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CHARACTERISTICS OF RETICULAR FORMATION PROJECTIONS FROM THE MEDULLA TO THE FOREBRAIN IN THE NORTH AMERICAN OPOSSUM

The Ohio State University

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CHARACTERISTICS OF RETICULAR FORMATION PROJECTIONS
FROM THE MEDULLA TO THE FOREBRAIN
IN THE NORTH AMERICAN OPOSSUM

DISSERTATION

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

By
Robert Paul Waltzer, B.A.

* * * * *

The Ohio State University
1985

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In Memory of My Mother
Geraldine Epstein Waltzer
ACKNOWLEDGEMENTS

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PUBLICATIONS


Waltzer, R.P. 1982 Evidence that neurons within specific reticular and raphe nuclei innervate either the cervical enlargement of the spinal cord or the anterior lobe of the cerebellum. Anat. Rec. 202: 198A

Waltzer, R.P. and Martin 1982 A double labelling study demonstrating that most cells in the nucleus reticularis gigantocellularis and adjacent raphe

iv
project to either the anterior lobe of the cerebellum or the spinal cord in the rat. Soc. For Neurosci. Abst. 8: 874


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# TABLE OF CONTENTS

DEDICATION .................................................... ii
ACKNOWLEDGEMENTS ............................................. iii
VITA. ............................................................ iv
LIST OF TABLES ............................................. viii
LIST OF FIGURES ............................................ ix
LIST OF ABBREVIATIONS .................................... xii
INTRODUCTION .................................................. 1
MATERIALS AND METHODS ........................................ 5
RESULTS .......................................................... 13
DISCUSSION ..................................................... 27
ILLUSTRATIONS ................................................ 38
LIST OF REFERENCES .......................................... 96
**LIST OF TABLES**

<table>
<thead>
<tr>
<th>Table</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. The area of labeled neurons from each area which received an HRP injection</td>
<td>39</td>
</tr>
<tr>
<td>II. Measurement of the amount of autoradiographic labeling in the thalamus</td>
<td>41</td>
</tr>
<tr>
<td>III. Measurement of the amount of autoradiographic labeling in the hypothalamus</td>
<td>43</td>
</tr>
</tbody>
</table>
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Line drawing of 5 sections through the medulla with the boundaries of the reticular nuclei indicated. Taken from Nissl-stained sections.</td>
<td>46</td>
</tr>
<tr>
<td>2</td>
<td>Line drawing of 5 sections through the medulla after a large injection of HRP in the diencephalon.</td>
<td>48</td>
</tr>
<tr>
<td>3</td>
<td>Photomontage of HRP-labeled cells within the ventrocaudal nucleus reticularis dorsalis.</td>
<td>50</td>
</tr>
<tr>
<td>4</td>
<td>Photomontage of HRP-labeled cells within the dorsal aspect of the nucleus reticularis dorsalis.</td>
<td>52</td>
</tr>
<tr>
<td>5</td>
<td>Plate of different size HRP-labeled and unlabeled cells in the medullary reticular formation.</td>
<td>54</td>
</tr>
<tr>
<td>6</td>
<td>Photomontage of HRP-labeled cells in the nucleus reticularis gigantocellularis.</td>
<td>56</td>
</tr>
<tr>
<td>7</td>
<td>Photomontage of HRP-labeled cells in the nucleus reticularis gigantocellularis; pars ventralis.</td>
<td>58</td>
</tr>
<tr>
<td>8</td>
<td>Line drawing of a sections from an experiment which had a large injection of True Blue into the diencephalon and was processed with the Falck-Hillarp technique.</td>
<td>60</td>
</tr>
<tr>
<td>9</td>
<td>Plate of pairs of photomicrographs taken from the same area under different filter optics to demonstrate both True Blue-labeled cells and monoaminergic cells.</td>
<td>62</td>
</tr>
<tr>
<td>Figure</td>
<td>Description</td>
<td>Page</td>
</tr>
<tr>
<td>--------</td>
<td>-----------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>10</td>
<td>Line drawing of sections from an experiment which had a large injection of</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>True Blue into the diencephalon and was processed with the immunofluorescence</td>
<td></td>
</tr>
<tr>
<td></td>
<td>technique to demonstrate serotonin.</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Photomontage of an area of the midbrain which contains a dense amount of</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>autoradiographic labeling.</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Line drawing of 5 sections through the forebrain after an injection of 3H-</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>leucine into the ventrocaudal nucleus reticularis dorsalis.</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Line drawing of 6 sections through the forebrain after an injection of 3H-</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>leucine into the nucleus reticularis gigantocellularis.</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Line drawing of 6 sections through the forebrain after an injection of 3H-</td>
<td>72</td>
</tr>
<tr>
<td></td>
<td>leucine into the nucleus raphe magnus.</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Line drawing of 5 sections through the forebrain after an injection of 3H-</td>
<td>74</td>
</tr>
<tr>
<td></td>
<td>leucine into the nucleus reticular gigantocellularis; pars ventralis.</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Photomontage of autoradiographic labeling in the pretectal nucleus after an</td>
<td>76</td>
</tr>
<tr>
<td></td>
<td>injection of 3H-leucine into the nucleus reticularis gigantocellularis;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>pars ventralis.</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>Plate of darkfield and lightfield photomontages of autoradiographic labeling</td>
<td>78</td>
</tr>
<tr>
<td></td>
<td>in the ventrobasal nucleus of the thalamus after an injections of 3H-leucine</td>
<td></td>
</tr>
<tr>
<td></td>
<td>into the caudal nucleus reticularis dorsalis.</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>Plate of darkfield and lightfield photomontages of autoradiographic labeling</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>in the nucleus of the diagonal band after an injection of 3H-leucine into</td>
<td></td>
</tr>
<tr>
<td></td>
<td>the caudal nucleus reticularis dorsalis.</td>
<td></td>
</tr>
<tr>
<td>Figure</td>
<td>Description</td>
<td>Page</td>
</tr>
<tr>
<td>--------</td>
<td>-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>19</td>
<td>Plate of darkfield and lightfield photomontages of autoradiographic labeling in the dorsal medial nucleus of the hypothalamus after an injection of 3H-leucine into the caudal nucleus reticularis dorsalis.</td>
<td>82</td>
</tr>
<tr>
<td>20</td>
<td>Plate of darkfield and lightfield photomontages of autoradiographic labeling in the dorsal paraventricular nucleus after an injection of 3H-leucine into the caudal nucleus reticularis dorsalis.</td>
<td>84</td>
</tr>
<tr>
<td>21</td>
<td>Line drawing of 5 sections through the medulla after an injection of HRP into the parafascicular and paracentral nuclei.</td>
<td>86</td>
</tr>
<tr>
<td>22</td>
<td>Plate of HRP injections sites into the forebrain from four separate cases.</td>
<td>88</td>
</tr>
<tr>
<td>23</td>
<td>Line drawing of 5 sections through the medulla after an injection of HRP into the hypothalamus.</td>
<td>90</td>
</tr>
<tr>
<td>24</td>
<td>Line drawing of 5 sections through the medulla after an injection of HRP into the septum and adjacent forebrain areas.</td>
<td>92</td>
</tr>
<tr>
<td>25</td>
<td>Line drawing of 5 sections through the medulla after an injection of HRP into the cerebral cortex.</td>
<td>94</td>
</tr>
</tbody>
</table>
LIST OF ABBREVIATIONS

A1 nucleus alaris
Apo area postrema
AV anterior ventral thalamic nucleus
cn anterior commissure
CA catecholamine
CcD dorsal cochlear nucleus
Cd caudate nucleus
Ce central thalamic nucleus
contra contralateral
CxA hippocampal cortex (ammon’s cortex)
CxH anterior hippocampal cortex
CxPa parietal cortex
CxPr preorbital cortex
CxPx postorbital cortex
DB nucleus of the diagonal band
DOR dorsal
Fac nucleus of the facial nerve
fx fornix
g genu of the facial nerve
G giant cell
GLD dorsal nucleus of the lateral geniculate
GP globus pallidus
Gr gracile nucleus
HAD anterior dorsal hypothalamic nucleus
HDM dorsal medial hypothalamic nucleus
Hg nucleus of the hypoglossal nerve
HRP horseradish peroxidase
HyD dorsal hypothalamic area
HyL lateral hypothalamic area
ipsi ipsilateral
ISTa interstitial nucleus of the stria terminalis: anterior part
L large cell
LAT lateral
LR lateral reticular nucleus
M medium sized cell
MD medial dorsal thalamic nucleus
OI inferior olivary nucleus
Pc paracentral nucleus
ped cerebral peduncle
PF parafascicular nucleus
PFP posterolateral parafascicular nucleus
PH  posterior hypothalamic nucleus
PHA anterior paraventricular nucleus
PHD dorsal paraventricular nucleus
Po posterior thalamic nucleus
POL lateral preoptic area
POM medial preoptic area
PrT pretectal nucleus
pyr pyramidal tract
RaM raphe magnus
RD nucleus reticularis dorsalis medullae oblongata
RF reticular formation
RGc nucleus reticularis gigantocellularis
RGcv nucleus reticularis gigantocellularis; pars ventralis
RL nucleus reticularis lateralis
RP nucleus reticularis pontis
RPC nucleus reticularis parvocellularis
RV nucleus reticularis ventralis medullae oblongata
S small cell
SI substantia innominata
SL lateral septal nucleus
sm stria medullaris thalami
SM medial septal nucleus
SO supraoptic nucleus
trs tract of the spinal trigeminal nerve
TS nucleus of the solitary tract
VA ventral anterior thalamic nucleus
VENT ventral
Vstl lateral vestibular nucleus
xVB ventral basal thalamic nucleus
ZI zona incerta
III third ventricle
3H-Leu tritiated leucine
S-HT serotonin
INTRODUCTION

It is becoming clear that cytoarchitecturally distinct regions of the medullary reticular formation (RF) differ in their connectional and chemical characteristics. For example, it has been shown that some regions project heavily to the spinal cord, whereas others do not (Martin et al. '81, '85b), that projections to lamina IX motoneurons and laminae I and II arise within different nuclei (Martin et al. '81, '85b), and that such projections are partly monoaminergic (Martin et al. '82). Differences in the projections of various medullary reticular nuclei to the forebrain may also exist. The ventrolateral RF of the medulla has been reported to innervate the paraventricular nucleus of the hypothalamus (the rat, Loewy et al. '81), whereas other reticular areas do not (the cat, Graybiel '77), and projections to the forebrain from the RPC were not reported by Holstege et al. ('77), Vertes, ('84), and Vertes et al. ('84). At least some of the forebrain projections of the medullary RF are monoaminergic (Sawchenko and Swanson '82). Although selected forebrain projections of the medullary RF have been studied (rat, Loewy et al. '81, Graybiel '77, Nakano et al. '85), there
are no reports which apply a variety of tracing techniques to systematically assess the projections of different nuclei in the same species.

The studies reported here were designed to determine in the North American opossum: 1) the location of all neurons within the medullary RF which project to the forebrain, 2) whether any such neurons are monoaminergic, 3) whether individual nuclei of the medullary RF innervate the same or different forebrain targets, and 4) the location of RF neurons which project to specific forebrain regions. The North American opossum was chosen as the experimental animal because: 1) its brain is relatively generalized, suggesting that patterns of connectivity observed within it may be present in other species; 2) the organization of reticular projections to certain cranial nerve nuclei (Panneton and Martin '83) as well as to the spinal cord (Martin et al, '81, '82) has been previously reported from earlier experiments; 3) and we hope to take advantage of the opossum’s unique embryology to study the development of reticular connections. The opossum is born 12 days after conception and is available in a very immature state for experimental manipulation (see review by Martin et al. '78).

We have attempted to identify all of the neurons in the medullary RF which project to the forebrain by
employing the retrograde transport of horseradish peroxidase (HRP) (Lavail and Lavail '72). In those experiments, we attempted to fill the diencephalon with multiple injections of HRP in order to obtain maximal incorporation of the tracer by axon terminals and to injury label axons passing through the diencephalon to other areas of the forebrain.

In order to establish if any of the medullary RF neurons which innervate the forebrain are monoaminergic, we employed two approaches. In the first, the retrograde transport of the fluorescent marker True Blue (TB) was used in conjunction with the Falck-Hillarp technique for identifying monoaminergic neurons (Falck '62) and in the second, the same marker was combined with the immunofluorescence method of Coons ('58) for serotonin (Sawchenko and Swanson, '81). Multiple injections of TB were made in order to label as many forebrain-projecting neurons as possible.

In order to determine whether individual nuclei of the medullary RF project to different forebrain targets, we used the autoradiographic technique. For these studies, we analyzed the forebrain labeling produced by injections of 3H-leucine into different nuclei of the medullary RF. Based on the autoradiographic results we made relatively restricted injections of HRP or WGA-HRP into specific
areas of the forebrain innervated by the medullary RF. Such cases were used to map the locations of RF neurons which innervate restricted forebrain targets.
MATERIALS AND METHODS

For the retrograde transport studies, injections of either 50% HRP (volumes of .02-.08 ul) or 2.5% WGA-HRP (volumes of .04 to .18 ul) were made into different areas of the diencephalon in 30 opossums. In 5 cases large, multiple injections were made so as to fill the diencephalon with HRP. Such injections were centered in areas shown by the autoradiographic method to contain bundles of reticular axons which distribute to the entire forebrain. In 30 cases small injections were made into specific areas innervated by axons of the medullary RF. All animals were anesthetized for the surgical exposure by sodium pentobarbital (40 mg/kg) and subsequently placed in a stereotaxic head frame for the injection. The location of structures in the opossum's brain relative to bony landmarks varies from animal to animal, making it necessary to establish injection coordinates from landmarks on the brain itself. In some animals the position of the needle in the midline at the inferior colliculus cerebellar junction was used as the "zero" point. The injection needle was then moved an appropriate distance rostrally from that landmark and then laterally before being lowered
to the desired depth. Coordinates were taken from the atlas of Oswaldo-Cruz and Rocha-Miranda ('68) and from our own collection of Nissl-stained material, which we also used to determine the boundaries of the reticular nuclei. In other cases the surface of the diencephalon was exposed by aspiration of the overlying cortex so that an initial touch point could be established on the diencephalon itself. These injections were made with the aid of a surgical microscope. After a three day survival all animals were deeply anesthetized and perfused intracardially with warm physiological saline followed by cold 1% paraformaldehyde and 1.25% glutaraldehyde. The brains were removed, blocked and placed in a 30% sucrose solution for cryoprotection. Sections were cut at 50um on a freezing microtome and processed for HRP using either tetramethylbenzidene (TMB) (Mesulam '78) or O-dianisidine (de Olmos '77) as the chromagen. In many cases alternate sections were processed using TMB and O-dianisidine. The tissues were mounted on subbed slides, counterstained, and coverslipped.

Sections were examined with a Leitz microscope using light and darkfield optics and the position of labeled cells was recorded on drawings of the sections using an X-Y plotter interfaced with the microscope stage. Representative photomicrographs were taken using light and
dark field optics. Some of the HRP material was analyzed through the use of the image analysis system "Ideas" (Computer Institute) equipped with a program (also called Ideas) for morphometry. In the case analyzed with the large diencephalic injection (P741 in Fig. 2), many neurons were labeled. Therefore, only one out of eighteen sections was chosen for morphometric analysis. This is the first case documented in table I. The other cases included in table I had smaller injections (some were illustrated in Figs. 21, 23, 24, and 25 and others were not illustrated). To maintain consistency across all the cases, neurons were measured and counted on every third section. A video image of each cell was analyzed was circled using a light sensitive pen so that its area could be calculated by the computer. Data on number, size and laterality of labeled cells are presented in tabular form (table I). Small cells range from 1 to 300 um (Fig. 5A,B); medium cells from 301 to 600 um (Fig. C-F); large cells from 601 to 900 um (Fig. 5E,F); and giant cells range from 901 to 1200 um (Fig. 5D).

The experiments designed to identify the origin of monoaminergic axons in the diencephalon were done as follows. Six animals were anesthetized and stabilized for sterile surgery as described previously. After the appropriate exposure, injections of 6% TB were made into
the diencephalon. The injections were intentionally large and centered in areas shown by the autoradiographic method to contain major bundles of reticular axons as described above. Quantities from 3 to 14 ul were delivered in multiple injections, so as to include both the thalamus and hypothalamus. Most animals were injected intraperitoneally with a monoamine oxidase inhibitor (pargyline, 200 mg/kg) and a serotonergic precursor (L-tryptophan, 100 mg/kg) prior to sacrifice in order to enhance serotonin levels.

Three of the above animals were allowed to survive for 7 days before being deeply anesthetized so that the brains could be removed quickly, blocked and placed in isopentane cooled with liquid nitrogen according to the procedure of Falck (‘62). The tissue blocks were lyophilized for 2 weeks before being exposed to humidified paraformaldehyde vapors, embedded in paraffin, and sectioned at 20 μm. The sections were mounted onto slides and coverslipped using a non-fluorescing mounting medium.

The remaining 3 animals were allowed to survive for 10-14 days before being anesthetized and perfused with warm physiological saline followed by ice cold 4% paraformaldehyde in neutral phosphate buffer. The brains were removed, blocked, and kept overnight in a 15% sucrose solution. Sections were cut at 25μm on a freezing microtome, rinsed in phosphate buffered saline (PBS) and
then placed in a primary antiserum generated against serotonin (5-HT) for 2 days at 4-10 degrees. The antiserum was diluted 1/5000 in a solution of phosphate buffered saline (PBS, pH 7.4), 0.3% Triton X-100, and bovine serum albumin. While in the primary antiserum, sections were kept on ice and placed on a shaker during the day. At night they were left in the refrigerator. After two days in the primary antibody, the sections were rinsed three times in PBS and then placed in the secondary antibody, goat antirabbit conjugated to fluorescein (Antibodies Incorporated). Sections were shaken in the secondary antibody for 1 hour at room temperature, rinsed three times in PBS, mounted onto slides, and coverslipped with PBS/glycerin 3:1. Alternate unreacted sections were taken for retrograde labeling and for Nissl staining. The control serum consisted of the same antibody, pretreated overnight (4 degrees C) with 0.05 mg. of serotonin/ml of the diluted antibody.

The primary antibody had been prepared and characterized in Dr. Robert Elde's laboratory at the University of Minnesota as follows. Rabbits received subcutaneous injections of 1 mg of serotonin creatinine sulphate conjugated to bovine serum albumin in combination with Freund's complete adjuvant. The serum of these animals was then tested for specificity of binding to 5-HT
using animals treated with 5-HT depleting drugs such as reserpine, parachlorophenylalanine (PCPA) and 5,7, dihydroxytryptamine. In all such cases immunostaining was markedly reduced. PCPA results in increased tryptamine in tissue, and since there was no increase in the staining, a lack of cross-reactivity with tryptamine was assumed. L-Dopa increases melatonin, but decreases 5-HT. L-Dopa treatment resulted in decreased staining in the pineal body, indicating a lack of cross-reactivity with melatonin.

Sections from the cases described above were analyzed on a Leitz orthoplan microscope equipped for epifluorescence. True Blue has a maximum excitation wavelength of 360nm and was visualized using the A filter system of Leitz. The fluorescence characteristic of catecholamines and serotonin in the Falck-Hillarp processed material (maximal excitation wavelength of 410 and 415 respectively) and that of fluorescein (maximal excitation wavelength between 480 and 490) could be observed using the I2 filter system. An X-Y plotter interfaced with the microscope stage was utilized to plot the location of cells containing TB and/or monoamines.

The autoradiographic cases were available from previous studies of reticulospinal (Martin et al '81) and propriobulbar (Panneton and Martin '83) projections. Using stereotaxic techniques, placements of 3H-leucine had been
made into various regions of the medullary reticular formation and raphe nuclei of 18 anesthetized animals. The obex was used as a visual reference, and the injections were delivered in volumes of 0.1 to 0.4 ul using concentrations varying from 100-400μCi/ul. The animals were allowed to survive for 8-14 days before being anesthetized again and perfused with physiological saline followed by 10% formalin. The brains were removed and placed in 10% formalin until processing. They were then blocked and sectioned on a freezing microtome at 32μm. The sections were mounted onto subbed slides, defatted, dipped in NTB-2 emulsion diluted with water, and placed into light tight boxes for refrigeration from 4—12 weeks. The slides were developed in D-19 and stained through the emulsion for Nissl substance.

Sections were examined and photographed using light and darkfield microscopy and the location of autoradiographic label was transposed onto drawings of the sections made with an overhead projector. Quantitative analysis of autoradiographic labeling was done through computer assisted densitometry using the Quandens program of the "Ideas" system. In regions of interest the densest labeling within a squared area was measured. Intensity limits were chosen so that the scans would selectively measure the area occupied by the silver grains. The
computer then calculated the area occupied by silver grains over that of the total area within the square to get percentages which are listed in tables II and III. Areas containing little or no labeling were measured to provide a baseline for comparison.
RESULTS

In the following account we will first describe the location and boundaries of reticular nuclei in the opossum's medulla. That will be followed, in order, by results obtained from cases subjected to large forebrain injections of HRP or WGA-HRP, large forebrain injections of TB and processing for monoamines, injections of 3H-leucine into different nuclei of the medullary RF and injections of HRP or WGA-HRP into restricted areas of forebrain innervated by the medullary RF.

NUCLEAR DIVISIONS OF THE MEDULLARY RETICULAR FORMATION

Sections corresponding to those used in other illustrations have been drawn from Nissl stained preparations of the opossum's brainstem and presented in Fig. 1. In each section we have outlined the boundaries of individual nuclei as described by Oswaldo-Cruz and Rocha-Miranda ('68) and verified by us.

The nucleus reticularis dorsalis (RD, Fig. 1B-E) is located medial to the spinal trigeminal nucleus and ventral to the nucleus of the solitary tract. It extends from the caudal end of the medulla to the facial nucleus.
and contains small and medium sized neurons. RV is located medial to RD and dorsal to the caudal one-third of the inferior olive.

The nucleus reticularis lateralis (RL, Fig. 1A-D) is located dorsolateral to the inferior olive and extends from the rostral one-third of that nucleus to the rostral end of the facial nucleus. Rostrally, RL occupies a position medial to the facial nucleus and dorsolateral to the pyramids. The rostral part of RL was indicated by an asterisk in Fig. 1A because it was not identified as such by Oswaldo-Cruz and Rocha-Miranda ('68). Neurons in RL are small and medium sized.

The nucleus reticularis gigantocellularis (RGc, Fig. 1A-D) extends from the RV caudally to the trapezoid body rostrally. It contains small, medium, large, and giant neurons. The presence of giant neurons aids in distinguishing this area from surrounding nuclei.

The nucleus reticularis gigantocellularis; pars ventralis (RGcv, Fig. 1A,B) extends from the inferior olive to the trapezoid body. It contains mostly medium to large cells, many of which have a medial to lateral polarity.

The nucleus reticularis parvocellularis (RPC, Fig. 1A,B) extends from a level just caudal to the facial nucleus to the trapezoid body and is composed of small to medium sized neurons. In sections through the facial
nucleus, the RPC dominates the lateral part of the reticular formation.

**BULBAR RETICULAR LABELING PRODUCED BY LARGE INJECTIONS OF HRP OR WGA-HRP**

Multiple, large injections of HRP or WGA-HRP were made into the diencephalon of 5 opossums. In the case with the largest injection the marker spread across the midline (Fig. 2) as well as into the preoptic area and ventral midbrain. The effectiveness of the injection was demonstrated by the presence of retrograde labeling in layer VI of the entire neocortex on the side of the injection, as well as within the deep nuclei of the cerebellum (Fig. 2A), the principal and spinal trigeminal nuclei (Fig. 2A-E), and the dorsal column nuclei (Fig. 2D,E) primarily contralateral to the injection.

Neuronal labeling was present within all nuclei of the medullary reticular formation (RF). That within the nucleus reticularis dorsalis medullae oblongata (RD) was found primarily on the side contralateral to the injections, and in some sections (Figs. 2E, 3, 4) it formed part of a band of labeling which extended from beneath the spinal trigeminal tract to the magnocellular nucleus of the solitary tract (TSc). Most of the labeled cells in RD were small, but some were medium sized. The
size, number, and location of labeled neurons in all the HRP and WGA-HRP cases to be described can be found in table I. Examples of labeled cells which varied in size are demonstrated in Fig. 5.

The nucleus reticularis ventralis medullae oblongata (RV) contained fewer labeled cells than RD (see Fig. 2E). Such cells were located predominantly contralaterally and most of them were small (table I). Labeled neurons within the nucleus reticularis lateralis (RL) were located mainly on the side of the injection and were small to medium-sized (Fig. 2B-E).

The nucleus reticularis gigantocellularis (RGc) was labeled predominantly contralateral to the injection. Labeled neurons were found throughout the rostral to caudal extent of RGc although they were most numerous rostrally (Fig. 2 A-D). Most of the labeled neurons were small, but some were medium sized (table I, Fig. 5A,D). No large or giant cells contained HRP. On the side contralateral to the injection, some of the labeled neurons were clustered ventral to the rostral extreme of the hypoglossal nucleus and the nucleus prepositus hypoglossi (see Figs. 2B,C and 6).

The nucleus reticularis gigantocellularis; pars ventralis (RGcv) was labeled predominantly ipsilaterally and rostrally (see Figs. 2A,B and 7). Most of the labeled
cells were small, but some were medium sized (table I, Fig. 5B, E, and F). The nucleus reticularis parvocellularis (RPc, Fig. 2A) was also labeled, predominantly ipsilaterally, but only small neurons showed evidence of the marker.

Small and medium sized neurons were labeled in the nuclei obscurus and magnus raphae but in the latter nucleus an occasional large neuron also contained HRP.

**STUDIES DESIGNED TO DETERMINE IF MONOAMINERGIC NEURONS IN THE MEDULLARY RF PROJECT TO THE FOREBRAIN**

Three opossums were employed for studies which combined the retrograde transport of TB with the Falck-Hillarp technique. In the case shown in Fig. 8, TB spread throughout the hypothalamus and included most of the intralaminar and medial nuclei of the thalamus. Such areas are major targets of axons from the medullary RF (see autoradiographic results) and are relatively rich in both catecholaminergic (unpublished results) and serotonergic (Martin et al. '85a) axons. Although TB labeled cells were present in the spinal trigeminal nucleus and cerebellum, few were found in the dorsal column nuclei.

TB labeling in the bulbar reticular formation and raphe was qualitatively comparable to that seen after large injections of HRP or WGA-HRP. TB labeled cells were
intermixed with catecholamine (CA) cells in areas of the ventrolateral medulla (see Fig. 8C-E) which included RD and RL. Roughly one eighth of the CA cells contained TB (Figs. 8C,D and 9A,B). In another case, neurons containing both TB and CA were also found within dorsal parts of RD. Spectrophotometric data corroborated the conclusions reached by visual examination. TB labeled cells were intermixed with indolamine cells in the RM, RGc, and the RGcv, but none contained both substances (Fig. 8A-C).

Three cases with TB injections of the diencephalon were processed immunocytochemically for serotonin. In the one to be described (P806, see Fig. 10), the injection included the dorsal half of the hypothalamus and most of the thalamus. TB-labeled neurons were numerous in rostral portions of the spinal trigeminal nucleus, the deep nuclei of the cerebellum, and the dorsal column nuclei. Neurons containing either TB or serotonin-like immunofluorescence were intermixed in the RGcv (Figs. 9C,D, and 10A) and RM, but none contained evidence for both markers. In another case, two double labeled cells were found in the RGcv, but the dye spread to the periaqueductal grey of the midbrain.
AUTORADIOGRAPHIC STUDIES OF FOREBRAIN PROJECTIONS FROM THE MEDULLARY RETICULAR FORMATION

Nine of the 18 autoradiographic cases available were used for this study because they had injections of 3H-leucine which included regions labeled after large diencephalic injections of HRP or TB. Plots of the labeling found in 4 cases are presented in Figs. 12-15 and the results of measurements of the percentage of area occupied by silver grains can be found in tables II and III. In all cases, except the one with an injection of the nucleus reticularis parvocellularis, bundles of labeled axons could be traced bilaterally through the brainstem to a position dorsomedial to the red nucleus (Fig. 11). At that level, the major bundle on each side spilt into a dorsal division, which became positioned dorsal to the retroflex fasciculus, and a ventral division located ventral to it.

Labeling associated with transport through the dorsal bundle was found in the parafascicular and pretectal nuclei (PF and PrT in Figs. 12E, 13F, 14F, 15E, and 16), as well as within the central nucleus of the thalamus (Ce in Figs. 12 D: 13D,E; 14D,E; and 15D). Labeling associated with axons in the ventral bundle was distributed more widely. In the diencephalon, labeled axons coursed primarily dorsal to the lateral hypothalamus although some
were present in that area (HyL, Figs. 12C-E, 13D-F, 14D-F, and 15C-E). Labeled axons also coursed laterally within the zona incerta (Z1, Figs. 12D,E; 13E,F; 14E,F; and 15D,E) to reach medial parts of the ventral lateral geniculate nucleus and, in some cases, the lateral part of the ventrobasal complex (xVB, Figs. 12D,E; 14F, 15D; and 17). Labeling from the ventral bundle extended rostrally from the hypothalamus into the lateral preoptic area and the dorsal limb of the nucleus of the diagonal band (DB, Figs. 12A,B; 13A,B; 14B,C; and 18). Qualitative (Figs. 14-16) and quantitative (tables II and III) analyses suggest that differences exist in labeling density in the same regions between cases.

Although there were a number of similarities between cases, differences were also noted. In brains with injections centered within ventrolateral RD, with some involvement of RL (Fig. 12), or with injections centered primarily within RL, labeling was found in several areas not labeled in the other cases. Such areas included the dorsomedial nucleus of the hypothalamus (HDM, Figs. 12D and 19), which was labeled heaviest ipsilaterally, the dorsal paraventricular nucleus (PHD, Figs. 12C and 20), which was labeled most densely contralaterally, as well as areas bordering the anterior paraventricular (PHA, Fig. 12B) and supraoptic (Fig. 12B) nuclei. The labeling related to the
contralateral dorsal paraventricular nucleus was found only over areas containing small cells. Light labeling, unique to this case, was also found just lateral to the pretectal nucleus in the lateral posterior nucleus of the thalamus (LP, Fig. 12E), in the dorsal lateral geniculate nucleus (Fig. 12E), and in the median eminence.

Areas labeled after R6c injections, which were not labeled in the other cases, included the globus pallidus and substantia innominata (Fig. 13C), as well as layers III and VI of the cerebral cortex (Fig. 13A-C). Although the cases shown in Figs. 14 and 15 contained no cortical labeling, such labeling was present in another brain with an injection which included the RGcv and the lateral edge of the raphe magnus.

Case 582 of our collection (not illustrated) contains an injection of 3H-Leucine which fills most of the nucleus reticularis parvocellularis (RPc). Cranial nerve nuclei of the brainstem are labeled heavily, but little evidence for forebrain labeling was found outside of the medial hypothalamus.
LABELING WITHIN THE BULBAR RETICULAR FORMATION PRODUCED BY INJECTIONS OF HRP OR WGA-HRP INTO SPECIFIC AREAS OF THE FOREBRAIN

Relatively small injections of HRP or WGA-HRP were made into forebrain areas shown by the autoradiographic method to contain axons from the medullary RF. The results of morphometric analysis of each case presented (Figs. 21-25) are provided in table I. In the case illustrated in Fig. 21 the injection (HRP) included the PF, the paracentral, and the mediodorsal nuclei of the thalamus (Fig. 22A). This case was chosen for presentation because PF is a major target of axons in the dorsal bundle. This relatively small injection labeled neurons in all nuclei of the medullary RF except Rpc. Neurons were labeled in RD and RV contralaterally (Fig. 21E), the RL and R6c predominantly ipsilaterally (Fig. 21B-D), the RGcv primarily ipsilaterally (Fig. 21A), and the RM (not illustrated). Labeled neurons in this case and in the other small cases were never labeled in areas which were unlabeled in the larger case.

After injections of PrT (e.g. Fig. 22B), another major target of the dorsal bundle, labeled neurons were found bilaterally within the central part of RL, within R6c and within lateral RGcv. Where present, neurons labeled after injections into either PF or PrT were generally
comparable in size, position, and laterality to those seen after large injections (table I).

The case illustrated in Fig. 23 contains an injection of WGA-HRP within the lateral hypothalamic area which spread to the dorsomedial hypothalamic nucleus. This case was chosen for illustration because it included several areas innervated by the ventral bundle. As might be expected from the autoradiographic results, labeled neurons were found in all nuclei of the medullary RF. Bilateral labeling was present in caudal RD (Fig. 23D,E) as well as throughout most of RV (not illustrated). The RL was sparsely labeled, bilaterally (Fig. 23B-D), and most of the labeled cells were found caudally. Labeled cells were found throughout RGc, particularly on the side of the injection, and in rostral sections they tended to cluster medial and ventral to the TSc. Labeled neurons were also present bilaterally within the RGcv (Fig. 23A,B). Only a few labeled neurons were found in RPC (not shown in Fig. 23), although they were present bilaterally. Labeled neurons were particularly numerous in the RM in sections through rostral parts of the facial nucleus (Fig. 23A).

Injections were made into several diencephalic areas supplied by axons of the ventral bundle. In case P824 (not illustrated) an injection of WGA-HRP was made which included the GLV and the adjacent ZI with some
involvement of the rostrolateral substantia nigra. Neurons were labeled within the RL bilaterally, the RGc contralaterally, and the RGcv ipsilaterally. Labeling was also found in the RM. Most of the labeled cells in the RGc were located near the hypoglossal nucleus and the nucleus prepositus hypoglossi. Injections of GLD (P840) or LP (P816) labeled neurons in RGcv laterally and injections of either the anteroventral or lateral intermediate nuclei (P814) labeled neurons in the contralateral RL. Injections restricted to the Po (P820) did not label the medullary RF.

In case 851 a WGA-HRP injection was centered in the ventrobasal nucleus (xVB) with spread to the ventrolateral (VL) and the ventral anterior (VA) nuclei. The effectiveness of the injection was reflected by extensive retrograde labeling within the spinal trigeminal, dorsal column, and deep cerebellar nuclei. In the bulbar reticular formation RD, RL, and RGc were labeled contralaterally, the RGcv was labeled bilaterally, with an ipsilateral predominance, and the RPC was labeled ipsilaterally. Some labeled neurons were present in the contralateral RD, where they were located beneath the spinal trigeminal tract. This pattern of labeling was similar to that seen after large diencephalic injections (Fig. 3).

In 2 animals HRP injections were made into the
preoptic area, another target of the ventral bundle. In P767 (Fig. 22D) the injection was limited to the lateral preoptic area (POL). Although relatively few in number, labeled neurons were found in all reticular nuclei of the medulla except the RPC. The RD contained the greatest number of such neurons (2 to 3 per section), which were found bilaterally, whereas RL contained only one on each side. The RV and RGc were only labeled ipsilaterally, but RGcv was labeled bilaterally. Only two labeled cells were found in the raphe magnus. After an injection of POM a few labeled neurons were found bilaterally in RD and RM.

HRP injections were centered in the septal-diagonal band region in two animals. In Fig. 24 we have presented results from the case with the larger injection (P781) which spread into adjacent telencephalic areas. Fairly extensive labeling was present in RD, bilaterally, but with an ipsilateral predominance (see Fig. 24D,E). In contrast, RV contained only one labeled cell which was located on the side opposite the injection. A few neurons were labeled in RL, RGc, and RGcv, primarily on the side of the injection (Fig. 24A-D). Labeling was sparse within the raphe magnus.

In two animals multiple injections of WGA-HRP were made into areas of the neocortex labeled in the autoradiographic experiments. The injections were intentionally large because relatively few cortical axons
were labeled in the autoradiographic material. The case with the most RF labeling is illustrated in Fig. 25. Labeled neurons were few in RD, but they were somewhat more numerous within RV, RL, RGc, and RGcv. Most of the labeled neurons were found ipsilaterally. An occasional neuron was even labeled within the RPC and the raphe magnus (see Fig. 25A, B).

Other telencephalic areas which received injections of HRP or WGA-HRP included the hippocampus and the striatum. Although there was no autoradiographic labeling in these areas after brainstem injections of 3H-leucine, an occasional neuron was labeled in the RGcv and the raphe magnus.
DISCUSSION

TECHNICAL CONSIDERATIONS

Each of the tracing techniques employed in this study has limitations. The use of markers such as HRP (Lavail and Lavail '72), WGA-HRP (Gonatas et al. '79), and TB (Van der Kooy et al. 78) is limited by the fact that there is evidence that they may be taken up injured axons passing through the injection site (Adams and Warr '76, Trojanowski et al. '82). This results in difficulty determining whether retrograde (or orthograde) labeling is due to uptake by axons of passage or by terminals. It is also difficult to judge the effective size of an injection and the critical density of axonal terminals needed to produce retrograde labeling (Jones '75). It is for these and other reasons that the results of retrograde transport studies should be interpreted in light of observations made using other techniques.

The use of the Falck-Hillarp method in conjunction with TB for retrograde labeling presents additional problems. For example, fluorescence is lost after exposure to ultraviolet light and over time, and the bright fluorescence of serotonergic cells often makes it difficult
to tell if they contain TB.

We chose to use TB labeling in conjunction with immunohistofluorescence for serotonin to confirm the absence of double labeled cells found with the Falck-Hillarp method. Immunohistofluorescence is not without problems, however. Most importantly, the validity of the results depends on the specificity of the antibody and the results of absorption controls. As in the above experiments, loss of fluorescence due to ultraviolet light exposure and time presents difficulties. Loss of TB labeling during processing for immunohistofluorescence can also occur, but is minimized by proper perfusion (Sawchenko and Swanson '81). In any case, the results of these approaches complement one another.

Although data from other experiments done in this laboratory suggest that the autoradiographic method is not as sensitive as the orthograde transport of WGA-HRP for tracing axons, the former method was chosen for this study because 3H-amino acids are not taken up by axons of passage in sufficient quantities to be demonstrated autoradiographically (Cowan et al. '72). This may not be true for WGA-HRP and is a particularly critical issue in studies of reticular connections. The limits of effective injection sites in autoradiographic studies are difficult to define. In our experiments we chose to be conservative
and include the halo around the area of greatest radioactivity.

Care must be taken in determining whether the absence of labeling in an area actually means that there are no pathways from the injection site to that area. In our studies, such determinations were aided by the use of more than one technique. For example, 3H-leucine injections into the RGcv failed to label RGcv axons in the neocortex, but cortical injections of WGA-HRP labeled RGcv neurons. Since the HRP technique is usually more sensitive than autoradiography, particularly for demonstrating axons over long distances, we assume that some RGcv axons do indeed reach the neocortex. On the other hand, injections of 3H-leucine into the RM resulted in axonal labeling within the PrT, but labeled neurons were not seen in RM after pretectal injections of HRP. In this case RM axonal collaterals may have been too few to incorporate and transport HRP in sufficient quantities for detection or the effective part of the injection may not have included RM axons.

**GENERAL CONCLUSIONS CONCERNING THE ORGANIZATION OF FOREBRAIN PROJECTIONS FROM THE MEDULLARY RF**

Five general conclusions can be drawn from our results: 1) all reticular nuclei of the medulla project to the forebrain, although the projections of Rpc are sparse,
2) most reticular nuclei innervate multiple and widely divergent forebrain targets, 3) axons from different nuclei of the medullary RF converge on common forebrain targets, 4) some nuclei of the medullary RF have projections which are unique to them and 5) some forebrain projections of the medullary RF are catecholaminergic. Each of these conclusions will be discussed separately.

Classical studies, using retrograde degeneration techniques, suggested that forebrain projections from the medullary RF arise primarily from its medial two-thirds (Brodal '56). In contrast, our results indicate that they originate within all medullary RF nuclei and that forebrain projecting neurons within some areas of the lateral medulla (RD and RL) are actually numerous. Our findings are not unique to the opossum, since comparable results have been obtained in the rat (Loewy et al. '81, Vertes '84). As suggested by Brodal ('56), the largest (giant) neurons of the medullary RF do not innervate the forebrain.

It is obvious from our studies and those of others (Graybiel '77, Loewy et al. '81, Vertes et al. '84, Peschanski and Besson '85), that individual nuclei of the medullary RF project to widely divergent forebrain areas. However, it is not clear whether axons of single neurons innervate such areas by collaterals. Preliminary results from double labeling studies suggest that neurons of the
medullary RF provide bilateral innervation to the diencephalon, presumably via collaterals, but no information is available on collateral projections to separate nuclei on the same side. Axons from separate nuclei of the medullary RF converge on common forebrain targets. For example, the PrT receives apparently overlapping projections from the Rgc, Rgcv, and RM. It is of interest that the RF axons project to those parts of PrT innervated by cerebellar axons (compare Figs. 16A, B and 17C of Martin et al. '73 with Figs. 13-15F and Fig. 16 of this communication). Scalia and Arango ('79) have suggested that the PrT is involved in visuomotor integration, but it appears that parts of it may be involved in sensory and/or motor function (Berkley and Mash '78, Robertson et al. '83). The functions of RF projections to PrT are unknown.

In the opossum, PF receives projections from all nuclei of the medullary RF except RPC. Many of these projections have also been documented in the rat (Loewy et al. '81, Peschanski and Besson, '84) and cat (Graybiel '77, Comans and Snow '81). In the opossum (unpublished observations, Hazlett and Bagley '83), as in the rat (e.g. Jones and Leavitt '74), and cat (Macchi et al. '77), PF innervates both the striatum and neocortex, suggesting that the medullary RF exerts a relatively global effect on forebrain activity through its projections to RF. This is
consistent with classical notions of RF function (Morison and Dempsey, '42, Moruzzi and Magoun '49). However, another possibility exists. Many neurons of PF project to either the striatum or the neocortex (Macchi et al. '84), suggesting that different reticular nuclei, or subsets of reticular neurons, might influence activity in restricted forebrain areas through projections to PF.

Another example of possible convergence is found in the projections of RD, RL, RGc, RGcv and RM to the ventral nuclei of the thalamus. Bobillier et al (‘76) have reported evidence for projections from the RM to the ventral posterior lateral nucleus in the cat and Nakano et al. (‘85) have described projections from various medullary RF nuclei to the ventral thalamus in the rat. It is well known that the medullary RF modulates somatosensory functions in the spinal cord (Basbaum et al. ‘78, Wolstencroft ‘80) and it now seems possible that it performs a comparable function in the thalamus.

The dorsal limb of the diagonal band receives converging projections from the RD, RGcv, and RL. Comparable results have been reported in the rat (Loewy et al. ‘81). If RF axons actually synapse with DB neurons, they may indirectly influence the hippocampus (Amaral and Kurz ‘85) and cerebral cortex (Mesulum et al. ‘83). Some of the DB neurons which innervate the hippocampus and
neocortex are cholinergic (Mesulam et al. '83, Amaral and Kurz '85) and it has been suggested that they affect pacemaker activity (Petsche et al. '62). Our material provides little evidence for projections to septal nuclei from the medullary RF, but such projections have been reported in the rat (Loewy et al. '81) and have been seen by us in that species (unpublished results).

It appears that several nuclei of the medullary RF have projections which are unique to them. Of particular note are the RL and ventrocaudal RD which project to HDM and PH. Projections from RD to HDM and PH have not been reported in previous autoradiographic studies in the rat (Loewy et al. '81, McKellar and Loewy '82), but labeled neurons have been found in ventrocaudal RD after injections of HRP into either the HDM or PH in the same species (Berk and Finklestein '81).

Other projections which appear unique to RL and ventrocaudal RD include those to the vicinity of PHD, PHA, and SO. Apparently comparable projections have been documented for the rat (Loewy et al. '81). Different parts of the paraventricular complex project to the pituitary gland, the dorsal vagal nucleus, and the intermediolateral cell column of the spinal cord (see Swanson and Kuypers '80). Projections from RL and ventrocaudal RD to the paraventricular complex may be one route by which the
medulla influences neuroendocrine and autonomic functions (Ciriello and Caverson '85). Although RF axons appear to distribute outside the boundaries of PHD, PHA, and SO (see also Sawchenko and Swanson '82), it is possible that they synapse on dendrites of neurons contained within them (Oldsfield '85).

Based on our autoradiographic material, the ventrolateral medulla, the area which includes RL and ventrocaudal RD, projects lightly to the lateral posterior, dorsal lateral geniculate, anterior ventral, and lateral intermediate nuclei of the thalamus. Although little evidence for such projections was found in our HRP experiments, it has been reported in the rat (Loewy et al. '81). In the cat the RGc apparently projects to the LP and LI (Rodrigo-Angulo and Reinoso-Suarez '82). Both thalamic nuclei have been considered as part of the extrastriate visual system (Kawamura '74, Symonds et al. '81).

In the opossum, RGc projects to the globus pallidus and substantia innominata, whereas other nuclei in the medullary RF do not. Similar projections have been reported in the cat (Graybiel '77). The globus pallidus and substantia innominata are best known for their roles in motor function (Delong and Georgopoulos '81, Turski et al. '84) and both areas contain cholinergic neurons, some of which project to the cerebral cortex (Mesulum et al. '83).
This study has provided evidence for direct
projections from RGC to the cerebral cortex. Evidence for
such projections has also been described for the cat
(Bentivoglio et al. '80) and it has been reported that RGC
modulates cortical activity (Dell et al. '61, Favale et al.
'61). It is possible, of course, that such modulation is
primarily indirect through projections to PF and DB.

Our finding that some projections of the medullary
RF to the forebrain are catecholaminergic is not
surprising, since similar results have been reported for
the rat (Lindvall and Bjorklund '78, Sawchenko and Swanson
'82). In the opossum, most of the neurons which provide
such projections were located ventrolaterally in a
region which includes RL and RD. It was of interest that
many of the forebrain projecting neurons in these nuclei
were not catecholaminergic. In other species, neurons in
apparently comparable nuclei contain substance P (Ljundjahl
et al. 78), somatostatin (Shiosaka et al. '81, Johansson et
al. '84), acetylcholine (Kimura et al. '81), enkephalin
(Sar et al. '78, Williams and Dockray '83), and
cholecystokinin (Zaborsky et al. '84) as well as
catecholamines.

Terminology for the ventrolateral medulla, the area
which includes RL and ventrolateral RD in the opossum,
seems to be in a state of flux. Because of its
cytoarchitectural and histochemical complexity, it has recently been referred to as simply the ventrolateral medulla (Loewy et al. '81) or the nucleus reticularis rostroventrolateralis (Ross et al. '84).

These results do not allow us to determine the exact area of forebrain innervated by medullary catecholamine cells, but they likely include those labeled autoradiographically after 3H-leucine injections of RL. Catecholamines have been implicated in reward (see Wise '78 for review), depression (Maas '75), and REM sleep (Morgane and Stern '74) and it has been suggested that they affect signal to noise ratio (Dismukes '79, Foote and Bloom '79).

These results provided little evidence for serotonergic projections from the medullary RF or raphe to the forebrain. In the opossum (Martin et al. '85) as in other species, axons from the dorsal raphe and superior central nucleus have strong projections to the forebrain (Bobillier et al '76, Azmitia and Segal '78) and some of these projections are serotonergic (Van de Kar and Lorens '79, Sawchenko et al. '83, Consolazione et al '84). It appears, however, that many of the non-serotonergic neurons of the RGcv, RaM and RaO, which are intermingled with serotonergic ones, project to the forebrain. Similar results have been reported in the rat (Takagi et al. '80). Raphe neurons are known to contain enkephalin, substance P,
and somatostatin, as well as serotonin (Bowker and Abbott '85).

CONCLUDING REMARKS

It has been traditionally thought that the RF is diffusely organized and that its major role is to provide divergent, non-specific influence over multiple areas of the neural axis. Our anatomical results, like those of others, suggest that the RF is not diffusely organized. Although many RF nuclei provide divergent projections, such projections may originate primarily from separate neurons. Many nuclei of the RF innervate common targets, but they also have projections which are unique to them. Such evidence suggests that the RF, like other areas of the brain, must be meticulously dissected before unifying hypotheses concerning its organization and functions can be generated.
TABLES
Table I Numbers of small (S), medium (M), and large (L) neurons labeled ipsilaterally (ipsi) and contralaterally (contra) in different nuclei of the medullary RF (top) after the HRP or WGA-HRP injections of the nuclei listed on the left. The definitions of S, M, and L neurons are given in the methods section. Only the sizes found in each nucleus are listed. No giant neurons were labeled in any case. In the case with the large diencephalic injection labeled neurons were counted from one out of eighteen sections, whereas in the remainder, they were counted in every third section. The lack of a number does not necessarily mean that no labeled neurons were present.
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| Large dienceph. |    |    |    |     |      |    |    |    |
| Parafascicular  | -  | -  | -  | -   | -    | -  | -  | -  |
| Pretectal       | -  | -  | -  | -   | -    | -  | -  | -  |
| Lateral Hipo.   | -  | -  | -  | -   | -    | -  | -  | -  |
| V. Lat. Genic.  | -  | -  | -  | -   | -    | -  | -  | -  |
| Lat. Posterior  | -  | -  | -  | -   | -    | -  | -  | -  |
| D. Lat. Genic.  | -  | -  | -  | -   | -    | -  | -  | -  |
| Ventralateral   | -  | -  | -  | -   | -    | -  | -  | -  |
| Ventralbasal    | -  | -  | -  | -   | -    | -  | -  | -  |
| Medial Preopt.  | -  | -  | -  | -   | -    | -  | -  | -  |
| Septum          | -  | -  | -  | -   | -    | -  | -  | -  |
| Cerebral Cortex | -  | -  | -  | -   | -    | -  | -  | -  |
| Hippocampus     | -  | -  | -  | -   | -    | -  | -  | -  |
| Caudate nucleus | -  | -  | -  | -   | -    | -  | -  | -  |
Table II  The results of measurements of the percentage of area occupied by autoradiographic labeling in selected thalamic nuclei of four cases. The individual numbers represent the percentage of lit pixels over the total number of pixels within a measured area.
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Table III  The results of measurements of the percentage of area occupied by autoradiographic labeling in selected areas of hypothalamus, basal forebrain and neocortex. The individual numbers represent the percentage of lit pixels over the total number of pixels within a measured area.
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FIGURES
Fig. 1  Line drawings of Nissl stained sections through medulla of the opossum (rostral A to caudal E), showing sections comparable to those used in subsequent figures. All nuclei of the medullary RF are indicated and the rostral continuation of the RL is indicated by an asterisk.
Fig. 2 Plot showing the locations of neurons (dots) labeled in selected sections of the medulla (rostral A through caudal E) in a case which had multiple injections of HRP into the diencephalon (upper right). Note that HRP labeled neurons are found in all nuclei of the medullary reticular formation. The tissue was processed with O-dianisidine. Scale bar equals 1 mm.
Fig. 3  Darkfield photomontage of HRP-labeled cells in the ventrolateral extreme of RD at level of Fig. 2E. The arrows indicate labeled cells. Similar labeling was seen after an xVB injection. Scale bar equals 100um.
Fig. 4 Darkfield photomontage of HRP-labeled cells in the dorsomedial part of RD at the level of Fig. 2E. HRP labeled cells (arrows) extend from the ventrolateral edge of the nucleus of the solitary tract into RD. This labeling was found primarily on the side contralateral to the injection. Scale bar equals 200um.
Fig. 5 Examples of labeled and unlabeled neurons in the medullary Rf of the the case illustrated in Fig. 2. Neurons of both types are designated as to their size (S, small; M, medium; L, large; G, giant). C was taken from the RD, A and D were taken from the RGe, and B, E, and F were taken from the RGev. Large cells in RGev are not listed in table I because we did not find them when carrying out the measurements of random sections. Scale bar equals 50um.
Fig. 6  Darkfield photomontage of HRP-labeled cells (arrows) in the dorsal medial aspect of RGc taken at the level of Fig. 2B. Scale bar equals 100um.
Darkfield photomontage of HRP-labeled cells (arrows) in the RGcv and the RM taken at a level of Fig. 2A. Neuronal labeling extends from the midline, primarily ipsilaterally, into the RGcv. Scale bar equals 200um.
Fig. 8  Plot showing the locations of medullary neurons (dots) labeled after multiple injections of True Blue into the diencephalon (upper right). The tissue was processed for Falck Hillarp histofluorescence to demonstrate monoaminergic neurons. Catecholamine cells are indicated by the squares, serotonin cells are indicated by the open circles, and cells which contain both TB and catecholamines are indicated by the stars set in black circles. Scale bar equals 1mm.
Fig. 9 Photomicrographs of neurons containing TB and/or monoamines taken from the ventrolateral medulla (A and B) and from an area rostral to RGcv (C and D). The filter system which demonstrates TB maximally was used in A and C, whereas that which demonstrates monoamines maximally was used in B and D. Neurons containing both TB and catecholamine histofluorescence are indicated by the solid block arrows in A and B. A cell which contains a catecholamine but not TB is indicated by the open arrow. C and D were taken from sections processed for serotonin immunofluorescence. In C TB-labeled cells are indicated by the curved arrows while in D a serotonergic neuron is indicated in the same section by the arrowhead. Both scale bars equal 25um.
Fig. 10  Plot showing the locations of neurons (dots) labeled in selected sections of the medulla (rostral A through caudal E) by multiple injections of TB into the diencephalon (upper right). Tissue was processed for serotonergic immunofluorescence. Serotonergic cells were indicated by the open circles. No double-labeled neurons were found. Scale bar equals 1mm.
Fig. 11 Darkfield photomontage of the autoradiographic labeling in the midbrain produced by a 3H-leucine injection of RGc. The section was taken from the area of the midbrain outlined in the brightfield photomontage at the upper right. Dense labeling in Tgl is present over the major ascending tract from RGc caudal to its split into dorsal and ventral subdivisions. Scale bar equals 200um.
Fig. 12 Plot of the autoradiographic labeling present in selected areas of the forebrain (rostral A to caudal E) after an injection of 3H-leucine into the ventrolateral medulla (lower left). Scale bar equals 1mm.
Fig. 13  Plot of the autoradiographic labeling present in selected areas of the forebrain (rostral A to caudal F) after an injection of $^{3}H$-leucine into the rostral R6c (lower left). Scale bar equals 1mm.
Fig. 14  Plot of the autoradiographic labeling present in selected areas of the forebrain (rostral A to caudal F) after an injection of 3H-leucine into the raphe magnus which spilled into the RGcv and RGc (lower left). Scale bar equals 1 mm.
Fig. 15  Plot of the autoradiographic labeling present in selected areas of the forebrain (rostral A to caudal E) after an injection of 3H-leucine into the RGcv (lower left). Scale bar equals 1 mm.
Fig. 16 Darkfield photomontage of autoradiographic labeling along the edge of the pretectum and within it. This was taken from the side contralateral to the injection in the case illustrated in Fig. 14. Scale bar equals 100μm.
Fig. 17  Darkfield  (A)  and  lightfield  (B) photomontages of autoradiographic labeling in xVB taken from the case illustrated in Fig. 12. Scale bar equals 50um.
Fig. 18  Darkfield (A) and lightfield (B) photomontages of autoradiographic labeling in DB taken from the case illustrated in Fig. 12. Scale bar equals 50um.
Fig. 19  Darkfield (A) and lightfield (B) photomontages of autoradiographic labeling in HDM taken from the case illustrated in Fig. 12. Scale bar equals 50um.
Fig. 20 Darkfield (A) and lightfield (B) photomontages of the autoradiographic labeling in PHD taken from the case illustrated in Fig. 12. Scale bar equals 100μm.
Fig. 21 Plot showing the locations of neurons (dots) labeled in selected sections through the medulla (rostral A through caudal E) by an HRP injection which includes the parafascicular and paracentral nuclei of the thalamus (the upper right). The labeling shown was taken from two adjacent sections. Scale bar equals 1mm.
Fig. 22 Plate showing selected photomicrographs of injection sites within the forebrain. A shows an injection of HRP which includes the PF and PC. Injections of HRP or WGA-HRP within the Prt (B), POM (C) and POL (D) are also shown. The bar in D equals 1mm.
Fig. 23  

Plot showing the location of neurons (dots) labeled in selected sections through the medulla (rostral A through caudal E) by a large WGA-HRP injection of the hypothalamus (upper right). The labeling shown was taken from two adjacent sections. Scale bar equals 1mm.
Fig. 24 Plot showing the location of neurons (dots) labeled in selected sections through the medulla (rostral A through caudal E) by an HRP injection which includes the nucleus of the diagonal band and septum. The labeling shown was taken from two adjacent sections. Scale bar equals 1mm.
Fig. 25 Plot showing the location of neurons (dots) labeled in selected sections through the medulla (rostral A through caudal E) by multiple WGA-HRP injections into the neocortex. The labeling shown was taken from two adjacent sections. Scale bar equals 1mm.
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