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The Organization of Projections From the Cortex, Amygdala, and Hypothalamus to the Nucleus of the Solitary Tract in Rat

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ABSTRACT

Direct projections from the forebrain to the nucleus of the solitary tract (NTS) and dorsal motor nucleus of the vagus in the rat medulla were mapped in detail using both retrograde axonal transport of the fluorescent tracer True Blue and anterograde axonal transport of wheat germ agglutinin conjugated to horseradish peroxidase (WGA-HRP). In the retrograde tracing studies, cell groups in the medial prefrontal cortex, lateral prefrontal cortex (primarily ventral and posterior agranular insular cortex), bed nucleus of the stria terminalis, central nucleus of the amygdala, paraventricular, arcuate, and posterolateral areas of the hypothalamus were shown to project to the NTS and in some cases also to the dorsal motor nucleus of the vagus. The prefrontal cortical areas projecting to the NTS apparently overlap to a large degree with those cortical areas receiving mediodorsal thalamic and dopaminergic input. The retrogradely labeled cortical cells were situated in deep layers of the rat prefrontal cortex.

The anterograde tracing studies revealed a prominent topography in the mediolateral termination pattern of forebrain projections to the rostral part of the NTS and to the dorsal pons. The projections to the NTS were generally bilateral, except for projections from the central nucleus of the amygdala and bed nucleus of the stria terminalis which were predominantly ipsilateral. The prefrontal cortical projections to the NTS travel through the cerebral peduncle and pyramidal tract and terminate throughout the rostrocaudal extent of the NTS. Specifically, the prefrontal cortex innervates dorsal portions of the NTS (lateral part of the dorsal division of the medial solitary nucleus, dorsal part of the lateral solitary nucleus and the caudal midline region of the commissural nucleus), areas which receive relatively sparse subcortical projections. These dorsal portions of the NTS receive major primary afferent projections from the vagal and glossopharyngeal nerves. In contrast, the subcortical projections, which travel through the midbrain and pontine tegmentum, terminate most heavily in the ventral portions of the NTS, i.e., the area immediately dorsal and lateral to the dorsal motor nucleus of the vagus. Only the paraventricular hypothalamic nucleus has substantial terminals throughout the dorsal motor nucleus of the vagus. Hypothalamic cell groups innervate the area postrema and, along with the prefrontal cortex, innervate the zone subjacent to the area postrema.

We suggest that direct projections to the NTS and dorsal motor nucleus of the vagus are one criterion that can be used to define the visceral forebrain. The organization of the forebrain terminations in the solitary complex described here may eventually allow a better understanding of how the brain controls visceral function.

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Key words: forebrain, autonomic control, axonal tracing

The nucleus of the solitary tract (NTS) is a visceroceptive cell group located in the dorsal medulla. The NTS receives primary afferent terminations of the facial, glossopharyngeal, and vagal nerves (Torvik, '56; Beckstead and Norgren, '79), which carry primarily gustatory and visceral information. The immediately subjacent dorsal motor nucleus of the vagus is one of the main origins of preganglionic parasympathetic fibers controlling cardiovascular, respiratory, and other visceromotor functions. Historically, the NTS has been defined and subdivided on the basis of a rough rostrocaudal ordering of the primary afferent terminations of the facial, glossopharyngeal, and vagal nerves (Torvik, '56). However, even these rough subdivisions have now been questioned by studies showing a more widespread terminal distribution of each primary afferent nerve throughout the NTS (Beckstead and Norgren, '79). Recently, research on the NTS has focused on the projections of specific brain systems to various parts of the nucleus. This has led to a concentration on two systems. First, catecholamine systems in the NTS have been studied (Doba and Reis, '74; Renaud et al., '79; Sawchenko and Swanson, '81; Armstrong et al., '81; Koda et al., '81; Koda and Bloom, '83) primarily because of the effectiveness of drugs that manipulate catecholamine systems in treating hypertension (Kaplan, '80). Second, because of the well-known hypothalamic control of autonomic function (Ciriello and Calaresu, '77; Kabat et al., '35; Nauta, '72), attention has been directed to the hypothalamic connections to the NTS.

Until recently, the hypothalamic projections to the NTS were believed to be multisynaptic, traveling by way of the reticular formation (Nauta, '72). However, modern neuroanatomical tracing techniques utilizing anterograde and retrograde axoplasmic transport have now established direct monosynaptic hypothalamic connections to the NTS (Conrad and Pfaff, '76; Hosoya and Matsushita, '81; Saper et al., '76; Swanson and Kuypers, '80). The role of these *direct* hypothalamic (and other forebrain) projections to the NTS and dorsal motor nucleus of the vagus in visceral control mechanisms could be a important as that played by local brainstem connections (Ross et al., '81). Some progress has been made in studying forebrain control of, for example, cardiovascular function by electrically stimulating various forebrain areas (Faiers et al., '75; Kaada, '56; Kabat et al., '35). However, this work was done prior to the development of neuroanatomical methods which are able to pinpoint unequivocally direct forebrain connections to the NTS.

The main objective of the present study is to provide a detailed anatomical description of forebrain projections to the NTS and dorsal motor nucleus of the vagus. This may help provide a solid hodological basis for defining and subdividing the NTS. In the present study fluorescent retrograde axonal tracing methods were used to localize the forebrain neurons (in the hypothalamus, bed nucleus of the stria terminalis, amygdala, and prefrontal cortex) projecting directly to the NTS in the rat. In addition, the patterns of termination of each of these forebrain projections within the nucleus of the solitary tract are described in detail using the anterograde transport of wheat germ agglutinin conjugated to horseradish peroxidase. The results allow a better understanding of the organization of the NTS and also lay a foundation for explicating forebrain control of visceral function. (Portions of the data on cortical-NTS projections have been previously reported; van der Kooy et al., '82a,b.)

MATERIALS AND METHODS

Retrograde tracing

The retrograde axonal transport of the fluorescent tracers Evans blue (Kuypers et al., '77) and True Blue (Bentivoglio et al., '79) was employed to map forebrain cells projecting to the NTS. Evans blue was used in early studies, but True Blue was used in all later studies because of its very sensitive retrograde-tracing properties (Sawchenko and Swanson, '81). Male Spraque-Dawley rats (250–350 g) were anesthetized, placed in a stereotaxic apparatus, and injected into the NTS with 0.1-0.3 μl of a 10% True Blue suspension (in distilled water) using a 1.0 μl Hamilton microsyringe held in a stereotaxic micromanipulator. The atlanto-occipital membrane and part of the occipital bone were removed so that the obex and area postrema were clearly visible. Injections were made under visual control in both the rostral (750–1,000 μm rostral to the obex) and caudal (250 μm rostral to the obex) portions of the NTS. After a survival of 3–7 days, rats were anesthetized and perfused transcardially with 10% formalin or 4% paraformaldehyde in 0.1 M phosphate buffer at pH 7.4. The brains were kept overnight in 20% sucrose at 4°C and then cut transversely at 30–40 μm on a freezing microtome. The sections were mounted on slides and air dried.

A Zeiss epifluorescence microscope was used to examine the sections. Filters which provided excitation light of 360- and 550-nm wavelength were used to examine the True Blue- and Evans blue-containing cells, respectively.

Anterograde tracing

Injections of the sensitive anterograde tracer wheat germ agglutinin conjugated to horseradish peroxidase (WGA-HRP) (Gonatas et al.,'79; Brushart and Mesulam, '80; Staines et al., '80) were made into the various forebrain areas that were revealed to project to the NTS in the initial retrograde tracing portion of this study. In anesthetized rats, 0.02–0.3 μl of a 2% WGA-HRP (Sigma) solution were stereotaxically injected through a 1.0-μl Hamilton microsyringe. Different rats were injected in each of the following areas: the medial prefrontal cortex, lateral prefrontal cortex, bed nucleus of the stria terminalis, paraventricular hypothalamus, lateral hypothalamus, and central nucleus of the amygdala. After a 3-day survival, rats were perfused and the brains cut and processed using the tetramethylbenzidine method for visualizing HRP (Mesulam, '78). Briefly, deeply anesthetized rats were intracardially perfused first with normal saline, then with a 1.0% paraformaldehyde-1.5% glutaraldehyde solution in 0.1 M phosphate buffer (pH 7.4) at room temperature, and finally with ice-cold 10% sucrose in 0.1 M phosphate buffer. Frozen sections (40 μm) were mounted on coated slides and reacted as outlined by Mesulam ('78) except that a higher concentration of H_2O_2 (0.01%) was used in the reaction. Most sections were examined under darkfield illumination without counterstaining. Some sections were studied under brightfield illumination

after using an Azur II-methylene blue counterstain (Richardson et al., '60). In most cases, tissue from 3–4 brains per injection site was examined, with one brain per injection site selected for detailed pathway and terminal field analyses.

RESULTS

Normal anatomy of the rat NTS

Photomicrographs of Nissl-stained sections at selected rostrocaudal levels through the medulla are presented in Figure 1 to facilitate the description of the experimental tracing studies. Our description of the rat NTS generally follows the cytoarchitectonic divisions of Cajal ('11) and Torvik ('56). However, we have demarcated some additional nuclear subdivisions based on the experimental tracing material described below as well as by acetylocholinesterase staining, enkephalin immunoreactivity, glyoxylic acid-induced catecholamine fluorescence, and intravenous sodium fluorescein-stained sections available in our laboratory.

Obex. The rat NTS extends over 3 mm in the rostrocaudal dimension. The caudal end of the nucleus is approximately 500 μm caudal to the obex, defined here as the caudal end of the area postrema. The term "foramen of the central canal" is used for the region at the rostral end of the area postrema where the central canal of the spinal cord opens up into the fourth ventricle (Fig. 1C).

Medial and midline solitary nuclei. The most caudal portion of the NTS appears as a small, round cell group dorsal to the central canal of the spinal cord and ventral to the white matter of the dorsal columns (Fig. 1A). Cajal ('11) and Torvik ('56) term most of this caudal area the nucleus commissuralis. We have divided their nucleus commissuralis into two portions. First, we have used the term "midline solitary nucleus" to refer to the small,cell-poor area situated on the midline, extending (Fig. 1A,B) from the dorsal commissural nucleus of the spinal cord (with which it is continuous) to the foramen of the central canal. The remaining major portion of the commissural nucleus (Fig. 1A) is here included in the medial solitary nucleus on the basis of its appearance in Nissl sections and its forebrain afferents (see below). The medial solitary nucleus is made up of small, densely packed neurons and is present through the entire extent of the NTS (Fig. 1A–F). It can be divided into a dorsal and a ventral division, the cells of the dorsal division being somewhat less densely packed. The dorsal division occupies a progressively more lateral portion of the medial solitary nucleus at more rostral levels (Fig. 1D,E), and is completely absent at the most rostral level of the NTS (Fig. 1F).

Dorsal motor nucleus of the vagus. The large-celled, deeply Nissl-stained, dorsal motor nucleus of the vagus is present from middle to caudal portions of the NTS. Also present over the same rostrocaudal extent is the nucleus intercalatus (Fig. 1A–D), interposed between the dorsal motor nucleus and the hypoglossal nucleus.

Lateral solitary nucleus. The lateral solitary nucleus is present through all but the most caudal portion of the NTS. It contains larger and less densely packed cells than the medial solitary nucleus. The solitary tract itself can be recognized at all but the most caudal levels of the NTS (Fig. 1B–F) and consists mainly of primary afferent fibers, espe-

Abbreviations

abl	basal lateral amygdala nucleus
abm	basomedial amygdala nucleus
AC	anterior commissure
ace	central nucleus of amygdala
aco	cortical nucleus of amygdala
agi	agranular insular cortex
al	lateral nucleus of amygdala
ap	area postrema
BC	brachium conjunctivum
bnst	bed nucleus of stria terminalis
c	cuneate nucleus
cc	corpus callosum
CHP	choroid plexus
cl	claustrum
cp	caudate-putamen
CP	cerebral peduncle
dr	dorsal raphe nucleus
dt	dorsal tegmental nucleus
F	fornix
FC	foramen of central canal
g	gracilis nucleus
gi	granular insular cortex
gp	globus pallidus
hpv	hypothalamic paraventricular nucleus
IC	internal capsule
ic	intercalatus nucleus
ip	interpeduncular nucleus
dt	lateral dorsal tegmental nucleus
lh	lateral hypothalamus
lp	lateral parvocellular paraventricular hypothalamic nucleus
mesV	mesencephalic nucleus of V
ML	medial lemniscus
mp	medial parvocellular paraventricular hypothalamic nucleus
MT	mammillothalamic tract
mV	motor nucleus of V
nts	nucleus of the solitary tract
PAG	periaqueductal gray
pfcl	prefrontal cortex lateral
pfcm	prefrontal cortex medial
pp	peripeduncular nucleus
PT	pyramidal tract
PTD	pyramidal tract decussation
pXII	prepositus hypoglossal nucleus
r	red nucleus
re	reuniens thalamic nucleus
RF	rhinal fissure
sl	lateral solitary nucleus
sm	medial solitary nucleus
smd	medial solitary nucleus, dorsal division
smid	midline solitary nucleus
smv	medial solitary nucleus, ventral division
sn	substantia nigra
spv	spinal trigminal nucleus
ST	stria terminalis
sze	subpostremal zona exterior
szi	subpostremal zona interior
tdml	medial dorsal thalamic nucleus, pars lateralis
tmdc	medial dorsal thalamic nucleus, pars centralis
tmdm	medial dorsal thalamic nucleus, pars medialis
T-mesV	tract of mesencephalic trigeminal nerve
tpt	thalamic paratenial nucleus
tpv	thalamic paraventricular nucleus
TS	tractus solitarius
tvb	thalamic ventrobasal complex
vl	lateral vestibular nucleus
vm	medial vestibular nucleus
vt	ventral tegmental nucleus of Gudden
vta	ventral tegmental area
Zi	zona incerta
I-VI	layers of cortex
X	dorsal motor nucleus of vagus
XII	hypoglossal motor nucleus

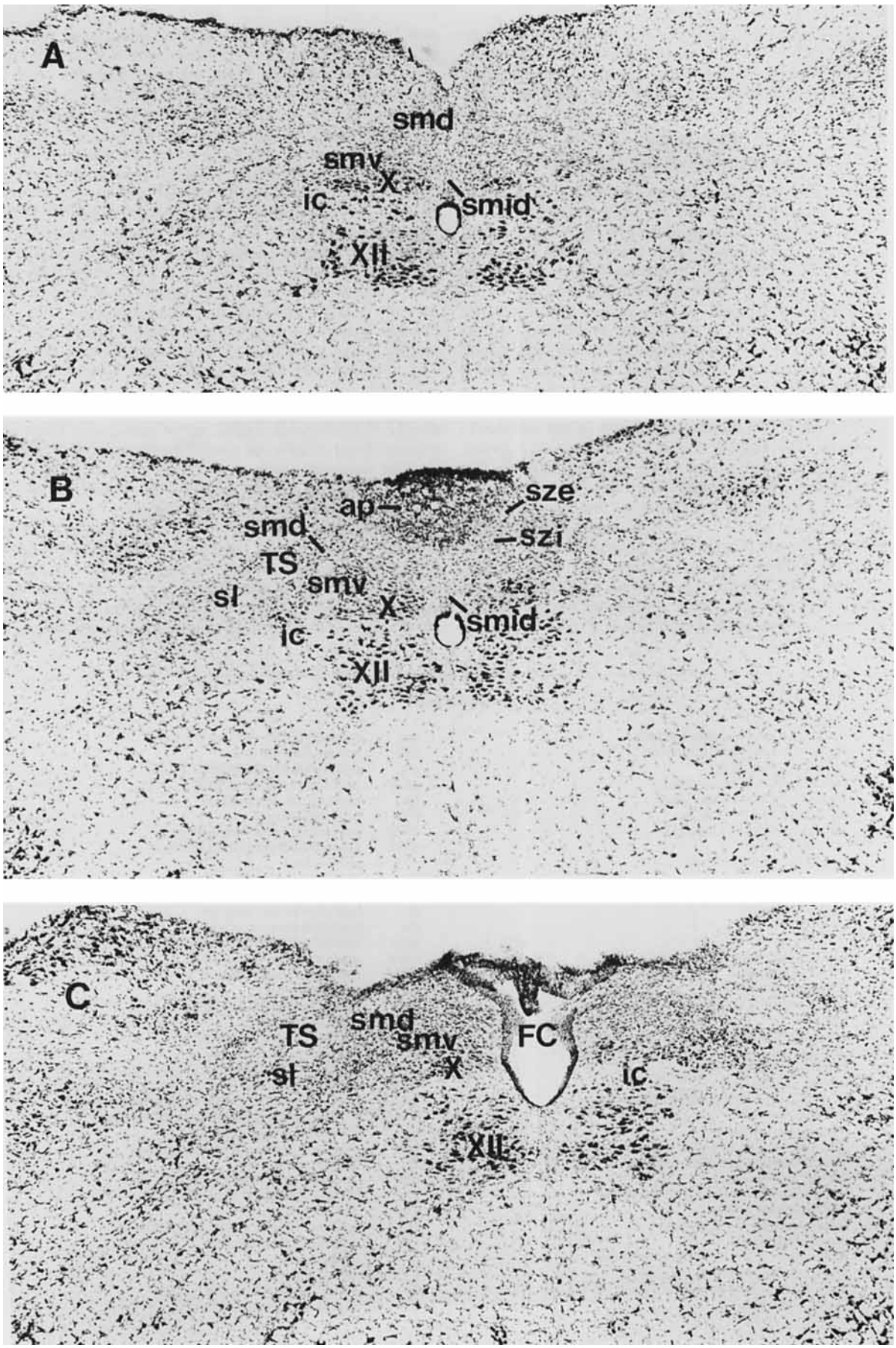
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Figure 1 A–C

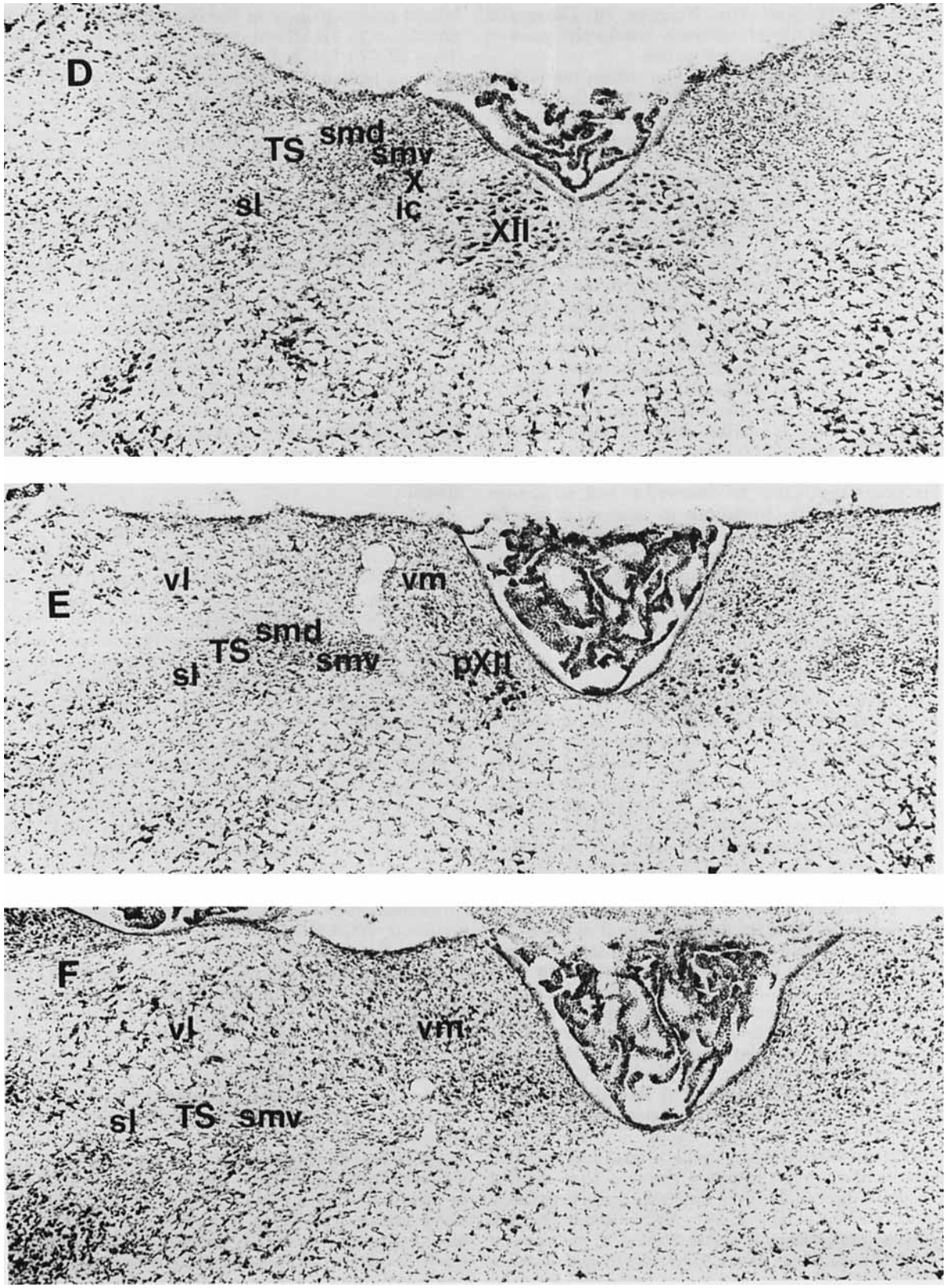

Fig. 1. A–F. Nissl-stained frontal sections through the caudorostral (A is most caudal) extent of the NTS in the rat medulla. The sections are approximately 400 μm apart although section C, which is included to illustrate the foramen of the central canal, is closer to the preceding and following sections. Abbreviations for this and all following figures are given in the list.

cially vagal fibers (Beckstead and Norgren, '79). The medial border of the solitary tract generally marks the point of division of the medial and lateral nuclei.

Area subpostrema. One final region within the NTS is the area subpostrema, defined in the cat by Gwynn and Wolstencroft ('68) as the tissue directly ventral to the area postrema, the blood vessels of which stain positive for cholinesterase. This area subpostrema has access to the weak blood brain barrier of the area postrema, and, although intravenous Evans blue, trypan blue, or bisbenzimid (Fig. 2A) do not stain it, it is filled with fluorescein in rats injected intravenously with 1 ml of 20% sodium fluorescein 5 minutes prior to sacrifice (Fig. 2B). At midcaudal levels of the NTS (Fig. 1B), this subpostrema region in the rat can be divided into two concentric zones (external and internal subpostremal zones) surrounding the ventral and lateral aspects of the area postrema. In Nissl sections these zones can be seen to have less densely packed cells than the adjacent divisions of the NTS. Although it is difficult to delineate the external from the internal zone in Nissl material, it seems that the smaller external zone (closest to and immediately surrounding the area postrema) is relatively cell poor compared to the much larger internal zone. The thin external zone can be observed to best advantage in tissue stained with antiserum to leucine-enkephalin (kindly provided by R.J. Miller, U. Chicago), which reveals enkephalin immunofluorescent staining of cell bodies and processes concentrated in the external solitary zone. We have previously referred to the external and internal subpostremal zones as the external and internal solitary zones (van der Kooy and Koda, '83).

Retrograde tracing

Cortex. Figure 3 (A–G) illustrates the distribution of retrogradely labeled forebrain perikarya in a representative case following an injection of True Blue in the midrostral NTS (Figs. 3H, 7A). In the cortex, labeled cells were localized bilaterally in three prefrontal areas: (1) The medial prefrontal cortex (medial to the forceps minor) contained a small number (< 10 per section) of labeled cells in deep cortical layers. These scattered cells were found both in prelimbic cortex (area 32) (Fig. 3A) and at more caudal levels in infralimbic cortex (area 25) (Fig. 3B) as well. (2) Very few labeled cell bodies were found scattered in the prefrontal cortex dorsal to the forceps minor (Fig. 3A,B). (3) The most numerous, heavily labeled, and densely clustered collection of cell bodies (Fig. 3A–E) was situated in deep layers of the lateral prefrontal or agranular insular cortex lateral to the rostral claustrum (Krettek and Price, '77). This group extended from the level of the rostral pole of the forceps minor (Fig. 3A) to a level just caudal to the crossing of the anterior commissure (Fig. 3E). The labeled cells were located dorsal to the rhinal sulcus at rostral levels (Fig. 3A,B), but more caudally, where they became more numerous (>20 cells per section), they appeared at the level of and ventral to the rhinal sulcus in the ventral and posterior agranular insular cortex. Figure 4 shows a Nissl-stained section at a similar caudal level, illustrating the location of the greatest number of labeled cells. NTS injections did not label cells in the prefrontal cortex rostral to the forceps minor or in the cingulate cortex. However, in some cases a few labeled cells were scattered dorsal and caudodorsal to the lateral prefrontal cortical area.

Subcortex. After NTS injections, retrogradely labeled forebrain cells were present bilaterally (with a strong ipsilateral predominance) in the bed nucleus of the stria terminalis (Fig. 3D,E) and central nucleus of the amygdala (Figs. 3F, 5A). Labeled cells were also seen bilaterally (with a strong ipsilateral predominance) in the hypothalamus, particularly in the medial and lateral parvocellular portions (Swanson and Kuypers, '80) of the paraventricular nucleus (Figs. 3F, 5B), in the perifornical area (Fig. 3F,G), the posterolateral hypothalamus (Fig. 3G), and the arcuate nucleus (Fig. 3G).

Injection site. Retrogradely labeled neurons were seen in all of the forebrain areas mentioned above after both rostral and caudal NTS injections of True Blue, but the cells were more heavily labeled and numerous after the rostral injections.

Control injections of True Blue into the fourth ventricle dorsal to the NTS did not label forebrain cell bodies. In addition, control injections were made in the spinal trigeminal complex (lateral to and without infringing on the NTS), because projections from the lateral cortex to the spinal trigeminal complex have been reported (Wise et al., '79). Spinal trigeminal injections retrogradely labeled cortical cell bodies dorsal to but not within the lateral prefrontal cortical area labeled after the NTS injections (described above).

Anterograde tracing

Injections of WGA-HRP were made into the various forebrain regions that were revealed to project to the NTS in the retrograde tracing studies. The distribution of label through the brain after a representative injection into each area is described below. It is important to mention that in certain of the anterogradely labeled nuclei, collections of peroxidase granules that appear to be retrogradely labeled cell bodies are seen (for example, the medial and lateral divisions of the mediodorsal thalamic nucleus after medial prefrontal cortex injections), but it is sometimes difficult unequivocally to identify labeled cell bodies because of the very dense anterograde label in these areas. This is a general problem associated with the use of WGA-HRP in sections that are not counterstained (the most sensitive procedure for revealing peroxidase staining).

Medial prefrontal cortex.

Injection site. Figure 6A illustrates a large bilateral injection of WGA-HRP in the medial prefrontal cortex, infringing ventromedially on both prelimbic and infralimbic regions (Krettek and Price, '77). This injection site did not infringe on the bed nucleus of the stria terminalis, the closest region also having projections to the area of the solitary complex. Bilateral WGA-HRP injections were employed in the case of the medial prefrontal cortex in order to increase the density of the faint labeling seen in the NTS after unilateral medial prefrontal injections.

Descending projections. The injection labeled fibers in the most medial portion of the internal capsule (Fig. 6B,C). In the rostral diencephalon (Fig. 6B), a dense terminal field was seen in the paratenial nucleus of the thalamus. Sparser terminal labeling was seen in the rhomboid and reuniens nuclei, with some retrogradely labeled cell bodies also present in the latter nucleus. More caudally in the thalamus, dense terminal labeling appeared in the lateral and medial (but not central) divisions of the mediodorsal nucleus (Fig. 6C). Some cell bodies in these divisions appeared to be retrogradely labeled, but the intensity of the anterograde label made this determination equivocal. Ter-

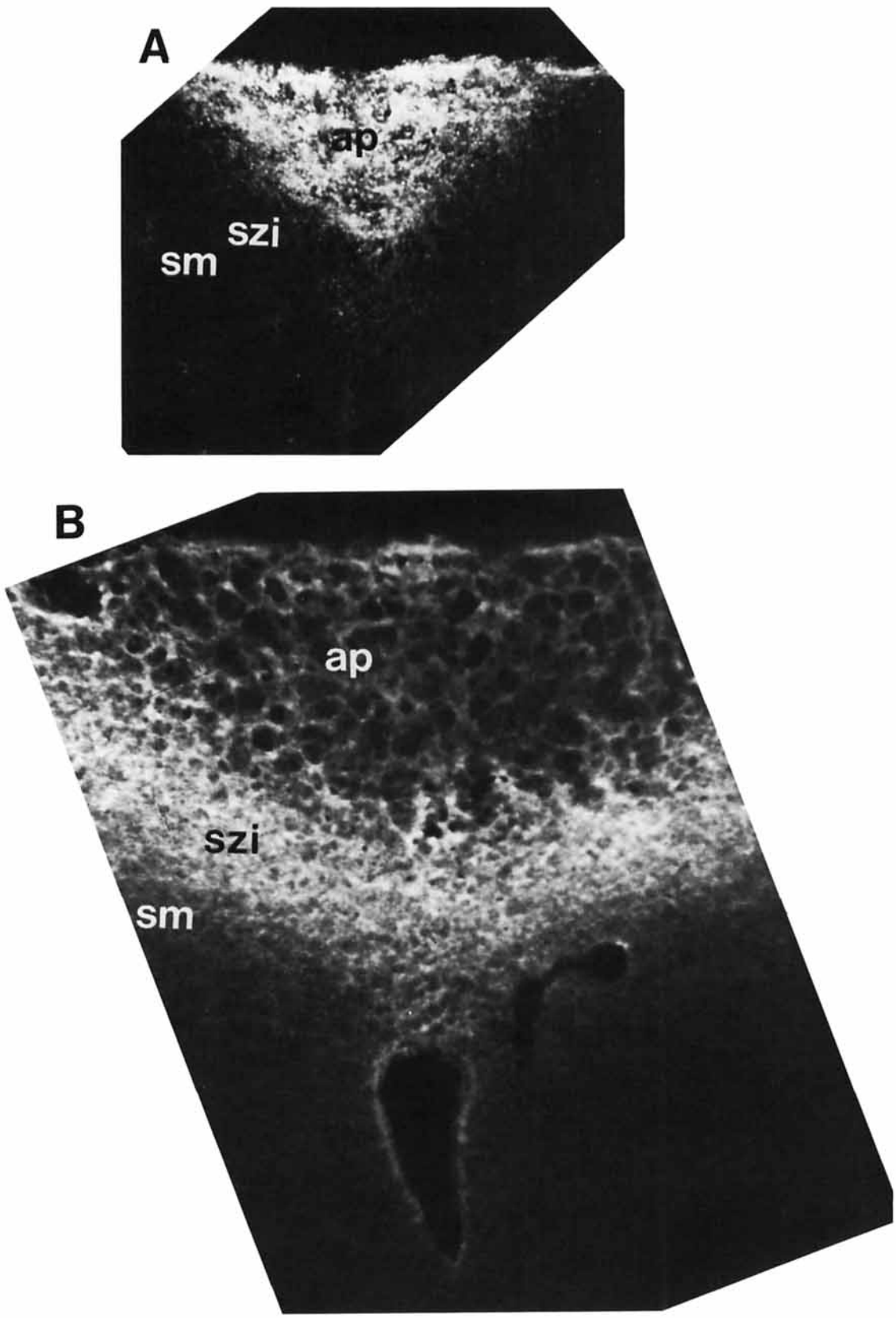

Fig. 2. Frontal sections showing fluorescent labeling through the area postrema and adjacent portions of the NTS. A. 5 minutes after intravenous injection of bisbenzimid (1.0 ml of 20%) only the area postrema (ap) is labeled. B. Note fluorescent labeling of the internal subpostremal zone (szi) 5 minutes after intravenous injection of sodium fluorescein (1.0 ml of 20%).

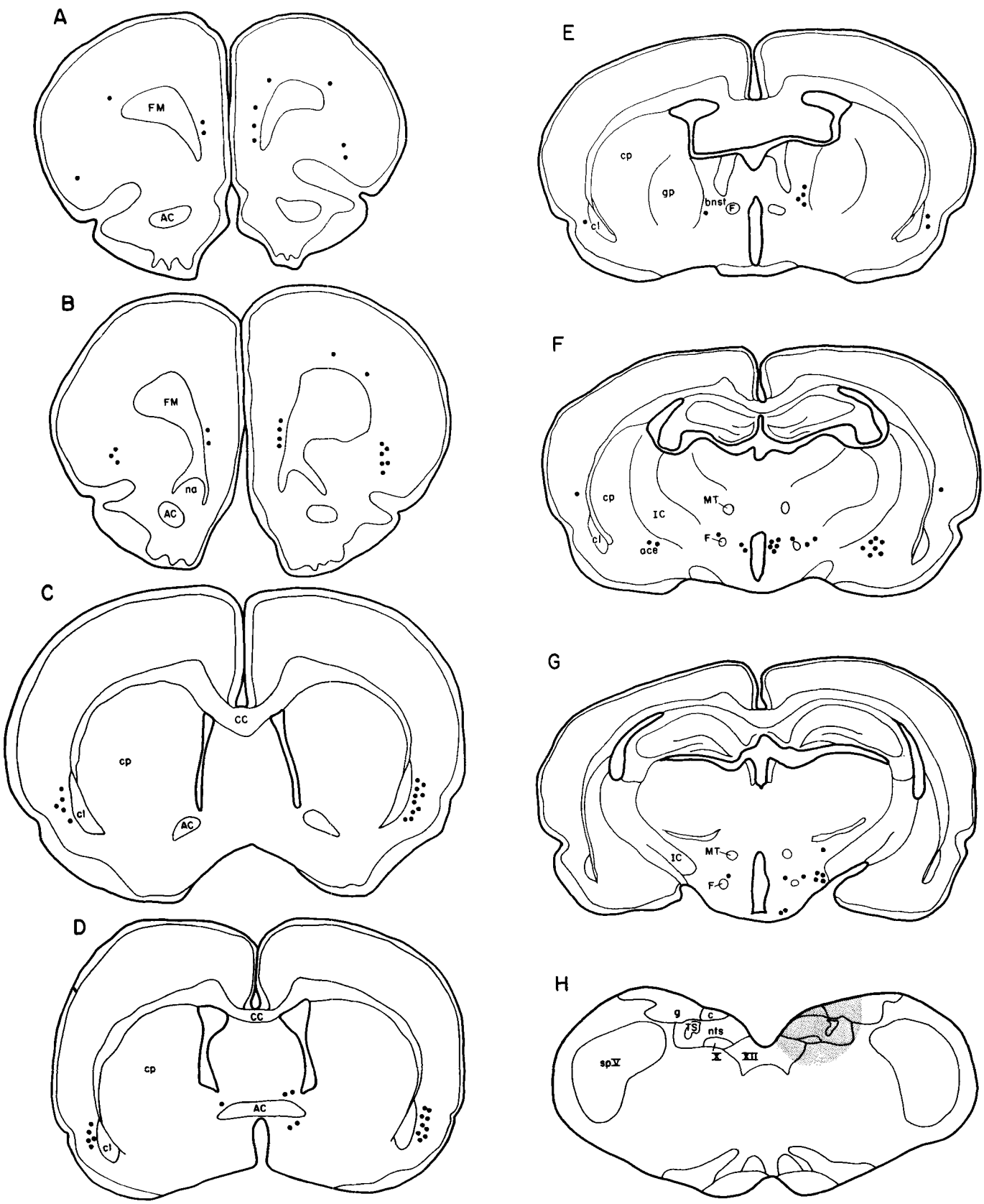

Fig. 3. Schematic diagrams of retrogradely labeled cell bodies in the forebrain from rostral to caudal (A–G) levels after injection of True Blue in the NTS (H).

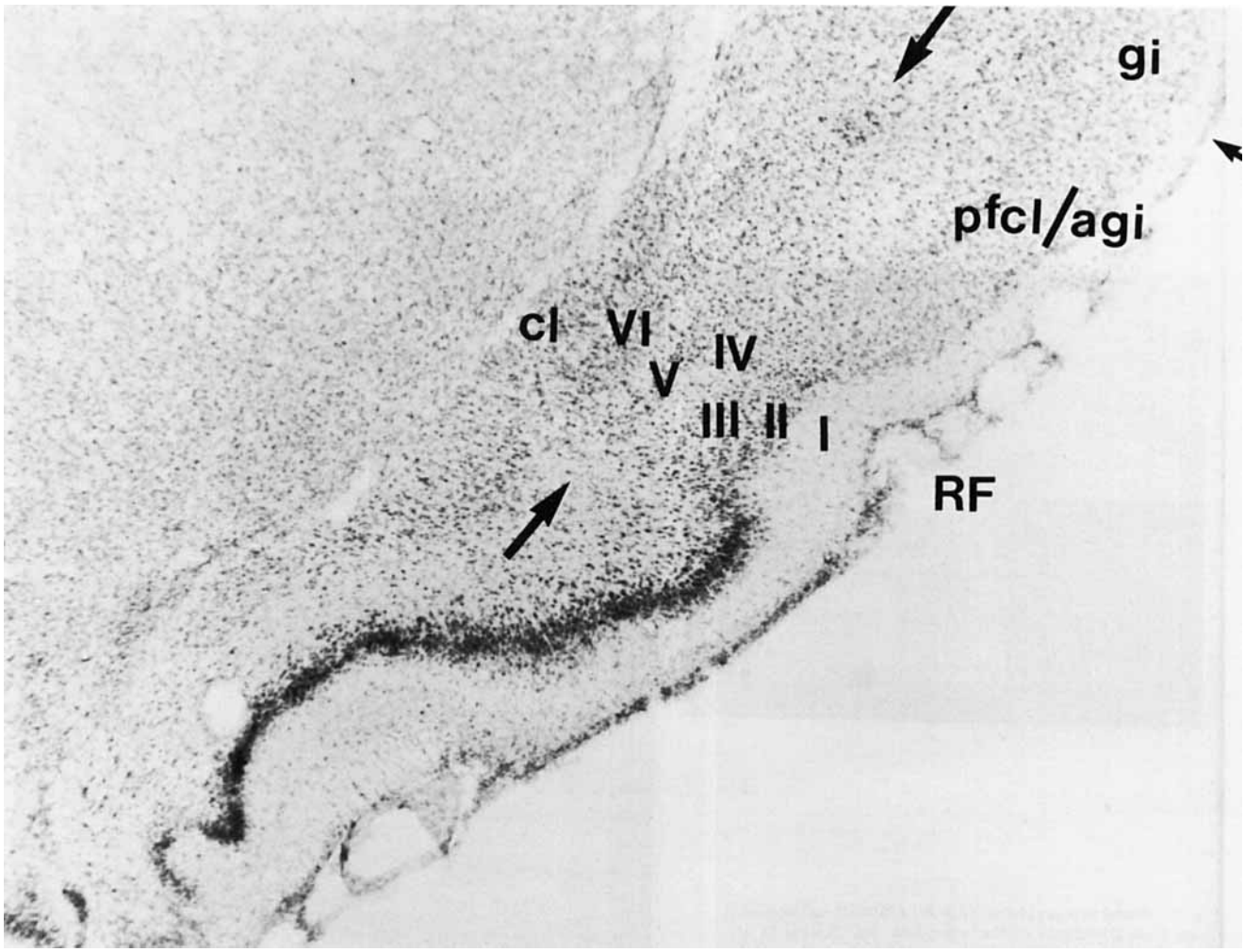

Fig. 4. A Nissl-stained section points out (between the large arrows) the deep cortical (layer V) area where fluorescent retrogradely labeled cells are situated after a True Blue injection in the NTS. The small arrow marks the division between granular insular cortex dorsally and agranular insular cortex ventrally. Within the agranular insular cortex, most of the labeled cell bodies are situated in the ventral and posterior (lateral prefrontal) portion (at and ventral to the rhinal fissure).

minal labeling was also seen throughout much of the medial thalamus at this rostrocaudal level, including the central, paracentral, and central lateral nuclei as well as the reuniens and ventromedial nuclei. Interestingly, the submedial nucleus (nucleus gelatinosus) contained less label than did surrounding areas.

Label was also present in other diencephalic and telencephalic regions. In the dorsolateral hypothalamus (dorsolateral to the fornix) some very sparse anterograde labeling and a few retrogradely labeled cell bodies appeared (Fig. 6C). In the amygdala, anterograde labeling marked the central nucleus and the basolateral nucleus, and a few retrogradely labeled cell bodies were found in the ventral portion of the basolateral nucleus (Fig. 6C). In addition, sparse anterograde labeling was seen throughout the claustrum, and a small number of retrogradely labeled cell bodies were found in the rostral claustrum (Fig. 6B,C), as well as lateral to it in the deep lateral prefrontal cortex.

In the mesencephalon, a major fascicle of labeled fibers continued in the most medial portion of the cerebral peduncle. Sparse anterograde labeling was seen in the substantia nigra pars compacta and medial pars reticulata. Considerable anterograde labeling was present in the peripeduncular nucleus (dorsolateral to the substantia nigra). This peripeduncular labeling seemed to tail off into the periaqueductal gray, an area which was most heavily labeled in its dorsal parts, i.e., lateral and dorsal to the aqueduct. Sparse labeling was also seen in the stratum intermedium of the superior colliculus. Retrogradely labeled cells were found scattered in the dorsal raphe nucleus and in the ventral tegmental area, including the interfascicular nucleus immediately dorsal to the interpeduncular nucleus.

In the pons (Fig. 6D), retrogradely labeled cell bodies were seen in the lateral portion of the dorsal parabrachial nucleus, as well as in the locus coeruleus and caudal dorsal raphe area. Anterograde labeling appeared in the most medial portion of the ventral parabrachial nucleus, in the locus coeruleus, in an area surrounding the lateral dorsal tegmental nucleus, and in the dorsal and ventral extremes

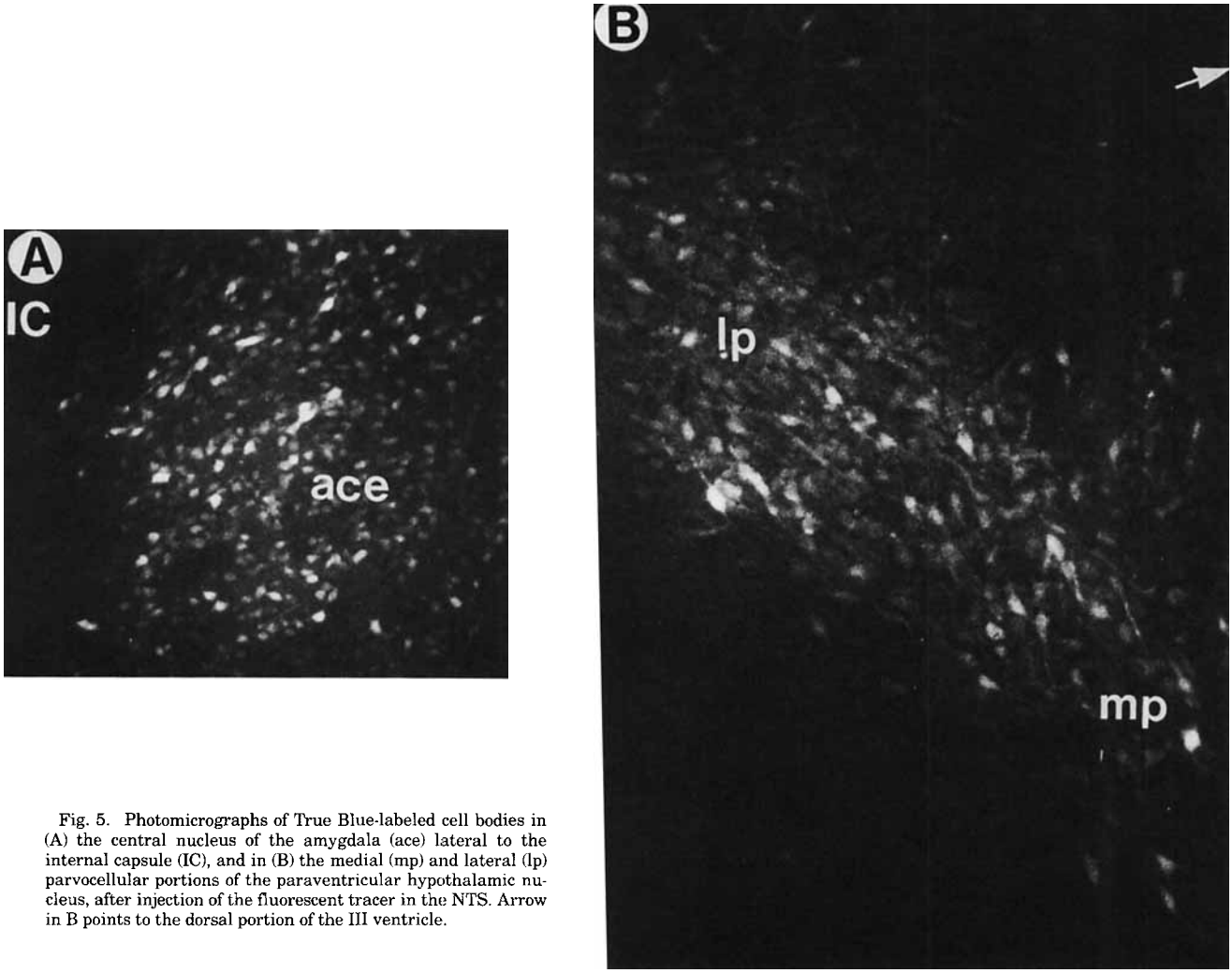

Fig. 5. Photomicrographs of True Blue-labeled cell bodies in (A) the central nucleus of the amygdala (ace) lateral to the internal capsule (IC), and in (B) the medial (mp) and lateral (lp) parvocellular portions of the paraventricular hypothalamic nucleus, after injection of the fluorescent tracer in the NTS. Arrow in B points to the dorsal portion of the III ventricle.

of the lateral periaqueductal gray. Anterograde labeling was also seen in the reticular formation immediately medial and lateral to the motor trigeminal motor nucleus. The fascicle of labeled axons noted above continued caudalward in the medial part of the pyramidal tract.

NTS terminations. After bilateral medial prefrontal cortex injections, terminal labeling in the NTS primarily marked medial parts of the nucleus. This was especially true at rostral levels of the NTS (Fig. 6E,F) where labeling was restricted to the medial solitary nucleus, continuing more sparsely ventralward into the region of the reticular formation medial to the spinal trigeminal nucleus. At mid-NTS levels labeling also included the lateral solitary nucleus, but this nucleus was not labeled as densely as the medial solitary nucleus and nucleus intercalatus (Fig. 6G). The lateral portion of the dorsal division of the medial solitary nucleus (immediately medial to the solitary tract) was relatively lightly labeled at this level. At caudal levels of the NTS (Fig. 6H,I), some labeling was seen through most of the nucleus, but the dorsal division of both the medial and the lateral solitary nucleus were labeled only very sparsely. The dorsal motor nucleus of the vagus was not labeled.

The most medial portion of the pyramidal tract contained labeled axons at all but the most caudal levels of the NTS. Anterograde labeling was also seen in the ventromedial reticular formation at all levels of the NTS; this labeling did not extend to the midline. A surprising finding was the presence of sparse anterograde labeling in the most ventrolateral portion of the hypoglossal motor nucleus. A few retrogradely labeled cell bodies were present in the ventromedial reticular formation at rostral levels of the NTS and in the ventrolateral reticular formation at more caudal levels.

Lateral prefrontal cortex.

Injection site. Figures 7C and 8A illustrate a large unilateral injection of WGA-HRP in the lateral cortex. The injection site encompasses the lateral prefrontal area which was retrogradely labeled in the first part of the study, as well as the cortex dorsal to this area, the claustrum, and the extreme lateral portion of the rostral caudate-putamen,

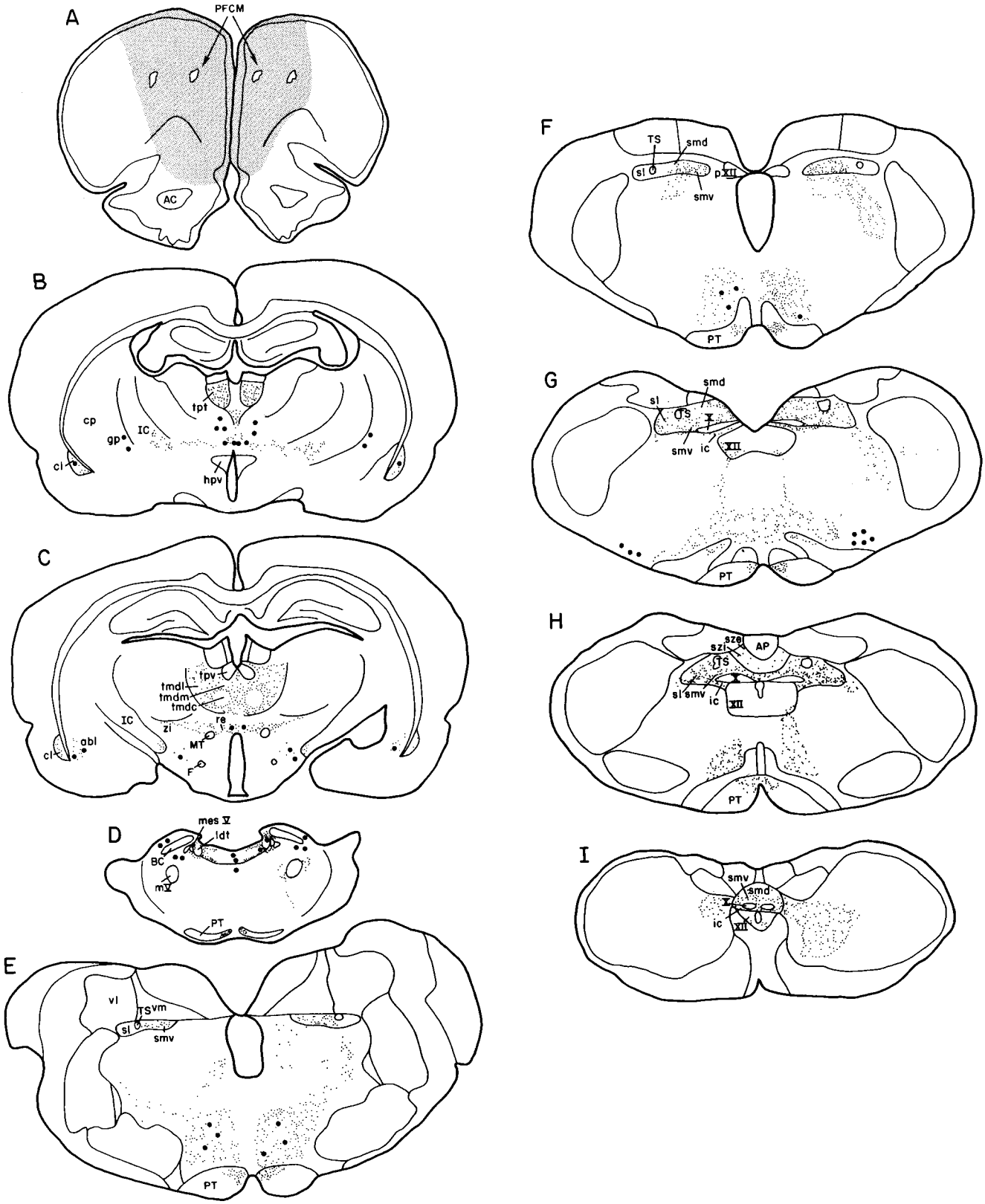

Fig. 6. Schematic diagram from rostral to caudal (A–I) of anterograde (small dots) and retrograde (large dots) labeling produced by a bilateral injection of WGA-HRP in the medial prefrontal cortex (A).

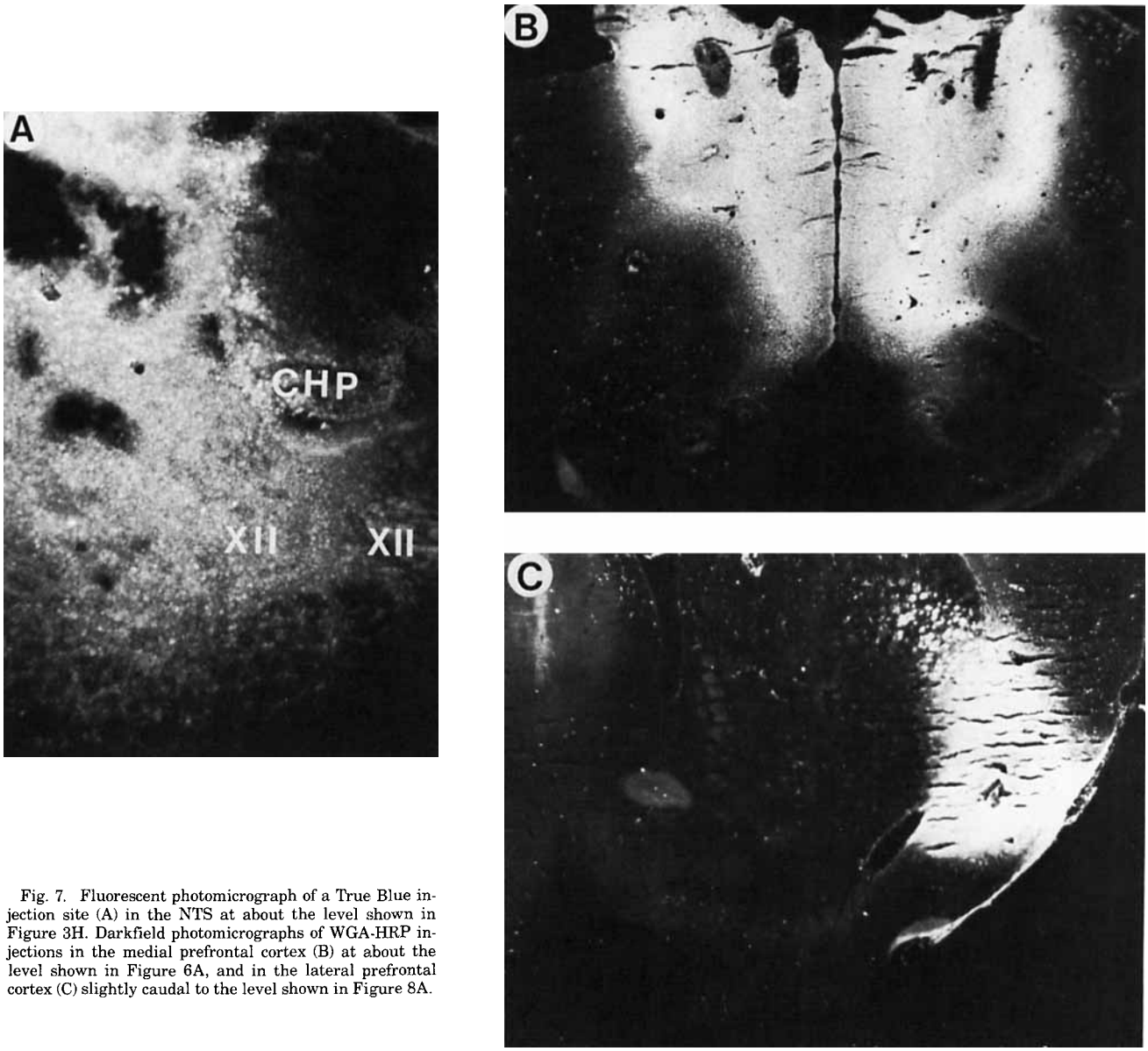

Fig. 7. Fluorescent photomicrograph of a True Blue injection site (A) in the NTS at about the level shown in Figure 3H. Darkfield photomicrographs of WGA-HRP injections in the medial prefrontal cortex (B) at about the level shown in Figure 6A, and in the lateral prefrontal cortex (C) slightly caudal to the level shown in Figure 8A.

but does not infringe on the central nucleus of the amygdala, the closest forebrain region that also projects to the NTS.

Descending projections. At the level of the injection site, numerous labeled fibers could be traced to the lateral prefrontal area of the opposite side, following the most ventral portions of the external capsule and corpus callosum. Retrogradely labeled cell bodies were seen especially in layers 3 and 5 of the contralateral lateral prefrontal cortex and dorsally adjacent cortex. Subcortical fibers for the most part followed ventrolateral portions of the ipsilateral internal capsule (Fig. 8B) and were distributed to a great variety of cell groups in forebrain, midbrain and hindbrain.

In the thalamus, relatively sparse anterograde labeling and a small number of retrogradely labeled cell bodies were seen primarily in the medial division of the ipsilateral mediodorsal nucleus. Substantial anterograde labeling was also seen in the medial portion of the ventrobasal complex (Fig. 8B), possibly due to spread of the injection into more dorsal portions of the cortex, and retrogradely labeled cells were found in the central and paracentral nuclei. Other ipsilateral telencephalic sites that contained anterograde label include the central and basolateral nuclei of the amygdala, the extreme lateral portion of the caudate-putamen, and the claustrum (Fig. 8B).

In the midbrain (Fig. 8C), labeled fibers were grouped in the lateral portion of the ipsilateral cerebral peduncle. Anterograde labeling was seen in the most ventrolateral portion of the substantia nigra pars reticulata, and a few retrogradely labeled perikarya were present in the ventral tegmental area, lateral substantia nigra pars compacta, and dorsal raphe nucleus. Labeled fibers continued ipsilaterally in the lateral portion of the pyramidal tract through the pons (Fig. 8D). Anterograde labeling was bilateral

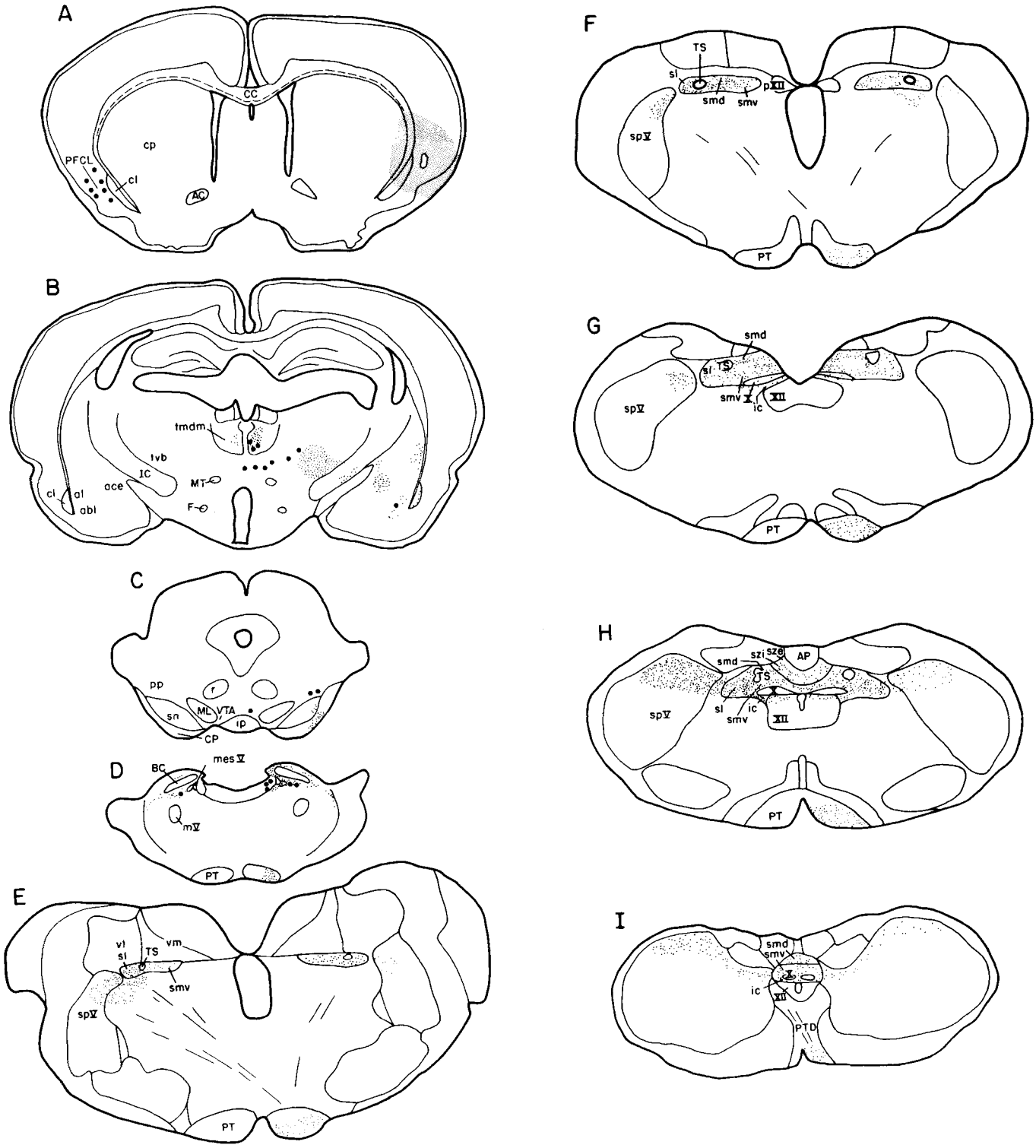

Fig. 8. Schematic diagrams from rostral to caudal (A–I) of anterograde (small dots and lines) and retrograde (large dots) labeling after injection of WGA-HRP in the lateral prefrontal cortical area (A).

throughout much of the parabrachial nuclei. Retrogradely labeled cell bodies were present, with a strong ipsilateral predominance, in the locus coeruleus, lateral-dorsal tegmental nucleus, and in the extreme ventral portion of the parabrachial nuclei beneath the mediolateral midpoint of the brachium conjunctivum.

NTS terminations. After the unilateral injection in the lateral prefrontal cortex, anterograde labeling was bilateral throughout much of the NTS. At rostral levels especially (Fig. 8E,F), labeled fibers could be seen leaving the ipsilateral pyramidal tract, extending dorsally and sometimes crossing the midline in the reticular formation (Fig. 9A). These fibers appeared to terminate bilaterally in the medial and lateral solitary nuclei, most densely in the lateral nuclei, especially at rostral levels. Dense fiber labeling was also seen in the dorsomedial part of the contralateral spinal trigeminal nucleus (Fig. 8E–I), continuous across the reticular formation with the fiber labeling in the NTS (Figs. 8H, 9B). Much sparser anterograde labeling appeared in the same region of the ipsilateral spinal trigeminal nucleus. At more caudal levels (Figs. 8G–I, 9B) most subdivisions of the NTS were anterogradely labeled, with the exception of a small label-free area in the ventral division of the medial solitary nucleus at mid-NTS levels (Fig. 8G). The dorsal motor nucleus of the vagus contained few if any labeled terminals. Interestingly, substantial labeling was seen in the internal subpostremal zone and in the lateral portion of the dorsal division of the medial solitary nucleus (Figs. 8G,H, 9B), unlike the termination patterns seen after many of the other forebrain injections described below.

Bed nucleus of the stria terminalis.

Injection site. Figure 10A illustrates a large unilateral injection of WGA-HRP in the rostral portion of the bed nucleus of the stria terminalis encompassing the area both dorsal and ventral to the anterior commissure. This injection site infringed upon the preoptic area of the hypothalamus and the caudal portion of the nucleus accumbens.

Descending projections. Dense fiber labeling appeared in the ipsilateral stria terminalis and in the central and cortical amygdaloid nuclei (Figs. 10B, 11). The basomedial amygdaloid nucleus showed less dense anterograde labeling. Retrogradely labeled cell bodies were seen in the central and cortical amygdaloid nuclei, and a further few appeared in the far lateral temporal cortex, mainly in layer V, and even more sparsely in layer III. It is important to note that these cells were situated well caudal to the lateral prefrontal cells labeled by injections of the NTS.

From the injection site labeled fibers coursed ipsilaterally through the entire lateral hypothalamus (Figs. 10B, 11). Sparse anterograde labeling appeared in the dorsomedial hypothalamic nucleus and the nucleus reuniens of the thalamus. In addition, anterograde fiber labeling was present bilaterally (with an ipsilateral predominance) in the medial division of the mediodorsal thalamic nucleus, and a few retrogradely labeled cell bodies were seen in the ipsilateral paraventricular thalamic nucleus.

Anterograde labeling in the ipsilateral caudal midbrain extended through the ventrolateral reticular formation up into the central gray substance, producing a bilateral field of terminal labeling (with an ipsilateral predominance) through the ventral half of the central gray. Retrogradely labeled cells appeared at this level in the dorsal raphe nucleus. In the pons, the anterograde labeling filled the entire central gray except for the dorsal tegmental nuclei (Fig. 10C). Anterograde labeling at this level was also seen bilaterally throughout the parabrachial nuclei, although here also in greater density on the ipsilateral side. Retrogradely labeled cells were seen mainly in the ipsilateral parabrachial nucleus, lateral dorsal tegmental nucleus, and locus coeruleus (Fig. 10C). In the caudal pons the bilateral anterograde labeling in parabrachial nuclei was mostly restricted to medial portions of the nuclei.

NTS terminations. In the rostral portion of the NTS, anterograde labeling appeared bilaterally in the medial solitary nucleus (Fig. 10D). Sparse ipsilateral labeling was seen in the lateral solitary nucleus. Some labeling also marked the reticular formation immediately ventral to the medial solitary nucleus, and to a lesser degree the ventrolateral reticular formation. At middle and caudal levels of the NTS, anterograde labeling (with a strong ipsilateral predominance) was found primarily in the ventral division of the medial solitary nucleus (Fig. 10E,F). Some weaker

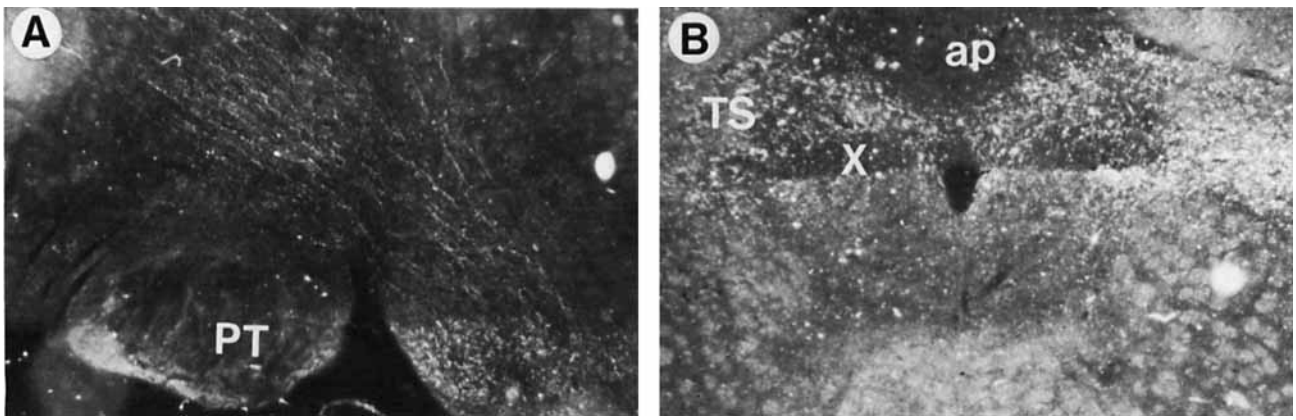

Fig. 9. Darkfield photomicrographs of WGA-HRP labeling in fibers in the medial reticular formation and pyramidal tract (rostral to the pyramidal decussation) at the rostral level of the nuclus of the solitary tract (A), and in terminals in the midcaudal NTS (B), after injection of the tracer in the lateral prefrontal cortical area.

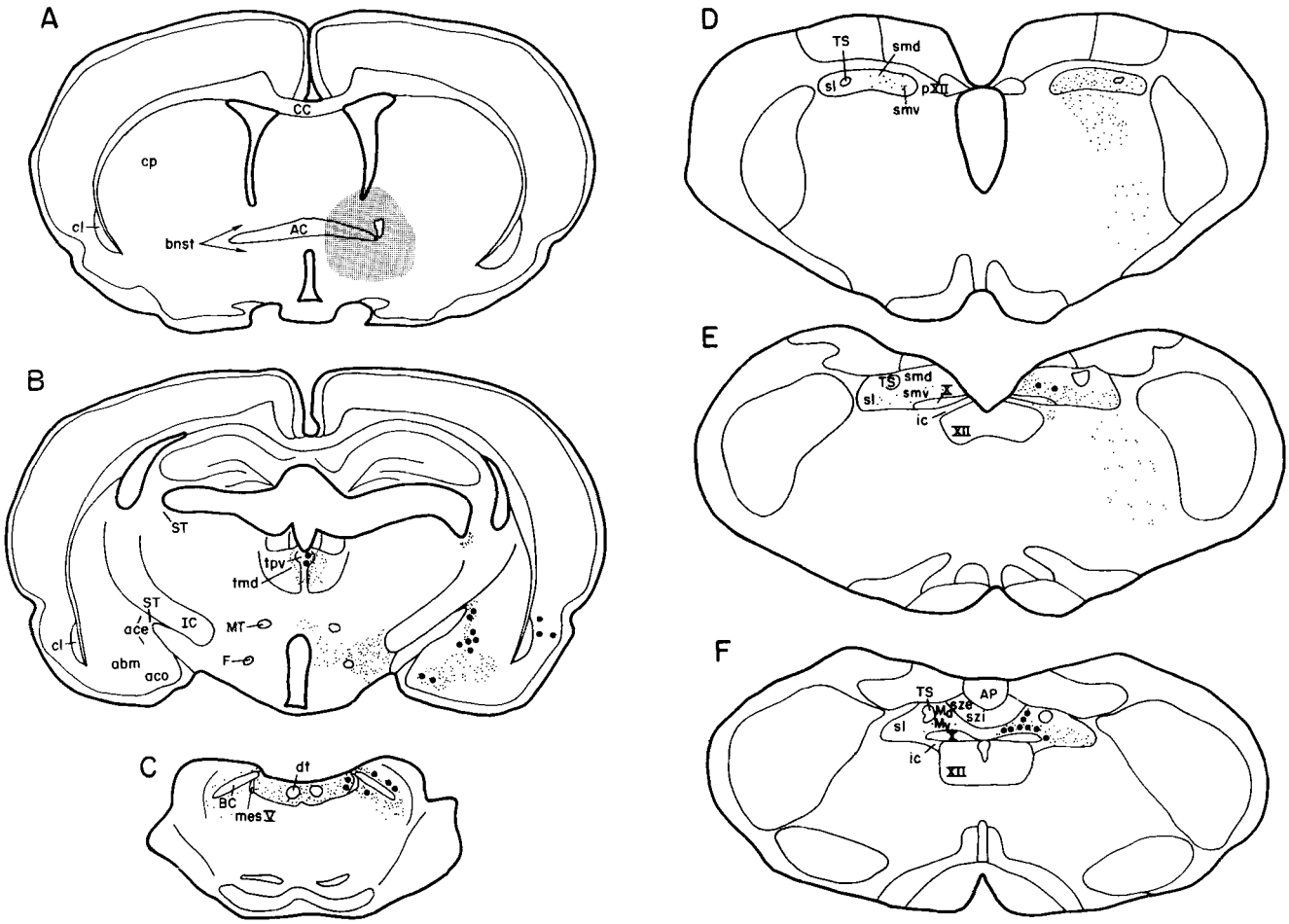

Fig. 10. Schematic diagrams from rostral to caudal (A–F) of anterograde (small dots) and retrograde (large dots) labeling after injection of WGA-HRP in the area of the bed nucleus of the stria terminalis (A).

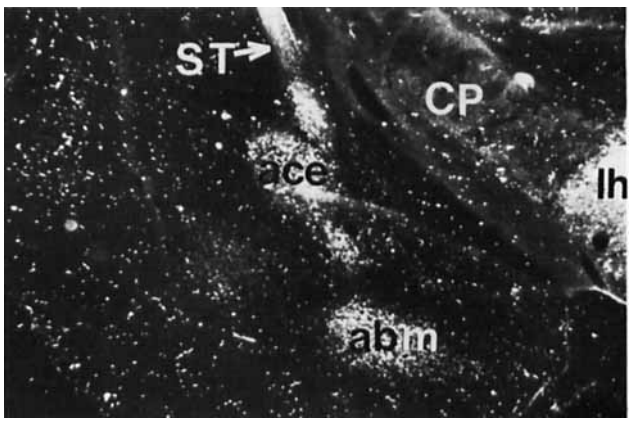

Fig. 11. Darkfield photomicrograph of WGA-HRP labeling in the stria terminalis (ST), central (ace) and basomedial (abm) amygdala, and lateral hypothalamus (lh) after injection of the tracer in the area of the bed nucleus of the stria terminalis.

ipsilateral labeling also appeared in the lateral portion of the nucleus intercalatus and in the ventral portion of the lateral solitary nucleus. The dorsal division of the medial solitary nucleus, the internal subpostremal zone, and the dorsal motor nucleus of the vagus (except for its extreme lateral zone at mid-NTS levels) (Fig. 10E) were notably free of anterograde labeling. Retrogradely labeled cell bodies appeared in caudal portions of the ipsilateral NTS (Fig. 10F), immediately dorsal to the dorsal motor nucleus of the vagus, primarily in the ventral division of the medial solitary nucleus.

Central nucleus of the amygdala.

Injection site. Figure 12A illustrates a large ipsilateral WGA-HRP injection centered on the central nucleus of the amygdala, but infringing upon the ventral edge of the internal capsule, the lateral part of the optic tract, the medial part of the lateral and basolateral amygdaloid nuclei, the ventrocaudal caudate-putamen, the lateral part of the medial amygdaloid nucleus, and dorsal portions of the basomedial and cortical amygdaloid nuclei. It must be recalled, however, that none of these other amygdaloid nuclei showed

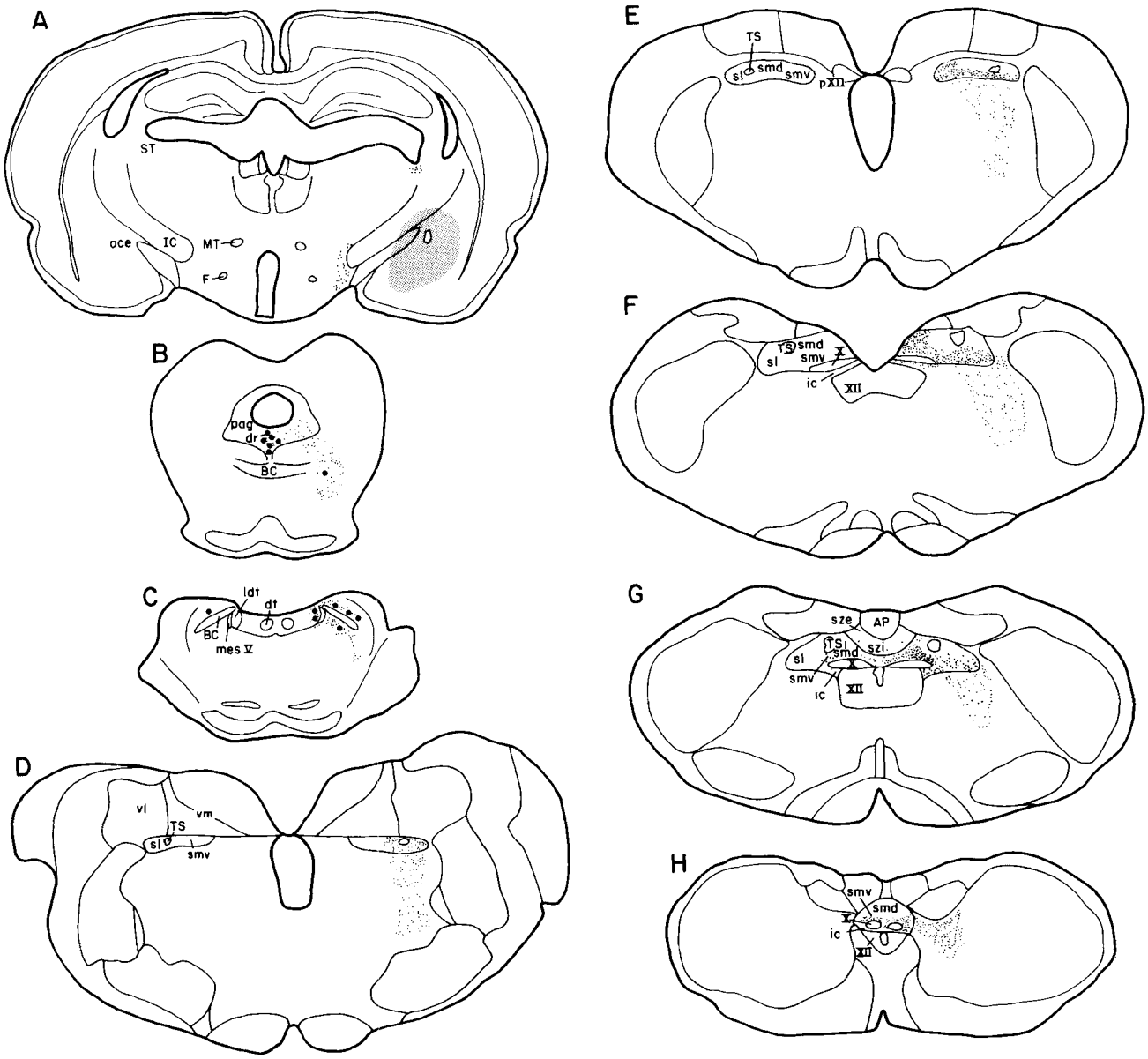

Fig. 12. Schematic diagrams from rostral to caudal (A–H) of anterograde (small dots) and retrograde (large dots) labeling after injection of WGA-HRP in the area of the central nucleus of the amygdala (A).

retrograde cell labeling following WGA-HRP injections in the NTS.

Descending projections. Anterogradely labeled fibers from the injection site were present in the stria terminalis and in the ventral amygdalofugal pathway. The labeled fibers of this latter pathway gathered ipsilaterally in the far lateral hypothalamus (Fig. 12A) and descended in the area dorsolateral to the substantia nigra pars reticulata. At this level, retrogradely labeled cell bodies were seen in the medial geniculate nucleus (presumably due to involvement of the ventrolateral part of the internal capsule at the injection site), and in lesser numbers in the ventral tegmental area and extreme lateral part of the substantia nigra pars compacta. Sparse anterograde labeling also appeared throughout the substantia nigra pars compacta. In the caudal midbrain (Fig. 12B), labeled fibers moved laterally around the brachium conjunctivum into the ventral part of the ipsilateral central gray substance. At this level, retrogradely labeled cell bodies were present in the dorsal raphe nucleus and occasionally in the ipsilateral A8 or retrorubral area. In the pons (Fig. 12C), considerable anterograde labeling appeared throughout the ipsilateral parabrachial nucleus and locus coeruleus. Both of these nuclei also contained ipsilateral and to a lesser extent contralateral retrogradely labeled cell bodies. Anterograde labeling in the pons was also seen surrounding the ipsilateral trigeminal motor nucleus and was especially heavy ventral to the nucleus.

NTS terminations. In the most rostral portion of the NTS (Fig. 12D), anterograde labeling from the amygdala

injection was almost exclusively ipsilateral and confined to the lateral part of the medial solitary nucleus, the lateral solitary nucleus, and the lateral reticular formation ventral to these areas. At midrostral levels of the NTS (Fig. 12E), labeling was again ipsilateral, being centered in the ventral part of the lateral solitary nucleus and particularly in the ventral division of the medial solitary nucleus. The ventral division of the medial solitary nucleus at this midrostral level is also the location of some rostral vagal motoneurons (Contreras et al., '80; Ritchie et al., '82); little anterograde labeling, however, was seen in the major portion of the dorsal motor nucleus of the vagus at more caudal levels. Anterograde labeling also extended ventrally from the solitary nuclei into the lateral reticular formation, with a small concentration of label present in the region of the nucleus ambiguus. At mid-NTS levels (Fig. 12F) fiber and terminal labeling was distributed throughout the ipsilateral NTS except for the lateral portion of the dorsal division of the medial solitary nucleus, and the dorsal part of the lateral solitary nucleus. The dorsal motor nucleus of the vagus likewise was free of label. The anterograde labeling in the lateral solitary nucleus continued ventrally into the lateral reticular formation. At more caudal levels of the ipsilateral NTS (Fig. 12G,H), fiber labeling was particularly concentrated in the ventral division of the medial solitary nucleus immediately dorsal to the dorsal motor nucleus of the vagus. Sparser labeling was also seen in the ventral division of the contralateral medial solitary nucleus, bordering the dorsal motor nucleus of the vagus. The dense labeling in the medial solitary nucleus made the absence of label in the midline solitary nucleus particularly noticeable in this case. At caudal levels of the NTS, ipsilateral fiber labeling was also seen in the lateral part of the intercalated nucleus, the ventral part of the lateral solitary nucleus, and the reticular formation ventral and lateral to the NTS. The internal subpostremal zone, the dorsal division of the medial solitary nucleus and the dorsal part of the lateral solitary nucleus were all relatively free of label at these caudal levels. At midcaudal levels, a few retrogradely labeled cells were observed in the ipsilateral ventral division of the medial solitary nucleus.

Paraventricular nucleus of the hypothalamus.

Injection site. Figure 13A illustrates a large, primarily unilateral WGA-HRP injection into the paraventricular area of the hypothalamus. The injection site includes much of the mediolateral extent of the hypothalamus at this rostral level and also infringes on the ventromedial part of the thalamus (especially the nucleus reuniens) and on the dorsomedial portion of the contralateral paraventricular hypothalamic nucleus. The large injection site does not infringe on extrahypothalamic or posterolateral hypothalamic cell groups that were shown in the initial portion of this study to project to the NTS, but it does involve the rostral region situated dorsolateral to the fornix where scattered cells were found labeled by injections of the NTS.

Descending projections. Labeled fibers from the injection site gathered in the midbrain mainly dorsolateral to the substantia nigra pars reticulata in the area of the peripeduncular nucleus, an area which also contained retrogradely labeled cell bodies. From this group of labeled fibers, anterograde labeling tailed off ventromedially into the ipsilateral substantia nigra pars compacta and ventral tegmental areas as well as dorsomedially into the central gray substance. In the last-mentioned of these regions, labeling was bilateral, with a strong ipsilateral predominance. Retrogradely labeled cells appeared in the region of the dorsal raphe nucleus (Fig. 13B).

In the pons (Fig. 13C), bilateral anterograde labeling occupied most of the central gray, including the locus coeruleus and lateral dorsal tegmental nucleus, but was absent in the region around the dorsal tegmental nuclei. The parabrachial nuclei were labeled bilaterally, but with an ipsilateral predominance. Retrogradely labeled cell bodies were also seen in the the locus coeruleus, lateral dorsal tegmental nucleus, and lateral portion of the parabrachial nuclei.

NTS terminations. In the rostral portion of the NTS (Fig. 13D,E), bilateral anterograde labeling was seen in all NTS subdivisions. The medial solitary nuclei appeared most densely innervated. Bilateral labeling was also seen in the area of the reticular formation immediately ventral to the medial solitary nuclei and in the ventromedial reticular formation immediately dorsal to the pyramidal tract. A few retrogradely labeled cell bodies were seen in the ventral reticular formation lateral to the pyramidal tract. At mid-NTS levels (Fig. 13F), bilateral anterograde labeling with an ipsilateral predominance involved most subnuclei of the NTS, except that the labeling was relatively sparse in the lateral portion of the dorsal division of the medial solitary nucleus and in the dorsal part of the lateral solitary nucleus. The labeling was heaviest in the ventral division of the medial solitary nucleus bordering the dorsal and lateral aspects of the dorsal motor nucleus of the vagus. Sparser labeling was also present in the dorsal motor nucleus of the vagus, especially in its lateral portion. At intermediate and caudal levels of the NTS, numerous retrogradely labeled cell bodies were seen, primarily in the ventral division of the ipsilateral medial solitary nucleus. At caudal levels likewise (Fig. 13G,H), bilateral fiber labeling occupied much of the NTS, but here it was striking that it included both the internal and external subpostremal zones as well as the area postrema proper. As was the case at more rostral levels, the two areas on either side of the solitary tract (the dorsal division of the medial solitary nucleus and the dorsal portion of the lateral solitary nucleus) were only sparsely labeled. Some bilateral fiber labeling was noted in caudolateral parts of the dorsal motor nuclei of the vagus, but the medial portions of these nuclei, as well as the midline solitary nucleus, were relatively free of label. In addition, considerable bilateral anterograde labeling with an ipsilateral predominance extended laterally and ventrally from the caudal NTS into the reticular formation.

Posterolateral hypothalamus.

Injection site. Figure 14A illustrates a large WGA-HRP injection in the posterolateral hypothalamus, situated mainly lateral to the fornix and mammillothalamic tract and medial to the optic tract and internal capsule. This injection site also infringed on the zona incerta and ventromedial portion of the thalamic ventrobasal complex. There was no spread from the injection site to other areas giving rise to NTS projections, except possibly to some cells situated dorsolateral to the fornix at the rostral edge of the injection site.

Descending projections. Labeled fibers from the injection site gathered in the A8 or retrorubral area caudal and dorsolateral to the substantia nigra and red nucleus, and

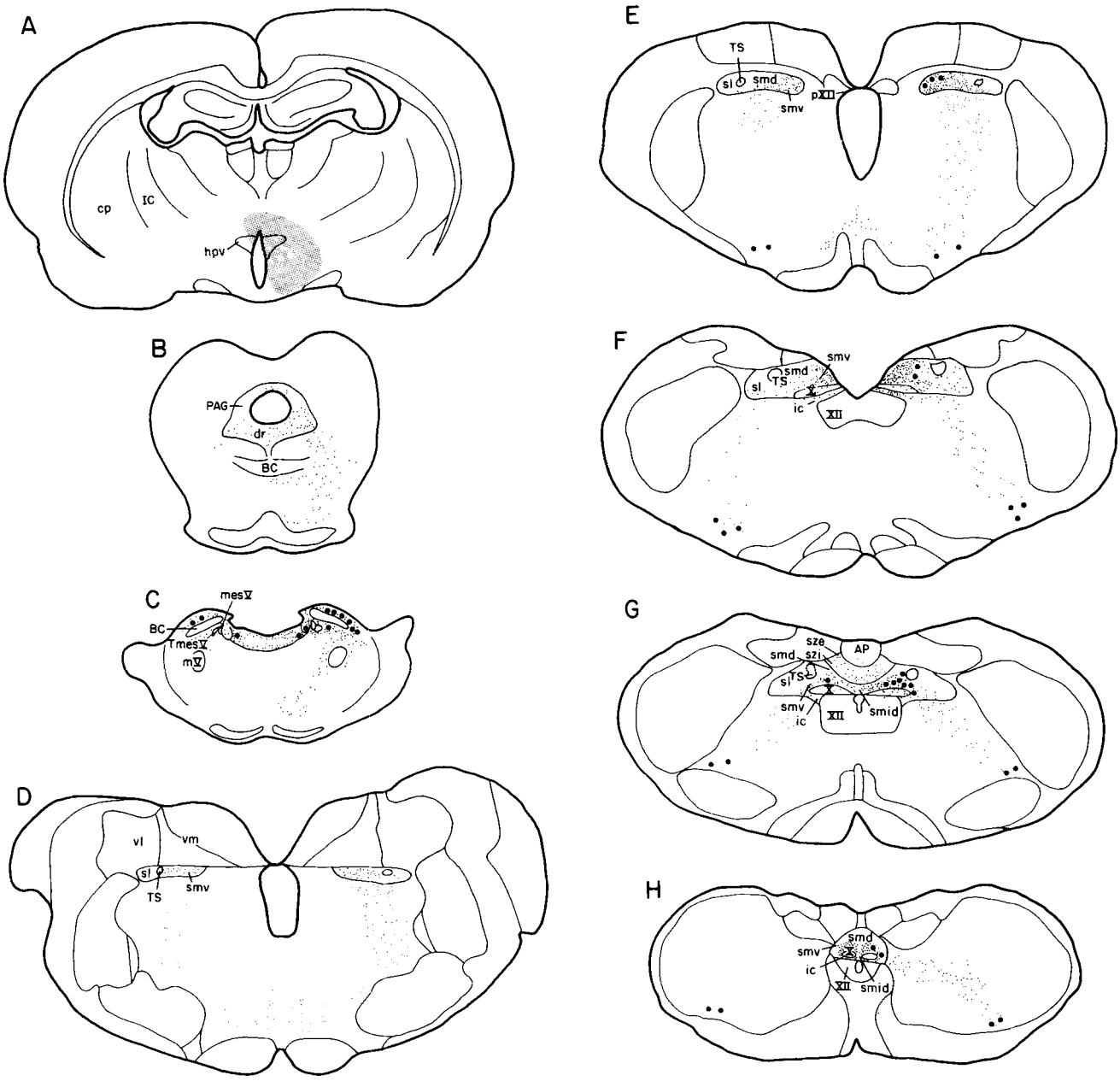

Fig. 13. Schematic diagrams from rostral to caudal (A–H) of anterograde (small dots) and retrograde (large dots) labeling after injection of WGA-HRP in the area of the paraventricular hypothalamic nucleus (A).

were concentrated just lateral to the brachium conjunctivum (Fig. 14B). At this midbrain level, anterograde labeling was present in the ipsilateral ventral midbrain area, the ipsilateral ventral tegmental nucleus of Gudden and bilaterally (with an ipsilateral predominance) in the ventral two-thirds of the central gray substance. Retrogradely labeled cell bodies were seen in the dorsal raphe nucleus.

In the pons (Fig. 14C), bilateral labeling (with an ipsilateral predominance) was seen throughout the central gray including the locus coeruleus and lateral dorsal tegmental nucleus, but avoided the dorsal tegmental nucleus of Gudden which was completely outlined in label. Anterograde label filled the ipsilateral parabrachial nucleus and medial portions of the contralateral parabrachial nucleus. Anterograde labeling was also seen ipsilaterally on the medial, lateral, and especially the ventral border of the trigeminal motor nucleus, extending into the ventromedial reticular formation. Retrogradely labeled cell bodies appeared in the ipsilateral locus coeruleus, in the dorsal part of the contralateral main sensory trigeminal nucleus, and bilaterally (with an ipsilateral predominance) in the parabrachial nuclei.

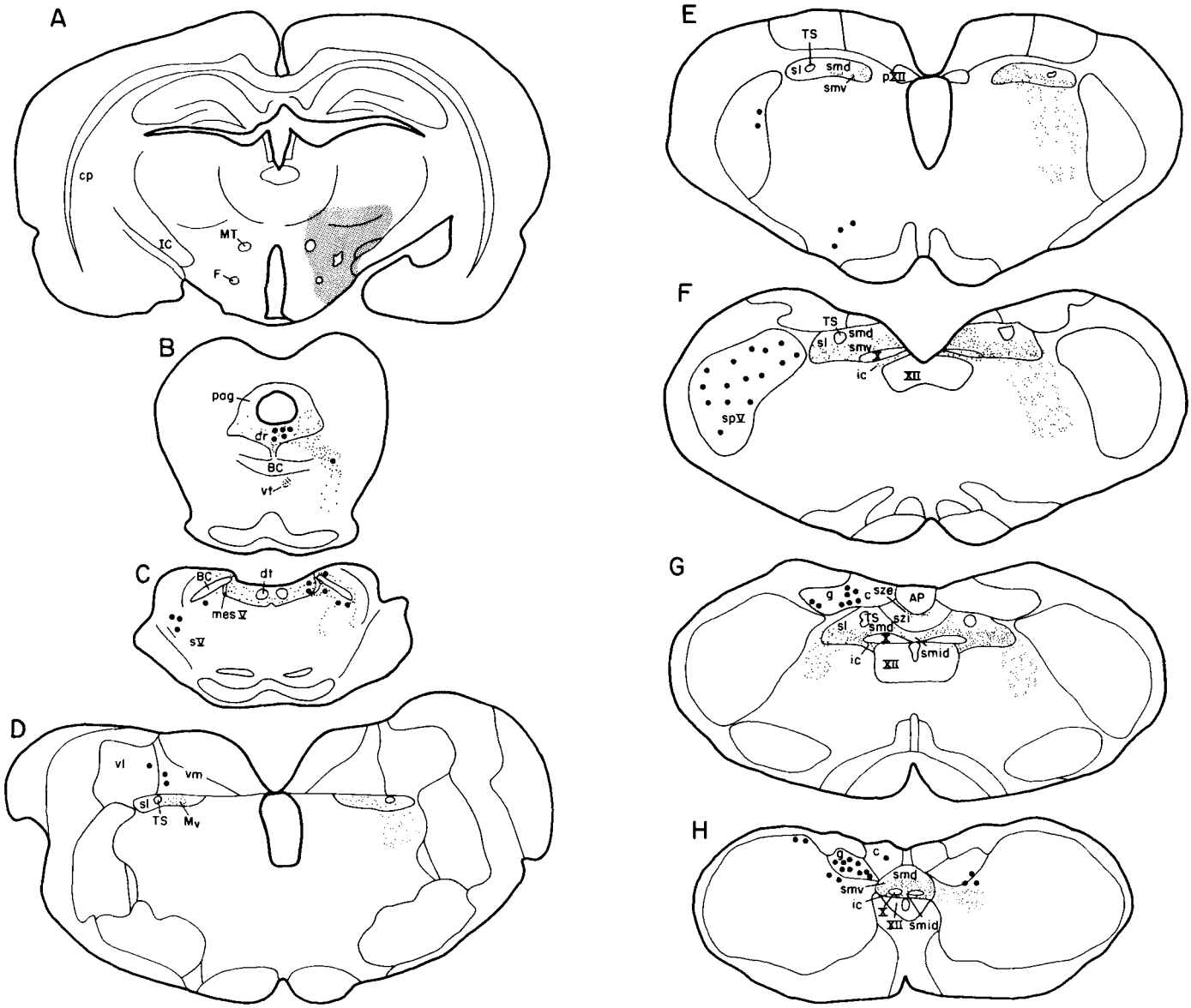

Fig. 14. Schematic diagrams from rostral to caudal (A–H) of anterograde (small dots) and retrograde (large dots) labeling after injection of WGA-HRP in the posteriolateral hypothalamus (A).

NTS terminations. At rostral levels of the NTS (Fig. 14D,E), bilateral anterograde labeling was seen mainly in the medial solitary nucleus. The ipsilateral NTS labeling trailed off into the ventrolateral reticular formation. This reticular formation labeling seemed somewhat heavier in the region of the nucleus ambiguus. At mid-NTS levels (Fig. 14F), bilateral anterograde labeling (with an ipsilateral predominance) occupied much of the NTS. The rostral portion of the ipsilateral dorsal motor nucleus of the vagus was also sparsely labeled. At this level, as well as further caudally (Fig. 14G,H), the areas on either side of the solitary tract (the lateral part of the dorsal division of the medial solitary nucleus and the dorsal portion of the lateral solitary nucleus) were relatively free of label, as was the internal subpostremal zone at the level of the area postrema (Fig. 14G). Throughout this caudal half of the NTS, anterograde labeling was concentrated dorsal, lateral, and ventral to the dorsal motor nuclei of the vagus, i.e., in the ventral divisions of the medial solitary nuclei as well as in the lateral and intercalated nuclei. Sparse fiber labeling was also seen in the external subpostremal zone. Caudal parts of the dorsal motor nuclei of the vagus and the midline nucleus of the NTS were free of label. Bilaterally, the anterograde labeling of the NTS extended (with an ipsilateral predominance) into the reticular formation lateral and ventral to the caudal NTS. Retrogradely labeled cell bodies were seen primarily in the contralateral vestibular, spinal trigeminal, and dorsal col-

umn nuclei (presumably due to ventral thalamic involvement at the injection site), and in the ventral reticular formation lateral to the pyramidal tract.

Both the posterolateral and paraventricular hypothalamic injections of WGA-HRP produced some anterograde labeling in the spinal cord (especially in the intermediate zone), but this labeling was not studied in detail.

DISCUSSION

Methodological considerations

In the present study the projections from different forebrain areas (prefrontal cortex, central nucleus of the amygdala, bed nucleus of the stria terminalis, and hypothalamus) to the NTS have been defined in the rat by a combination of retrograde and anterograde tracing methods that can make hodologic conclusions more certain. For example, the retrograde data demonstrate that the forebrain areas that provide NTS afferents are sufficiently separate from each other to prevent even large WGA-HRP injection sites from involving more than one source of afferents. The fluorescent dye injections were not confined exactly to the boundaries of the NTS and may have been taken up by fibers of passage. But the actively distribution of forebrain projections to the NTS and surrounding areas could be confirmed convincingly by anterograde labeling with WGA-HRP.

Nonetheless, one difficulty with the present methods remains: the evidence that fibers of passage can be labeled with WGA-HRP (Gerfen et al., '82). Although this is not a likely problem in cases of cortical injection, it could be a source of confusion in interpreting the results of, for example, posterolateral hypothalamic WGA-HRP injections which must involve some fibers descending to the NTS from the amygdala, bed nucleus of the stria terminalis, and anterior hypothalamus. The differences in the termination patterns in the NTS after these various injections obviates the problem to a certain degree, but it is impossible to be certain that none of the anterograde labeling caused by posterolateral hypothalamic injections resulted from uptake by fibers of passage.

Forebrain projections to the NTS

The present study has confirmed earlier reports of projections to the NTS from the hypothalamus (Ciriello and Calaresu, '77; Conrad and Pfaff, '76; Hosoya and Matsushita, '81; Saper et al., '76; Swanson and Kuypers, '80) and amygdala (Hopkins and Holstege, '78; Price and Amaral, '81). However, the projections from the bed nucleus of the stria terminalis and prefrontal cortex have not been detailed before.

Previously there has been scant evidence for the existence of cortical projections to the NTS. From studies by the Glees method in the rat, lesions involving most of the rat cortex except the prefrontal areas werre reported to produce terminal degeneration in the lateral solitary nucleus (Torvik, '56), and similar findings were made in cats with large cortical lesions which sometimes included medial and basal portions of the cortex (Brodal et al., '56). In addition, there are two brief reports of a projection from the cat anterior coronal gyrus to the NTS (Kawana and Kusama, '68; Flindt-Egelak and Olsen, '81). Injections of tritiated amino acids in the sensory gustatory area, situated dorsal and caudodorsal to the lateral prefrontal cortex (Wolf, '68; Krettek and Price, '77; Yamamoto et al., '80) were reported to produce medullary labeling that "envelops" the NTS in the rat (Norgren and Grill, '76). Norgren et al. ('82) have recently suggested that taste in the rat is primarily represented in the agranular insular cortex dorsal to the rhinal fissure, rather than in the granular insular cortex as was previously believed. Notably, it is the ventral and posterior agranular insular cortex (situated ventral to this dorsal agranular insular cortex) that has been shown in the present report to be the major origin of the lateral prefrontal cortical projections to the NTS. From the cortex dorsal to the lateral prefrontal area in the rat, projections have been traced to the sensory trigeminal nuclei (Wise et al., '79; van der Kooy et al., unpublished observations), but a cortical projection to the NTS comparable in volume to the one suggested by our observations after prefrontal cortical WGA-HRP injections has not been reported previously. Recently, however, Shipley ('82) has traced a substantial corticosolitary projection in the mouse by the aid of the anterograde transport of WGA-HRP from the insular cortex. Shipley's injection sites probably include the area homologous to the ventral and posterior agranular insular cortex (or lateral prefrontal) region that we have shown to be the main cortical origin of this projection in the rat.

Prefrontal cortical inputs to the NTS

The original definition of the prefrontal cortex as that part of the frontal cortex containing a distinctive granular layer IV (Brodmann, '09) has evolved into a definition based on the cortical projection areas of the mediodorsal thalamic nucleus (Rose and Woolsey, '48). While such a designation may be compromised to some extent by reports that the paralamellar portion of the mediodorsal nucleus in primates projects to the frontal eye fields (area 8 of Brodmann) (Scollo-Lavizzari and Akert, '63; Kievit and Kuypers, '77) and to the anterior insular cortex (Locke, '67), areas arguably outside functionally defined regions of the prefrontal cortex, such a definition lends itself more readily to comparative studies. Thus, although markedly agranular, the cortical regions in the rat receiving afferents from the mediodorsal nucleus, particularly from the medial and central segments of the nucleus, may be defined as prefrontal (Leonard, '69, and see Reep and Winans, '82 for discussion). Since the cortical neurons that project to the NTS are located for the most part in these particular regions, they must be considered components of the prefrontal cortex. The medial prefrontal group (Fig. 3A,B) is situated in the cortex termed prelimbic by Rose ('28) and receives afferents from the medial subdivision of the mediodorsal nucleus (Leonard, '69; Krettek and Price, '77). Occasionally labeled neurons are found adjacent to this prelimbic area: ventral, in the infralimbic cortex which receives afferents from the nucleus reunions, and dorsal, in the medial precentral cortex which receives afferents from the paralamellar subdivision of the mediodorsal nucleus (Leonard, '69; Krettek and Price, '77).

The lateral prefrontal cell group that provides NTS afferents is situated near the rhinal sulcus and extends from the level of the forceps minor to the rostral portion of the claustrum (Fig. 3A–E). A small number of neurons in this group are located dorsally in what is termed the dorsal agranular insular cortex (Rose, '28) that receives afferents from the paralamellar mediodorsal thalamic nucleus (Leonard, '69; Krettek and Price, '77; Gerfen and Clavier, '79; Reep and Winans, '82), but a majority are located more ventrally in the ventral agranular insular cortex projected upon by the central segment of the mediodorsal nucleus (Leonard, '69; Krettek and Price, '77; Gerfen and Clavier,

'79; Reep and Winans, '82), and in the posterior agranular insular area (Rose, '28; Krettek and Price, '77). The thalamic connections of this last-mentioned area have not been studied in detail; our present findings suggest that it has reciprocal connections with the central and medial subdivisions of the mediodorsal nucleus, but no experiments controlling for possible spread of WGA-HRP from the injection site were done. Thus, a definitive designation of the posterior agranular insular areas as prefrontal cortex will have to wait until the afferent connections of this perirhinal cortex are better known. It should be recalled that the cells labeled in the caudal area in question appear to be a caudal continuation of the group of neurons in the ventral agranular insular cortex that provide NTS afferents.

Although a small number of neurons in the frontal motor cortex appear to project to the NTS, other cortical areas of the frontal pole such as the medial orbital and ventrolateral orbital cortex which receive afferents from the paratenial and submedial thalamic nuclei, respectively (Krettek and Price, '77), do not contribute afferents to the NTS.

The distribution of prefrontal cortical cells projecting to the NTS overlaps with the dopamine innervation of the cortex. Dopaminergic cortical innervation has been claimed as a marker for prefrontal cortex in view of the similarities between the respective distributions of dopamine terminals and thalamocortical fibers from the mediodorsal nucleus (Lindvall et al., '78; Divac et al., '78). In fact, the cortical region providing NTS afferents seems to match the distribution of the mesocortical DA system more closely than it does the projection of the mediodorsal nucleus. For example, the infralimbic, medial precentral, and dorsal agranular insular, in which a few neurons were labeled by NTS injection, receive dopaminergic innervation (Lindvall et al., '78). Moreover, possibly in contrast to mediodorsal thalamic afferents (Krettek and Price, '77), dopamine terminals are found in the posterior agranular insular cortex at the level of the claustrum, where a relatively large number of NTS projecting neurons are located. In all cortical areas providing NTS afferents, the cell bodies giving rise to these projections are located in the same deeper layers which also receive dopaminergic terminals (Lindvall et al., '78; Morrison et al., '79). It would be of interest to determine at the electron microscopic level whether dopamine terminals make synaptic connections with the deep prefrontal cortical cells that project to the NTS. Such direct connections would be of particular interest in light of studies demonstrating a selective activation of the cortical dopaminergic system by stress (Thierry et al., '76). Since stress (Kaplan, '80), catecholamine agonists (Kaplan, '80), or electrical stimulation of the prefrontal cortex (Kaada, '56; Morrison et al., '80) all produce cardiovascular changes (hypotensive responses were observed after electrical stimulation of the lateral prefrontal cortex, unpublished observations), the direct prefrontal-NTS pathway here described may prove to be an important conveyor of forebrain influences on cardiovascular as well as general autonomic function.

Distribution of forebrain afferents in the NTS

A number of generalizations can be made concerning the patterns of forebrain terminations in the NTS. First, there is a gross mediolateral topography in the forebrain projections to the rostral part of the NTS. Thus, the more medially situated forebrain sites (medial prefrontal cortex, bed nucleus of the stria terminalis, paraventricular hypothalamus) project to the medial solitary nucleus (mainly the latter's ventral division) at rostral levels, whereas the more lateral forebrain sites (lateral prefrontal cortex and central nucleus of the amygdala) project to the rostral NTS with predominant termination in the lateral solitary nucleus. It is easier to define the limits of the NTS at these rostral levels by the forebrain projection patterns than by cytoarchitectonic criteria. At middle and caudal levels of the NTS, both medial and lateral solitary nuclei are innervated by all of the forebrain regions examined. A certain mediolateral topography is also seen in forebrain projections to the dorsal pons. Although all the forebrain areas examined project to the locus coeruleus regions, medial forebrain areas such as the medial prefrontal cortex tend to project to the pontine central gray and avoid the parabrachial nuclei, whereas more lateral areas such as the lateral prefrontal cortex and amygdala tend to project to the parabrachial nuclei and avoid the pontine central gray.

Second, the direct projections from forebrain regions to the NTS are generally bilateral. The ipsilateral and contralateral projections to NTS from the lateral prefrontal cortex are of similar density. In contrast, the central nucleus of the amygdala and the bed nucleus of the stria terminalis project to the NTS with a strong ipsilateral predominance. With respect to the volume of the various forebrain projections to the NTS, the most massive innervation from the cortex originates in the lateral prefrontal area, and the heaviest subcortical afferentation of the NTS originates from the central nucleus of the amygdala and the paraventricular hypothalamic nucleus.

The third generalization is that in several respects the distribution patterns of the subcortical NTS afferents differ from those of the cortical afferents. These differences are seen in certain axon trajectories and dorsal pontine projections, as well as in the terminations within NTS proper. The cortical projections to the NTS travel primarily through the internal capsule, cerebral peduncle, and pyramidal tract, whereas the subcortical projections pass through the substance of the midbrain and brainstem tegmentum. The subcortical projections to the NTS traverse the far lateral substantia nigra and the retrorubral area and innnervate portions of the periaqueductal gray, as also do the afferents from the medial prefrontal cortex. The subcortical forebrain sites also project to the parabrachial nuclei, as does the lateral prefrontal cortex. Of particular interest are some of the subcortical projections to the caudal (pontine) central gray. The subcortical (but not the cortical) projections appear to innervate the lateral dorsal tegmental nucleus, the so-called pontine micturition center (Loewy et al., '79). The lateral dorsal tegmental nucleus also projects to the NTS (Loewy et al., '79) in a pattern resembling the subcortical rather than cortical projections (see below). Further, the bed nucleus of the stria terminalis and the paraventricular and posterolateral hypothalamus project throughout the pontine central gray, except for the dorsal tegmental nucleus of Gudden. This subcortical projection pattern is best exemplified by the posterolateral hypothalamic projections which completely outline the dorsal tegmental nucleus of Gudden but, surprisingly, avoid the latter and instead terminate in the ventral tegmental nucleus of Gudden. A similar distribution of label in the pontine central gray, avoiding the dorsal tegmental nucleus, is seen with autoradiographic muscarinic receptor binding (Wamsley et al., '81), with serotonin immunohistochemistry (Steinbusch, '81), and with anterograde tracing of interpeduncular nucleus projections (Carter and van der Kooy, unpublished

observations). The dorsal tegmental nucleus itself has been shown to project rostrally through thel mammillary peduncle to the mammillary bodies and into the medial forebrain bundle (Morest, '61).

Probably the most notable differences between cortical and subcortical projections appear in the caudal NTS. The prefrontal cortical regions project to dorsal parts of the NTS (the dorsal division of the medial solitary nucleus, especially its lateral part, and the dorsal portion of the lateral solitary nucleus), as well as to the midline nucleus. These are areas largely avoided by the subcortical projections. It is the more dorsal and lateral regions of the caudal NTS which receive the most massive primary afferent innervation from the glossopharyngeal and vagus nerves (Torvik, '56; Beckstead and Norgren, '79). However, the specific synaptic relationships between cortical and vagal-glossopharyngeal afferents in the NTS await electron microscopic analysis.

In contrast to the cortical afferents, subcortical projections to the NTS terminate most heavily in the ventral division of the medial solitary nucleus. The prefrontal cortex also sends some projections directly to this division, and some indirectly by way of the central nucleus of the amygdala which has an especially heavy projection to the NTS area just dorsal and lateral to the dorsal motor nucleus of the vagus (both the medial and lateral prefrontal cortex project to the central nucleus of the amygdala). All the subcortical regions projecting to the NTS have a particularly heavy projection to the NTS area bordering the dorsal motor nucleus dorsally and laterally. It is not known if this area has short projections into the dorsal motor nucleus of the vagus itself, but dendrites of the vagal motoneurons probably do not extend dorsally into this ventral division of the medial solitary nucleus (McLean and Hopkins, '81). The ventral division of the medial solitary nucleus also contains the catecholamine cells in the NTS (Koda et al., '81; Koda and Bloom, '83), which, according to Sawchenko and Swanson ('82), are by far the major source of the direct NTS projection to the forebrain.

The paraventricular nucleus of the hypothalamus is the only forebrain region with substantial direct projections into the dorsal motor nucleus of the vagus. These projections involve mainly the lateral part of the motor nucleus. In agreement with studies in the monkey (Price and Amaral, '81) but not in the cat (Hopkins and Holstege, '78), little evidence of direct projections from the central nucleus of the amygdala to the dorsal motor nucleus was found in the rat, although dorsally and laterally adjacent NTS areas were heavily labeled. The central nucleus of the amygdala and other subcortical regions do project to the rostral portion of the ventral division of the medial solitary nucleus, in which a few of the most rostral vagal motoneurons are scattered (Contreras et al., '80; Ritchie et al., '82). The NTS area immediately dorsal to the dorsal motor nucleus of the vagus receives relatively few primary afferents from the vagus and glossopharyngeal nerves (Torvik, '56), although it seems to receive in particular gastric afferents (Leslie et al., '82). Given the heavy termination of various subcortical afferents in this NTS region, and of projections from the paraventricular hypothalamic nucleus to the dorsal motor nucleus itself, the subcortical projections might be posited in general to have more to do with motor than with sensory functions of the NTS.

All the cortical and subcortical forebrain sites appear to have some projections to the nucleus intercalatus and especially to the ventral part of the lateral solitary nucleus, areas which are thought, on the basis of electrophysiological studies, to be involved in respiration (Merrill, '81). All of the cortical and subcortical forebrain regions examined also appear to project to the rostral one-third of the NTS, which is held to be the primary reception site for taste afferents (Torik, '56), although taste afferents are now thought to have a wider rostrocaudal distribution in the NTS (Beckstead and Norgren, '79). The prefrontal cortex and, to a lesser extent, the paraventricular hypothalamic nucleus project to a caudolateral area of the dorsal division of the medial solitary nucleus (around the solitary tract) that receives primary vagal afferents from the aortic nerve (Kalia and Welles, '80). Given that electrical stimulation within the lateral prefrontal cortex produces decreases in blood pressure (Koda and van der Kooy, unpublished observations), the lateral prefrontal cortex afferents to this portion of the NTS may play a role in modulating the baroreceptor reflex and other cardiovascular functions.

The visceral forebrain

The cortical and subcortical areas that project directly to the NTS (i.e., medial and lateral prefrontal cortex, paraventricular, arcuate and posterolateral hypothalamic nuclei, bed nucleus of the stria terminalis, and central nucleus of the amygdala) could be thought collectively to compose a visceral forebrain system. It remains to be investigated, however, whether the efferents of this system primarily synapse on peripheral sensory afferents, interneurons, or efferents of the NTS. As for visceral sensory input, it is interesting that all of the subcortical components of the system (bed nucleus of the stria terminalis; central nucleus of the amygdala; and paraventricular, arcuate, and posterolateral hypothalamic nuclei) have been shown to receive direct projections from the NTS (Ricardo and Koh, '78). The prefrontal cortex is not known to receive such direct visceral input, but it may nonetheless receive information from the NTS over a number of indirect pathways. The only known direct projection from the NTS to the thalamus in the rat is to the paraventricular nucleus (Ricardo and Koh, '78; Sawchenko and Swanson, '82), although in the monkey the ventral posteromedial thalamic nucleus also receives a direct projection from the NTS (Beckstead et al., '80). In the present study, only sporadic paraventricular thalamic neurons were found to project to the medial (but never to the lateral) prefrontal cortex. A large projection from the gustatory thalamic nucleus to the insular taste cortex does exist (Yamamoto et al., '80; Norgren and Wolf, '75; Beckstead et al., '80; Norgren et al., '82), but there are no comparable connections between the thalamus and cortex for other modalities of visceral information. A more prominent indirect pathway for visceral input to the cortex involves the parabrachial nuclei. These nuclei receive direct inputs from the NTS (Norgren, '78; Beckstead et al., '80) and are reciprocally connected with the visceral forebrain areas, including the prefrontal cortex, that provide NTS afferents (Norgren, '78; Saper and Loewy, '80; Saper, '82; Lasiter et al., '82; present report). Thus, it appears that medial and lateral prefrontal cortex; the central nucleus of the amygdala; the bed nucleus of the stria terminalis; and the paraventricular, arcuate, and posterolateral hypothalamic nuclei, through their reciprocal connections with the NTS and/or parabrachial nucleus, may compose a significant visceral forebrain system. This system (excluding taste) is similar to the olfactory forebrain system (Haberly and

Price, '78a,b) in that it does not receive its main sensory input by way of the thalamus.

It is our contention that the visceral forebrain (as decribed above) sends direct monosynaptic inputs to the NTS that may have a common influence on cardiovascular, respiratory and possibly other autonomic functions which are processed in the NTS. The individual components of the visceral forebrain system may not all convey reflex visceral information. On the contrary, many of these visceral forebrain influences may actually interfere with or override brainstem homeostatic mechanisms during periods of stress or emotional activity.

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