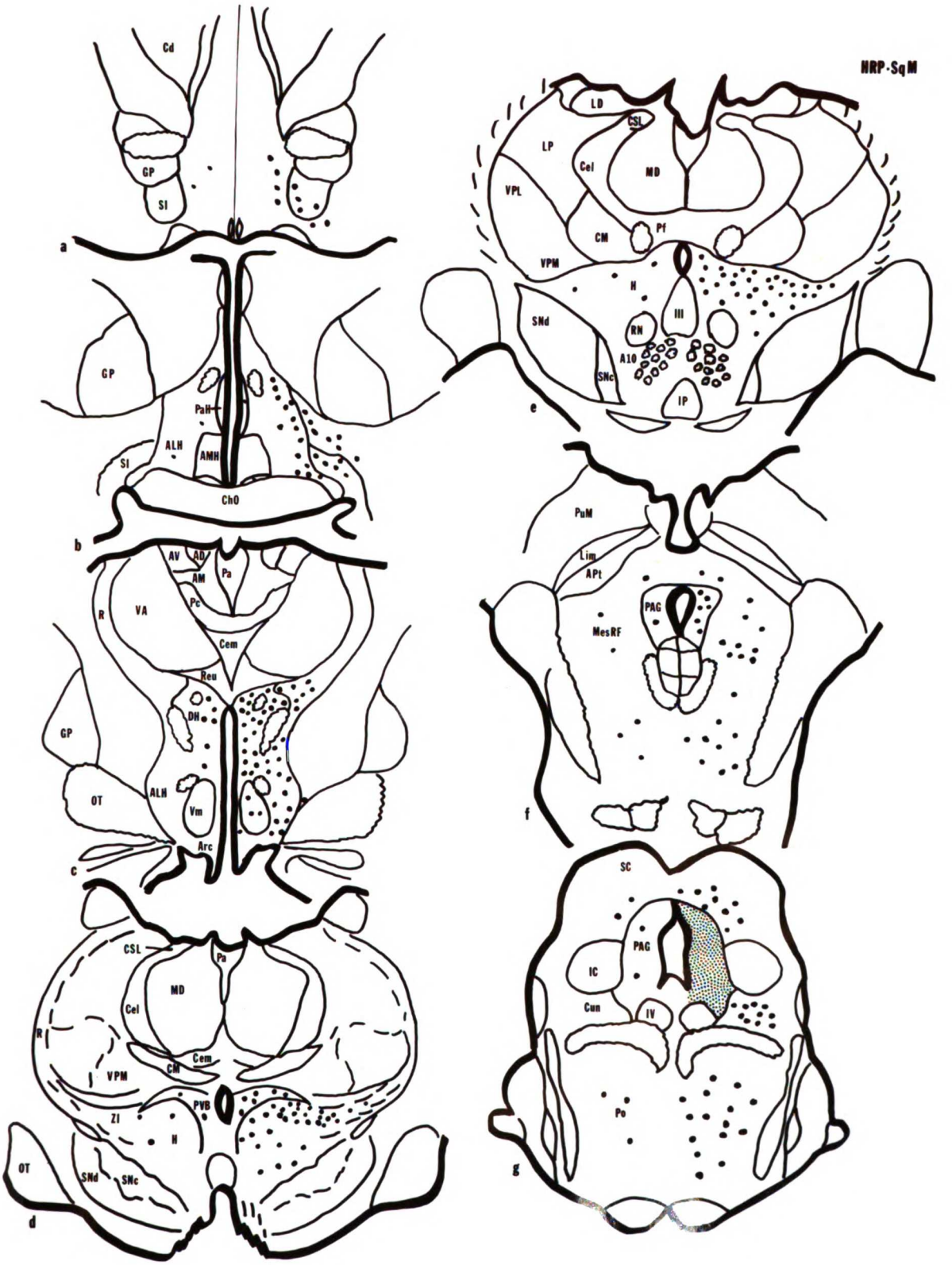
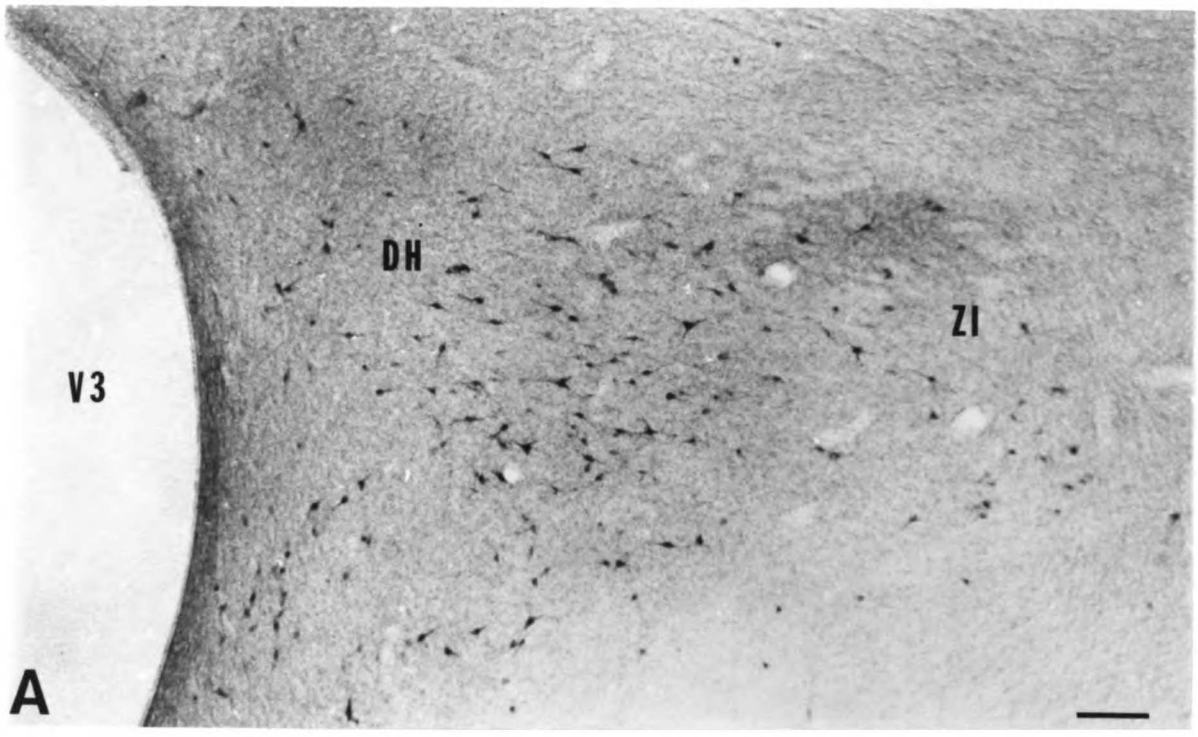

HRP-SqM
Cd
GP
SI
a
GP
PaH
ALH
AMH
SI
ChO
b
AV
AD
AM
Pa
Pc
R
VA
Cem
Reu
DH
GP
OT
ALH
Vm
Arc
c
CSL
Pa
MD
Cel
R
CM
Cem
VPM
PVP
ZI
H
OT
SNd
SNc
d
LD
CSL
LP
Cel
MD
VPL
CM
Pf
VPM
H
III
SNd
RN
A10
SNc
IP
e
PuM
Lim
APt
PAG
MesRF
f
SC
PAG
IC
Cun
IV
Po
g

Figure 21. A. A photomicrograph to show the distribution of HRP labeled neurons in the dorsal hypothalamus (DH) and the more lateral zona incerta (ZI) after an injection into the dorsal PAG; saimiri sciureus, (experiment SM-8). Scale 125μm.

B. A darkfield photomicrograph to show the distribution of HRP filled neurons in the dorsal hypothalamus (DH) and the zona incerta (ZI) after an injection into the ventrolateral PAG; saimiri sciureus, (experiment SM-7). Scale 125μm.


DH
ZI
V3
A

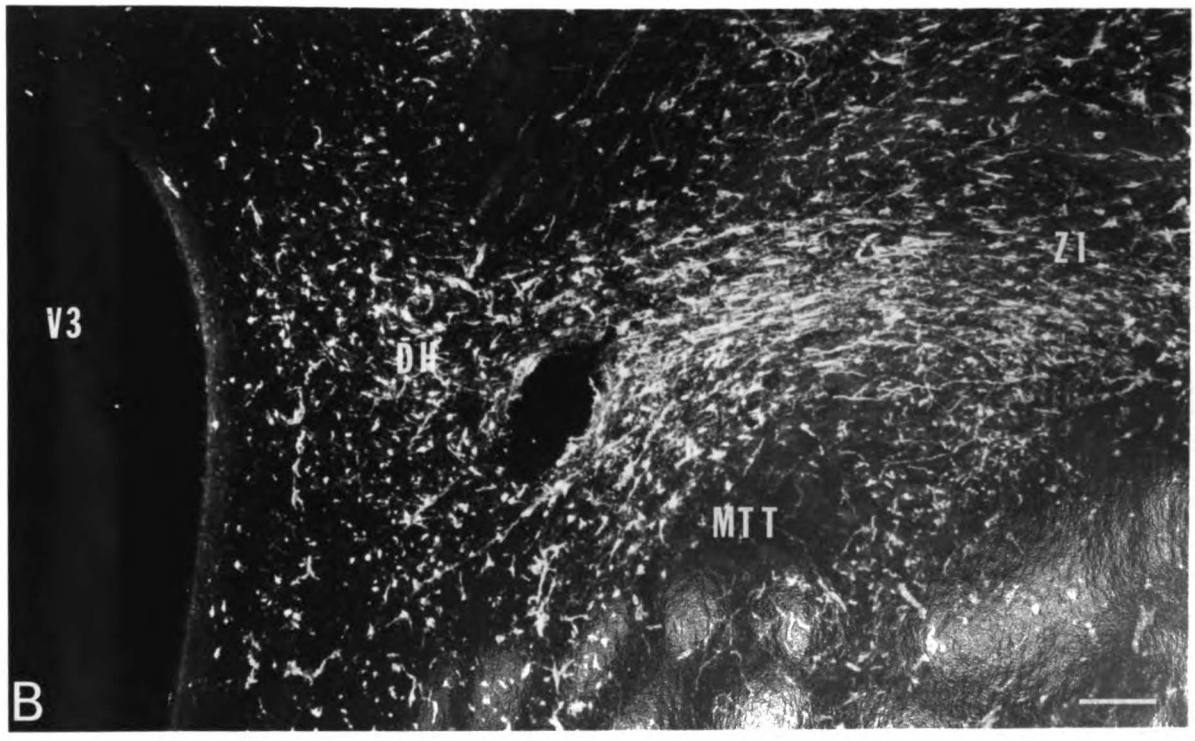
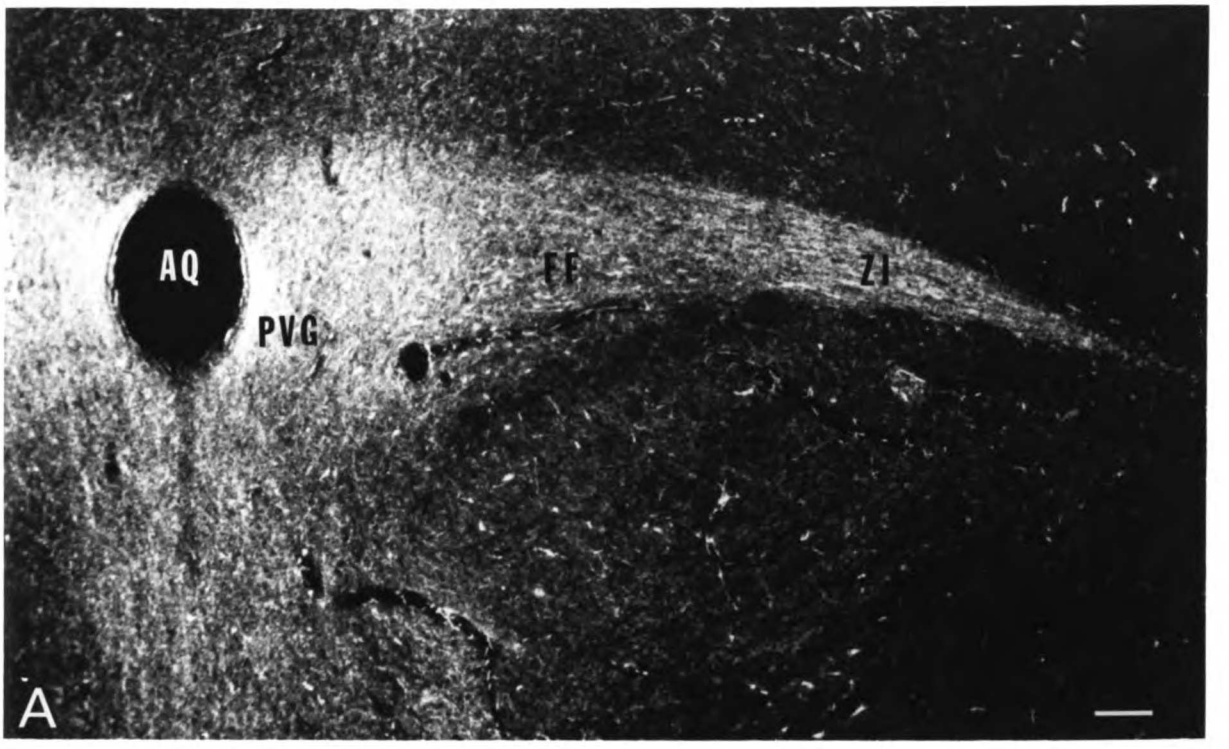

ZI
V3
DH
MTT
B

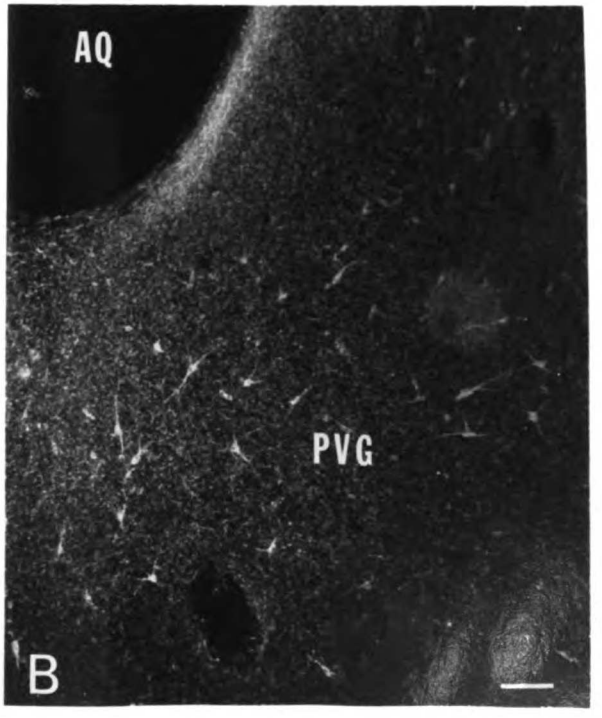
Figure 22.

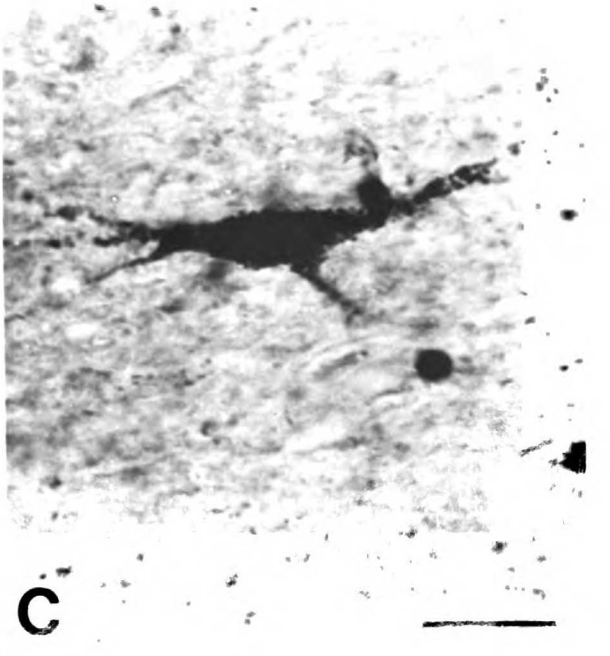
A. A darkfield photomicrograph to show te distribution of HRP filled axons and neurons in the periventricular grey (PVG), the Fields of Forel (FF), and the zona incerta (ZI) after an injection into the lateral PAG; cat, (experiment WC-3). Scale = 100μm.

B. A darkfield photomicrograph to show the HRP filled neurons in the periventricular grey (PVG) after an injection into the dorsal PAG; saimiri sciureus, (experiment SM-8). Scale = 100μm.

C. A light field photomicrograph to show the typical fusiform shape of an HRP filled zona incerta cell; cat, (experiment WC-1). Scale 25μm.


AQ
PVG
FF
ZI
A


AQ
PVG
B


C

CHAPTER 3: FOREBRAIN PROJECTIONS FROM THE PERIAQUEDUCTAL GREY THE ASCENDING EFFERENT CONNECTIONS OF A PAIN MODULATORY CENTER

INTRODUCTION

The periaqueductal grey (PAG) has been implicated in a variety of physiological functions as reviewed in the previous paper. One physiological function the PAG plays a role in, modulation of nociceptive input into the central nervous system has recently elicited a great deal of interest in the PAG. This involvement of the central grey in the modulation of nociceptive input has been demonstrated in both stimulation produced analgesia (SPA) (Reynolds, 1969) and opiate induced analgesia (OA) (Mayer and Hayes, 1975). However the anatomical substate and the mechanisms of action of both SPA and OA remain relatively obscure. Physiological and pharmacological data indicate that it is doubtful that SPA or OA acts by blocking the transmission of nociceptive information through the PAG since lesion of this area does not provide analgesia (Melzack et. al., 1958; Rhodes 1979). Rather it appears that both SPA and OA are obtained by a common activation of central grey modulatory influences over the CNS areas. While most previous investigators have emphasized the importance of the descending PAG brainstem projections in the mechanisms of SPA and OA, evidence suggests that diencephalic and telencephalic structures, which have also been implicated in the neural processing of pain, might also be involved in the modulation of nociception (Kerr and Wilson, 1978).

Previous anatomical investigations, using degeneration methods, of the ascending efferent projections of the PAG have revealed relatively few connections (Hamilton, 1974). However the silver degeneration techniques employed in this previous investigation preferentially stain larger axons. Since one of the prominent

features of the PAG is its content of fine caliber unmyelinated axons, previous investigations might have overlooked fine unmyelinated projection fibers of the PAG. In the present investigation modern anatomical techniques are employed which labeled fibers without regard to their size or degree of myelinization (Cowan et. al., 1972). By using these autoradiographic techniques a more complete picture of the PAG's efferent connections should be possible. The present investigation will therefore identify anatomical substrates by which the PAG could influence and modulate rostral brain areas, some of which have previously been identified to be involved in the processing of visceral and/or nociceptive information (Kerr and Wilson, 1978).

MATERIALS AND METHODS

Ten adult squirrel monkeys, four cats, and fifteen rats were used in this study. The animals were anesthetized with sodium pentobarbital (20-35mg/kg) and placed in an appropriate stereotoxic head holder. Then with usual aseptic conditions the skull was exposed and a small burr hole made at the appropriate level. The dura was then incised and a specially insulated and beveled 1µl syringe was lowered to a predetermined set of coordinates determined from the atlas of Emmers and Akert (1963) and Gergen and MacLean (1962) for the squirrel monkey, Mehler (unpublished) for the cat, and Albe-Fessard et. al. (1966) for the rat. In some animals, to help verify the correct placement of the electrode, current was passed through the end of the insulated needle and involuntary eye movements and vocalization (Jurgens and Pratt, 1979) helped confirm the proper placement of the injection needle.

Once the injection needle had been positioned properly a small injection (.01-.1µl) was made into the PAG over the period of 1 hour. For the monkey the injection solution consisted of 5-10µc of ^{3}H-leucine in a 30% HRP solution. To obtain this mixture a commercially obtained isotope was evaporated to dryness in a dessicating jar and then reconstituted to a known concentration using a 30% HRP solution in sterile saline. For the cat and rat a 30% HRP sterile saline solution alone was injected into the PAG. After an injection period of 1-2 hours the injection needle was carefully removed, gelfoam was placed in the burr hole, the wound closed and the animal returned to its cage. Two to three days later the animal was reanesthetized, its chest opened and its heart exposed. The animal was then perfused transcardially, first with a heparinized phosphate buffered (pH 7.4) saline solution (37°C), followed by a phosphate buffered (pH 7.4) solution of fixative (4°C) consisting of 1.0% paraformaldehyde and 2.0% gluteraldehyde. After the fixation procedure, the brain was left in situ for 30 minutes to allow for a more complete penetration of the fixative. After this time had elapsed a 10% sucrose solution (4°C) in phosphate buffer (pH 7.4) was perfused through the vasculature to rid the brain of any excess fixative which might depress the enzymatic HRP activity and to provide cryoprotection for the brain. The brain and spinal cord were then removed from the animal, blocked in sterotaxic plane and placed in a 30% sucrose solution in phosphate buffer (pH 7.4) until the brain sank. After this time the brains were cut on a freezing microtome in serial section in a 50-25-50 thickness cycle: one 50µm section was developed for the HRP reaction product alone; one 25µm section for

autoradiography when appropriate, and the final 50μm section was kept in reserve in case a more complete series was warranted.

Autoradiographic (ARG) processing in the monkey followed the technique of Cowan et. al. (1972). 25mm sections were collected in distilled water and were thoroughly washed in at least three changes of distilled water. After this washing the sections were mounted on gelatinized slides and allowed to air dry in a dust free container. The sections were then dehydrated in graded alcohols, cleared in xylol, rehydrated, and allowed to air dry in a dust free container. The slides were then dipped (in the dark) in Kodak NTB-2 emulsion diluted 1:1 with distilled water at 40°C, and allowed to dry in the slide racks for 2 hours. Slides were then placed in light-tight boxes with capsules of Drierite in the dark in a 4°C refrigerator. Both injection sites and areas receiving a projection were exposed in the dark for 8-36 weeks. Following development in half strength D-19 (Kodak) for 3 minutes at 10-12°C, the slides were rinsed briefly in tap water and fixed in full strength Kodak Rapid Fix for 3 minutes. After a two hour rinse to insure removal of the fixer, the sections were counter stained lightly in cresyl violet, dehydrated through graded alcohols, cleared in xylol, and coverslipped with Permount.

The 50μm sections from the monkeys, cats, and rats were processed for HRP according to the tetramethyl benzidine (TMB) protocol of Mesulum (1978) as detailed in the preceding paper. By using this TMB technique one can observe the anterogradely transported HRP which appears as fine grained pinkish colored deposits.

Drawings were then made of both the ARG and HRP reacted brain sections using a microprojector. Sections were then viewed under both

dark and light field for the presence of either silver grains (ARG) or the fine grained HRP reaction product. These deposits were then plotted onto the drawings.

RESULTS

The data presented here will emphasize the autoradiographic results obtained in the primate material. Cat and rat HRP material will only be discussed when significant differences were observed in this material when compared to the primate. These cat and rat experiments were undertaken in order to determine if significant differences were present in PAG connectivity between these commonly used laboratory species. After examining the material obtained in these experiments it does not appear that significant differences in the rostral efferent connections of the PAG occur between the monkey, cat, and rat.

One of the most difficult problems in this study was to determine if the effective spread of the tracers at the injection site was limited to a restricted region. To overcome this problem we made very small injections (average of .025µl of 30% HRP and 7µc leucine), waited a long period after the injection before withdrawing the electrode to minimize the electrode track, used the most sensitive chromagen avaiable to visualize the extent of the HRP spread, and used the same long exposure time for the injection site as for the projection areas.

The injection sites obtained in the primate are shown in figure 16. As can be seen from the diagram a variety of discrete injections were made into the various regions of the primate PAG. Figure 23

shows a representative injection site with 23a having been reacted for the presence of HRP with TMB and 23b an autoradiogram of an adjacent section. As can be seen from these sections the injection sites were well localized and the diffusion of HRP was roughly equal to the spread of the tritiated leucine. Although different regions of the PAG were injected in different animals (Figure 16) nearly all of the projection areas receiving PAG efferent connections were consistently labeled in the different cases. In other words no matter what restricted region of the PAG one injected tracers, a similar set of efferent connections were observed with both the HRP and the autoradiographic techniques employed in this study. Because of this finding we shall present a summary of our results using the representative injection site of SM-2. This injection site was in the dorsolateral aspect of the PAG with a rostral to caudal spread of approximately 2mm. As previously stated no consistent differences were observed between rostral and caudal injection sites.

In the following presentation of results we shall describe the areas in which silver grains were found and in which anterogradely HRP reaction product was observed in adjacent sections. Of the two methods the autoradiographs proved to be superior to the HRP technique because of the lack of contamination by fibers of passage and because the autoradiographs gave a clearer and more sharply defined picture of the projection areas than did the HRP material.

Dorsal Thalamus

The dorsal thalamus was consistently labeled after tritiated amino acid injections into the PAG. This labeling was most prominent

in the ipsilateral thalamus although lesser but consistent labeling was observed in the contralateral thalamus. This labeling was seen in some of the intralaminar and midline thalamic nuclei and also in the nucleus reticularis thalami. The nucleus reticularis thalami contained silver grains after injection into any region of the PAG with more lateral PAG injections giving the most intense labeling of this structure (Figures 24c-e and 25). The silver grains appeared to be distributed in an even fashion throughout the entire length of this cellular mantle which surrounds the dorsal thalamus. It should be emphasized that the zona incerta labeling seemed continuous with the reticularis thalami and at least in the pattern of the efferent projection from the PAG, there two structures appeared be contiguous (Figure 24d-e). The ventrobasal complex amd other sensory relay nuclei were never observed to contain silver grains (Figures 24d, 24e and 25).

The intralaminar nuclei which were consistently labeled included, paracentralis, the centralis lateralis, centralis superior lateralis, and parafasiculus. The fibers which appeared to terminate in the intralaminar regions seemed to arise from the periventricular bundle. These fibers could be observed to be exiting this central bundle laterally to form a wing like structure which bordered on the lateral edge of the nucleus medialis dorsalis (Figures 24d, 24e and 25b). This lateral band of silver grains was present in a continuous sheet over the nucleus paracentralis and nucleus parafascularis and moving more laterally over the nucleus centralis lateralis and finally its rostral most extension in the nucleus centralis superior lateralis. The silver grains over these intralaminar regions appeared to be

present in a contiguous fashion from one intralaminar nucleus to the other.

The midline nuclear groups which had silver grains localized over them were nucleus centralis medialis, centralis inferior, intramedialis dorsalis, and the paraventricular thalami (Figure 24d and 24e). The fibers which sent projections to these midline areas appeared to again arise from this periventricular bundle and were present in a contiguous fashion over these midline nuclei (Figure 26a).

The nucleus medialis dorsalis also had silver grains over part of its nucleus. This projection was only seen after larger lateral injections and when present the silver grains appeared to terminate in patches in a discontinuous fashion over this nucleus (Figures 24d and 24e).

Hypothalamus

The anterograde labeling seen in the hypothalamus was both heavy and consistent after injections in the PAG. This labeling tended to overlap regions in which HRP positive cells were present as seen in adjacent sections that have been described in the previous paper. The degree of receprocity in the hypothalamus was striking. This is to say that whenever HRP positive cells were observed in an area, adjacent sections which had been processed for autoradiography would demonstrate anterograde labeling in the same areas.

Anterior Group

The position of the anterogradely transported tritiated amino acids as demonstrated by the presence of silver grains was in exact register with the location of HRP positive cells as described in the previous paper in this and all regions of the hypothalamus. Briefly the silver grains were found in the most rostral areas of the medial-preoptic hypothalamus and then swung laterally into the lateral preoptic nucleus and even more laterally into the region of the substantia innominata (Figure 24a and 24b). As one moved caudally these silver grains continued in a contiguous fashion and finally merged with the rostral anterior hypothalamic areas. Few if any silver grains were observed in the supraoptic, paraventricular or suprachiasmatic hypothalamic nuclei. Injections into various regions of the PAG did not show any significant variation in the distribution of silver grains seen in the anterior hypothalamic region.

Medial Group

The medial region of the hypothalamus had a moderate to consistent labeling of silver grains which was nearly identical to the distribution of HRP positive cells described in the previous paper. The distribution of silver grains was heaviest in the dorsal nucleus, the periventricular nucleus, and the more lateral zona incerta (Figures 24c, 24d, 24e and 27a). This pattern of silver grains ran in a continuous fashion from the periventricular hypothalami, through the dorsal hypothalamic nucleus to the zona incerta as did the HRP positive cells seen in adjacent sections. What differed however between the areas in which HRP cells were found and the silver grains

was that the silver grains continued to sweep up into the nucleus reticularis thalami while in adjacent sections HRP positive cells were rarely found in the reticularis thalami. This emphasizes the fact that while reciprocity was maintained between the location of HRP positive cells and the distribution of silver grains in the hypothalamus no such reciprocity was present in the dorsal thalamus. Other areas in the medial hypothalamus which contained silver grains were the ventromedial (Figure 24c and 27b) and periarcuate region (Figure 24c). No silver grains were found over the tear shaped arcuate nucleus as defined by Kreig (1932). Other medial hypothalamic regions such as the tuberal or tubermammillary nuclei contained few if any silver grains.

Lateral Region

The lateral region of the hypothalamus contained moderate numbers of silver grains (Figure 24c), and extended to the same lateral regions in which the variety of labeled HRP cells were found as described in the previous paper.

Posterior Region

The posterior region of the hypothalamus contained a heavy concentration of silver grains (Figures 24d and 28). This posterior area seemed to blend imperceptibly with the more caudal periventricular bundle. The labeling was quite heavy with large numbers of labeled fibers coursing through this area. Few if any silver grains were found to overlay the well delineated mammillary nuclei which were immediately ventral to the well labeled posterior

nuclei. This unlabeled mammilary nuclei served as convenient indicators of the level of background labeling (Figure 28).

Zona Incerta

The zona incerta which was described in the previous paper as having large numbers of HRP labeled cells was also heavily overlain with silver grains. This labeling seemed to be present in a contiguous fashion between the more medial dorsal hypothalamic nucleus the zona incerta and the reticularis thalami (Figures 24c, 24d and 27a). The subthalamus which is ventral to the zona incerta was unlabeled except when the deep tegmentum was also involved such as in case SM-5. In these instances the subthalamus and medial globus pallidus were moderately labeled. PAG injections alone however consistently labeled the zona incerta, with few if any silver grains being present in the subthalamus.

Mesencephalic Reticular Formation

This ill defined nuclear group contained moderate to heavy numbers of silver grain (Figures 24e and 24f). This projection was diffuse in nature and did not seem to arise from the periventricular bundle but rather ascended through the deep tectum and tegmentum. The area in this region corresponding to the AIO dopaminergic region was also overlain with silver grains. No significant labeling was observed in the adjacent habenula nuclei or the pretectal region.

DISCUSSION

Before discussing the anatomical and functional implications of the present study it is important to realize the limitations and caution one should take in interpreting these results. The injected tritiated amino acids are known to be taken up by cells, incorporated into proteins, and anterogradely transported to axons and axonal terminals (Cowan et al., 1972). Since axons apparently are incapable of protein synthesis inadvertant labeling of axons of passage is not a problem with this technique. This lack of contamination of axons of passage is not true of the anterograde transport of HRP. Anterograde transport of HRP can apparently occur equally well between damaged axons of passage and cell bodies (Mesulam and Mufson, 1980). Therefore the anterograde transport of HRP, which should label both damaged fibers of passage and the true projections of the PAG, was used for confirmatory purposes only with the major stress on this paper being made on the grounds of the autoradiographic (ARG) analysis.

A note of caution however should be made in attempting to interpret even the anterograde transport of tritiated proteins as revealed by the presence of silver grains. The fast transport component moves at about 100-200mm per day (Cowan et. al., 1972). Since no area of the squirrel monkey brain is more than 200mm away from the PAG and the survival times used in this study were between 2-3 days, all efferent projections made by the PAG neurons had adequate time for terminal labeling using TAAs. The difficulty however is in differentiating between a terminal field of a PAG projection and those silver grains which are in axons which give rise

to the terminals. Because silver grains overlie both axons and terminals, one cannot distinguish between them with light microscopy ARG, although the distribution of grains over fiber bundles and terminal fields can be differentiated.

Therefore by carefully noting in serial sections when projecting fibers terminate in an area, that is they proceed no further, one can judiciously assume, given the rate of transport and the survival time, that one is viewing a terminal projection area.

Previous reports on the PAG's efferent connections have focused almost exclusively on its descending projections. While these descending connections have been hypothesized to exert powerful effects on nociceptive and other sensory input (Basbaum and Fields, 1978), the areas of termination and the possible function of these ascending PAG projections has received scant attention. In light of the numerous ascending connections that have demonstrated been in this study we shall discuss the possible functional significance of these midbrain diencephalic connections.

The nucleus reticularis thalami which receives a heavy projection from the PAG is thought to be the diencephalic extension of the reticular formation of the brainstem (Scheibel and Scheibel, 1966). Most of the neurons comprising this structure have been shown to contain GABA (Houser et. al., 1980) a putative inhibitory neurotransmitter (Roberts and Hammerschlag, 1976). These reticularis thalami neurons are unique in the thalamus for while they receive a cortical input they are one of the only neuronal groups in the thalamus that does not send back a projection to the cortex (Jones, 1975). These reticularis thalami neurons are known to have a high

spontaneous discharge rate (Peschanski et. al., 1980) which is potentiated by morphine administration (Peschanski, personal communication) and reduced upon noxious stimulation (Peschanski et. al., 1980). Previous investigators have also suggested that these reticularis neurons are in some way involved in regulating sensory input in the thalamus, particularly to the ventrobasal complex which recieves spinothalamic inputs. The demonstration of a PAG-reticularis thalami projection establishes a possible anatomical substrate by which stimulation of the PAG could have an effect on regulating sensory input, in this case at the thalamic level.

The intralaminar nuclei, especially the nucleus centralis lateralis are thought to be involved in a poorly localized sensory perception (Albe-Fessard, 1968). The nucleus centralis lateralis is unique in that it is the only intralaminar nucleus which is known to recieve an input from the spinothalamic tract (Mehler et. al., 1960; Boivie, 1979). This overlap of the projection from the PAG and that of the spinothalamic tract is therefore unique for while stimulation of the spinothalamic tract is known to cause pain (Mayer et. al., 1975), stimulation of the PAG is known to cause a multitude of physiological changes including a naloxone reversible analgesia (Adams, 1976). Whether an interaction occurs between the PAG projection to the centralis lateralis and the spinothalamic tract is unknown although a possible anatomical substrate presents itself here by which the PAG could regulate incoming spinothalamic input at the thalamic level.

The midline and intralaminar thalamic nuclei with the exception of centralis lateralis are not currently thought to receive a direct

projection from the spinothalamic tract (Mehler et. al., 1960; Boivie, 1979). Rather they are thought to connect indirectly with the spinothalamic tract via the brain stem reticular formation (Scheibel and Scheibel, 1958). Stimulation of these thalamic nuclei is known to cause a diffuse and extremely noxious pain that is often accompanied with unpleasant emotional overtones (Albe-Fessard, 1968). Interestingly it is this type of unlocalized pain that is most effectively blocked by morphine or PAG stimulation (Hosobuchi et. al., 1977), whereas the more localized pin prick pain, thought to be carried by the lateral spinothalamic tract and terminating in the ventrobasal complex, is relatively unaffected by opiates (Hosobuchi et al., 1977). The projection of the PAG intralaminar and midline thalamic nuclei could provide a further anatomical substrate by which the PAG is able to modulate nociceptive sensory input.

The unique aspect of this dorsal thalamus PAG connection is the lack of reciprocity shown by this connection. The PAG-hypothalamus connections are strikingly reciprocal and in marked contrast to the lack of any afferent projection from the dorsal thalamus to the PAG as shown in the previous paper. While such a dichotomy may be anatomically intriguing the functional significance of this difference remains unknown.

As mentioned above the reciprocity between the connections of the PAG and the hypothalamus is impressive. By processing alternate sections for either HRP histochemistry or autoradiography one can readily and precisely compare adjacent sections for such reciprocity. As discussed in the previous paper the massive hypothalamic inputs to the PAG could be responsible for part of the wide variety of visceral

and emotional changes seen upon stimulation of the periventricular bundle or the PAG. The function of these reciprocal projections back to the hypothalamus is unknown but could serve as direct feedback loops to regulate the output of the hypothalamus.

The zona incerta and the mesencephalic reticular formation have also been shown in this study to be reciprocally connected to the PAG. Unfortunately, however, the functional roles of these structures is unknown and therefore the possible significance of these reciprocal connections with the PAG cannot be addressed.

Previous anatomical studies on the efferent connections of the central grey have suggested that this central grey region can be subdivided into three subnuclei, a medial, a lateral, and a dorsal, each possessing a distinct and nonoverlapping set of connections (Hamilton, 1973, 1974). The present investigation did not find such regional differences in the pattern of efferent connections made by each subnuclei. Rather all regions of the PAG seemed to have the majority of their efferent connections in common. These differences in projection areas may be due to the different techniques utilized, that is the present technique of the autoradiographic localization of TAA's does not involve fibers of passage and does not preferentially stain larger axons. It is also noteworthy that we did not find any projection from the PAG to the ventrobasal complex of the thalamus, the habenula, the pretectum, or the inferior colliculus all of which have been previously described by Hamilton and Skultety (1970) as receiving a projection from the PAG.

As previously noted the central grey region has been implicated in the production of stimulation produced analgesia (SPA) and central

opiate induced (OA) analgesia. However the neuronal pathways involved and the mechanisms of action of both SPA and OA remain relatively obscure. Physiological and pharmacological data indicate that it is doubtful that SPA or OA acts by blocking the transmission of nociceptive information through the PAG, since lesion of this area does not provide any analgesia (Melzack et. al., 1958; Rhodes, 1979). Rather it appears that both SPA and OA are obtained by the activation of central grey modulatory influences over other CNS areas. Several investigators (Liebeskind et. al., 1973; Basbaum et. al., 1976) have proposed both SPA and OA are obtained by descending connections of the PAG which project to brainstem areas which in turn project to the spinal cord to block nociceptive input. While these descending PAG projections seem likely to be responsible for some of the SPA and OA affects as will be discussed in the two following papers, experiments to date have not ruled out the possibility that SPA or OA may be produced partly via the extensive PAG ascending system as shown in this study. Central grey SPA and OA are both most effective in the relief of extremely noxious pain that is often accompanied by strong emotional overtones (Liebeskind and Mayer, 1971). SPA and OA however seem to be much less effective in reducing highly localized pain such as pin prick pain (Hosobuchi et. al., 1977). In fact during human SPA there often is no change in the ability to discern response to this localized pain (Hosobuchi et. al., 1977). Several investigators have suggested that this finely localized pain is conveyed by the portions of the spinothalamic tract which project to the ventrobasal complex (neospinothalamic tract - Walker, 1942; Mehler, 1960; Spiegel and Wycis, 1962) while the diffuse noxious pain whcih is often accompanied

by emotional overtones is conveyed by the medial or paleospinothalamic tract (Mehler, 1974; Spiegel and Wycis, 1962). The paleospinothalamic tract terminates directly upon the nucleus centralis lateralis of the thalamus (Mehler et. al., 1960) and indirectly on other midline and intralaminar thalamic nuclei via the brainstem reticular formation (Bowsher, 1957). The PAG for its part in SPA or OA does not directly project to the termination site of the neospinothalamic tract, the ventrobasal complex. It does however have a moderate to heavy projection to the midline and intralaminar thalamic nuclei which have been hypothesized to receive either directly or indirectly a projection from the paleospinothalamic tract. It is therefore interesting to note that while central grey SPA or OA does not seem to significantly interfere with finely localized nociceptive inputs associated with the ventrobasal complex which it does not directly project to, it is highly efficatious in the relief of the type of nociception associated with the midline or intralaminar nuclei which it does project to. It is somewhat paradoxical however that while stimulation of the PAG can bring about analgesia it can also produce a highly noxious emotional and fearful pain in patients (Nashold et. al., 1969). Thus under different stimulation parameters the PAG seems to be able to elicit either the modulation of pain or under different stimulation parameters the production at the conscious level of an extremely noxious pain. The mechanism by which the PAG is able to produce such dichotomous effects is unknown but as has been shown cytoarchitecturally (Mantyh, 1980) and pharmacologically the PAG is a very heterogeneous structure. Possibly under one set of stimulation parameters an ascending opiate projection from the PAG could be

activated while under a different set of stimulation parameters other PAG neurons with ascending projections utilizing a different neurotransmitter could be activated whose release in these forebrain areas would cause the noxious emotional pain also associated with stimulation of this structure. While the mechanism by which the PAG is able to participate in both analgesia and the production of pain is unknown it should be appreciated that the PAG plays a highly complex and heterogeneous physiological role, even when one examines a single modality of sensation such as that of nociception.

Stimulation of the PAG has also revealed it to be involved in a wide variety of other functions besides the production or modulation of nociception. Changes in gastric function, emotional tone, breathing rate, heart beat, and sexual function have all been shown to take place in humans during central grey stimulation (Nashold et. al., 1969). Just what the common thread is among this wide variety of physiological changes that occur during PAG stimulation is unknown, but all the effects appear to have emotional overtones and/or to be centered on the midline of the body. This observation has been made by clinicians (Nashold et. al., 1969) and experimenters alike and the term "limbic midbrain region" has been used to describe the central grey (Nauta, 1958). The anatomical connections as shown in these two papers presented here support this loose "limbic" generalization by demonstrating the anatomical substrate by which some of the PAG's functions could occur. Thus some of the forebrain areas which the PAG projects preferentially to are the, midline, intralaminar, and reticularis thalami which are mainly "reticular" in nature (Brodal, 1969) or the phylogentically quite ancient hypothalamus, and Mes RF

(Ranson, 1943), that is those regions which are hypothesized to modulate man's more primitive instincts and reflexes.

SUMMARY

In summary the present investigation has stressed four points on the structure and function of the periaqueductal grey.

1) The PAG projects to a variety of rostral structures including: the reticularis thalami, some intralaminar and midline thalamic groups, hypothalamus, zona incerta, and the mesencephalic reticular formation.

2) Of these structures receiving projections from the PAG, the hypothalamus, zona incerta, and mesencephalic reticular formation are reciprocally connected with the PAG. The efferent projection to the dorsal thalamus from the PAG is not reciprocally related as the PAG receives no significant input from the dorsal thalamus.

3) The different regions of the PAG have in common the majority of their efferent connections. This coupled with the previous cytoarchitectural and afferent connection studies of the PAG suggests subdivision schemas based on each subnuclei having its own distinctive cytoarchitecture and connectivity are inappropriate.

4) In view of the diverse forebrain areas to which the PAG projects, hypothesis on how PAG stimulation exerts its antinociceptive and other physiological functions, might be more realistic in their scope if their ascending PAG connections were taken into consideration.

BIBLIOGRAPHY

Albe-Fessard, D., F. Stutinski, and S. Libouban 1966 Atlas sterotaxique du Diencephate du Rat Blanc. CNRS, Paris.

Albe-Fessard, D. 1968 Central nervous mechanisms involved in pain and analgesia. In: Pharmacology of Pain. vol. 9., Proceed. 3rd International Pharm. Meeting 1966, pp 131-168 Permagon Press, Oxford-New York.

Adams, J.E. 1976 Naloxone reversal of analgesia produced by brain stimulation in the human. Pain 2:161-166.

Basbaum, A.I., C.H. Clanton, and H.L. Fields 1976 Opiate and stimulus produced analgesia: functional anatomy of a medullospinal pathway. Proc. Natl. Acad. Sci. USA 73:4685-4688.

Basbaum, A.I. and H.L. Fields 1978 Endogenous pain control mechanism: a review and hypothesis. Annals of Neurology 4:451-462.

Boivie, J. 1979 An anatomical reinvestigation of the termination of the spinothalamic tract in the monkey. J. Comp. Neurol. 186:343-370.

Bowsher, D. 1957 Termination of the central pain pathway in man: the conscious appreciation of pain. 80:606-622.

Cowan, W.M., D.I. Gottlieb, A.E. Hendrickson, J.L. Price, and T.A. Woolsey 1972 The autoradiographic demonstration of axonal connections in the central nervous system. Brain Res., 37:21-51.

Emmers, R. and K. Akert 1963 A stereotaxic atlas of the brain of the Squirrel monkey (Saimiri sciureus). Univ. of Wisconsin Press, Madision.

Gergen, J.A. and P.D. MacLean 1962 A stereotaxic atlas of the Squirrel monkey's brain. Public Health Service, Bethesda, MD.

Hamilton, B.L. and F.M. Skultety 1970 Efferent connections of the periaqueductal grey matter in the cat. J. Comp. Neurol. 139:105-114.

Hamilton, B.C. 1973 Cytoarchitectural subdivisions of the periaqueductal grey matter in the cat. J. Comp. Neurol. 149:1-28.

Hamilton, B.C. 1974 Projections of the nuclei of the periaqueductal grey matter of the cat. J. Comp. Neurol. 152:45-58.

Hosobuchi, Y., J.E. Adams, and R. Linchitz 1977 Pain relief by electrical stimulation of the central grey matter in humans and its reversal by naloxone. 197:183-186.

Houser, C.R., J.E. Vaughn, R.P. Barber, and E. Roberts 1980 GABA neurons are the major cell type of the nucleus reticuarlis thalami. Brain Res. 200:341-354.

Jones, E.G. 1975 Some aspects of the organization of the thalamic reticular complex. J. Comp. Neurol. 162:285-308.

Jurgens, U.D. and R. Pratt 1979 Role of the periaqueductal grey in vocal expression of emotion. Brain Res., 167:367-378.

Kerr, F. and P. Wilson 1978 Pain. In: Annual Review of Neuroscience 1:83-102.

Krieg, W.J.S. 1932 The hypothalamus of the albino rat. J. Comp. Neurol. 55:19-89.

Liesbeskind, J.C. and D.J. Mayer 1971 Somatosensory evoked responses in the mesencephalic central grey matter of the rat. Brain Res. 27:133-151.

Liesbeskind, J.C., G. Guilbaud, J.M. Besson, and J.L. Oliveras 1973 Analgesia from electrical stimulation of the periaqueductal grey matter in the cat: behavioral observations and inhibitory effects on spinal cord interneurons. Brain Res. 50:441-446.

Mantyh, P.W. 1980 Nissl, weil, and golgi analysis of the periaqueductal grey in the rat, cat, and monkey. Neurosci. Abstracts.

Mayer D.J., D.D. Price, and D.P. Becker 1975 Neurophysiological characterization of the anterolateral spinal cord neurons' contribution to pain perception in man. Pain 1:51-58.

Mayer, D.J. and R. Hayes 1975 Stimulation-produced analgesia: development of tolerance and cross-tolerance to morphine. Science 188:941-943.

Mehler, W.R. 1974 Central pain and the spinothalamic tract. Advances in Neurology 4:127-146.

Mehler, W.R., M.E. Feferman, and W.J.M. Nauta 1960 Ascending axon degeneration following anterolateral cordotomy. An experimental study in the monkey. Brain 83:718-751.

Melzack, R., W.A. Stotler, and W.K. Livingston 1958 Effects of discrete brainstem lesions in cats on perception of noxious stimulation. J. Neurophys. 21:353-367.

Mesulam, M.M. and E.J. Mufson 1980 The rapid anterogarde transport of horseradish peroxidase. 5:1277-1286.

Nashold, B.S., W.P. Wilson, and D.G. Slaughter 1969 Sensations evoked by stimulation in the midbrain of man. J. Neurosurg. 30:14-24.

Nauta, W.J.H. (1958) Hippocampal projections and related neural pathways to the midbrain in the cat. Brain. 81:319-340.

Peschanski, M., G. Guilbaud, and M. Gautron 1980 Neuronal responses to cutaneous electrical and noxiuos mechanical stimuli in the nucleus reticularis thalami of the rat. Neurosci. Letters. 20:165-170.

Rhodes, D.L. 1979 Periventricular system lesions in stimulation-produced analgesia. Pain 7:31-51.

Ranson, S.W. 1943 The anatomy of the nervous system. 7th ed. W.B. Saunders Co., Philadelphia-London.

Reynolds, D.V. 1969 Surgery in the rat during electrical analgesia induced by focal brain stimulation. Science 164:444-445.

Roberts, E. and R. Hammerschlag 1976 Amino acid transmitters. In: G.J. Siegel et. al., (eds), Basic Neurochemistry, 2nd ed. Little, Brown, Boston, pp. 218-245.

Schiebel, M.E. and A.B. Scheibel 1958 Structural substrates for integrative patterns in the brain stem reticular core. In: Reticular Formation of the Brain. (Henry Ford Hosp. Symp.), H.H. Jasper et. al., eds.) pp. 31-55 Little Brown and Co., Boston.

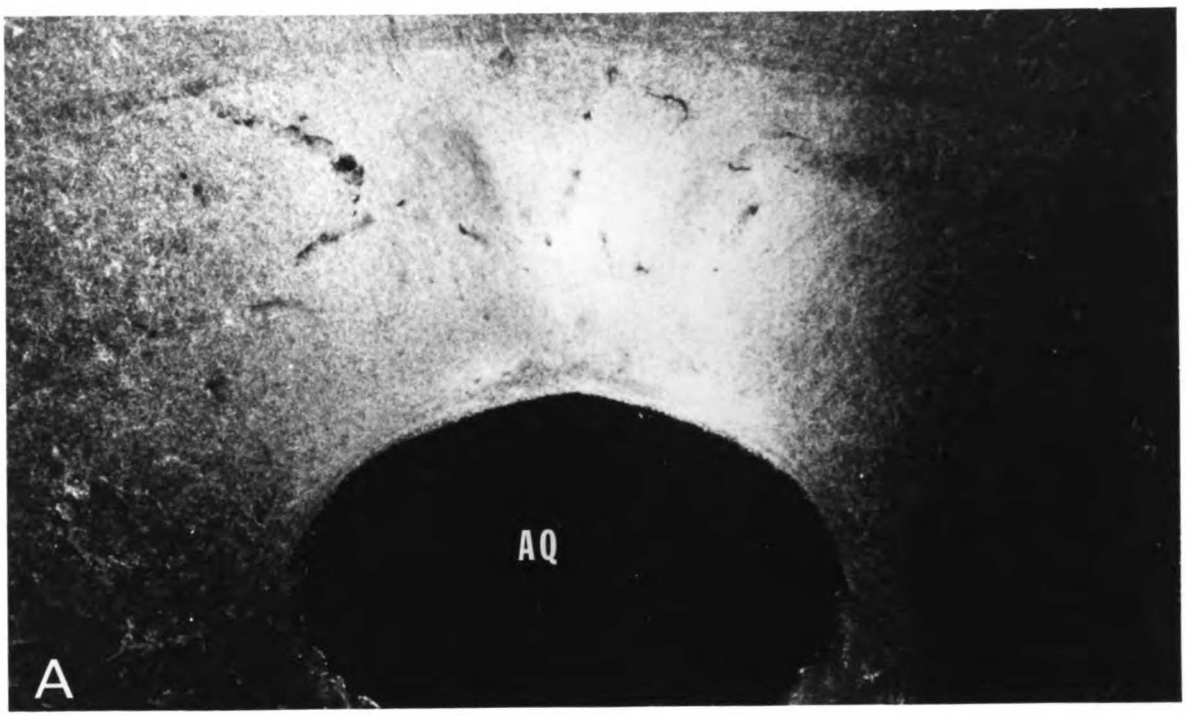
Scheibel, M.E. and A.B. Schiebel 1966 The organization of the nucleus reticularis thalami: a golgi study. Brain Res. 1:43-62.

Spiegel, E.A. and H.T. Wycis 1962 Stereoencephalotomy. Pt. II Clinical and physiological applications. New York: Grune and Stratton.

Walker, A.E. 1942 Somotopic localization of the spinothalamic and secondary trigeminal tracts in the mesencephalon. Archs. Neurol. Psychiol. 48:884-889, Chicago.

Figure 23. A. A darkfield photomicrograph of a TMB reacted section to show the extend of the HRP spread at the injection site in a typical injection; saimiri sciureus, (experiment SM-8).

B. A darkfield photomicrograph of a nearby section to show the extent of the spread of the tritiated amino acids in a typical injection saimiri sciureus, (experiment SM-8).


AQ
A

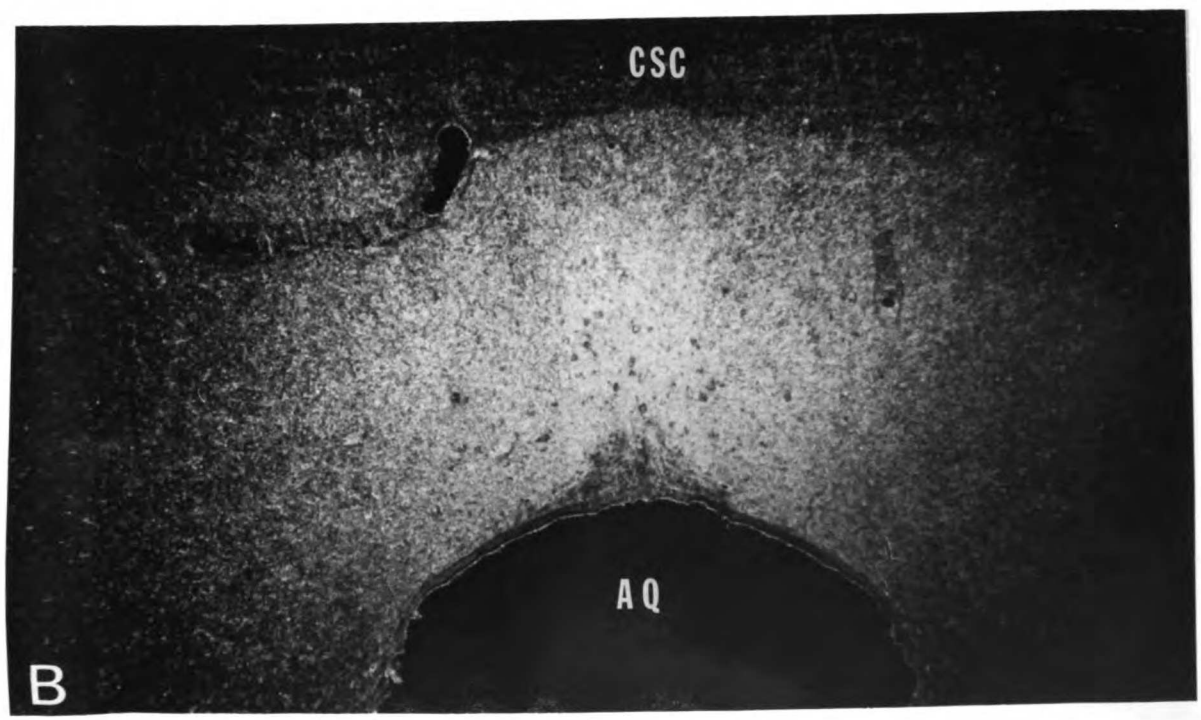

CSC
AQ
B

Figure 24. A series of tracings illustrating the forebrain projections labeled following an injection involving the entire right PAG. In this and similar diagrams of autoradiographic sections to follow, the injection site is shown in stippling and the labeled projections and terminal projection fields by dots. All figures are arranged from rostral to caudal as if the brain were cut in coronal section; saimiri sciureus, (experiment SM-2).

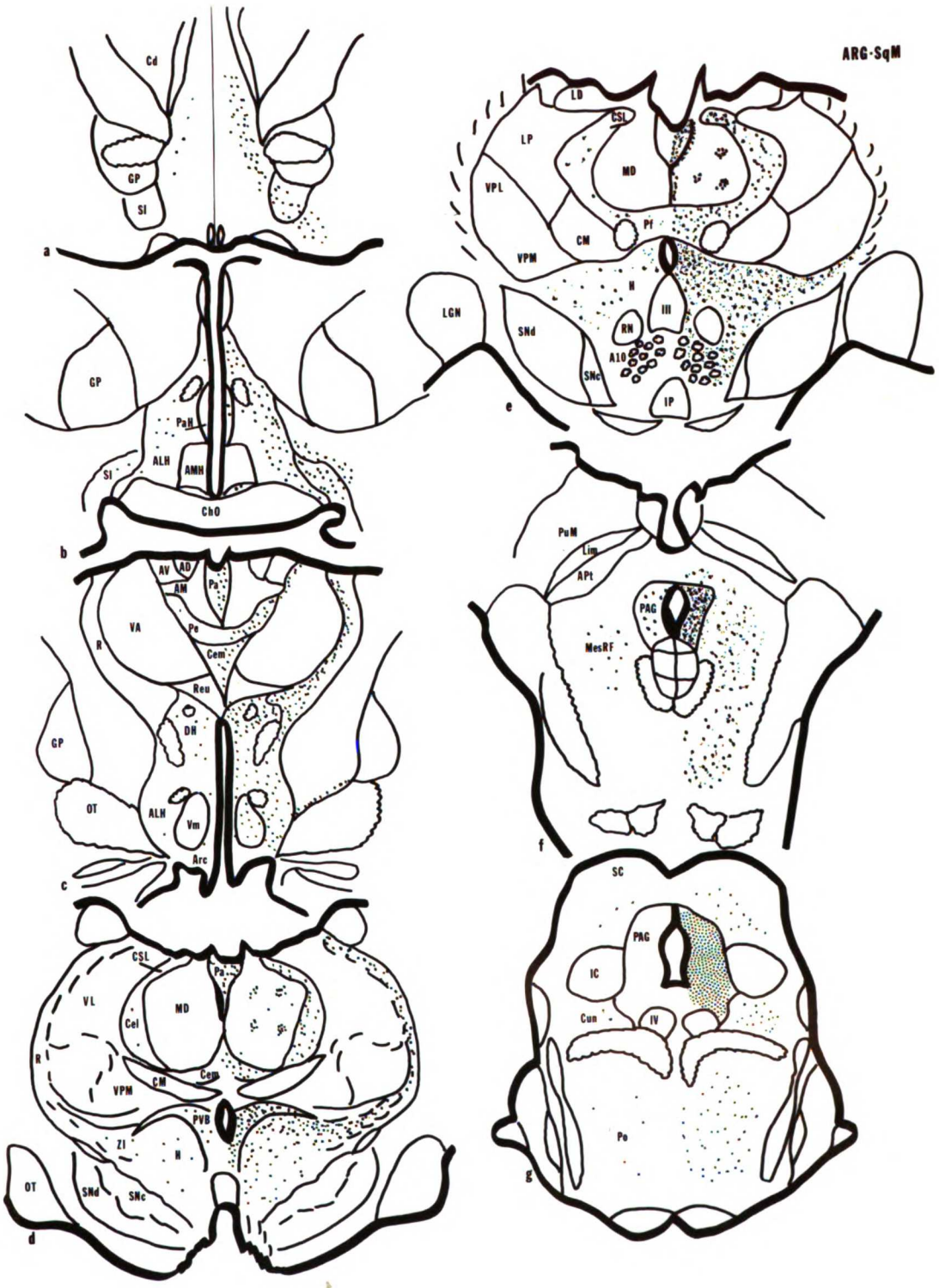
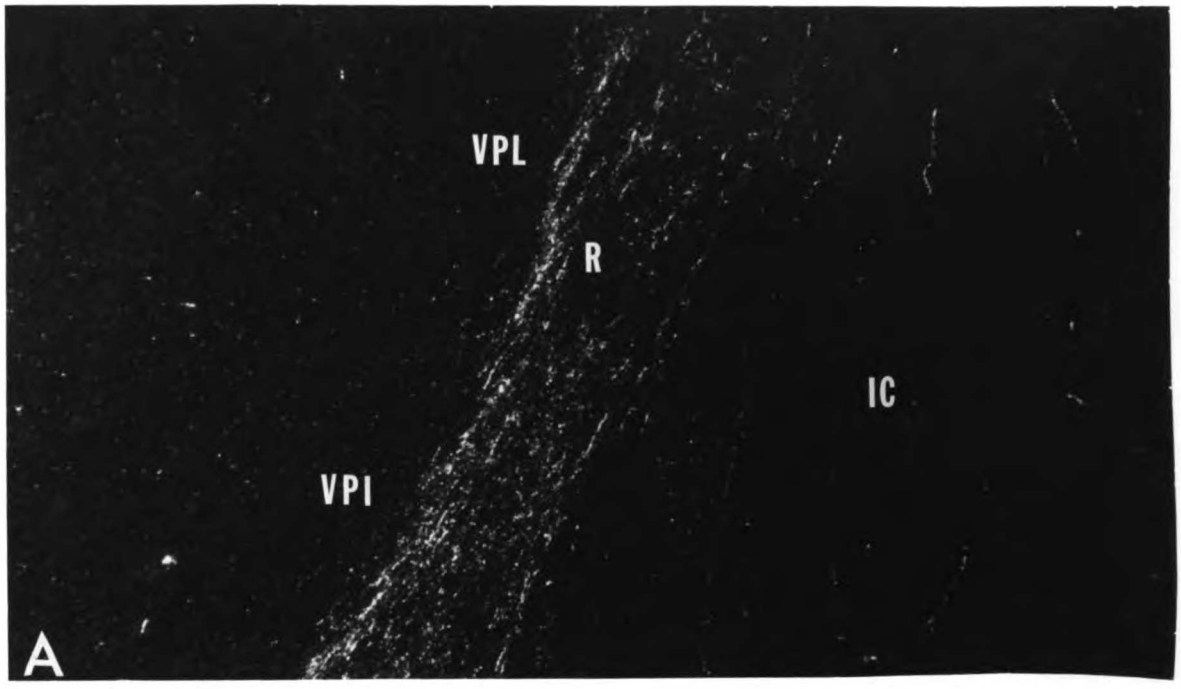

ARG·SqM
Cd
GP
SI
a
GP
PaH
ALH
AMH
SI
ChO
b
AV
AD
AM
Pa
VA
R
Pe
Cem
Reu
DH
GP
OT
ALH
Vm
Arc
c
CSL
Pa
VL
MD
Cel
R
CM
Cen
VPM
PVP
ZI
H
OT
SNd
SNc
d
LD
CSL
LP
MD
VPL
CM
Pf
VPM
H
III
LGN
SNd
RN
A10
SNc
IP
e
PuM
Lim
APt
PAG
MesRF
f
SC
PAG
IC
Cun
IV
Po
g

Figure 25. A. A darkfield photomicrograph to show the distribution of silver grains over the ipsilateral nucleus reticularis thalami as seen in a coronal section following an injection of the ventrolateral PAG. In this and the other darkfield photomicrographs of the autordiographs, the silver grains appear as bright dots. Note the relatively sparse background labeling over the ventral posterior lateral nucleus (VPL) compared to the high density of silver grains over the nucleus reticularis thalami (R); saimiri sciureus, (experiment SM-7).

B. Photomicrograph of the above Nissl-stained autoradiograph as seen in lightfield. Note the area which contain relatively sparse labeling in the photomicrograph (A) corresponds to the ventral posterior lateral nucleus (VPL) the ventral posterior inferior nucleus (VPI) and the internal capsule (IC); saimiri sciureus, (experiment SM-7).


VPL
R
IC
VPI
A

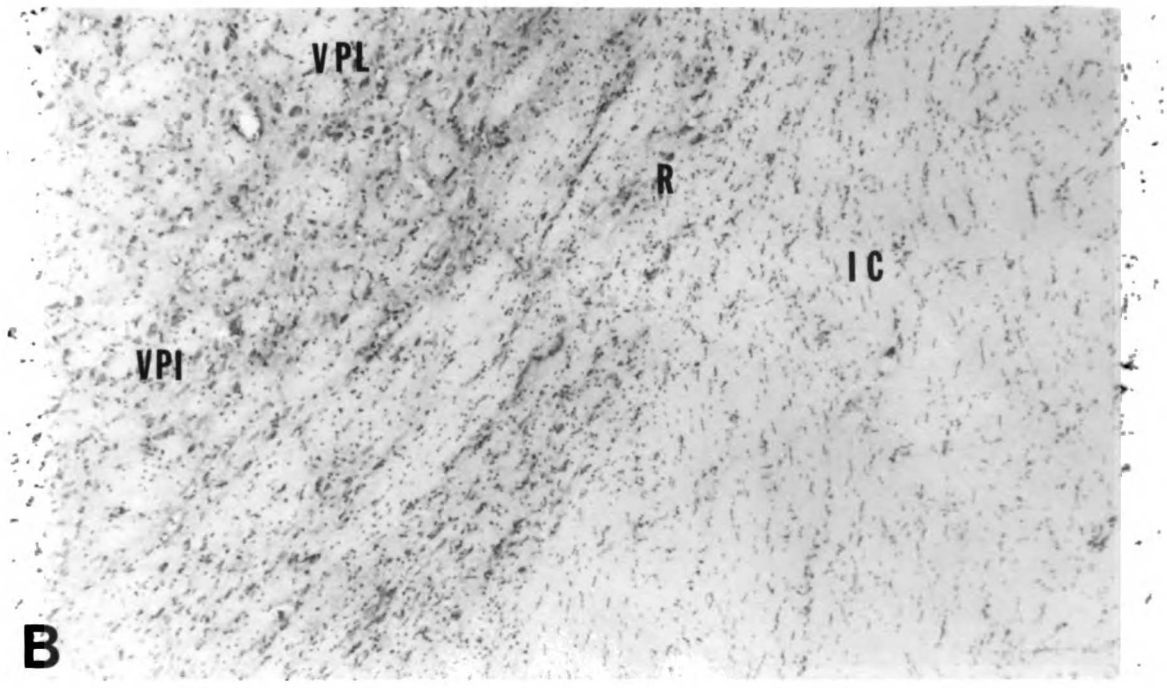
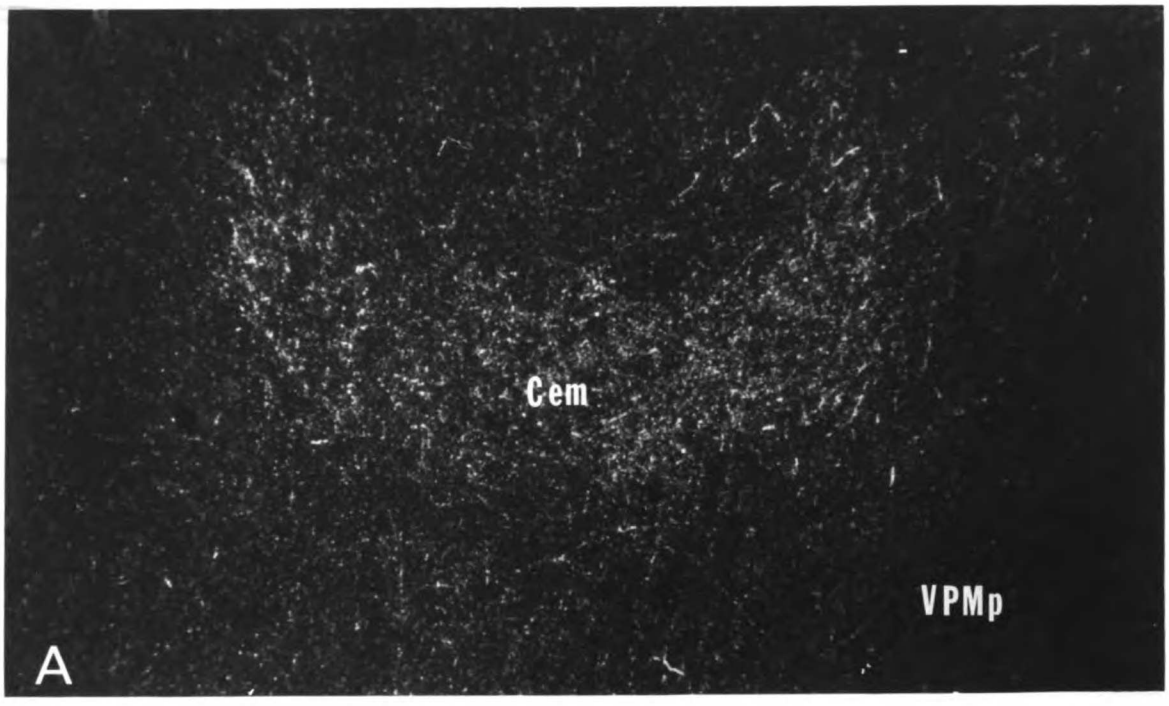

VPL
R
IC
VPI
B

Figure 26. A. A darkfield photomicrograph showing the midline thalamic nucleus centralis medialis overlain with silver grains after an injection into the ventrolateral PAG; saimiri sciureus, (experiment SM-7).

B. A darkfield photomicrograph of the nucleus paracentralis which is one of the thalamic intralaminar nuclei overlain with silver grains after an injection into the dorsolateral PAG; saimiri sciureus, (experiment SM-11).


Cem
VPMp
A

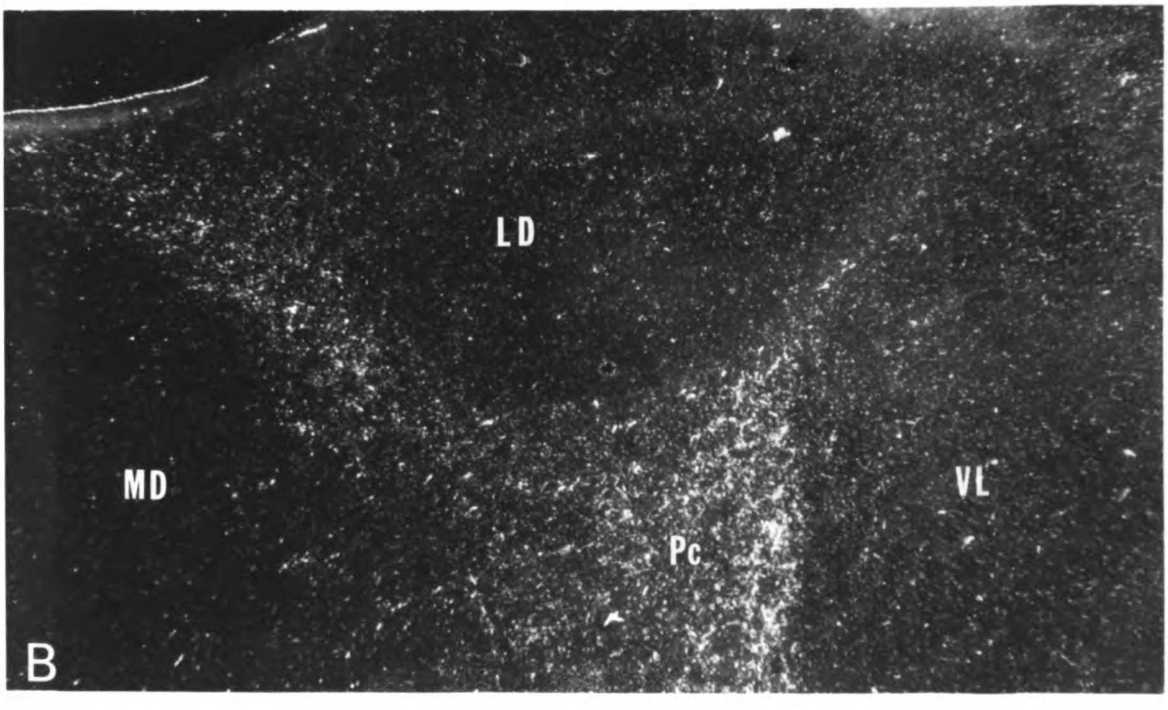

LD
MD
VL
Pc
B

Figure 27. A. A darkfield photomicrograph of a coronal section to show the dorsal hypothalamus (D-) and the adjacent zona incerta (ZI) heavily overlain with silver grains after an injection into the ventrolateral PAG; saimiri sciureus, (experiment SM-7).

B. A darkfield photomicrograph of a coronal section to show the periventricular and ventromedial hypothalamic nuclei heavily overlain with silver grains after an injection involving most of the PAG; saimiri sciureus, (experiment SM-2).

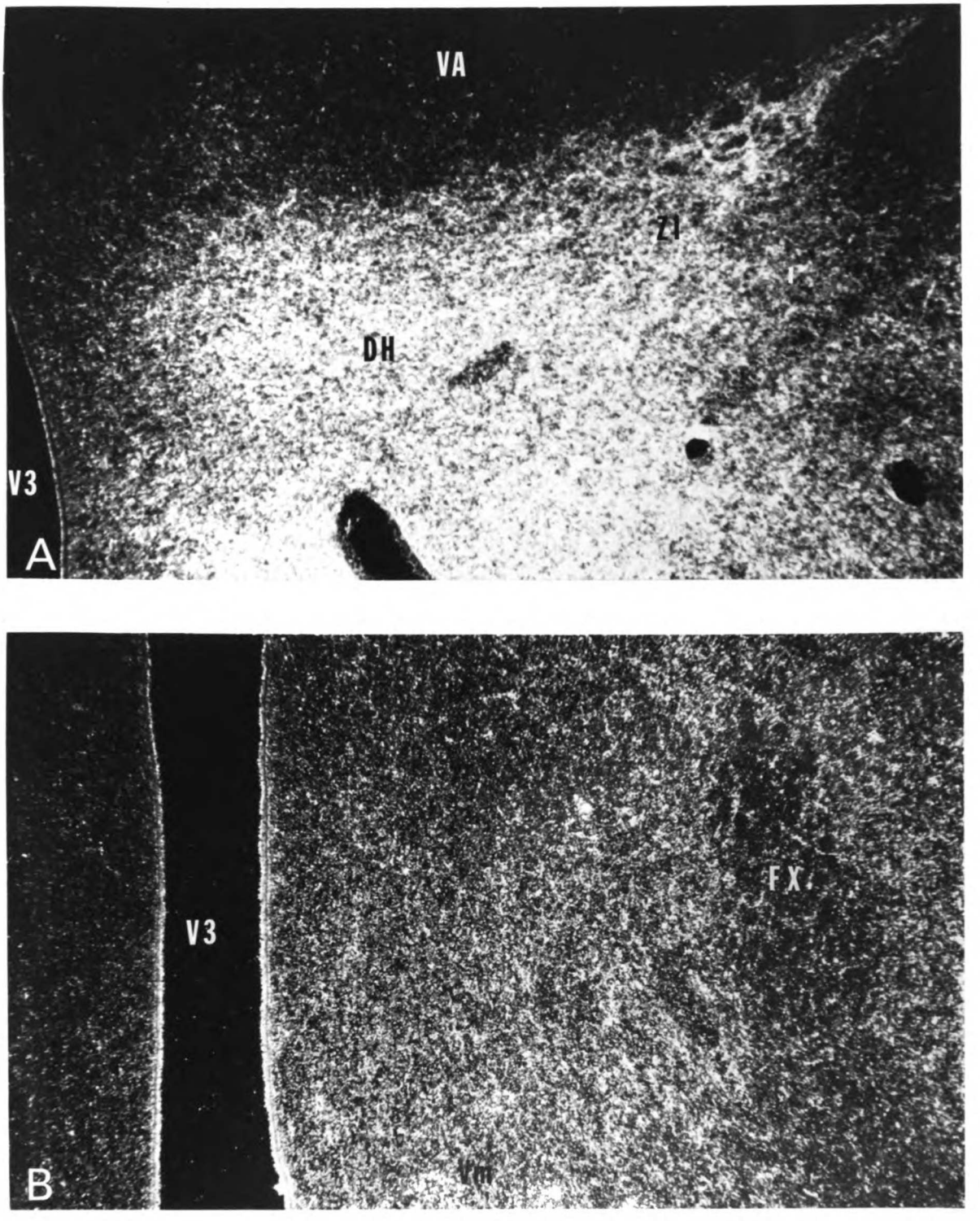
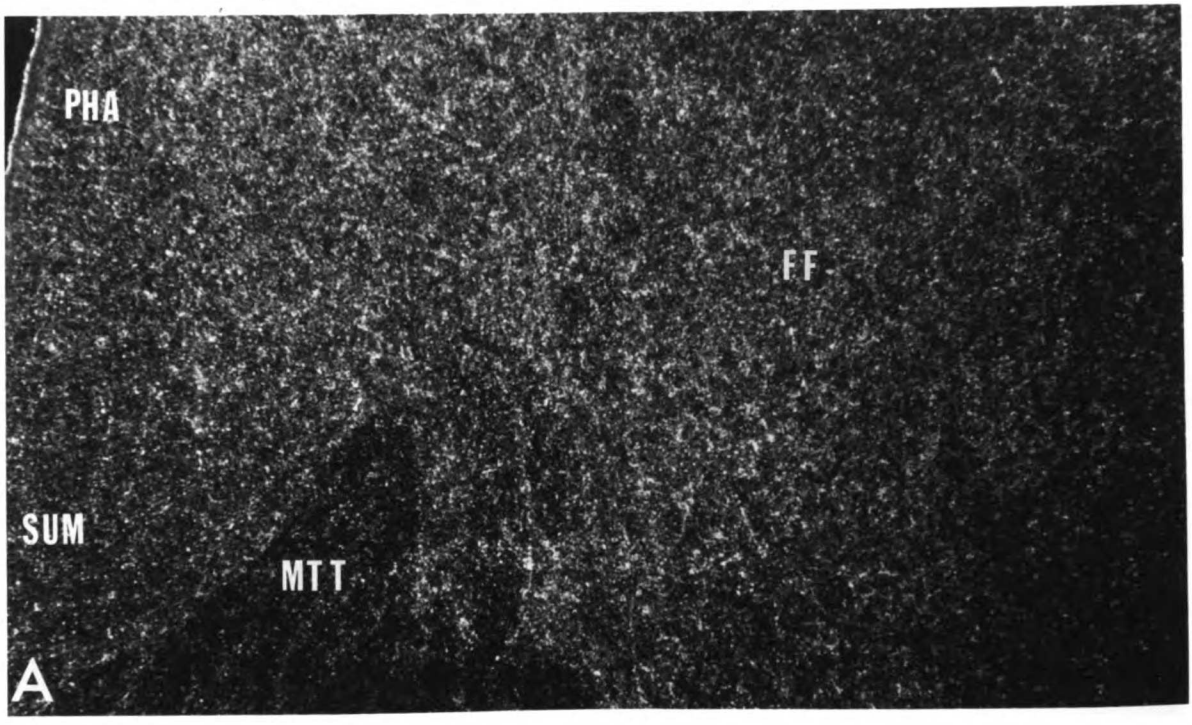
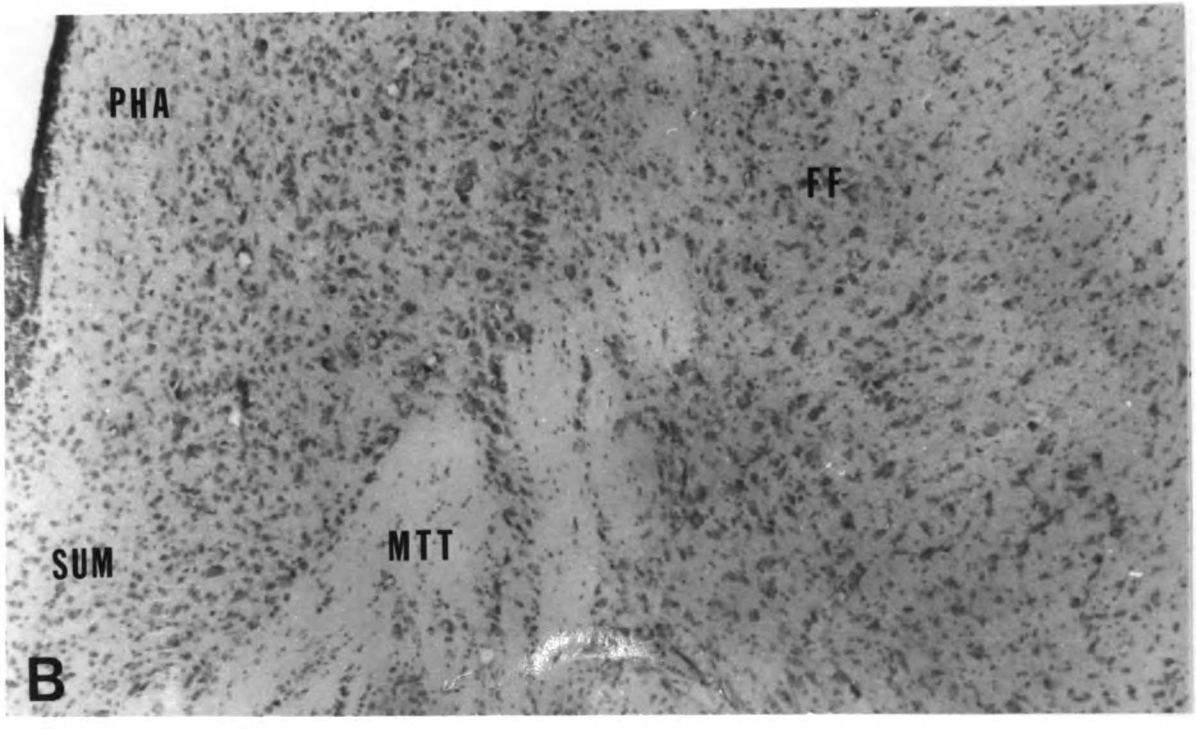

VA
ZI
DH
V3
A
V3
FX
B

Figure 28. A. A darkfield photomicrograph to demonstrate the diffuse labeling in the posterior hypothalamic area (PHA) and the Field of Forel (FF) after an injection in the dorsolateral PAG; saimiri sciureus, (experiment SM-11).

B. A lightfield photomicrograph of the above Nissl-stained autoradiograph to show the cellular boundaries of the above section; saimiri sciureus, (experiment SM-11).


PHA
FF
SUM
MTT
A


PHA
FF
SUM
MTT
B

CHAPTER 4: THE ASCENDING AFFERENT INPUT TO THE PERIAQUEDUCTAL GREY OF THE PRIMATE: THE CONNECTIONS OF A PAIN MODULATORY CENTER

INTRODUCTION

In the previous papers we examined the cytoarchitecture and the afferent and efferent forebrain connections of the periaqueductal grey (PAG). The PAG studies demonstrated that the PAG is a convergent point for a wide variety of structures involved in limbic and visceral activities. In turn our autoradiographic studies demonstrated that the PAG projected in a reciprocal fashion to all the hypothalamic areas from which it received a projection from. In addition, the central grey projected to midline and intralaminar groups in the dorsal thalamus in a non-reciprocal fashion. Thus in the previous papers we have demonstrated that the PAG is directly connected with a variety of forebrain structures which are generally thought to be involved in nociceptive and visceral functions.

A major question concerns the anatomical substrate by which the PAG communicates with the brainstem and spinal cord. Stimulation of the PAG is hypothesized to activate brainstem structures which in turn descend to the level of the dorsal horn where they exert an anti-nociceptive action (Basbaum and Fields, 1978). The anatomical substrates by which the PAG accomplishes its descending activation will be dealt with in the next paper. In the present paper we will explore which brainstem and spinal neurons directly project to the PAG in order to influence central grey function. Previous anatomical and physiological studies have described afferent inputs into the PAG from the spinal cord (Mehler, 1960), the superior colliculus (Graham, 1977), the locus coeruleus and the reticular formation (Bowsher, 1976). However there has been no global evaluation of some potential inputs from the brainstem and spinal cord into the PAG, particularly

from those areas that the PAG is known to modulate. We therefore have examined the ascending afferent inputs into the PAG using the horseradish peroxidase (HRP) technique in the primate. From these studies we hope to derive a better understanding of how the PAG modulates visceral and/or nociceptive input, particularly as it relates to chronic pain in humans, and we therefore chose to investigate these anatomical substrates of visceral and pain modulation in sub-human primates.

On the basis of the afferent input into the PAG, this central grey region can be described as a nociceptive integrator, for the PAG receives input from structures involved in both the transmission and modulation of nociceptive input. It should be emphasized however that while we shall stress the relevance of these connections to nociception, many of these afferent inputs are also probably involved in modulating the PAG in regard to the variety of visceral functions it is known to be involved in.

MATERIALS AND METHODS

The materials and methods used in this aspect of this investigation are the same as those used and outlined in Chapter 2 of this study.

RESULTS

The data presented here will emphasize the results obtained in the primate. Rat and cat material will only be discussed when significant differences were observed between this material and the primate material. The rat and cat experiments were undertaken to

determine if significant differences occur in PAG connectivity among the commonly used laboratory species. After examining the histological material obtained in this study it can be stated that few significant differences occur in the projections from the brainstem to the PAG among monkey, cat, and rat.

The placement of the horseradish peroxidase (HRP) injection sites in the primates used in this study are shown in figure 16. As can be seen in figure 16 a variety of discrete injection sites were placed in the various regions of the primate PAG. Although different regions of the PAG were injected in different animals and even different species the great majority of the areas containing HRP positive cells were consistently labeled. In other words, no matter where in the PAG one injected HRP, the majority of connections observed remained constant. Because of this finding we shall present a summary of our results obtained in this study using a representative injection site obtained in case SM-2. This injection was in the dorsolateral area of the PAG with a rostral to caudal spread of approximately 2.0mm. No consistent differences were noted between rostral and caudal injection sites.

In the following presentation of results we shall describe the brainstem and spinal regions in which HRP positive cells were consistently found, beginning with the rostral most areas of the brainstem and moving caudal to the spinal cord.

Superior Colliculus

After an HRP injection into any region of the PAG, HRP positive cell bodies were found in the stratum intermedium and profundum of the superior colliculus (Figure 29a and 29b). The labeled cells were

variable in size (15-25μm) but tended to have a fusiform or pyramidal shaped cell body. These labeled cells were located predominantly ipsilateral to the injection but a small but consistent number of deep tectal cells were found contralateral. Although these labeled deep tectal cells blended imperceptibly with the adjacent tegmental cells few if any labeled cells were observed in the superficial layers of the superior colliculus.

Central Tegmental Field

The central tegmental field consistently contained a moderate number of well labeled cells (Figure 29a). These labeled cells were approximately 15-25μm in diameter and ranged from stellate to pyramidal in shape. As noted above these labeled tegmental neurons seemed quite similar in shape, size, numbers, and distribution to the dorsally adjacent deep tectal neurons. As with the labeled deep tectal neurons most labeled tegmental cells were located ipsilaterally with a consistent few being present contralateral to the injection site.

Periaqueductal Grey

After injections into the PAG ipsilateral PAG cells were present both rostral and caudal to the injection site (Figure 29a and 29b). These labeled neurons included nearly all the types seen in Golgi material (Mantyh, 1980) and were not distributed in any identifiable subgroups. Interestingly, relatively few contralateral PAG cells were labeled, when compared with the same section in which moderate numbers of labeled contralateral tectal and tegmental cells could be observed.

Also of note was the lack of any significant number of labeled dorsal raphe neurons after PAG injections.

Cuneiformis

The ipsilateral nucleus cuneiformis contained moderate numbers of well labeled cells after HRP injection into any region of the PAG (Figure 29a). The borders of this nuclear zone could be clearly delineated in the cat whereas in the rat and monkey its borders are more difficult to define. The presence of HRP labeled cells in the cuneiformis was in marked contrast to the total lack of labeling in the caudally adjacent inferior colliculus (Figure 29a). As with the deep tectal and tegmental groups a few but consistent number of well labeled neurons were observed in the contralateral cuneiformis. A few but consistent number of labeled cells were also observed in both the ipsilateral and contralateral subcuneiformis.

Locus Coeruleus

The locus coeruleus contained the largest concentration of labeled cells per square micron of any region of the brain after injection of HRP into the central grey. The HRP filled cells were intensely labeled and were approximately 15-18μm in diameter. These labeled cells were stellate in shape and formed a tight cluster immediately ventral to the PAG and immediately lateral to Gudden's dorsal nucleus (Figure 29c). While most of the labeled coeruleus neurons were present ipsilaterally a few were observed contralaterally. Compared to the more dorsal locus coeruleus relatively few subcoeruleus neurons were labeled (Figure 29c).

Reticularis Pontis Oralis and Caudalis

This diffuse reticular group which runs in a continuous fashion throughout the pons as defined by Meesen and Olszewski (1949) and Taber (1961) consistently contained a scattering of labeled cells. No pattern or distinct nuclear clustering of the labeled cells was observed among the labeled pontine reticular neurons (Figure 29a and 29b). These labeled cells were quite similar in size, shape, and spatial distribution to the labeled mesencephalic reticular formation neurons of which this is its caudal extension.

Raphe Groups

In describing the labeling seen in the raphe groups after HRP injections into the PAG we shall use the nomenclature as defined by Taber et. al., (1960) in the cat. Although analogous midline raphe groups exist in the monkey and rat the precise delimitation of these raphe groups is more difficult in these species due to the differences in the cephalic flexure which gives a different plane of section to the brainstem when cut in coronal stereotaxic plane. Therefore while this presentation of results will again stress the primate material, all observations on raphe groups have been confirmed in the cat.

The heaviest projection to the PAG from the midline raphe groups comes from the raphe magnus and raphe pallidus (Figure 29b-e). In the nucleus raphe magnus (NRM) moderate numbers of well filled neurons were observed after injection into any region of the PAG. These labeled cells were large fusiform cells (Figure 30b) which did not remain solely on the midline but rather swept laterally from the midline to blend in with the adjacent nucleus reticularis

magnocellularis (Rmc) (Figure 30a). The more caudal midline raphe group, the nucleus raphe pallidus (NRP) also contained moderate numbers of well labeled cells. These labeled NRP cells however were slightly smaller than the labeled NRM cells and tended to remain closer to the midline than the NRM cells which merged with the lateral Rmc cells. Other raphe groups such as the raphe obscuris, centralis superior, and the dorsal raphe contained few if any labeled neurons.

Medullary Reticular Formation

Scattered labeled cells were observed in the medullary reticular formation in a similar pattern as observed in the mesencephalic and pontine reticular formation. A few scattered cells were observed in the reticularis gigantocellularis and both subdivisions of the nucleus medulla oblongatae centralis as defined by (Taber, 1961). These labeled cells were variable in size (15-25μm) and shape. One reticular area where large numbers of labeled cells were consistently observed was the nucleus paragigantocellularis lateralis (Taber, 1961) and the nucleus reticularis magnocellularis (Rmc) (Figure 29c). As previously mentioned these labeled Rmc cells appeared to be part of a band of cells which extended from the midline raphe magnus and extended laterally to include the Rmc. These labeled Rmc neurons were fusiform in shape. In all the reticular groups the majority of labeled cells were ipsilateral to the injection but a consistent few were present contralaterally.

Spinal cord

In the spinal cord only the rat and monkey material was analyzed in detail. In the primate labeled neurons could be found at all spinal levels (Figure 31). The majority of these labeled cells were present in the deeper lamina which corresponds to Rexed's lamina V-VIII (Rexed, 1951). The labeled spinal neurons were quite large (15-80μm) and were usually heavily labeled (Figure 32). No significant difference was noted in the pattern of distribution, size or numbers of labeled neurons in any spinal level. The majority of labeled cells were present contralateral to the injection (70%) but a significant percentage of the labeled cells (30%) were present ipsilateral to the injection (Figure 31).

DISCUSSION

The periaqueductal grey (PAG) is involved in a wide variety of physiological functions most of which might be termined "limbic" in nature. These functions include rage ractions (Hunsperger, 1956), oxytocin release (Aulsebrook and Holland, 1969), gastric motility (Skultety, 1963), vocalization during stress (Jurgens and Pratt), and the processing (Nashold et. al., 1969) and modulation (Reynolds, 1969) of nociceptive input into the central nervous system. This last function, that of modulating pain, has elicited the most interest in this heterogeneous structure. It is known that the PAG brings about this analgesia by way of its extrinsic connections (Rhodes, 1979). Therefore the purpose of this study is to explore which spinal and brainstem areas directly influence and modulate this annular midbrain structure, in an attempt to understand the anatomical substate by which these PAG functions are brought about.

Before discussing the possible morphological and functional implications of the afferent PAG connections revealed here it is important to briefly state the limitations and care one needs to take in interpreting such a study. HRP is known to be taken up by both axon terminals and damaged axons of passage (LaVail, 1975). The dorsal bundle of Schutz (Schutz, 1891), otherwise known as the periventricular bundle (Ranson, 1943), courses through the PAG. Neurons with fibers in this bundle, which pass through but do not synapse within the PAG, could have these axons of passage cut by the injection needle and be retrogradely labeled with HRP. These labeled neurons might therefore be erroneously thought to terminate in the PAG when in fact they pass through it without synapsing. This possible source of error has been minimized by using a small injection needle, by using a variety of approach angles, and by noting the consistency of a projection in the different cases. However even with these precautions the inadvertant labeling of fibers of passage can not be completely eliminated. Therefore previous anterograde studies which might prove or disprove the projections observed here will be cited and discussed whenever possible. It should be emphasized however that while the HRP technique might lead to false positives as far as true axonal terminations within the PAG, it does accurately reflect those afferent structures which would be affected if a stimulating electrode were placed in the same brain area.

Superior Colliculus

The superior colliculus, which has been shown in this study to project to the PAG, is a midbrain structure subdivisable into 3-7

laminae (Edwards, 1980) depending on the species of animal and the investigator. The most dorsal laminae (superficial and opticum) do not project to the PAG. These laminae have been hypothesized to be involved exclusively in the processing of visual inputs. The deeper lamina (intermedium and profundum) do project to the PAG. These laminae, unlike the superficial laminae, receive input from structures involved in a wide variety of sensory modalities (Grafova et. al., 1978; Baleydier and Mauquire, 1978; Blomquist et. al., 1978; Edwards et. al., 1979). The neural areas which project to these deep tectal laminae include the inferior colliculus, the retina, and the dorsal column nuclei which are involved in transmitting auditory, visual, and somatosensory information respectively. These multisensory inputs are organized in a tonotopic, retinotopic, and somatotopic manner with each modality occupying a particular laminae in the deep layers of the superior colliculus (Gordon, 1973; Drager and Hubel, 1975; Stein et. al., 1975).

Previous anatomical studies have suggested that these deep tectal lamina project to the central grey. Using anterograde autoradiographic techniques Graham (1977) has shown a projection to the PAG from the intermediate and profundum laminae of the superior colliculus (S.C.). Golgi studies (Kolliker, 1891; Laemle, 1979; Mantyh, 1980) have also suggested that the intermediate and profundum laminae send processes into the central grey region and vice versa. Thus the present study is in good correlation with previous anatomical studies which suggested that only the intermediate and deep laminae send a projection to the PAG.

In attempting to ascribe a functional significance to this projection from the superior colliculus to the central grey, it is noteworthy to examine some of the similarities and differences of these adjacent structures. The S.C. and the PAG have in common some of their afferent inputs including the frontal granular cortex (Goldman and Nauta, 1976), the zona incerta, the locus coeruleus, and the midbrain tegmentum (Edwards et. al., 1979). The deep S.C. also shares with the PAG a similar cytoarchitecture as revealed by the Golgi stain (Mantyh, 1980). Both areas have a marked heterogenicity of different sized neurons, a primary dendritic arborization in the transverse plane, and a dense meshwork of overlapping dendritic fields. In fact the lateral border of the PAG is often difficult to visualize in Golgi stained material because these two regions look so alike and intermingle so freely.

It is important to realize however that while the S.C. and PAG have some similarities they also have several noteworthy differences. Firstly and most marked is the fact that the S.C. is organized into laminae with well defined topography whereas only a very crude topography has been suggested to be present in any part of the PAG (Liebeskind and Mayer, 1971). This difference in the degree of topographical organization is also reflected in the different structures which project to each. Whereas the S.C. receives projections from areas known to have a precise topography such as the dorsal column nuclei, the retina and the inferior colliculus (Edwards, 1980), the PAG receives projections from brain structures such as the zone incerta (Boivie, 1979), reticular formation (Bowsher, 1978) and raphe nuclei (Watkins et. al., 1980) which are hypothesized to have only a loose topographical organization.

A further difference between the PAG and the S.C. is evident in the massive hypothalamic projection received by the PAG whereas the S.C. receives little if any direct hypothalamic input. This differential hypothalamic projection has been repeatedly found using both autoradiographic (Conrad and Pfall, 1976; Saper et. al., 1979) and HRP techniques.

To understand the possible functional relationship of the S.C. and the PAG it is important to review the role of tectum in submammalian species. In such species the tectum plays an important role in mediating fairly complex reflexes which require a coordination between sensory input and a motor output. The superior colliculus then serves as a crude neocortex with interactions occuring between several topological maps each representing a modality of sensation or motor function. The PAG for its part remains primarily an interface between the hypothalamus and the brainstem. In this role it serves primarily a visceral and central endocrine function. The relationship between the PAG and the superior colliculus then is possibly an interface between the sensory and motor input the brain receives and the vegetative or state of internal homeostasis. How and if the PAG performs such a function is not known but it is of interest that the S.C. receives no direct hypothalamic inputs while the PAG receives a massive hypothalamic input and the S.C. is the only known region of the brain possessing a discrete topographical organization which projects to the PAG. Since the PAG receives a significant projection from regions concerned with both internal homeostasis (hypothalamus) and the external environment (superior colliculus) it is in a unique position to serve as a focal site for the integration of these two sensory worlds.

Cuneiformis

The nucleus cuneiformis is a rectangular shaped reticular region of the midbrain-pons which sends a projection to the central grey. This projection has been reported by previous authors using autoradiographic techniques (Edwards, 1975). The function of the cuneiformis area or the projection to the PAG is unknown.

Reticularis pontis oralis and pontis caudalis

These two reticular regions are the caudal extensions of the mesencephalic reticular formation and the nucleus cuneiformis. That these pontine reticular regions project to the PAG has been previously suggested in an autoradiographic study (Graybeil, 1977). While this reticular projection to the PAG was moderate to light in the present study, it was a typical reticular projection, composed by scattered cells of heterogeneous size and shape which are not grouped in any identifiable pattern.

Locus coeruleus

The locus coeruleus an adrenergic cell group (Dahlstrom and Fuxe, 1965) sends a moderate to heavy projection to the PAG. After injection of HRP into the PAG this group is very well labeled whereas the more ventral subcoeruleus contains few retrogradely labeled cells. Noradrenaline has been reported to be present in the PAG (Versteeg et. al., 1976) and these labeled locus neurons could be the origin of this neurotransmitter in the PAG. Felten et. al., (1974) however has described a unique group of noradrenergic cell bodies within the PAG of the squirrel monkey which are not seen in the rat brain. Therefore

whether these locus cells do indeed provide the adrenergic input in the PAG is uncertain. It should be emphasized that these locus cells are probably not labeled due to lesioning the dorsal adrenergic bundle, for the injection sites were always dorsal and medial to the described location of this tract (Felton et. al., 1974). Further support for a locus projection to the PAG comes from an autoradiographic tracing study on the locus which also demonstrated a projection from locus to the PAG (Jones and Moore, 1977). The function of this locus projection to the central grey is unknown although a descending adrenergic projection to the spinal cord is known to exert an antinociceptive effect at the dorsal horn level (Reddy and Yaksh, 1980). Since stimulation of the PAG can be analgesic, and since the locus receives a projection from the PAG, this reciprocal projection could provide a feedback loop back to the PAG to modulate a possible central grey antinociceptive output via the locus coeruleus.

Raphe nuclei

Of the raphe nuclei as defined by Taber et. al. (1960) only the nucleus raphe magnus (NRM) and nucleus raphe pallidus (NRP) send a significant projection to the PAG. These raphe groups are known to project to the spinal cord where upon stimulation they can exert antinociceptive effects at the level of the dorsal horn (Fields et. al., 1977; Basbaum et. al., 1978; Fields and Andersen, 1979). Unlike the locus neurons which are known to contain mainly noradrenalin, these raphe magnus and pallidus neurons are known to contain a variety of putative neurotransmitters. These include enkephalin, substance P,

and serotonin (Hokfelt et. al., 1980), all of which are found in both the NRM neurons and the PAG. The significance of this raphe projection to the PAG is unknown although it has been hypothesized that some of SPA produced by PAG stimulation is mediated through the serotonergic raphe magnus and pallidus (Basbaum and Fields, 1979) and this projection back to the central grey might provide a feedback loop for regulating the descending inhibition. Few if any labeled cells were observed in the nucleus raphe centralis, dorsalis, pontis, or obscuris after HRP injections into the PAG.

Medullary reticular formation

The medullary reticular formation has been divided into a number of fairly discrete units by Meesen and Olszewski (1949). These medullary nuclei, the nucleus reticularis magnocellularis (Rmc) and the paragigantocellularis lateralis (pgl), send a moderate projection to the central grey. This region is known to project to the spinal cord (Basbaum and Fields, 1979) where it is hypothesized to play a role in stimulation produced analgesia (SPA) similar to NRM. It is noteworthy that these labeled medullary reticular neurons are part of a continuous band of labeled cells stretching from the NRM through the Rmc to end laterally in the pgl. A few cells were also found in the reticularis gigantocellularis (Rgc) itself but many fewer labeled cells were present in this area compared to the more ventral NRM, Rmc, and pgl.

Spinal Cord

That spinal neurons project to the PAG has been previously demonstrated using silver degeneration techniques (Mehler et. al., 1960; Boivie, 1979). Their experiments suggested that only the dorsal peripheral edge of the central grey received spinal inputs. However this dorsolateral rim of the PAG is also the only area of the PAG which is relatively myelinated as revealed by the Weil technique (Mantyh, 1980). Since silver degeneration techniques preferentially stain large axons which are often those that are myelinated, the suggestion that only the dorsolateral part of the PAG receives spinal input is probably due to a staining bias. That this is in fact so is suggested by the observation in the present experiments that injections of HRP into any region of the PAG reveals labeled neurons in the spinal cord. For example in case SM-8 when HRP was injected in the dorsal-most area of the PAG, which is not shown to receive spinal input using silver degeneration techniques (Mehler, 1974; Boivie, 1979) one still observes well labeled spinal cord neurons. Recently it has been suggested that the placebo response is mediated by the frontal cortex projection to the PAG inhibiting the spinal cord input to the PAG (Hardy and Leichnetz, 1980). In support of this hypothesis the authors felt that the frontal cortex projection into the PAG overlapped exactly with the spinal terminations, and that this anatomical overlap intimated a functional interaction, resulting in pain modulation. That this is not the case is suggested by two observations. First if the frontal cortex projection did indeed inhibit the input into the PAG from the spinal cord neurons, ablation of the frontal cortex should result in decreased pain thresholds or a

hyperalgesia. In fact the opposite occurs. Ablation of the frontal cortex results in the reduction in a patient's chronic pain (Foltz and White, 1962; Freeman and Watts, 1946) especially the affective aspects of the pain. Second the frontal cortex projection to the PAG and the spinal cord terminations in the PAG, do not exactly overlap. Injections of HRP into the ventromedial aspect of the PAG result in labeled spinal cord neurons in the cord but few labeled frontal cortex neurons. Such labeling of one set of neurons without concomitant labeling of the other suggests that these two projections do not exactly overlap, thus removing the one anatomical observation upon which the authors based their hypothesis.

It is noteworthy in the present investigation that the spinal neurons which were labeled after HRP injections into the PAG were located primarily in lamina V-VIII. Spinothalamic tract (STT) neurons located in these lamina are known to have wide receptive fields (Willis et. al., 1975), to have multimodal inputs, and to project to both brainstem and thalamic regions (Rustioni and Hanes, 1980). More dorsally located STT neurons are more apt to have narrow receptive fields (Willis et. al., 1975), to have only nociceptive inputs and to project primarily to the thalamus (Rustioni and Hayes, 1980). Previous investigators have reported mostly lamina I cells being labeled after HRP injections into the PAG (Trevino, 1976; Willis et. al., 1979). In contrast, the present investigation has found that the deeper lamina V-VII are the predominant location in which labeled neurons are found in the spinal cord after HRP injections into the PAG. The probable reason for this discrepency between this and previous investigations is that the injections made by previous

investigators were larger and more laterally located than those used in this investigation, and thus possibly included the ascending spinothalamic tract itself. If the STT itself was involved one would expect the results previously reported, that is an extremely large number of spinal neurons labeled, and a predominance of the lamina I cells, which are projecting up to the ventrobasal thalamus. It is therefore likely that HRP injections confined to the PAG alone predominately label spinal neurons in the deeper lamina. This observation is in agreement with the results obtained by others (Giesler et. al., 1979; Willis et. al., 1979; Abols and Basbaum, 1979) that HRP injections into medial regions of the brainstem and thalamus predominately label spinal neurons in deeper lamina while more lateral injections label predominately the more dorsally located spinal neurons in the spinal cord (Giesler et. al., 1979; Willis et. al., 1979). This medial to lateral dichotomy in the brainstem and thalamus supports the loose generalization of a medial paleospinothalamic tract and a lateral neospinothalamic tract (Mehler, 1969). The paleospinothalamic tract would be predominately composed of fibers from STT neurons located in the deeper laminae (V-VIII) while the neospinothalamic tract would arise chiefly from neurons located in the ventral lamina (I, II, and V).

OVERVIEW

Nociception

In the preceeding discussion we have reviewed the possible morphological and functional significance of the brainstem and spinal projections to the central grey. An important aspect of this study,

in the context of nociception, is the convergence of input into the PAG from both structures which transmit and those that modulate nociceptive input. Such a convergence is unique in the CNS and suggests that the PAG receives a direct feedback to modulate its function from those structures which it in turn modulates. In addition to its modulatory actions, the afferent inputs revealed in this study also suggest that the PAG may be involved in the transmission of pain. Stimulation of the PAG is known to cause analgesia at low currents (Hosobuchi et. al., 1977) and to cause an extremely noxious "fearful" pain with emotional and visceral overtones at higher currents (Nashold et. al., 1969). While such a dichotomy of action is at first somewhat surprising it is less so when viewed in the light of its afferent inputs, for the PAG appears to possess the anatomical substrate necessary for both functions.

Visceral functions

Although this discussion has emphasized the involvement of these anatomical connections in nociception functions there is good evidence that these afferent inputs into the PAG are also concerned with visceral functions. Two of these functions, the lordosis reflex in females and vocalization of emotion are known to be mediated in part via the central grey. The lordosis reflex, a dorsiflexion of the vertebral column, is elicited in sexually receptive female rodents by somatosensory stimuli (Pfaff et. al., 1977). Results from stimulation and lesion experiments (Sakuma and Pfaff, 1979a, 1979b) suggest that the PAG may help to integrate somatosensory and motor components of a reflex loop for lordosis. It has been hypothesized that projections

to the central grey by branches of the paleospinothalamic system (Zemlan et. al., 1978) would provide any somatosensory information involved. In the present study we have identified a significant input from spinal cord neurons which are probably part of this paleospinothalamic system (Mehler, 1969). Therefore it appears that these labeled spinal neurons identified here might also be responsible for providing sensory input into the PAG for this necessary reproductive function.

The involvement of the periaqueductal grey in vocalization has been experimentally obtained via stimulation in monkeys (Jurgens and Muller-Preuss, 1977), rats (Waldbilig, 1975), birds (Brown, 1972), frogs (Schmidt, 1966) and fish (Dewski and Gerald, 1974). It has been hypothesized that the function of the PAG is to couple the diverse motivational states to the appropriate vocal expressions (Jurgens and Pratt, 1979). It appears that the external and internal stimuli which normally give rise to vocalization must activate this central grey area in order to initiate this type of behavior. In the present study a wide variety of anatomical substates have been demonstrated which could activate the PAG to produce this vocalization. Structures which project to the PAG which might activate the PAG to produce vocalization include the frontal cortex, the superior colliculus, spinal neurons, and the reticular formation. Whether these structures actually are those that activate the PAG to produce vocalization must await physiological confirmation.

SUMMARY

(1) The PAG receives a wide variety of afferent projections from caudal areas of the brain and spinal cord. These include the deep layers of the superior colliculus, the nucleus cuneiformis, the locus coeruleus, raphe magnus and pallidus, medullary reticular formation, and the spinal cord.

(2) The spinal neurons projecting to the PAG were predominately located in the deeper laminae (V-VIII). Some of these ventrally located spinal neurons are known to conduct nociceptive information. Thus a possible anatomical substate exists by which pain itself could turn on the PAG's antinociceptive output.

(3) Nearly all the proposed subdivisions of the PAG receive a common set of ascending afferent projections. This finding does not support the hypothesis that each subdivision has a distinct cytoarchitecture and a distinct set of nonoverlapping connections.

(4) The PAG receives afferent projections from brainstem and spinal cord areas known to be involved in the modulation and conduction of nociceptive input, and in the regulation of visceral functions. These connections support the hypothesis that the periaqueductal grey is a visceral, nociceptive, and cognitive integrator.

BIBLIOGRAPHY

Abols, A.I. and A.I. Basbaum 1979 Afferent input to the medullary reticular formation and nucleus raphe magnus of the cat. An HRP study. Anat. Rec., 193:467.

Aulsebrook, L.H. and R.C. Holland 1969 Central inhibition of oxytocin release. Am. J. Physiol., 216:830-840.

Baleydier, C. and F. Mauquiere 1978 Projections of the ascending somesthetic pathways to the cat superior colliculus visualized by the horseradish peroxidase technique. Exp. Brain Res., 31:43-50.

Basbaum, A.I., C.H. Clanton, and H.L. Fields 1978 Three bulbospinal pathways from the rostral medulla of the cat: An autoradiographic study of pain modulating systems. J. Comp. Neurol., 178:209-224.

Basbaum, A.I. and H.L. Fields 1978 Endogenous pain control mechanisms: Review and hypothesis. Ann. Neurol., 4:451-462.

Basbaum, A.I. and H.J. Ralston III 1978 Projections from nucleus raphe magnus in the primate. Pain Abstr. 1:259.

Basbaum, A.I. and H.L. Fields 1979 The orogin of descending pathways in the dorsolateral funiculus of the spinal cord of the cat and rat: Further studies on the anatomy of pain modulation. J. Comp. neurol., 187:513-532.

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

Boivie, J. 1979 An anatomical reinvestigation of the termination of the spinothalamic tract in the monkey. J. Comp. Neurol., 186:343-370.

Bowsher, D. 1976 Role of the reticular formation in responses to noxious stimulation. Pain, 2:361-378.

Brodal, A. and G.F. Rossi 1955 Ascending fibers in brain stem reticular formation of cat. Arch. Neurol. Psychiat., (Chic.) 74:68-87.

Brown, J.L. 1972 An exploratory study of vocalization areas in the brain of the Redwinged Blackbird (Agelaius phoeniceus). Behavior, 39:91-127.

Conrad, L.C.A. and D.W. Pfaff 1976 Efferents from medial basal forebrain and hypothalamus in the rat. II: An autoradiographic study of the anterior hypothalamus. J. Comp. Neurol., 169:221-262.

Conrad, L.C., C.M. Leonard, and D.W. Pfaff 1974 Connections of the median and dorsal raphe nuclei in the rat: An autoradiographic and degeneration study. J. Comp. Neurol., 156:179-206.

Dahlstrom, A. and K. Fuxe 1965 Evidence for the existence of monoamine neurons in the central nervous system. Acta. Physiol. Scand., (Suppl. 247) 64:1-30.

Demski, L.S. and J.W. Gerald 1974 Sound production and other behavioral effects of midbrain stimulation in freeswimming toadfish, Opsanus beta. Brain, Behav. Evol., 9:41-59.

Drager, V.C. and Hubel, D.H. 1975 Responses to visual stimulation and relationship between visual, auditory, and somatosensory inputs in the mouse superior colliculus. J. Neurophysiol., 38:690-713.

Edwards, S.B. 1975 Autoradiographic studies of the projections of the midbrain reticular formation: Descending projections of nucleus cuneiformis. J. Comp. Neurol., 161:341-358.

Edwards, S.B., C.L. Ginsburgh, C.K. Henkel, and B.E. Stein 1979 Sources of subcortical projections to the superior colliculus in the cat. J. Comp. Neurol., 184:309-330.

Edwards, S.B. 1980 The deep cell layers of the superior colliculus: Their reticular characteristics and structural organization. In: The reticular formation revisted. ed. J.A. Hobson and M.A.B. Brazier, Raven Press, New York.

Felten, D.L., A.M. Laties, and M.B. Carpenter 1974 Monoamine containing cell bodies in the squirrel monkey brain. Am. J. Anat., 139:153-166.

Fields, H.L. and S.D. Anderson 1979 Evidence that raphe-spinal neurons mediate opiate and midbrain stimulation-produced analgesia. Pain, 5:333-350.

Fields, H.L., A.I. Basbaum, C.H. Clanton, and S.D. Anderson 1977 Nucleus raphe magnus inhibition of spinal cord dorsal horn neurons. Brain Res., 126:441-454.

Foltz, E.L. and L.E. White Jr. 1962 Pain "relief" by frontal cingulumotomy. J. Neurosurg. 19:89-100.

Freeman, W. and J.W. Watts 1946 Pain of organic disease relieved by prefrontal lobotomy. Proc. Roy. Soc. Med., 39:445-447.

Giesler, G.J. Jr., D. Menetrey, and A.I. Basbaum 1979 Differential origins of spinothalamic tract projections to medial and lateral thalamus in the rat. J. Comp. Neurol., 184:107-126.

Goldman, P.S. and W.J.H. Nauta 1976 Autoradiographic demonstration of a projection from prefrontal association cortex to the superior colliculus. Brain Res., 116:145-149.

Gordon, B. 1973 Receptive fields in deep layer of cat superior colliculus. J. Neurophysiol., 36:157-178.

Graham, J. 1977 An autoradiographic study of the efferent connections of the superior colliculus in the cat. J. Comp. Neurol., 173:629-654.

Graybiel, A.M. 1977 Direct and indirect preoculomotor pathways of the brainstem: An autoradiographic study of hte pontine reticular formation in the cat. J. Comp. Neurol., 175:37-78.

Grotova, I., Ottersen, O.P., and E. Rinvik 1978 Mesencephalic and diencephalic afferents to the superior colliculus and periaqueductal grey substance demonstrated by retrograde axonal transport of horseradish peroxidase in the cat. Brain Res., 146:205-220.

Hamilton, B.L. 1973 Cytoarchitectural subdivisions of the periaqueductal grey matter in the cat. J. Comp. Neurol., 149:1-28.

Hardy, S.G.P. and G.R. Leichnetz 1980 Cortical projection to the periaqueductal grey in monkeys: A potential route for influencing nociception. Neurosci. Abstr., 5:

Hokfelt, T.O., O. Johansson, A. Ljungdahl, J.M. Lundberg, and M. Schultzberg 1980 Peptidergic neurons. Nature, 284:515-521.

Hopkins, D.A. and L.W. Niessen 1976 Substantia nigra projections to the reticular formation, superior colliculus, and central grey in the rat, cat, and monkey. Neuroscience Letters, 2:253-259.

Hosobuchi, Y., J.E. Adams, and R. Linchitz 1977 Pain relief by electrical stimulation of the central grey matter in humans and its reversal by naloxone. Science, 197:183-186.

Hunsperger, R.W. 1956 Role of substantia gresea centralis mesencephali in electrically induced rage reactins. In: Progress in Neurobiology. Vol. I. J. Ariens Kappers, ed. Elsevier, New York pp. 289-294.

Jones, B.E. and R.Y. Moore 1977 Ascending projections of the locus coeruleus in the rat. Brain Res., 127:23-53.

Jurgens, U. and P. Muller-Preuss 1977 Cerebral representation of vocalization in the squirrel monkey. Exp. Brain Res., 10:532-554.

Jurgens, U. and R. Pratt 1979 Role of the periaqueductal grey in vocal expression of emotion. Brain Res., 167:367-378.

Kolliker, A. 1896 Handbuck der qewebelehre des Menschen, Verlag von Wilhelm Engelmann, Leipzig, p. 335.

Laemle, L.K. 1979 Neuronal populations of the human periaqueductal grey, nucleus lateralis. 186:93-108.

LaVail, J.H. 1975 Retrograde cell degeneration and retrograde transport techniques. In: The use of axonal transport for studies of neuronal connectivity. eds. Cowan, W.M. and Cuenod, M. pp. 217-248 Elsevier, Amsterdam.

Liebeskind, J.C. and D.J. Mayer 1971 Somatosensory evoked responses in the mesencephalic central grey matter of the rat. Brain Res., 27:133-151.

Mantyh, P.W. 1980 Nissl, weil, and Golgi analysis of the periqueductal grey in the rat, cat, and monkey. Neuroscience Abstr., 7

Meesen, H. and J. Olszewski 1949 A cytoarchitectonic atlas of the rhombencephalon of the rabbit. S. Karger, Basel.

Mehler, W.R., M.E. Feferman, and W.J.H. Nauta 1960 Ascending axon degeneration following anterolateral cordotomy. An experimental study in the monkey. Brain, 83:718-751.

Mehler, W.R. 1969 Some neurological species differences - a posteriori. Ann. N.Y. Acad. Sci., 167:424-468.

Mehler, W.R. 1974 Central pain and the spinothalamic tract. Adv. in Neurol., 4:127-146.

Nashold, B.S. Jr., W.P. Wilson, and D.G. Slaughter 1969 Sensations evoked by stimulation in the midbrain in man. J. Neurosurg., 30:14-24.

Pert, A. and M. Walter 1976 Comparison between reversal of morphine and electrical stimulation induced analgesia in the rat mesencephalon. Life Sci., 19:1023-1032.

Pierce, E.T., W.E. Foote, and J.A. Hobson 1976 The efferent connection of the nucleus raphe dorsalis. Brain Res., 197:137-144.

Pfaff, D.W., M.O. Montgomery, and C. Lewis 1977 Somatosensory determinants of lordosis in female rats: Behavioral definition of the estrogen effect. J. Comp. Physiol. Psychol., 91:134-145.

Ranson, S.W. 1943 The anatomy of the nervous system. 7th ed. W.B. Saunders Co., Philadephia-London.

Reddy, S.V.R. and T.L. Yaksh 1980 Spinal noradrenergic terminal system mediates antinociception. Brain Res., 189:391-401.

Rexed, B. 1952 The cytoarchitectonic atlas of the spinal cord in the cat. J. Comp. Neurol., 96:415-495.

Reynolds, D.V. 1969 Surgery in the rat during electrical analgesia induced by focal brain stimulation. Science, 164:444-445.

Rhodes, D.L. 1979 Periventricular system lesions in stimulation produced analgesia.

Rustioni, A. and Hayes, N.L. 1980 Spinothalamic and spinomedullary neurons in macques: A single and double retrograde tracer study. 5:861-874.

Sakai, K., M. Touret, D. Salvert, L. Leger, and M. Jouvet 1977 Afferent projections to the cat locus coeruleus as visualized by the horseradish peroxidase technique. Brain Res., 119:21-41.

Saper, C.B., L.W. Swanson, and W.M. Cowan 1979 Some efferent connections of the rostral hypothalamus in the squirrel monkey (samiri sciureus) and cat. J. Comp. Neurol., 184:205-242.

Schmidt, R.S. 1966 Central mechanisms of frog calling. Behavior, 26:251-285.

Schutz, H. 1891 Anatomische Untersuchunge ȳber den faserverlauf im Zentralen Hohlengrau und den Nervenfaser schu und indemselken bei der progressiven paralyse der Irren. Arch. Psychiat., 22:527-587.

Skultety, F.M. 1959 Relation of periaqueductal grey matter to stomach and bladder motility. Neurol., 9:190-197.

Sukuma, Y. and D.W. Pfaff 1979 Facilitation of female reproductive behavior from mesencephalic central grey in the rat. Am. J. Physiol., 237:R278-R284.

Sakuma, Y. and D.W. Pfaff 1979b Mesencephalic mechanisms for integration of female reproductive behavior in the rat. Am. J. Physiol., 237:R285-R290.

Taber, E., A. Brodal, and F. Walberg 1960 The raphe nuclei of the brainstem in the cat. I. Normal topography and cytoarchitecture and general discussion. J. Comp. Neurol., 114:161-188.

Taber, E. 1961 The cytoarchitecture of the brainstem of the cat. I. Brain stem nuclei of the cat. J. Comp. Neurol., 116:27-70.

Trevino, D.L. 1976 The origin and projections of a spinal nociceptive and thermoreceptive pathway. In: Sensory functions of the skin in primates, with special reference to man. Y. Zotterman ed. Pergamon Press, New York. pp. 367-376.

Versteeg, D., J. Vander Gugten, W. de Jung, and M. Palkovits 1976 Regional concentrations of noradrenaline and dopamine in rat brain. Brain Res., 113:563-574.

Waldbilig, R.J. 1975 Attach, eating, drinking, and gnawing elicited by electrical stimulation of rat mesencephalon and pons. J. Comp. Physiol. Psychol., 89:200-212.

Watkins, L.R., G. Griffin, G.R. Leichnetz, and D.J. Mayer 1980 The somatotopic organization of the nucleus raphe magnus and surrounding brainstem as revealed by HRP slow-release gels. Brain Res., 181:1-16.

Willis, W.D., R.A. Maunz, R.D. Foreman, and J.D. Coulter 1975 Static and dynamic responses of spinothalamic tract neurons to mechanical stimuli. J. Neurophysiol., 38:587-600.

Willis, W.D., D.R. Kenshalo, and R.B. Leonard 1979 The cells of origin of the primate spinothalamic tract. J. Comp. Neurol., 188:543-574.

Zemlan, F.P., C.M. Leonard, L.M. Kow, and D.W. Pfaff 1978 Ascending tracts of the lateral columns of the rat spinal cord using the silver impregnation and horseradish peroxidase techniques. Exp. Neuro., 62:298-334.

Figure 29. A series of tracings illustrating the location of retrogradely labeled HRP neurons following an injection involving most of the PAG; saimiri sciuresu, (experiment SM-2).

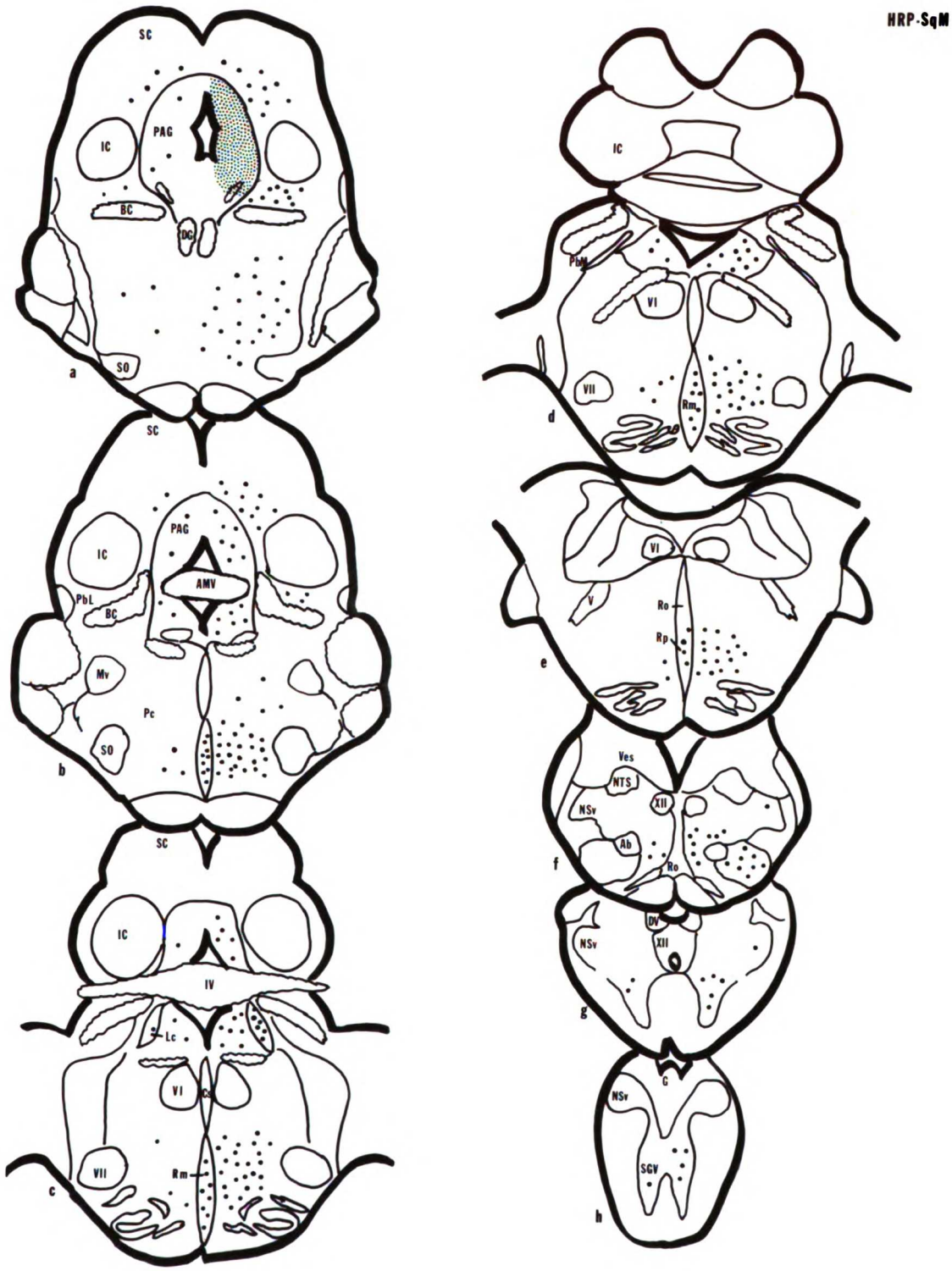

HRP-SqM
SC
PAG
IC
BC
DG
SO
a
SC
PAG
IC
AMV
PbL
BC
Mv
Pc
SO
b
SC
IC
IV
Lc
VI
VII
Rm
c
IC
PbM
VI
VII
Rm
d
VI
V
Ro
Rp
e
Ves
NTS
XII
NSv
Ab
Ro
f
DV
NSv
XII
g
NSv
SGV
h

Figure 30. A. A photomicrograph of the rostral nucleus raphe magnus (NRM) to show the density and type of retrograde HRP labeling seen after an injection into the ventrolateral PAG; saimiri sciureus, (experiment SM-12). Scale = 100µmm.

B. A photomicrograph of rostral NRM neurons to show the characteristic fusiform shape of the retrogradely labeled NRM neurons after an injection into the ventrolateral PAG; saimiri sciureus, (experiment SM-12). Scale = 100µm.

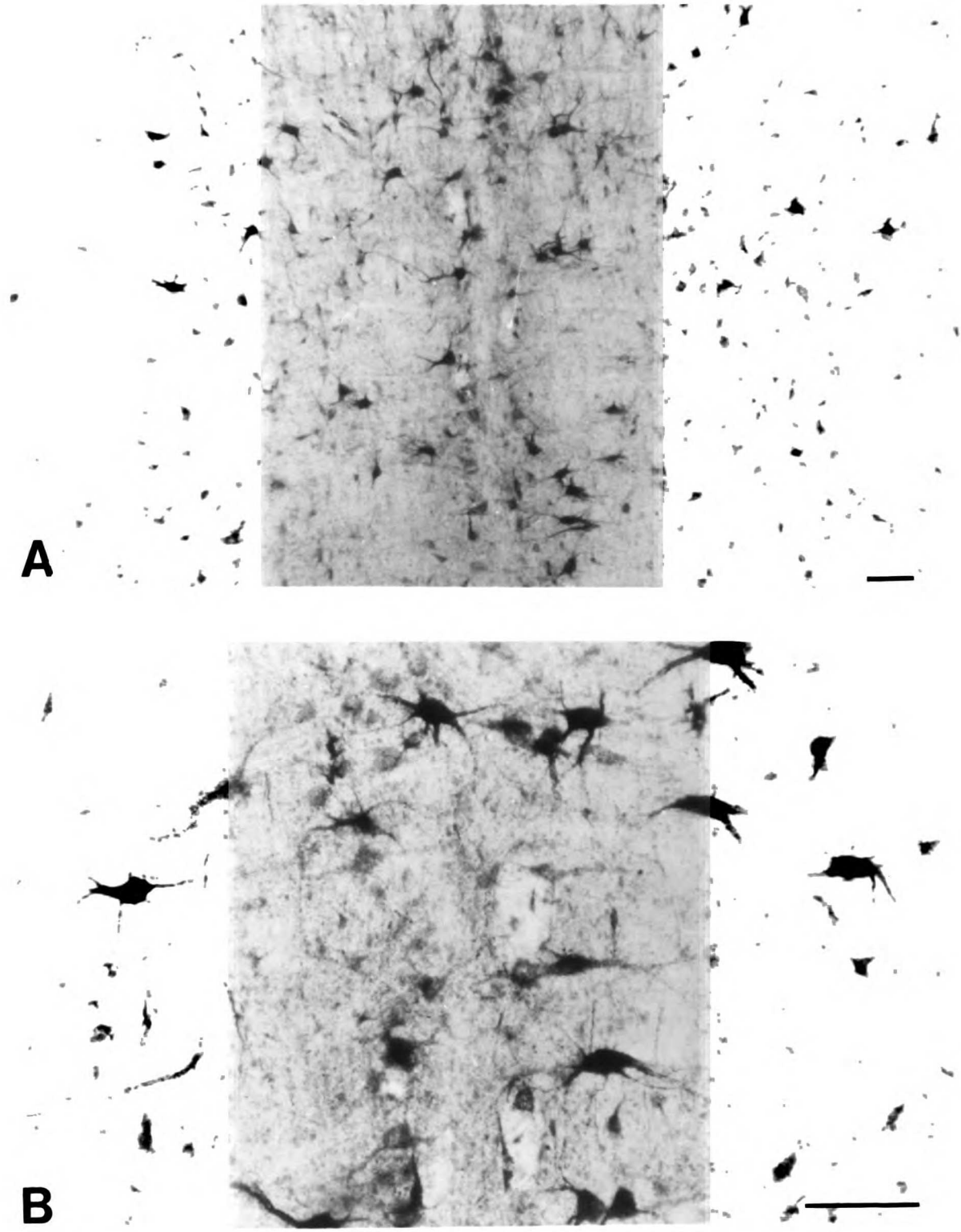

A
B

Figure 31. A series of tracings illustrating the regions in the various segments of the spinal cord in which HRP positive cells were found after an injection into the entire right half of the PAG; saimiri sciureus, (animal SM-2).

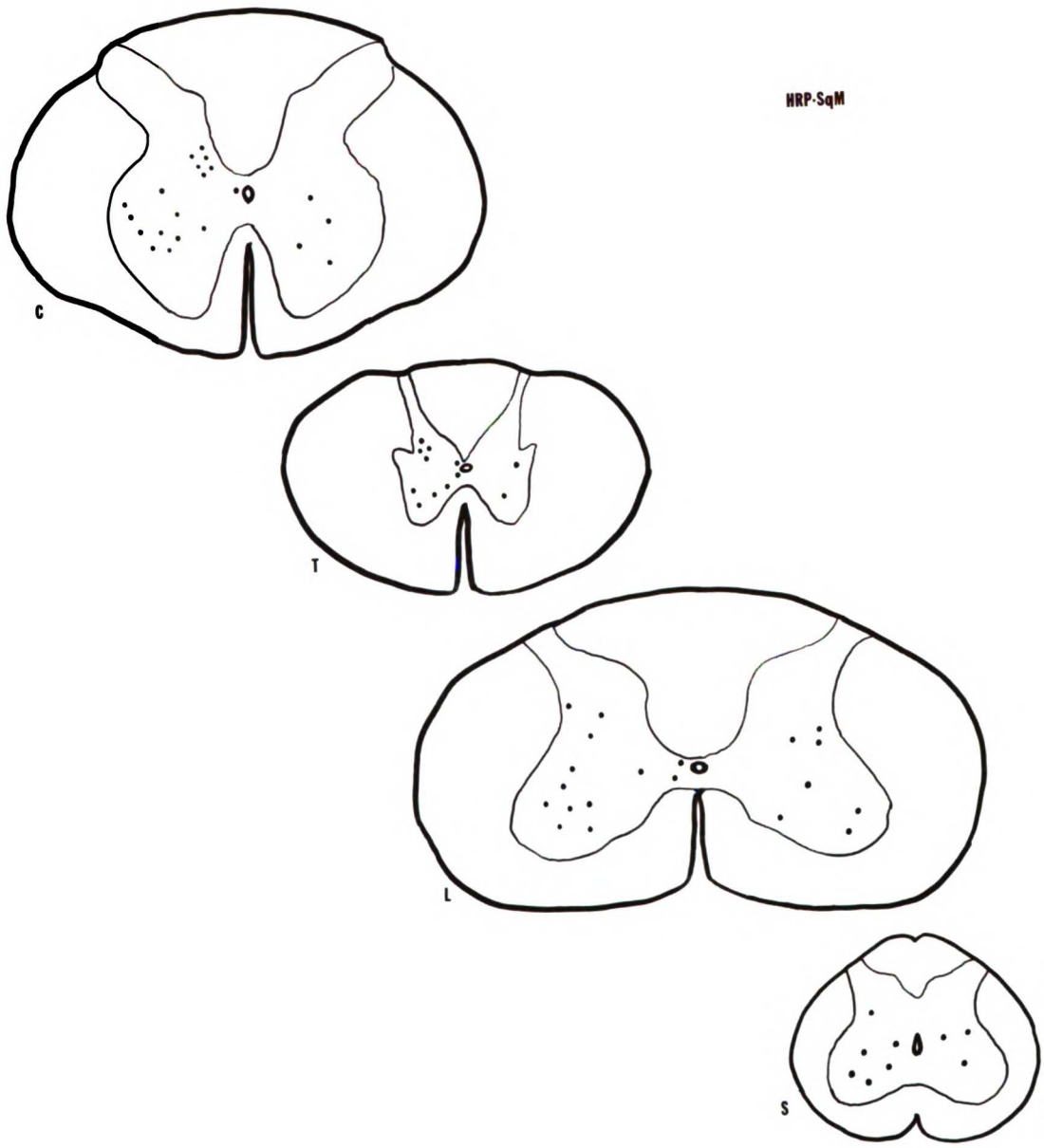

HRP-SqM
C
T
L
S

Figure 32. A. Darkfield photomicrograph of a coronal section showing a cervical cord in which HRP positive cells (circled) are found; saimiri sciureus, (experiment SM-12). Scale = 100μm.

B. Darkfield photomicrograph of a coronal section showing 2 HRP labeled neurons located in laminae VIII of Rexed (1951); saimiri sciureus, (experiment SM-12). Scale = 100μm.

C. Darkfield photomicrograph of a coronal section showing the HRP labeled lamina V cell seen at the upper right in Figure 23A; saimiri sciureus, (experiment SM-12). Scale 100μm.


A


B


C

CHAPTER 5: THE DESCENDING EFFERENT PROJECTIONS OF THE PERIAQUEDUCTAL GREY: CONNECTIONS OF A PAIN MODULATORY CENTER

INTRODUCTION

The periaqueductal grey (PAG) has been implicated in a variety of visceral activities as revealed by stimulation (Skultety, 1963) (Hunsperger, 1956) and ablation (Skultety, 1966) experiments. The functions the PAG is possibly involved in was expanded in 1969 when Reynolds discovered that a profound analgesia without an accompanying general behavioral depression resulted from stimulation of the PAG in the rat. This observation has since been confirmed in a wide variety of species including cat (Oliveras et. al. 1974) monkey (Goodman and Holcombe, 1975) and man (Hosobuchi et. al. 1977). Subsequent research into the anatomy, physiology and pharmacology of stimulation produced analgesia (SPA) suggests that it operates via a similar mechanism as opiate analgesia (OA) (Basbaum and Fields, 1978). Thus experimental SPA and OA have been hypothesized to utilize an endogenous pain modulatory system which has its own opiates (Hughes et. al. 1975) (β-endorphin, leu-enkephalin and met-enkephalin) and its own opiate receptors (Pert et. al. 1976). Furthermore, it appears that SPA in the central grey exerts its effects via its extrinsic connections and not by blocking nociceptive input at the level of the PAG (Rhodes, 1979).

The purpose of the present study is to investigate the descending connections of the central grey which might provide the anatomical substrate for the descending modulation of nociceptive input known to occur during central grey SPA (Basbaum and Fields, 1978). Previous anatomists and physiologists alike have attempted to determine the origin and distribution of the central grey's descending fiber system (Hamilton, 1973; Chi, 1970). Their attempts were severely hampered by

difficulties in staining fine and unmyelinated fibers and selective lesioning or stimulating a specific and determinable population among the rich mixture of cell bodies and interlacing axons within the central grey. This problem is particularily evident in the PAG were there pass extensive fiber systems known collectively as the dorsal bundle of Schutz (Schutz, 1891) or the periventricular bundle (Ranson, 1943). Thus the previous techniques employed did not permit an accurate and realistic view of the efferent connections by which this midbrain region is able to exert its descending modulation.

The introduction of autoradiographic tracing methods has greatly increased the degree of resolution in anterograde tracing studies, for it eliminates both the staining bias towards larger axons and is not complicated by labeled fibers of passage (Cowan et. al. 1972). Therefore, the present investigation will explore the descending projections from the central grey in order to determine the anatomical substrate by which the PAG exerts its descending modulatory effects.

MATERIALS AND METHODS

The materials and methods used in tracing the descending projections of this structure have previously been described in Chapter 3 of this study.

RESULTS

The results presented here will emphasize the autoradiographic results obtained in the primate material. Cat and rat HRP material will only be discussed when significant differences were observed in this material when compared to the primate. These cat and rat

experiments were undertaken in order to determine if significant differences were present in PAG connectivity between commonly used laboratory species. After examining the material obtained in these experiments it does not appear that significant differences in the caudal efferent connections of the PAG occur between the monkey, cat, and rat.

One of the most difficult problems in this study was to determine if the effective spread of the tracers at the injection site was limited to a restricted region. To overcome this problem we made very small injections (average of .025ml of 30% HRP and 7mc leucine), waited a long period after the injection before withdrawing the electrode to minimize the electrode track, used the most sensitive chromagen available to visualize the extent of the HRP spread, and used the same long exposure time for the injection site as for the projection areas.

The injection sites obtained in the primate are shown in figure 16. As can be seen from the diagram a variety of discrete injections were made into the various regions of the primate PAG. Figure 23 shows a representative injection site with 23a having been reacted for the presence of HRP with TMB and 23b an autoradiogram of an adjacent section. As can be seen from these sections the injection sites were well localized and the diffusion of HRP was roughly equal to the spread of the tritiated leucine. Although different regions of the PAG were injected in different animals (Figure 16) nearly all of the projection areas receiving PAG efferent connection were consistently labeled in the different cases. In other words, no matter what restricted region of the PAG one injected tracers, a similar set of

efferent connections were observed with both the HRP and the autoradiographic techniques employed in this study. Because of this finding we shall present a summary of our results using the representative injection site of SM-2. This injection site was in the dorsolateral aspect of the PAG with a rostral to caudal spread of approximately 2mm. As previously stated no consistent differences in the projections were observed between rostral and caudal injection sites.

In the following presentation of results we shall describe the areas in which silver grains were found and in which anterogradely HRP reaction product was observed in adjacent sections. Of the two methods the autoradiographs proved to be superior to the HRP technique because of the lack of contamination by fibers of passage and because the autoradiographs gave a clearer and more sharply defined picture of the projection areas than did the HRP material.

Superior Colliculus

After tritiated amino acid injections into any region of the PAG labeled fibers could be seen streaming out of the PAG into the adjacent stratum intermedium and stratum profundum of the ipsilateral superior colliculus (Figures 33a and 33b). These labeled fibers project only as far dorsal as the stratum intermedium. Few if any silver grains were observed in the stratum superficiale. Labeled fibers can also be traced to the contralateral superior colliculus where the deep tectal layers are overlain with silver grains. The opposite superior colliculus is consistently labeled after PAG injection although it is significantly less dense than the ipsilateral projection.

Central tegmental field

The central tegmental field, a large poorly defined reticular area extending between the mesencephalic reticular formation and the reticularis pontis oralis, also received a moderate projection from the ipsilateral PAG. This labeled tegmental area blended imperceptably with the stratum profundum of the superior colliculus. The fibers projecting to this region projected laterally from the PAG and then spread out in a diffuse manner over the entire region (Figure 33a and 33b). A lesser but consistent contralateral projection to the opposite central tegmental field was also observed.

Periaqueductal Grey

After injections into the periaqueductal grey (PAG) ipsilateral regions both rostral and caudal to the injection site contained heavy deposits of silver grains. These silver grains were grouped in bundles or fascicles intermixed with diffuse "clouds" of silver grains. The fascicles were presumably the PAG neurons contribution to the periventricular fiber bundle while the cloud-like pattern may indicate regions of termination. The contralateral PAG contained relatively few silver grains even when compared to the light labeling observed in the contralateral deep tectum and tegmentum (Figure 33a-c).

Cuneiformis

The ipsilateral nucleus cuneiformis was heavily overlain with silver grains after injections of TAA into the PAG. This rectangular-shaped nucleus could be clearly delineated from the

laterally adjacent inferior colliculus which contained few if any silver grains and therefore served as a convenient aid to indicate the level of background silver grains (Figure 33a). The contralateral cuneiformis along with both the ipsilateral and contralateral subcuneiformis receive only a light projection from the PAG.

Locus Coeruleus

The locus coeruleus received a heavy projection from the PAG as revealed by the heavy concentration of silver grains which overlaid this nucleus (Figure 33c). This labeling was quite heavy and ran along the lateral border of the caudalmost ventral central grey and dorsal to the Guddens nucleus. The nucleus subcoeruleus contained a few silver grains and seemed to have the same density of labeling as the more ventral reticularis pontis oralis. The labeling of both the locus coeruleus and subcoeruleus was primarily ipsilateral.

Reticularis pontis oralis and caudalis

The pontine reticular formation contained light to moderate concentrations of silver grains. This labeling was in marked contrast to the more medial central superior nucleus which contained few if any silver grains and the more dorsal locus coeruleus which contained heavy concentrations of silver grains (Figures 33b and 33c). Labeling was predominately ipsilateral but some contralateral label was present.

Raphe Region

The raphe nuclei which were particularily well labeled with silver grains were the nucleus raphe magnus (NRM) and the nucleus raphe pallidus (NRP). The NRM labeling was heavy over the midline and then swept laterally in a dense and continuous fashion into the more lateral medullary reticular formation (Figures 33b-d, 34 and 35). When observed with light field microscopy one can clearly see the characteristically large fusiform shaped raphe neurons in a dense swath of silver grains. The raphe pallidus also received a heavy projection from the PAG and also displayed a dense concentration of silver grains around its neurons. When viewing both of those raphe groups the more lateral inferior olive and the pyramidal tract was markedly unlabeled and served as a convenient indicator of the level of background noise (Figures 34 and 35).

Medullary Reticular Formation

Parts of the medullary reticular formation receive a moderate projection from the PAG. These regions receiving a projection from the PAG were those just lateral to the NRM. The reticularis magno-cellularis (Rmc) and the nucleus paragigantocellularis lateralis (pgl) were consistently well labeled after injections into any region of the PAG. This labeling appeared to be part of a band of silver grains which streched from the medial NRM through the Rmc to the lateral pgl (Figures 33c, 33d, 34 and 35). The more dorsal gigantocellularis proper contained only a few silver grain and those silver grains present appeared to be organized in fascicles, probably representing fibers passing through the area.

Spinal cord

Few if any silver grains were observed above background in the spinal cord although some spinal cord sections were exposed for up to nine months with low background still being present.

DISCUSSION

Before discussing the anatomical and functional implications of the present study it is important to realize the limitations and caution one should take in interpreting these results. The injected tritiated amino acids are known to be taken up by cells, incorporated into proteins, and anterogradely transported to axons and axonal terminals (Cowan et. al., 1972). Since axons apparently are incapable of protein synthesis inadvertant labeling of axons of passage is not a problem with this technique. This lack of contamination of axons of passage is not true of the anterograde transport of HRP. Anterograde transport of HRP can apparently occur equally well between damaged axons of passage and cell bodies (LaVail, 1975). Therefore, the anterograde transport of HRP, which should label both damaged fibers of passage and the true projections of the PAG, was used for confirmatory purposes only with the major stress on this paper being made on the grounds of the autoradiographic analysis.

A note of caution however should be made in attempting to interpret even the anterograde transport of tritiated amino acids (TAA) as revealed by the presence of silver grains. Labeled proteins have been reported to be transported between 100-200mm per day (Cowan et. al. 1972). Since no area of the squirrel monkey brain is more than 200mm away from the PAG and the survival times used in this were

between 2-3 days all efferent projections made by the PAG neurons had adequate time for terminal labeling using TAAs. The difficulty however is in differentiating between a terminal field of a PAG projection and those silver grains which are in axons which project to another area. Since all that one sees under the microscope is silver grains it is potentially difficult to differentiate between these two possibilities. This difficulty in interpretation however can usually be overcome by careful analysis of the material and by noting when a projection terminates in an area and does not proceed any further than a particular nuclear zone. Therefore by carefully noting in serial sections when projecting fibers terminate in an area, that is they proceed no further, one can judiciously assume, given the rate of transport and the survival time, that one is viewing a terminal projection area.

Previous published reports on the central grey's descending efferent connections have employed silver degeneration techniques. Two technical problems severely limit the conclusions which can be drawn from these previous experiments. First, silver degeneration techniques preferentially stain larger fibers. Unfortunately, one of the PAG's most distinctive features is its lack of large myelinated fibers, as seen with a Weil myelin stain. In light of these two facts there is a distinct possibility that projections of the PAG which are composed of relatively fine or amyelinated axons will not be stained when these techniques are employed. A second problem is that the initial lesion which produces the resulting Wallerian degeneration does not discriminate between cell bodies and axons of passage. Because of the lesioning of fibers of passage, false positives might

be included in the efferent projections of the cell bodies of the lesioned area. This is especially true in the PAG for the dorsal bundle of Schutz runs through the entire length of the PAG (Schutz, 1891). Of these two methodological problems faced by the previous investigators, the former appears to have caused the greatest problems in attempting to define the efferent connections of the PAG. Whereas the present study reports a wide spectrum of descending efferent projections from the central grey, previous authors reported that the PAG projected to only one caudal area, the central tegmental field (Hamilton, 1973; Chi, 1970). As reviewed above the present study does not suffer from either of these technical problems (Cowan et. al., 1972) and it is probably for this reason that the results of the present investigation differ so markedly from the previous investigators' reports.

The most rostral brainstem area the PAG projects to is the superior colliculus. This PAG to tectum projection has previously been reported by investigators using the HRP retrograde tracing technique (Grofova, 1978; Edwards et. al., 1979). This PAG projection is confined to the deep tectal layers composed of the stratum intermedium and stratum profundum. Within these deep tectal layers there are three to seven sublaminae (Edwards, 1980). These sublaminae are organized according to sensory modalities and motor functions with each modality or function being confined to one laminae and being organized in a topographical fashion (Gordon, 1973; Drager and Hubel, 1975; Stein et. al., 1975). It is interesting to note that this central grey-deep tectal projection is reciprocal in nature, that is, anterogradely transported TAA's represented by silver grains overlie

the same laminae in which HRP positive cell bodies are observed in adjacent sections. This finding suggests that both regions might be able to influence and modulate each other, and thus provides an anatomical substrate by which external stimuli and motor functions known to be mapped in the superior colliculus could be integrated with the visceral activities of the body which are known to be modulated by the PAG.

The nucleus cuneiformis is also reciprocally connected with the central grey. This "reticular" region displayed the characteristic pattern observed in labeled reticular regions. This labeling of silver grains was moderate in concentration and distributed in a scattered fashion to all parts of the nucleus. The caudally adjacent reticularis pontis oralis and caudalis were also reciprocally connected with the PAG and demonstrated a similar type of labeling in both the anterograde and retrograde direction as seen in the nucleus cuneiformis which is the rostral extension of these cell groups.

The locus coeruleus is an adrenergic containing cell group (Dahlstrom and Fuxe, 1965; Felten et. al., 1974) which is reciprocally connected with the PAG. The moderate to heavy efferent projection from the PAG to the locus has been previously suggested by a number of authors using retrograde HRP tracing techniques (Sakai et. al., 1979; Cedarbaum and Aghajanian, 1978). Locus neurons are known to project to extremely widespread areas of the brain including the neocortex (Jones and Moore, 1977) and the spinal cord (Basbaum and Fields, 1979). The locus-spinal cord projection has been postulated to be involved in modulating nociceptive input at the dorsal horn level (Basbaum and Fields, 1979). PAG stimulation is also known to modulate

nociceptive transmission at the dorsal horn level and in light of this PAG-locus coeruleus connection, it is possible that some of the PAG's modulatory effects may be mediated through a PAG-locus, locus-spinal cord pathway.

Two midline raphe groups, the nucleus raphe magnus (NRM) and the nucleus raphe pallidus (NRP) are also reciprocally connected with the PAG. Silver grains in moderate to heavy numbers overlie these raphe regions and alternate sections reacted for the presence of HRP have moderate numbers of HRP labeled neurons in these same nuclei. Previous retrograde HRP studies have also demonstrated a heavy projection from the PAG to these raphe groups (Gallagher and Pert, 1978; Abols and Basbaum, 1979). These raphe groups in turn project to the spinal cord via the dorsolateral fasciculus to terminate in the Rexed's (1952) lamina I, II, V, VI and medial VII of the dorsal horn (Basbaum and Fields, 1978). Stimulation of the PAG which projects to these raphe nuclei produces a potent analgesic effect which is partially reversible by p^{CPA} (Akil and Mayer, 1972), a serotonin synthesis inhibitor. Such evidence suggests that NRM and NRP project to the cord where they may exert a powerful inhibition of nociceptive input. The PAG-raphe projection therefore provides a further anatomical substrate by which the PAG can exert its known analgesic action. This hypothesis is supported by physiological evidence which demonstrates that stimulation of the PAG causes a marked increase in the firing rate of raphe neurons which project to the spinal cord (Behbehani and Fields, 1979). It is interesting to note however that central grey SPA is only <u>partially</u> reversed by p^{CPA} (Akil and Mayer 1972). This suggests that other non-serotonergic pathways are

involved in this descending inhibition. A variety of non-serotonergic putative neurotransmitters including enkephalin and substance P have been described as being present in these medullary neurons. Some of these medullary enkephalin containing neurons project to the spinal cord (Hokfelt et. al. 1979) where they may participate in the inhibition of nociceptive input. Therefore, while serotonergic NRM neurons are known to be involved in central grey SPA, a variety of other neurotransmitters, even in NRM neurons, may also be involved in this descending modulation.

The medullary reticular formation lateral to the NRM demonstrated reciprocal connections with the central grey. Particularily well labeled were the nucleus reticularis magnocellularis (Rmc) and the more lateral nucleus paragigantocellularis lateralis (pgl). Like the locus and NRM neurons, Rmc and pgl neurons project to the spinal cord (Basbaum and Fields, 1979) and thus provide a further anatomical substrate through which the PAG can exert its effects on the spinal cord.

One region noticeably devoid of silver grains above background level was the spinal cord. Previous investigators have observed a PAG-spinal projection (Castiglioni et. al., 1978; Basbaum and Fields, 1979; Mantyh and Mehler, unpublished obs.). However, even in light of this projection and with extremely long exposure times no projection was observed using autoradiographic techniques. Therefore, the laminae in which the PAG-spinal projection terminates remains unknown.

Overview nociception

The earliest evidence for the existence of an endogenous analgesia system was the observation that electrical stimulation of the midbrain periaqueductal grey produced a profound analgesia without an accompanying general behavioral depression (Reynolds, 1969). This phenomena is known as stimulation produced analgesia (SPA). Numerous sites in the brain are known to produce analgesia upon stimulation (Adams et. al., 1974, Gol, 1976; Fields and Basbaum, 1978; Lineberry and Vierck, 1979) but the PAG is consistently the most efficatious site for SPA (Mayer and Liebeskind, 1974; Fields and Basbaum, 1978). Interestingly this central grey SPA appears to be operating via a similar set of connection that underlie opiate-induced analgesia (OA). Several lines of evidence support this hypothesis. Moderate concentrations of both opiate receptor (Pert et. al., 1976) and the endogenous opiates β-endorphin (Dupont et. al., 1980) and leu enkephalin (Simantov et. al., 1976) are present in the central grey. The same sites which are effective in OA are effective in producing SPA (Pert and Walter, 1976; Lewis and Gebhart, 1977). There is a significant cross tolerance between SPA and OA (Hosobuchi et. al., 1977). Lastly, and probably most significantly, the PAG is the most effective site for both (Mayer and Liebeskind, 1974; Yaksh et. al., 1976) and SPA is partially reversed by the opiate antagonist naloxone (Akil et. al. 1976).

Physiological and pharmacological data indicate that it is doubtful that SPA or OA work by blocking pain transmission at the level of the central grey (Rhodes, 1980). Rather it appears the PAG exerts its antinociceptive effects by its extrinsic connections. The

descending connections of the PAG have been hypothesized to be part of the anatomical substrate by which descending modulation of nociception at the dorsal horn level is brought about (Basbaum and Fields, 1978). The PAG itself possesses a direct projection to the spinal cord (Castiglioni et. al., 1978) but pharmacological and physiological data suggests that descending brainstem projections also play a role in central grey SPA. It is therefore noteworthy that the PAG has a strong projection to most brainstem nuclei which project to the spinal cord. These brainstem groups which both receive a projection from the PAG and project to the spinal cord include the locus coeruleus, the raphe magus and pallidus, the reticularis magnocellularis and the paragigantocellularis lateralis (Tohyama et. al., 1979; Basbaum and Fields, 1979).

It is possible that locus coeruleus modulates nociceptive input at the spinal level. Noradrenaline which is found in high concentrations in locus coeruleus cell bodies (Versteeg et. al., 1976) has been demonstrated to mediate antinociception at the dorsal horn level (Reddy and Yaksh, 1980). This combined with the heavy locus projection to the cord has raised the possibility that the locus inhibits nociceptive input. The demonstration of a PAG locus projection could be a possible source of locus activation in its descending modulation.

NRM and NRP are also known to send a significant projection to the spinal cord (Basbaum et. al., 1978). Serotonin, a neurotransmitter found in many NRM and NRP neurons, is known to modulate nociceptive input. The PAG projection to NRM and NRP therefore provides an anatomical substrate by which raphe neurons

could be activated to produce a modulation of nociceptive input. It should be emphasized however, that while serotonin is found in some NRM neurons, other putative neurotransmitters are also present in these neurons and these may also play a significant role in SPA.

The PAG, Rmc and the pgl have direct projections to the spinal cord and it is conceivable that these neurons also play a role in mediating SPA. The important point to be made in reviewing these brainstem areas which receive a PAG input and project to the spinal cord is that a multitude of descending pathways are possibly involved in SPA. This is underscored by the observation that neither serotonin or noradrenalin antagonists alone will completely block SPA (Akil and Mayer, 1972; Reddy and Yaksh, 1980). It is then possible that a variety of neurotransmitters are present in these brainstem neurons which have descending projections and could play a role in SPA. Therefore, it appears that the PAG does not exert its descending antinociceptive effects via one set of brainstem neurons employing one type of neurotransmitter, but rather, a variety of brainstem nuclei receive a projection from the PAG which in turn project to the spinal cord. This neuroanatomical arrangement provides a heterogeneous anatomical substrate for the descending modulation of nociception as seen during SPA.

It has been hypothesized that only the ventrolateral quadrant of the PAG is involved in the descending inhibition of noxious input (Yaksh et. al., 1976). Such hypothesis stems from physiological and pharmacological observations that stimulation or injection of opiates into the ventrolateral quadrant produces the most efficacious analgesia at the lowest current and dose respectively (Mayer and

Liebeskind, 1974; Yaksh et. al., 1976). It might therefore be hypothesized that only the ventrolateral quadrant of the PAG would project to brainstem areas involved in the descending modulation of nociceptive input. However, this and previous investigations have failed to demonstrate any significant difference in the anatomical connections of the various regions of the PAG with lower brainstem areas. The anatomical observation that most regions of the PAG project to lower brainstem regions involved in SPA and OA has been confirmed physiologically (Dostrovsky and Shah, 1980). However, such anatomical and physiological demonstrations of a projection from all regions of the PAG to brainstem areas does not prove that all these PAG neurons are involved in the same function. Indeed, it is quite possible that only a particular subset of the PAG neurons projecting to locus coeruleus, NRM, NRP, Rmc or pgl are involved in activating descending antinociceptive units, while the other projection neurons could be involved in the myriad of other functions the PAG has been implicated in. Therefore, the present demonstration that all regions of the PAG have in common their efferent brainstem projections does not prove or disprove the suggestion that only the ventrolateral quadrant of the PAG is involved in antinociception. Rather, it suggests that if other regions of the PAG are not involved in the descending modulation of nociception, they probably are using similar brainstem connections for modulating another physiological functions that the PAG is known to be involved in.

The central grey is known to play a pivotal role in SPA and OA. These exogenous experimental manipulations however, are hypothesized to be activating an endogenous pain control system. A variety of

evidence supports the hypothesis that such an endogenous pain control system exists within the CNS. Naloxone the opiate antagonist which partially blocks both SPA and OA enhances visceral pain in rats (Kokka and Fairhurst, 1977) blocks acupuncture in experimental subjects (Mayer et. al., 1977), enhances post-operative pain in patients (Levine et. al., 1978) and lowers the latency for both the tail flick and hot-plate tests in rats (Jacob et. al., 1974). Such results suggest that pain itself activates a endogenous pain modulatory system. Since the PAG is the most efficacious site for both SPA and OA it is quite possible that the PAG plays an equally central role in the endogenous pain modulatory system. In light of the extensive extrinsic connections of the PAG as demonstrated in this study it appears that the central grey possesses the anatomical substrates to fill this role.

Visceral

While we and other investigators have emphasized the antinociceptive aspects of PAG function, it is important to realize that the PAG is also involved in a wide spectrum of visceral functions. These include oxytocin release (Aulsebrook and Holland, 1969), vocalization of emotion (Jurgens and Pratt, 1979), emotional rage (Hunsperger, 1956), control of gastric and bladder motility (Skultety, 1959), and the lordosis relfex in sexual behavior (Sakuma and Pfaff, 1979). The neural pathways by which the PAG modulates these functions is largely unknown although the present investigation has demonstrated a phethora of routes by which the PAG might influence other brain structure known to be involved in these functions,

particularly the massive reciprocal PAG-hypothalamic connections. However several descending projections from the PAG might also regulate some of these visceral functions.

The lordosis relfex, a dorsiflexion of the vertebral column is elicited in sexually receptive female rodents by somatosensory stimuli (Pfaff et. al., 1977). Results from stimulation and lesion experiments (Sukuma and Pfaff, 1979a and 1979b) suggest that the PAG may help integrate somatosensory and motor components of a reflex loop. In the previous paper we identified a spinothalamic input into the PAG which might provide the somatosensory input for this reflex. The descending component from the PAG which would direct the lordosis reflex is still unknown, although it could be the direct PAG-spinal projection or an indirect pathway through the variety of brainstem areas the PAG has been shown to project to.

Another visceral reflex which the PAG has been implicated in is vocalization (Jurgens and Pratt, 1979). That the PAG is needed for this function has been demonstrated in a wide variety of animals from monkeys (Jurgens and Muller-Preuss, 1977) to fish (Demski and Gerald, 1974). Previous investigators demonstrated that the PAG serves as a point of convergence for cortical and hypothalamic areas which are also known to be involved in this vocalization. These authors have hypothesized that the PAG projects to the nucleus ambiguous, which innvervates the striated muscle fibers of the pharynx and the vocal cord muscles of the larynx, and through excitation of this connection the vocalization would occur (Jurgens and Pratt, 1979). In the present experiments this connection was observed. This projection was mainly to the retro-ambiguous area although the more rostral ambiguous proper was also moderately overlain with silver grains.

The PAG has also been implicated in the control of gastric motility and bladder motility (Skultety, 1959). In the present investigation no direct projection was observed from the PAG to sympathetic or parasympathetic brainstem nuclei which are known to regulate such visceral activates. However the PAG does send a heavy projection to the NRM and the locus coeruleus both of which are known to project to the solitary nuclei, the dorsal motor nucleus of the vagus and its spinal equivalent, the intermediolateral horn of the thoracolumbar cord (Basbaum and Ralston, 1978). Thus the PAG may exert control of gastric and bladder motility through these secondary brainstem and spinal connections.

General Conclusions

The present investigation has demonstrated that the PAG is reciprocally connected with a variety of brainstem areas. These reciprocal connections provide a possible anatomical substrate for direct feedback loops to the PAG. Thus areas that the PAG is hypothesized to modulate such as NRM, project back to the PAG and provide a direct route for feedback onto the PAG modulatory neurons. Such an anatomical organization provides the possibility for a tight and immediate feedback loop.

A second general point of this study is that the various regions of the PAG have in common the majority of their brainstem projections. This finding is also confirmed by retrograde studies of the afferents to brainstem areas which recieve a projection from the PAG. In light of this data subdivision of the PAG into discrete units on the grounds that each area has a distinct and non overlapping connectivity seems inappropriate.

Finally the PAG is connected with a wide variety of brainstem structures most of which are known to have descending projections to the spinal cord. This hetergeneous projection pattern of the PAG is in accordance with the wide variety of physiological functions the PAG has been implicated in. These efferent connections thus provide an anatomical substrate by which the PAG can influence a wide variety of other neuronal structures.

SUMMARY

(1) The PAG projects to a wide variety of brainstem regions including the locus coeruleus, superior colliculus, cuneiformis, reticular formation, and the raphe magnus and pallidus.

(2) The brainstem areas the PAG projects to are heterogeneous but most have projections to the spinal cord. This secondary spinal projection provides an anatomical substrate by which PAG stimulation can profoundly effect nociceptive and visceral functions at the spinal level.

(3) The PAG establishes reciprocal connections to all brainstem areas to which it projects. These efferent projections discussed in this study are in exact register with adjacent sections reacted for neurons that project to the PAG. This arrangement provides an anatomical substrate for a possible direct feedback to the PAG from the regions the PAG is hypothesized to modulate.

(4) The PAG does not seem subdivisible into discrete subnuclei on the basis of its efferent descending connections. Rather the different quadrants of the PAG have in common the great majority of their descending connections.

BIBLIOGRAPHY

Abols, A.I. and A.I. Basbaum 1979 Afferent input to the medullary reticular formation and nucleus raphe magnus of the cat. An HRP study. Anat. Rec., 193:467.

Adams, J.E., Y. Hosobuchi, and H.L. Fields 1974 Stimulation of internal capsule for relief of chronic pain. J. Neurosurg. 41:740-744.

Akil, H. and D.J. Mayer 1972 Antagonism of stimulation produced analgesia by p-CPA a serotonin synthesis inhibitor. Brain Res., 44:692-697.

Akil, H., D.J. Mayer, and J.C. Leibeskind 1976 Antagonism of stimulation-produced analgesia by naloxone, a narcotic antagonist. Science, 191:961-962.

Aulsebrook, L.H. and R.C. Holland 1969 Central inhibition of oxytocin release. Am. J. Physiol., 216:830-840.

Basbaum, A.I., C.H. Clanton, and H.L. Fields 1978 Three bulbo-spinal pathways from the rostral medulla of the cat: An autoradiographic study of pain modulating systems. J. Comp. Neurol., 178:209-224.

Basbaum, A.I. and H.L. Fields 1978 Endogenous pain control mechanisms: Review and hypothesis. Ann. Neurol., 4:451-462.

Basbaum, A.I. and H.L. Fields 1979 The origin of descending pathways in the dorsolateral funiculus of the spinal cord of the cat and rat: Further studies on the anatomy of pain modulation. J. Comp. Neurol., 187:513-532.

Basbaum, A.I. and H.J. Ralston III 1978 Projections from nucleus raphe magnus in the primate. Pain Abstr., 1:259.

Behbehani, M.M. and H.L. Fields 1979 Evidence that an excitatory connection between the periaqueductal grey and nucleus rphe magnus mediates stimulation-produced analgesia. Brain Res., 170:85-94.

Castiglioni, A.J., M.C. Gallaway, and J.D. Coulter 1978 Spinal projections from the midbrain in monkey. J. Comp. Neurol., 178:329-346.

Cedarbaum, J.M. and Aghajanian 1978 Afferent projections to the rat locus coeruleus as determined by a retrograde tracing technique. J. Comp. Neurol., 178:1-16.

Chan-Palay, V., G. Jonsson, and S. Palay 1978 Serotonin and substance P coexist in neurons of the rat's central nervous system. Proc. Natl. Acad. Sci. USA, 75:1582-1586.

Chi, C.C. 1970 An experimental silver study of the ascending projections of the central grey substance and adjacent tegmentum in the rat with observations in the cat. J. Comp. Neurol., 139:259-272.

Cowan, W.M., D.I. Gottlieb, A.E. Hendrickson, J.L. Price, and T.A. Woolsey 1972 The autoradiographic demonstration of axonal connections in the central nervous system. Brain Res., 37:21-51.

Dahlstrøm, A. and K. Fuxe 1965 Evidence for the existence of monoamine-containing neurons in the central nervous system. I. Demonstration of monoamines in the cell bodies of brainstem neurons. Acta. Physiol. Scand. 62 Suppl. 232 pp. 1-55.

Demski, L.S. and J.W. Gerald 1974 Sound production and other behavioral effects of midbrain stimulation in freeswimming toadfish, Opsanus beta. Brain Behav. Evol., 9:41-59.

Drager, U.C. and Hubel, D.H. 1975 Responses to visual stimulation and relationship between visual, auditory, and somatosensory inputs in the mouse superior colliculus. J. Neurophysiol., 38:690-713.

Edwards, S.B., C.L. Ginsburgh, C.K. Henkel, and B.E. Stein 1979 Sources of subcortical projections to the superior colliculus in the cat. J. Comp. Neurol., 184:309-330.

Edwards, S.B. 1980 The deep cell layers of the superior colliculus: Their reticular characteristics and structural organization. In The Reticular Formation Revisited. J.A. Hobson and M.A.B. Brazier, eds., Raven Press, New York.

Felten, D.L., A.M. Laties, and M.B. Carpenter 1974 Monoamine containing cell bodies in the squirrel monkey brain. Am. J. Anat., 139:153-166.

Gallagher, D.W. and A. Pert 1978 Afferents to brainstem nuclei (brainstem raphe, n. reticularis pontes caudalis, and n. gigantocellularis) in the rat as demonstrated by microiontophoretically applied horseradish peroxidase. Brain Res., 144:257-275.

Gol, A. 1967 Relief of pain by electrical stimulation of the septal area. J. Neurol. Sci., 5:115-120.

Goodman, S.J. and V. Holcombe 1975 Selective and prolonged analgesia in monkey resulting from brain stimilation. In: First World Congress on Pain, 264.

Gordon, B. 1973 Receptive fields in deep layer of cat superior colliculus. J. Neurophysiol., 36:157-178.

Grofova, I., Otterson, O.P., and E. Rinvik 1978 Mesencephalic and diencephalic afferents to the superior colliculus and periaqueductal grey substance demonstrated by retrograde axonal transport of horseradish peroxidase in the cat. Brain Res., 146:205-220.

Hamilton, B.L. 1973 Projections of the nuclei of the periaqueductal grey matter in the cat. J. Comp. Neurol., 152:45-58.

Hokfelt, T., L. Terenius, H.G.J.M. Kuypers, and O. Dann 1979 Evidence for enkephalin immunoreactive neurons in the medulla oblongata projecting to the spinal cord. Neuroscience Letters, 14:55-60.

Hosobuchi, Y., J.E. Adams, and R. Linchitz 1977 Pain relief by electrical stimulation of the central grey matter in humans and its reversal by naloxone. Science, 197:183-186.

Hughes, J., T.W. Smith, and H.W. Kosterliz 1975 Identification of two related pentapeptides from the brain with potent opiate agonist activity. Nature, 258:577-579.

Hunsperger, R.W. 1956 Role of substantia gresea centralis mesencephali in electrically induced rage reactions. In: Progress in Neurobiology. Vol. I J. Ariens Kappers, ed., Elsevier, New York, pp. 289-294.

Jacob, J.J., E.C. Tremblay, and M. Colombel 1974 Facilitation de reactions nociceptures par la naloxone chez le souris et chez le rat. Psychopharmacologia, 37:217-223.

Jones, B.E. and R.Y. Moore 1977 Ascending projections of the locus coeruleus in the rat. Brain Res., 127:23-53.

Jürgens, U. and P. Muller-Preuss 1977 Cerebral representation of vocalization in the squirrel monkey. Exp. Brain Res., 10:532-554.

Jurgens, U. and R. Pratt 1979 Role of the periaqueductal grey in vocal expression of emotion. Brain Res., 167:367-378.

Kokka, N. and A.S. Fairhurst 1977 Naloxone enhancement of acetic acid induced writhing in rats. Life Sci., 21:975-980.

LaVail, J.H. 1975 Retrograde cell degeneration and retrograde transport techniques. In: The Use of Axonal Transport for Studies of Neuronal Connectivity. Cowan, W.M. and Cuenod, M., eds., Elsevier, Amsterdam, pp. 217-248.

Levine, J.D., N.C. Gordon, R.T. Jones et. al. 1978 The narcotic antagonist naloxone enhances clinical pain. Nature 272:826-827.

Lewis, V.A. and G.F. Gebhart 1977 Evaluation of the periaqueductal central grey (PAG) as a morphine-specific locus of action and examination of morphine-induced and stimulation-produced analgesia at coincident PAG loci. Brain Res., 124:283-303.

Lineberry, C.G. and C.J. Vierck 1975 Attenuation of pain reactivity by caudate nucleus stimulation in monkeys. Brain Res., 98:110-134.

Mayer, D.J. and J.C. Liebeskind 1974 Pain reduction by focal electrical stimulation of the brain: an anatomical and behavioral analysis. Brain Res., 68:73-93.

Mayer, D.J., D.D. Price, and A. Rafir 1977 Antagonism of acupuncture analgesia in man by the narcotic antagonist naloxone. Brain Res., 121:36-73.

Oliveras, J.L., J.M. Besson, G. Guilbaud, and J.C. Liebeskind 1974 Behavioral and electrophysiological evidence of pain inhibition from midbrain stimulation in the cat. Exp. Brain Res., 20:32-44.

Pert, A. and M. Walter 1976 Comparison between reversal of morphine and electrical stimulation induced analgesia in the rat mesencephalon. Life Sci., 19:1023-1032.

Pert, C.B., M.J. Kuhar, and S.H. Snyder 1976 Opiate receptor: autoradiographic localization in rat brain. Proc. Natl. Acad. Sci. USA, 73:3729-3733.

Pfaff, D.W., M.O. Montgomery, and C. Lewis 1977 Somatosensory determinants of lordosis in female rats: behavioral definition of the estrogen effect. J. Comp. Physiol. Psychol., 91:134-145.

Ranson, S.W. 1943 The anatomy of the nervous system. 7th ed. W.B. Saunders Co., Philadelphia-London.

Reddy, S.V.R. and T.L. Yaksh 1980 Spinal noradrenergic terminal system mediates antinociception. Brain Res., 189:391-401.

Rexed, B. 1952 The cytoarchitectonic atlas of the spinal cord in the cat. J. Comp. Neurol., 96:415-495.

Reynolds, D.V. 1968 Surgery in the rat during electrical analgesia induced by focal brain stimulation. Science, 164:444-445.

Rhodes, D.L. 1979 Periventricular system lesions in stimulation produced analgesia.

Sakai, K., M. Touret, D. Salvert, L. Leger, and M. Jouvet 1977 Afferent projections to the cat locus coeruleus as visualized by the horseradish peroxidase technique. Brain Res., 119:21-41.

Schutz, H. 1891 Anatomische Untersuchugen ϕber den Faserverlaug im Zentralen Hohlengrau und den Nervenfaser schiv und indemselken bei der progressiven Paralyse der Irren. Arch. Psychiat. 22:527-587.

Shah, Y. and J.O. Dostrovsky 1980 Electrophysiological evidence for a projection of the periaqueductal grey matter to nucleus raphe magnus in cat and rat. Brain Res., 193:534-538.

Simantov, R., M.J. Kuhar, G.W. Pasternak, et. al. 1976 The regional distribution of a morphine-like factor enkephalin in monkey brain. Brain Res., 106:189-197.

Skultety, F.M. 1963 Stimulation of periaqueductal grey and hypothalamus. Arch. Neurol., 8:38-50.

Skultety, F.M. 1966 Changes in caloric intake following brainstem lesions in cats. Arch. Neurol., 14:670-680.

Skultety, F.M. 1959 Relation of periaqueductal grey matter to stomach and bladder motility. Neurology, 9:190-198.

Stein, B.E., B. Magalhaes-Castro, and L. Kruger 1975 Superior colliculus: visuotopic-somatotopic overlap. Science, 189:224-226.

Sakuma, Y. and D.W. Pfaff 1979a Facilitation of female reproductive behavior from mesencephalic central grey in the rat. Am. J. Physiol., 237:R278-R284.

Sakuma, Y. and D.W. Pfaff 1979b Mesencephalic mechanisms for integration of female reproductive behavior in the rat. Am. J. Physiol. 237:R285-R290.

Tohyama, M., K. Sakai, D. Salvert, M. Touret, and M. Jouvet 1979 Spinal projections from the lower brainstem in the cat as demonstrated by HRP technique. I. Origins of the Reticulospinal tracts and their funiculus trajectories. Brain Res., 173:383-404.

Versteeg, D., J. Van der Gugten, W. de Jong, and M. Palkovits 1976 Regional concentrations of noradrenaline and dopamine in rat brain. Brain Res., 113:563-574.

Willis, W.D., L.H. Haber, and R.F. Martin 1977 Inhibition of spinothalamic tract cells and interneurons by brainstem stimulation in the monkey. J. Neurophys., 40:968-981.

Yaksh, T.L., J.C. Yeung, and T.A. Rudy 1976 Systemic examination in the rat of brain sites sensitive to the direct application of morphine: observation of differential effects within the periaqueductal grey. Brain Res., 114:83-103.

Figure 33. A series of tracings illustrating the efferent projections labeled in the brainstem following an injection primarily involving the entire right PAG; saimiri sciureus, (experiment SM-2).

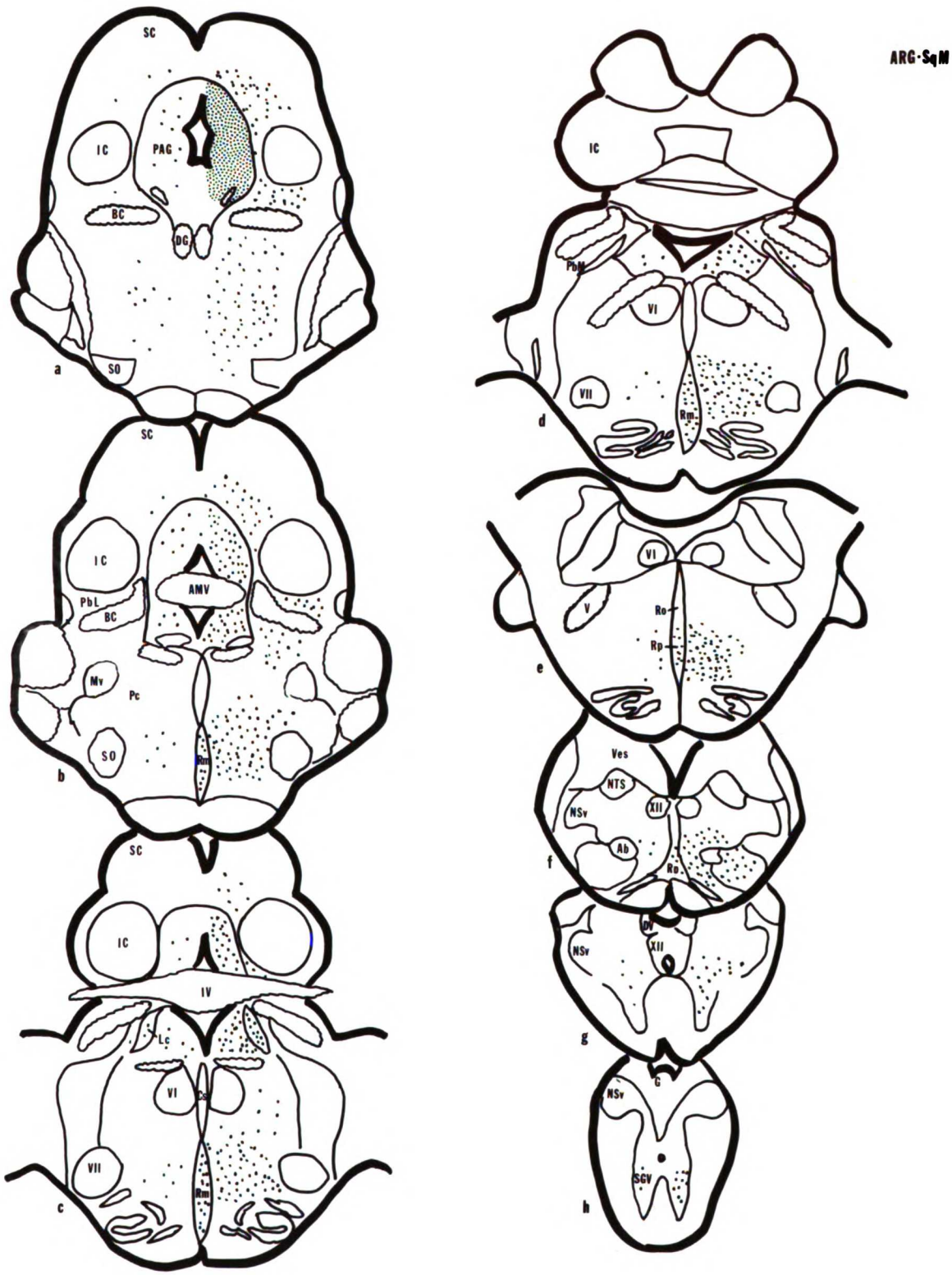
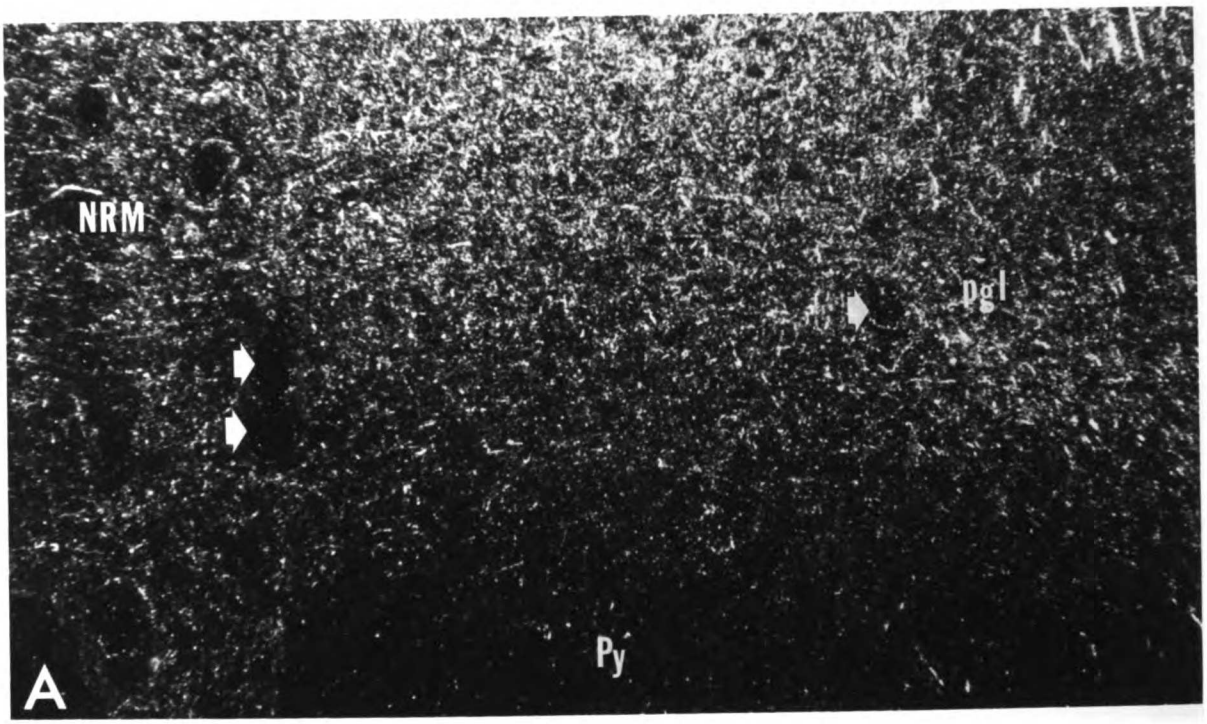

ARG·SqM
SC
IC
PAG
BC
DG
SO
a
SC
IC
PbL
BC
AMV
Mv
Pc
SO
Rm
b
SC
IC
IV
Lc
VI
Cb
VII
Rm
c
IC
PbM
VI
VII
Rm
d
VI
V
Ro
Rp
e
Ves
NTS
NSv
XII
Ab
Ro
f
DV
XII
NSv
g
C
NSv
SGV
h

Figure 34. A. A darkfield photomicrograph to demonstrate the heavy projection to the rostral nucleus raphe magnus (NRM) and the more lateral paragigantocellularis lateralis (pgl) after an injection into the ventrolateral aspect of the PAG. Note the heavy concentration of grains over the NRM and pgl whereas the more ventral pyrmaidal tract is unlabeled and serves as a convenient indicator of the level of background; saimiri sciureus, (experiment SM-7).

B. A lightfield photomicrograph of the above Nissl-stained autoradiograph showing the cellular boundaries of the above section. Arrows indicate prominent blood vessels visible in both photomicrographs; saimiri sciureus, (experiment SM-7).


NRM
Pgl
Py
A

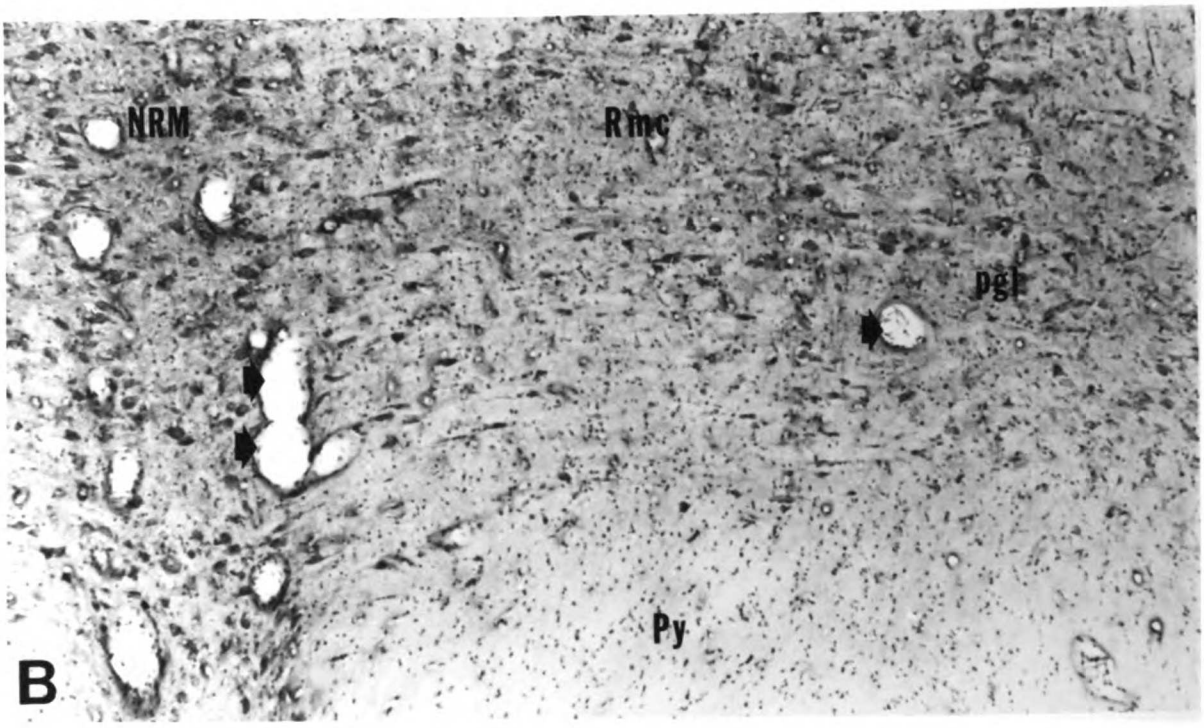
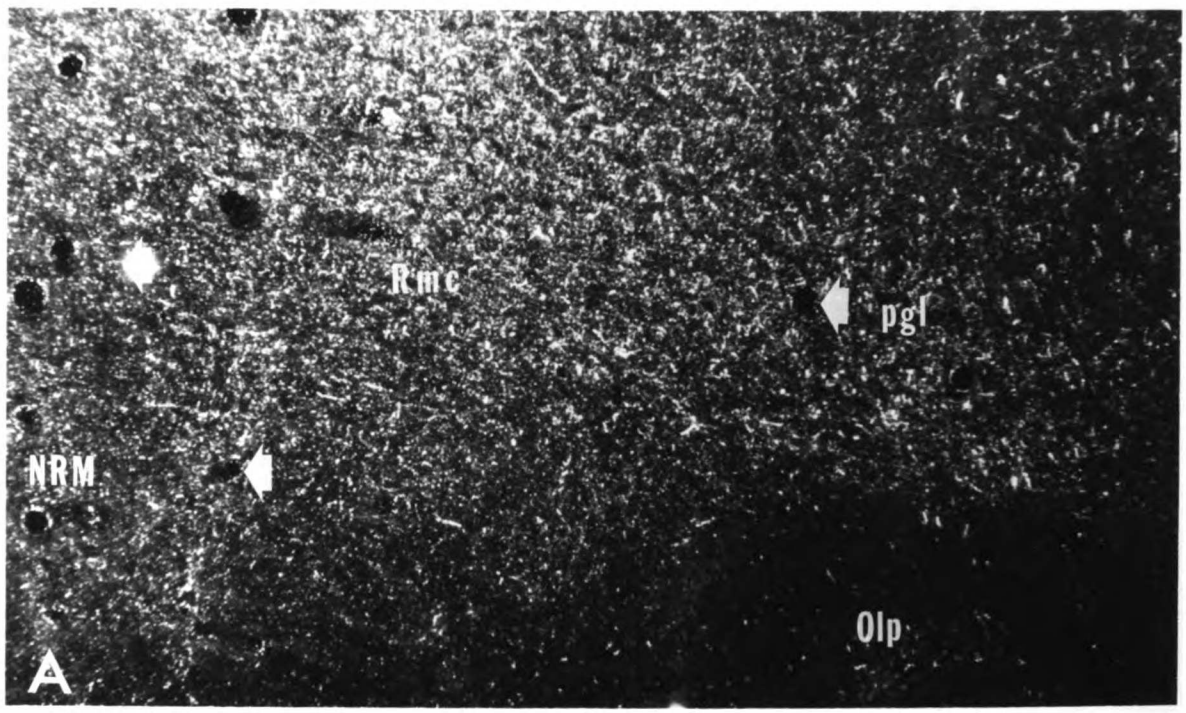
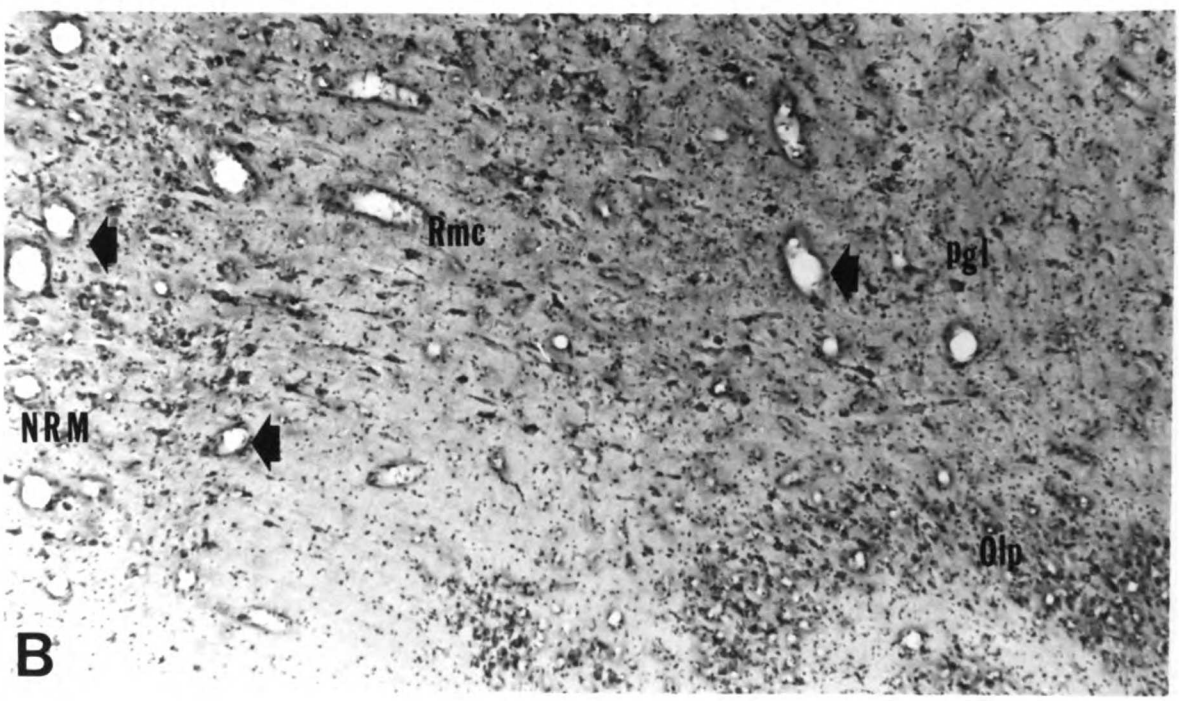

NRM
Rmc
pgl
Py
B

Figure 35. A. A darkfield photomicrograph showing the degree of labeling in the more caudal NRM, pgl, and reticularis magnocellularis (Rmc) after an injection in the ventrolateral aspect of the PAG; saimiri sciureus, (experiment SM-7).

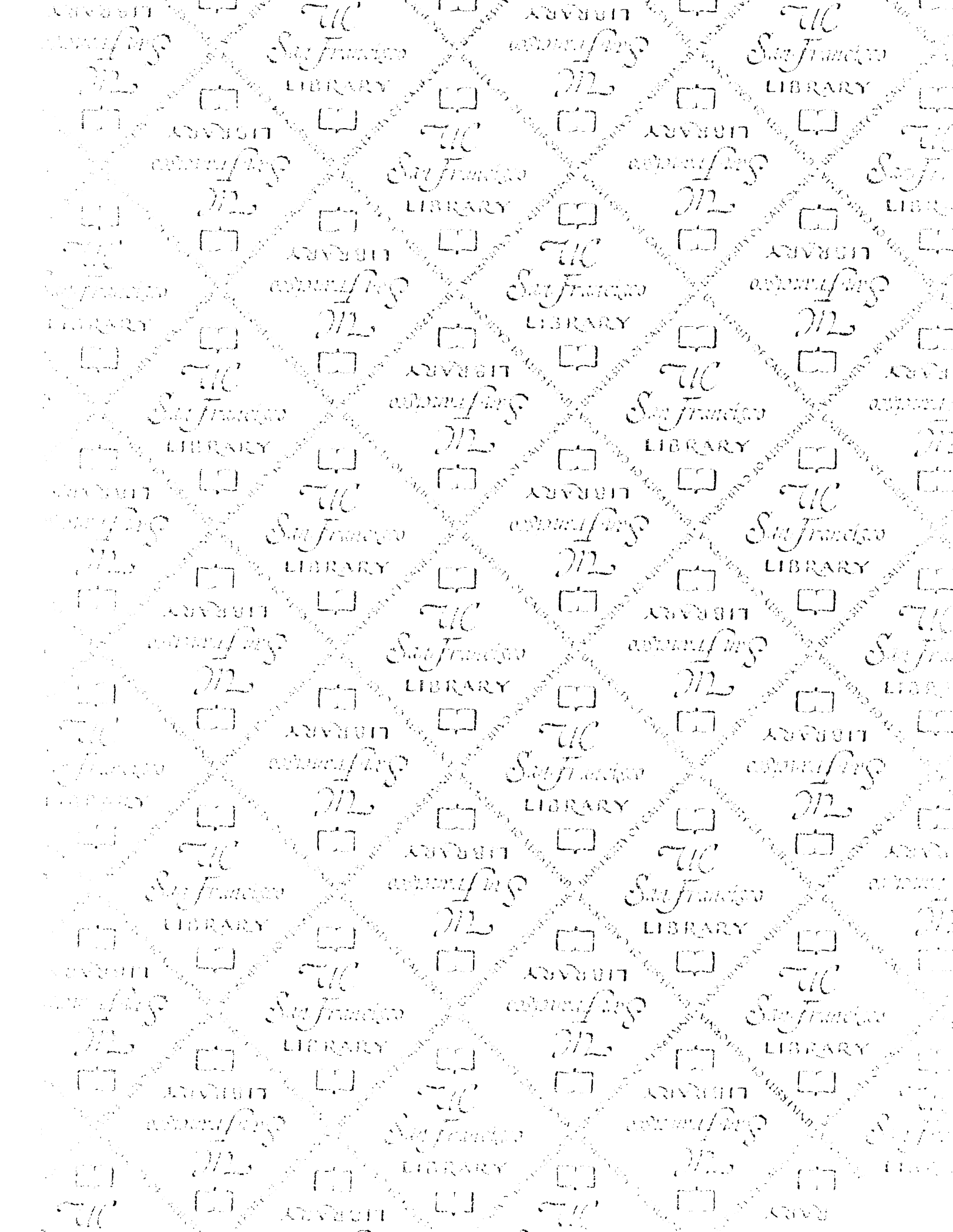
B. A photomicrograph showing the above Nissl-stained autoradiograph under lightfield illumination. Arrows indicate prominent blood vessels for orientation purposes; saimiri sciureus, (experiment SM-7).


Rmc
pgl
NRM
Olp
A


Rmc
pgl
NRM
Olp
B

Acknowledgements:

The execution of this dissertation has been made possible through the kindness, support, constructive criticism and encouragement of my mentors and friends. Dr. Peter Ralston has afforded me the opportunity to do this research and granting me the independence required to do this work; Dr. William Mehler has been a joy to work with and has provided me with a continuing source of inspiration as how to be both a wise and compassionate scientist in the true sense of the word; Dr. Allan Basbaum for his constructive criticism and his infectious scientific intensity; Dr. Howard Fields for his continuous encouragement and his well seasoned philosophy of life; Dr. Doug Schmucker for teaching me how to be an effective and compassionate teacher; Diane Ralston for teaching me how to do surgery; Dave Akers for his photographic skills and patience; Mary Fisher for her always generous attitude; Chong Lam Lee, Marc Peshanski, Jeffery Prior and Joe Rubertone for their friendship and humor; Dr. Steve Colwell for his ideas and his totally cynical sense of humor; and last but not least, Ingrid Abols for being the delightful French physician that she is. (Supported by NASA task 199-05-02-07 to William R. Mehler and NS-11614 to Henry J. Ralston).


San Francisco
LIBRARY