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The Projections of the Dorsomedial Hypothalamic Nucleus in the Rat

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TER HORST, G. J. AND P. G. M. LUITEN. *The projections of the dorsomedial hypothalamic nucleus in the rat.* BRAIN RES BULL **16**(2) 231–248, 1986.—The dorsomedial hypothalamic nucleus (DMH) output pathways are revealed by using autoradiographic tracing of tritium labeled Leucine and by the recently introduced Phaseolus vulgaris leuco-agglutinin immunocytochemical method. Terminal labeling appears in the dorsal motor nucleus of the vagus, nucleus ambiguus and in the parvocellular reticular formation at the lower medullary level. Mesencephalic labeling is found in the periaqueductal gray at the level of the oculomotor nucleus. In the hypothalamus labeled terminal boutons are identified in the lateral and ventromedial hypothalamic nuclei but also in the parvocellular paraventricular nucleus. Furthermore, the circumventricular organs are found to receive a dense DMH input, particularly the organum vasculosum of the lamina terminalis and the subfornical organ. These findings are discussed in relation to the dorsomedial nucleus involvement in the control of feeding and pancreatic hormone release. It appears that the DMH participates in this control via descending pathways to the preganglionic pancreas innervating neurons but also via a neuroendocrine route. The latter connection is indicated by terminal labeling in the parvocellular paraventricular nucleus in the area that contains the corticotropin-releasing factor positive cells.

Dorsomedial hypothalamic nucleus PHA-L tracing Pancreatic hormone release
Descending autonomic pathways Lateral hypothalamic area Ventromedial hypothalamic nucleus
Parvocellular paraventricular nucleus Organum vasculosum of the lamina terminalis

OF all nuclei in the mammalian hypothalamus that are reported to influence feeding behavior and metabolism the dorsomedial hypothalamic nucleus (DMH) may be considered as the anatomically least clearly understood. There is a conspicuous lack of information on its neural connections, although several investigations have been performed to reveal the functional role of DMH. Bilateral lesioning of the DMH is described to result in a temporary inhibition or reduction of daily food- and water-intake [2, 3, 4, 5, 6, 8, 10]. Simultaneously the body-weight decreases but it does not return to control values after the reappearance of feeding [4, 6, 8, 10]. The lesioned animals maintain a moderate hypophagia, which appears to be accompanied by unchanged body-compositions [4, 6, 8].

A major mechanism by which the hypothalamus exerts its influence on feeding and body-weight appears to be the modulation of the pancreatic hormone release. In an elegant neurophysiological experiment [54] considerable changes in the electrical activity in the pancreatic branches of the vagal and splanchnic nerves are found as a result of bilateral destruction of the DMH. Physiological research, however, does not demonstrate abnormal plasma insulin levels in such DMH lesioned animals [8]. Stimulation of the DMH by electrical current [11] or by norepinephrine infusions (Caffe, van der Gugten and Steffens, in prep.), on the other hand, produces hyperglycemic reactions that are accompanied by increases in the plasma catecholamine levels. Taken together these physiological data suggest nervous connections of the DMH, that relate this nucleus to the autonomic nervous system. Therefore we have studied the efferent connections of the DMH with particular attention to the descending pathways towards the preganglionic cellgroups in the lower medulla oblongata that innervate the pancreas on the one hand, and to intrahypothalamic connections on the other hand. Autonomic cellgroups which innervate the endocrine pancreas are previously demonstrated in the dorsal motor vagus (DMV) and ambiguus (AMB) nuclei [26,52]. Although connections of the DMH with lower medullary structures have been studied with retrograde tracer techniques [40,50], there is a lack of knowledge on the mode or the site of projections of the hypothalamus to the parasympathetic nuclei of the lower brainstem.

Likewise it is not clearly understood how the DMH is related to other hypothalamic structures in general and to the neuroendocrine structures at this level in particular. Here also some data are available from retrograde transport studies [22, 23, 24]. The latter tracing techniques although useful to demonstrate the connections afferent to a certain brain structure, have only limited capacity to demonstrate efferents and certainly offer injection problems in delicate complicated structures like the hypothalamus. Therefore, efferents of the DMH were studied with autoradiographic trac-

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ABBREVIATIONS

AC	anterior commissure
AHA	anterior hypothalamic area
AMB	ambiguus nucleus
AP	area postrema
aq	cerebral aqueduct
BL	basolateral amygdaloid nucleus
BM	basomedial amygdaloid nucleus
BST	bed nucleus stria terminalis
C 2	crus 2 ansiform lobule
Ca 1	field CA 1 of Ammon's horn
Ca 2	field CA 2 of Ammon's horn
Ca 3	field CA 3 of Ammon's horn
Ca 4	field CA 4 of Ammon's horn
CAI	internal capsule
CC	corpus callosum
CE	central amygdaloid nucleus
CG	periaqueductal gray
CPU	caudate putamen
D	nucleus of Darkschewitsch
DMH	dorsomedial hypothalamic nucleus
DR	dorsal raphe nucleus
DT	dorsal tegmental nucleus
DpMe	deep mesencephalic nucleus
EN	endopiriform nucleus
F	fornix
FI	fimbria hippocampus
FL	flocculus
FrPaM	frontoparietal cortex, motor area
FrPaSS	frontoparietal cortex, somatosensory area
GP	globus pallidus
Gr	gracile nucleus
HDB	horizontal diagonal band of Broca
HI	amygdalohippocampal area
IC	inferior colliculus
INT	interpositus cerebellar nucleus
IO	inferior olive
KF	kölliker-fuse nucleus
La	lateral amygdaloid nucleus
LC	locus coeruleus
LH	lateral hypothalamic area
LHB	lateral habenular nucleus
LL	lateral lemniscus
LR	lateral reticular nucleus
LS	lateral septal nucleus
LV	lateral cerebral ventricle
M 5	motor trigeminal nucleus
MD	mediodorsal thalamic nucleus
ME	medial amygdaloid nucleus
MG	medial geniculate nucleus
ML	medial lemniscus
MLF	medial longitudinal fasciculus
MS	medial septal nucleus
MSO	medial superior olive
MT	mammillo-thalamic tract
MdB	dorsal medullary reticular nucleus
MdV	ventral medullary reticular nucleus
Me 5	mesencephalic trigeminal nucleus
NTS	solitary tract nucleus
OVLT	organum vasculosum lamina terminalis
	third ventricle
OX	optic chiasm
P	pyramidal tract
PB	parabrachial nucleus
PCG	post cingulate cortex
PMV	ventral premammillary nucleus
PNC	caudal pontine reticular nucleus
PNO	oral pontine reticular nucleus
PO	preoptic area
PPT	pedunculopontine tegmental nucleus
PVM	magnocellular paraventricular nucleus
PVP	parvocellular paraventricular nucleus
Pr 5	principal sensory trigeminal nucleus
R	red nucleus
RE	reuniens thalamic nucleus
RF	rhinal fissure
RGI	gigantocellular reticular nucleus
RO	raphe obscurus nucleus
RP	raphe pallidus nucleus
RPC	parvocellular reticular nucleus
RPN	raphe pontis nucleus
RRF	retrorubral field
rH	retrohippocampal area
SFO	subfornical organ
SM	stria medullaris
SNr	substantia nigra, pars reticulata
ST	stria terminalis
Sp 5	spinal trigeminal nucleus
TO	optic tract
TS	solitary tract
VMH	ventromedial hypothalamic nucleus
VP	ventral pallidum
V III	third ventricle
Xscp	decussation superior cerebellar peduncle
ZI	zona incerta
III	oculomotor nucleus
X	dorsal motor nucleus of the vagus nerve
XII	hypoglossal nucleus
7	facial nucleus

ing of tritiated leucine (^{3}H-Leu) and with the recently introduced Phaseolus vulgaris leuco-agglutinin (PHA-L) tracing method [12]. The latter immunocytochemical method bears the great advantage of demonstrating the entire morphology of small populations of nerve cells from soma to synapse, irrespective of the length of the projection pathway [12, 49, 53].

METHOD

Fourty-five male Wistar rats were used for the present investigation. In fifteen cases iotophoretic ^{3}H-Leu injections were made within and around the dorsomedial hypothalamic nucleus. The rats were anesthetized with an intraperitoneal Sodiumpentobarbital (30 mg/kg) and an intramuscular Hypnorm (Duphar) (0.4 ml/kg) injection and placed in a Kopf-stereotaxic apparatus adjusted to the coordinate system of Paxinos and Watson [33]. Bevelled glass-micropipettes (10–18 μm) were filled with a 20–40 μCi/μl solution of tritiated Leucine (Amersham) dissolved in 0.01 N acetic-acid and positioned in the brain stereotactically. A pulsed DC-current of 0.5–0.8 μA was applied to the pipette for 30 minutes, using a Midgard CS-3 constant current source. Following iontophoresis the pipette was left in situ for 10 minutes to avoid loss of tracer in the pipette-track.

After a 7 to 18 days post-operative survival-time the

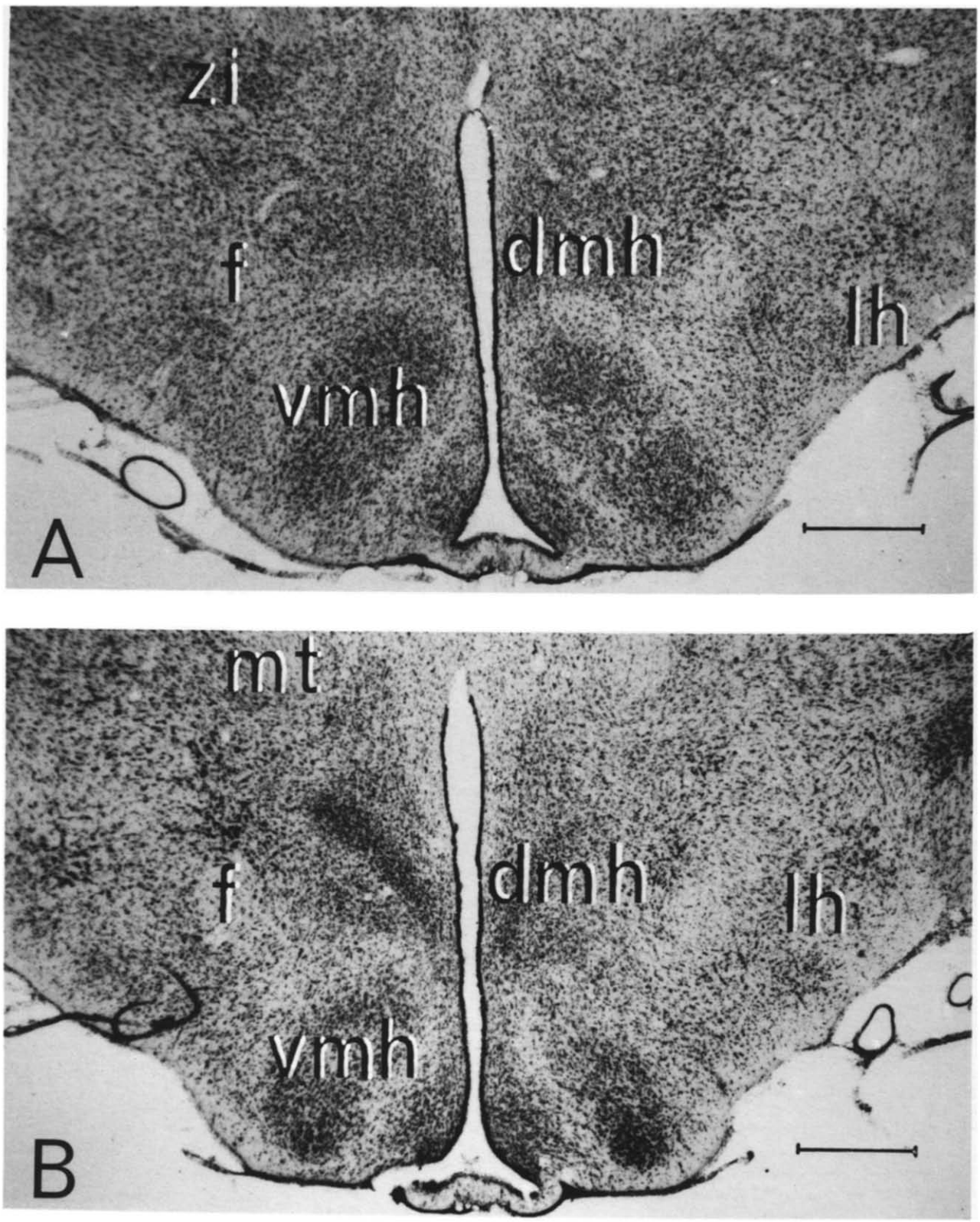

FIG. 1. Photomicrographs showing the position of the rostral (A) and caudal (B) dorsomedial nucleus (DMH) in the rat hypothalamus. Note the pars compacta in (B) Bar=500 μm.

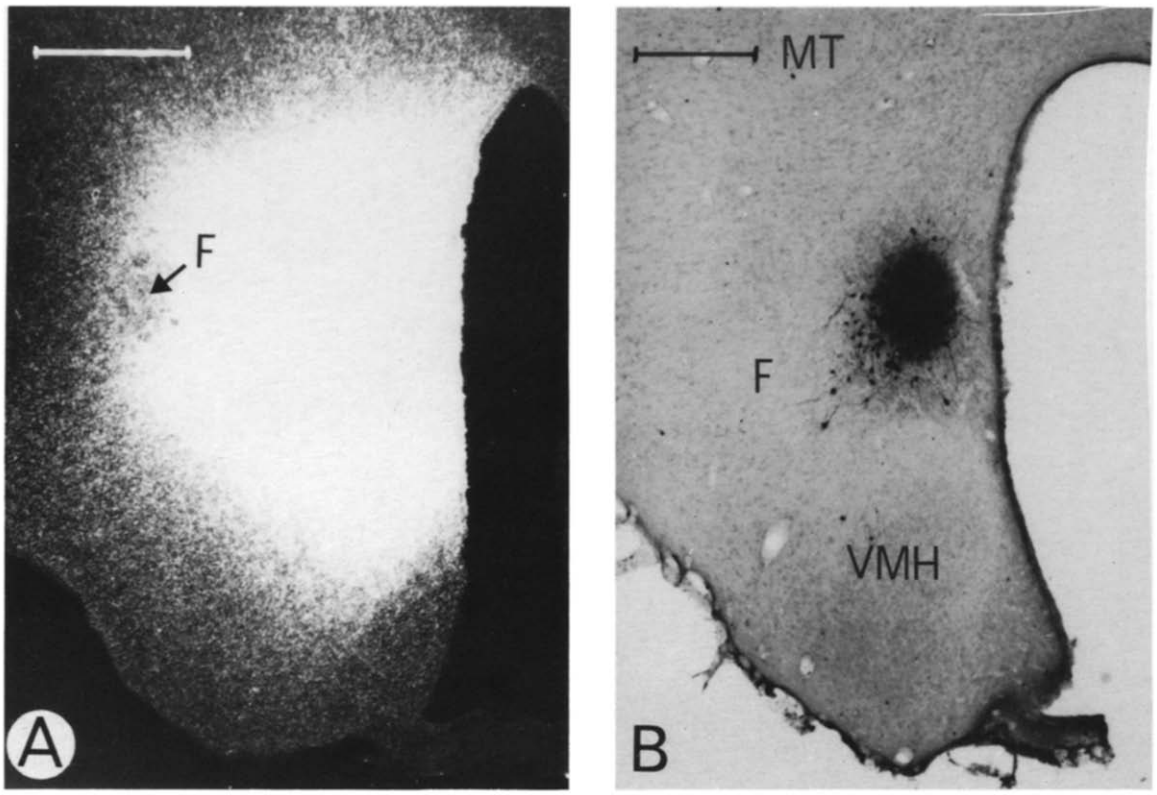

FIG. 2. (A) Tritiated leucine iontophoretic deposit in the dorsomedial hypothalamus (Case 8315). (B) Phaseolus vulgaris leucoagglutinin iontophoretic injection in the DMH. Bar=500 μm.

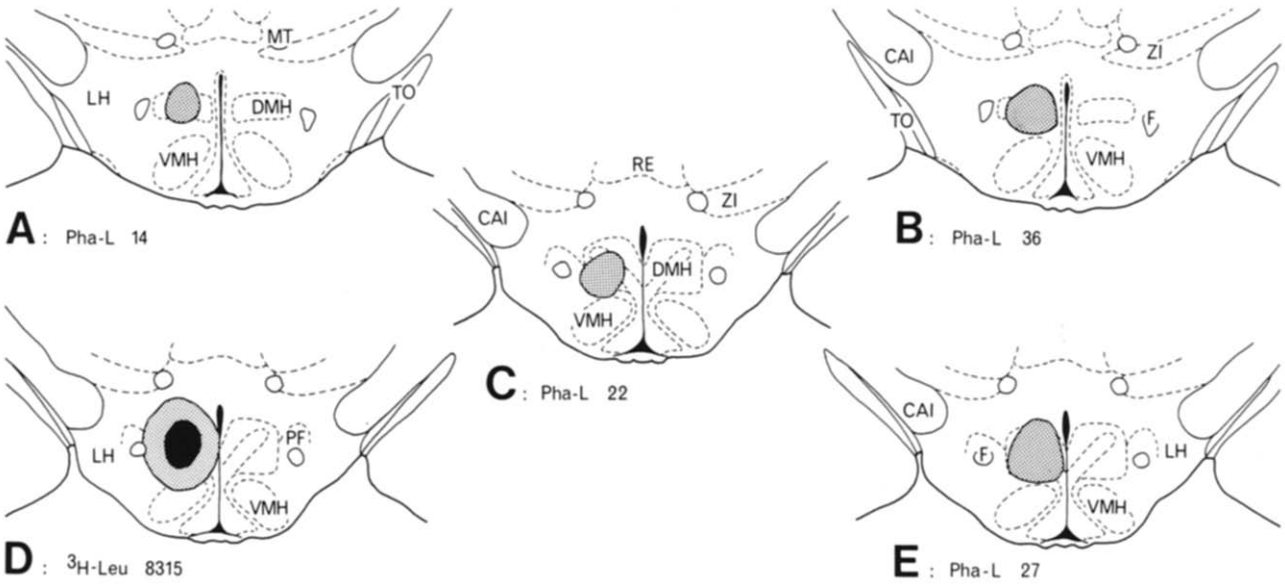

FIG. 3. Locations of PHA-L (A, B, C, E) and ^{3}H-Leu (D) injection-sites in the dorsomedial hypothalamic nucleus that are discussed in the text.

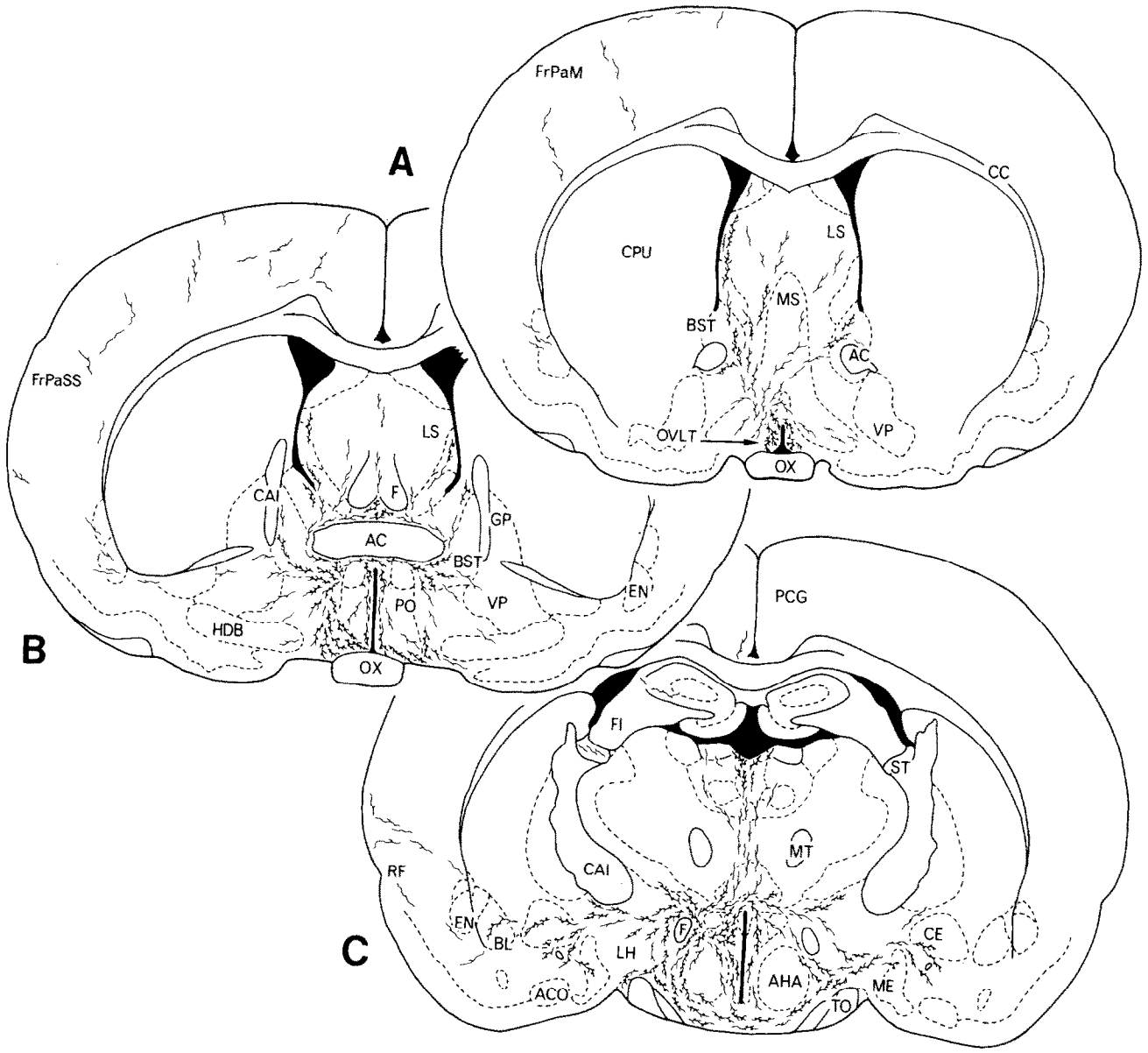

FIG. 4. A series of transverse sections, from case 27, from anterior (A) to posterior (L) in which the PHA-L injection (◆), the course of labeled fibers and terminal labeling (small dots) is indicated. Drawings come from Paxinos and Watson [33] (Fig. 4, D–G and H–L on following pages).

animals were deeply anesthetized (100 mg/kg Sodiumpentobarbital IP) and perfused transcardially with a 10% formalin solution. The brain was removed and post-fixed in 10% formalin for 2 weeks, dehydrated in a 30% sucrose-formalin solution for 3 days and sectioned at 40 μm on a cryostatmicrotome. Every second section was mounted onto gelatin-coated slides and air dried. Next, the slides were defattened and coated with a Kodak NTB-3 nuclear track emulsion. After being exposed in the dark, at 4°C for 12 to 24 weeks, the slides were developed in Kodak D19b at 15°C, counterstained with cresyl-violet and coverslipped. The sections were examined by using dark-field microscopy.

A second series of thirty rats was used in the PHA-L experiments. For a detailed account on the PHA-L immunocytochemical tracing procedure, we refer to previous papers [12,49]. In summary, injections of PHA-L were made iontophoretically with a solution containing 2.5% PHA-L (Vector Labs.) in tris-buffered saline (pH=7.4). Following a 7 days post-operative survival-time, the animals were fixed, after a short transcardial pre-rinse, with a solution made up of 0.5% paraformaldehyde, 2.5% glutardialdehyde and 4% sucrose in 0.05 M phosphate buffer (pH=7.4). Brains were cut in 40 μm sections on a cryostat-microtome and the free floating sections were thoroughly rinsed in tris-buffered saline (TBS). Subsequently, the sections were incubated for 48 hr, at room-temperature, in goat-anti-PHA-L (1:2000) (Vector Labs.) dissolved in TBS to which 0.5% Triton X-100 was added (TBS-T). Next, the sections were rinsed in TBS-T

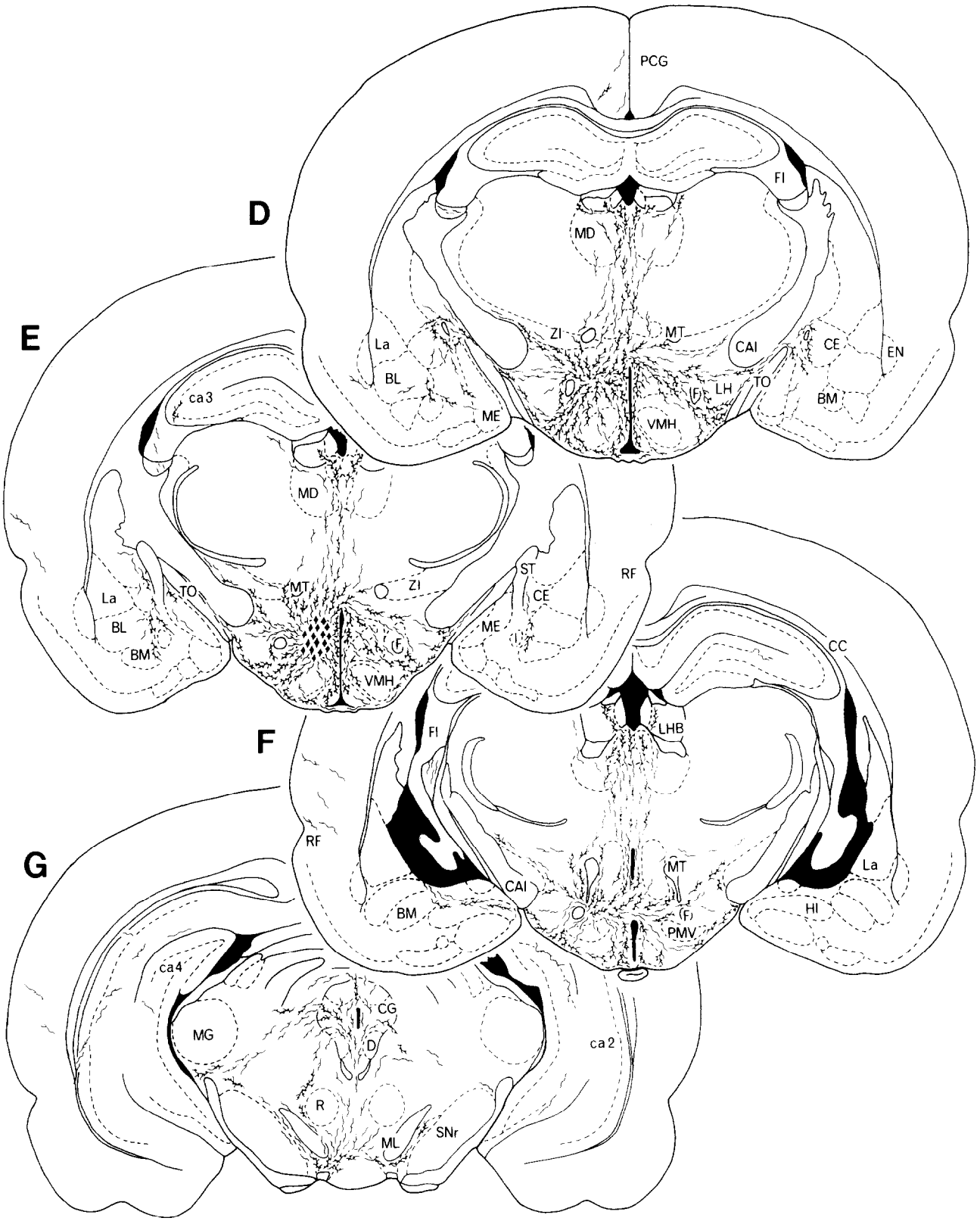
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FIG. 4. D–G

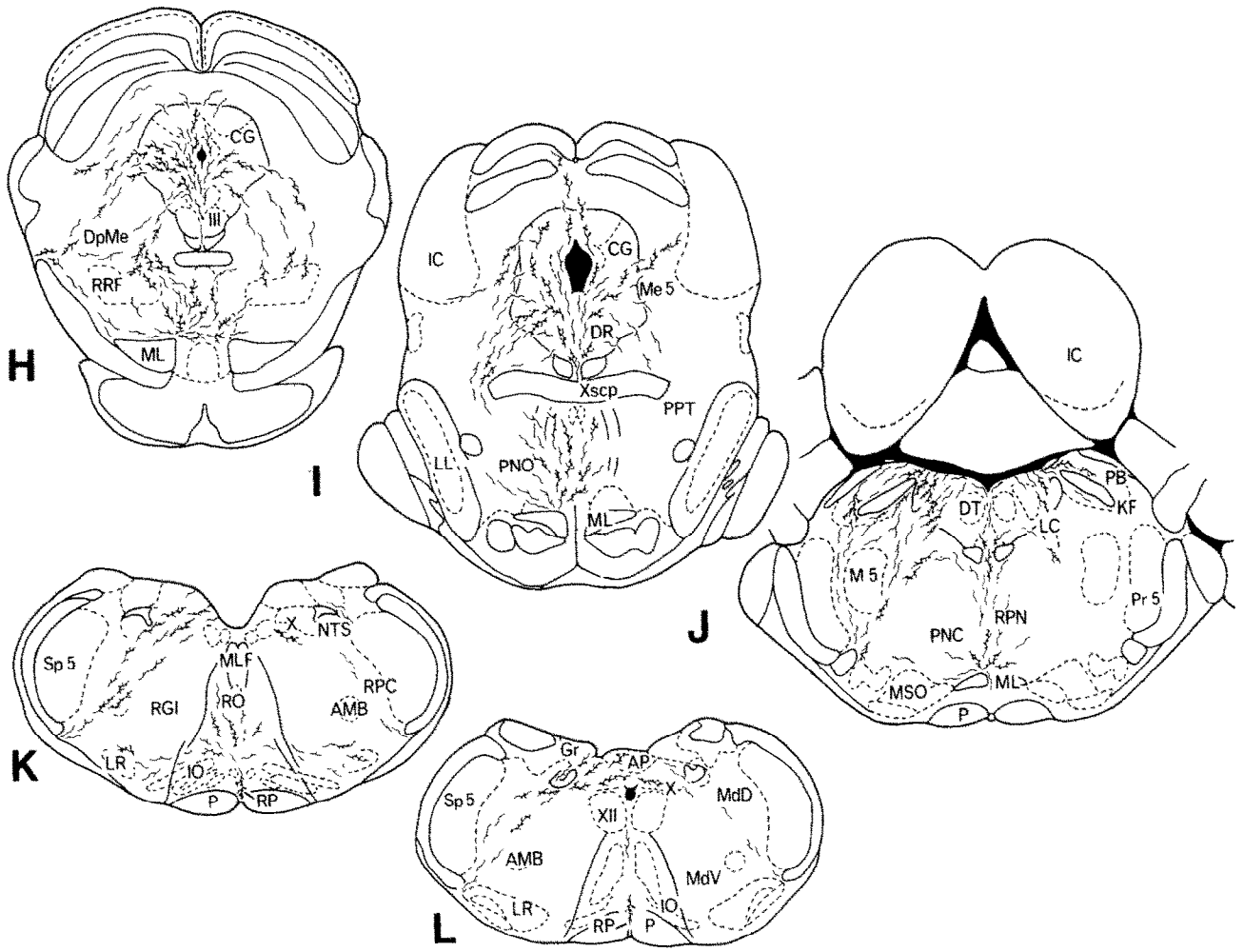


FIG. 4. H–L

again and incubated for 16 to 24 hr, at room temperature, with rabbit-anti-goat IgG (1:200) (Sigma). After a subsequent rinse the sections were transferred for 1 to 4 hr, to a solution containing goat peroxidase-anti-peroxidase complex (1:400) (Dako). Finally, the sections were reacted for 1 hr in a tris-buffered diamino-benzidine solution (40 mg/100 ml), to which 0.8 ml 1.5% H_2O_2 was added. Subsequently, the sections were rinsed in distilled water, mounted, counterstained with cresylviolet and coverslipped. The labeling was examined by using both light- and dark-field microscopy.

RESULTS

General Description

The DMH (Fig. 1) is a rather heterogeneous structure that constitutes the medial wall of the hypothalamus dorsal to the ventromedial hypothalamic nucleus (VMH) and central to the zona incerta (ZI). The anterior hypothalamic area (AHA) and paraventricular nucleus (PV) are marking the rostral limits, whereas it is caudally bordered by the posterior hypothalamic nucleus and the premammillary nuclei. On the lateral side the DMH meets the fornix (F) and the perifornical nucleus and medially the periventricular cell layers of the third ventricle.

The DMH is made up of medium-sized, multipolar cells of 14–18 μm in diameter that are, however, not evenly distributed over the nucleus. In the anterior half of the DMH we counted, in 40 μm thick transverse sections, an average of 124 cells per 100 μm^2. In the posterior part of the nucleus a conspicuous band of smaller neurons of approximately 10 μm in diameter in an oblique position divides the DMH in three parts. The band itself called the pars compacta [33] is more cell-dense and contains 219 cells per 100 μm^2 in 40 μm sections. Dorsal and ventral to the pars compacta the larger somata—approximately 15 μm in diameter—appear at a lower density of 155 per 100 μm^2 dorsal and 150 per 100 μm^2 ventral, again counted in 40 μm thick sections.

Comparison of the PHA-L and ^{3}H-Leu Material

As was described in more detail in previous papers [12,49] the PHA-L method yields a greater morphological detail than the autoradiographic method and enables the study of neuronal connections on a cellular level and even on very

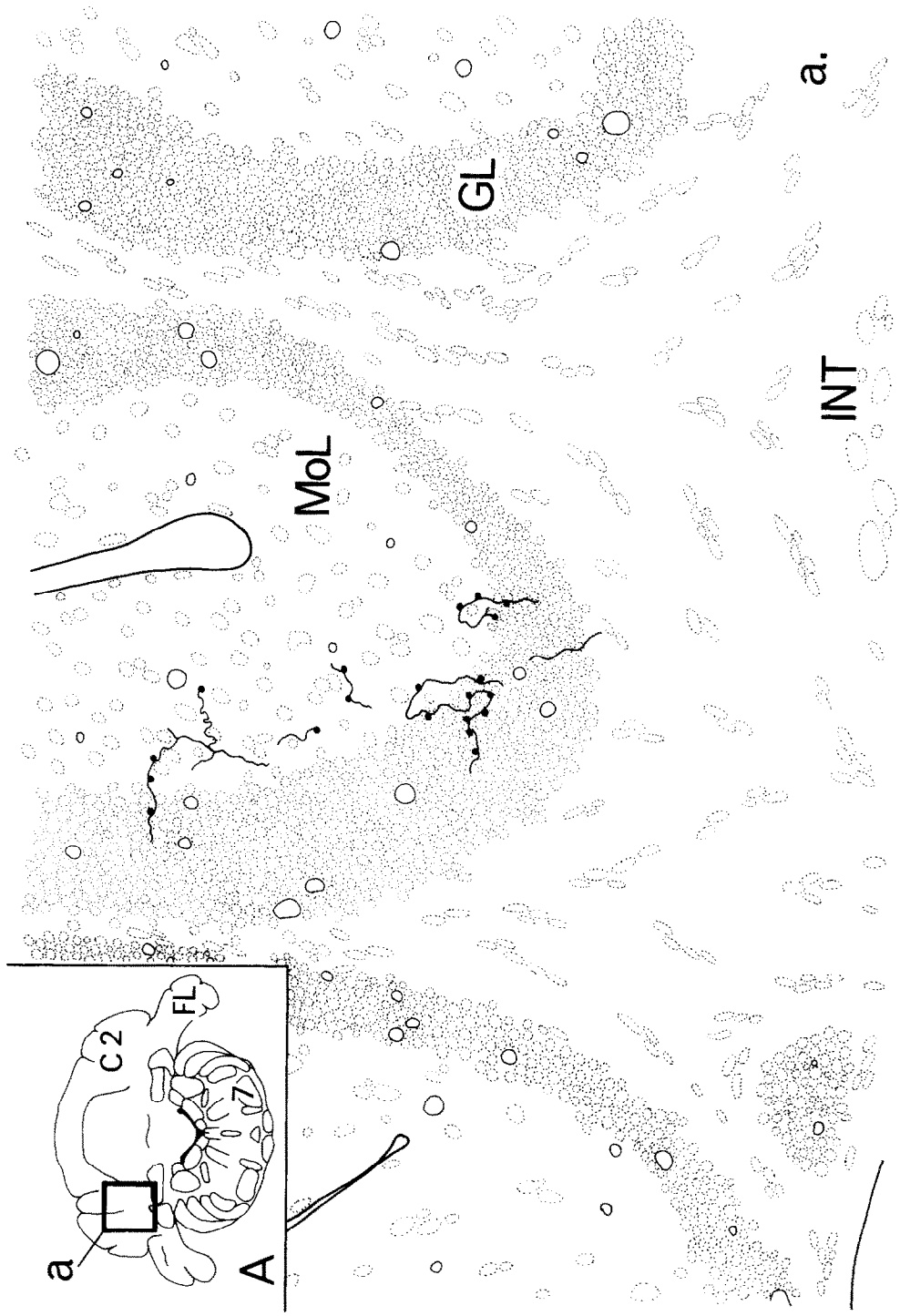


FIG. 5. Camera lucida drawing of Phaseolus lectin immunoreactive fibers and terminal boutons in the molecular (Mol) and granular (GL) layers of the cerebellar ansiform lobe. The inset (A) indicates the location of the cerebellar projections that are presented in detail in a.

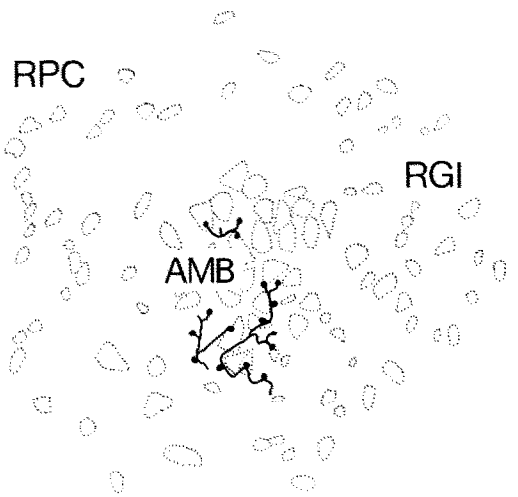


FIG. 6. Dorsomedial hypothalamic projections in the Ambiguus nucleus. Camera lucida drawing.

short distance from the injection locus. The injection itself can be recognized as a cluster of labeled cellbodies from which labeled dendrites and axons can be traced (Fig. 2). The appearance of the characteristic varicosities, that have been identified as terminal, presynaptic boutons [53], mark the sites of interneural contacts even on a very short distance. Such identifications in the PHA-L preparations are generally not obscured by untransported diffusion precipitates that are common for both autoradiographic and horseradish peroxidase material. For these reasons the description of DMH efferent connections will be primarily based on the PHA-L material. Furthermore, several of the injections with Phaseolus vulgaris lectin are confined to the dorsomedial nucleus and very well suited for a description of efferent connections. In particular the description of the long-distance projections will be complemented with the results of the ^{3}H-Leu experiments.

The DMH efferent connections are described by using the projections of case 27 with a PHA-L injection in the caudal portion of the nucleus. Topographical differences, furthermore, will be mainly presented by comparison with the cases 14, 22, 36 and with case 8315 of the autoradiographic tracing experiments. The locations of the injection-sites are depicted in Fig. 3.

Descending Pathways

The majority of the descending pathways emerge dorsally from the DMH injection site and follow a medial course through the thalamus. At the dorsal diencephalic level these fibers bend in a caudal directions and enter the periaqueductal gray (CG).

Mesencephalic projections (Fig. 4 G, H and I). In the mesencephalon the descending pathway continues in the CG and can be followed toward levels through the locus coeruleus (LC). In their caudal course through the midbrain the efferent fibers ramify and give rise to varicosities that terminate in the CG and in the adjacent reticular formation. A particularly strong projection appears in the medial CG, at the level of the oculomotor nucleus (III) (Fig. 10 D), and in the mesencephalic reticular formation dorsal to the retrorubral field (RRF). This latter projection, however, is situated more lateral to the RRF in cases 14 and 36, and is indicative of a topographic organization in DMH output to the mesencephalic reticular formation.

At the caudal mesencephalic level the terminal boutons are found in the dorsal raphe nucleus (DR) and in the ventromedial periaqueductal gray adjacent to the mesencephalic trigeminal nucleus (Me5). Small calibre PHA-L positive fibers, at this level, leave the CG in a ventrolateral direction and course around the brachium conjuctivum (Xscp) towards the medial raphe nucleus. Terminal boutons in the pontine reticular formation are predominantly found lateral to the medial raphe nucleus. In general the above mentioned projections appear bilaterally but are far less numerous on the contralateral side.

Lower brainstem (Fig. 4 J). Posterior to the pontine level the DMH efferent fibers leave the periaqueductal gray and course through the locus coeruleus. Here particularly strong projections appear in the LC and the dorsal portion of the nucleus subcoerulcus. The adjacent mesencephalic trigeminal nucleus, on the other hand, also contains some terminal boutons. Most fibers, however, are descending in a ventrolateral direction. Small projections are found in the ventrolateral parabrachial nucleus (PB), the Kölliker-Fuse nucleus (KF), the reticular formation at this level and in the pontine raphe nucleus (RPN).

In the caudal myelencephalon terminal boutons are identified in the nucleus raphe magnus (RM) and raphe obscuris (RO), and in the gigantocellular division of the reticular formation (RGi). Furthermore, at this level a few thin fibers emanate from the periventricular gray in a dorsolateral direction, traverse the vestibular nuclei and can be traced to target structures in the molecular and granular layers of the cerebellar ansiform lobe (Fig. 5 A, a). The projections to the cerebellum are only found in cases with PHA-L deposits in the posterior DMH, in the area dorsal to the pars compacta.

With respect to the myelencephalic projections it is found that the anterior DMH injections (e.g., cases 14, 36) contribute only moderately to projections to the LC, subcoeruleus and adjacent areas.

Projections to the medulla oblongata (Fig. 4 K, L). Continuing in the lower medulla the DMH descending connections run between Sp5 and the lateral reticular nucleus (LR). During their descending course these axons give off branches at various levels that travel in dorsomedial and medial directions. The dorsomedially coursing fibers run close to the nucleus ambiguus (AMB) which receives some terminal boutons (Fig. 6), and in the parvocellular reticular formation (RPC). A fair number of terminal structures can be seen in this reticular division especially in its ventral aspects lateral to the AMB nucleus. The remaining efferent fibers continue towards the nucleus of the solitary tract and dorsal motor vagus complex. Here the PHA-L positive fibers end in the nucleus of the solitary tract (NTS), the dorsal motor vagus nucleus (X) (Fig. 7) and in the area postrema (AP) (Fig. 8). Contralateral projections at this level appear in the dorsal motor vagus nucleus apart from a single fiber in the NTS.

A number of descending fibers, maintain a ventral position in the medulla oblongata and terminate in the area of the reticular formation situated dorsal to the lateral reticular nucleus and in the raphe pallidus nucleus (RP).

In contrast to the extensive projections in the lower medulla after injections in the posterior parts of the DMH,

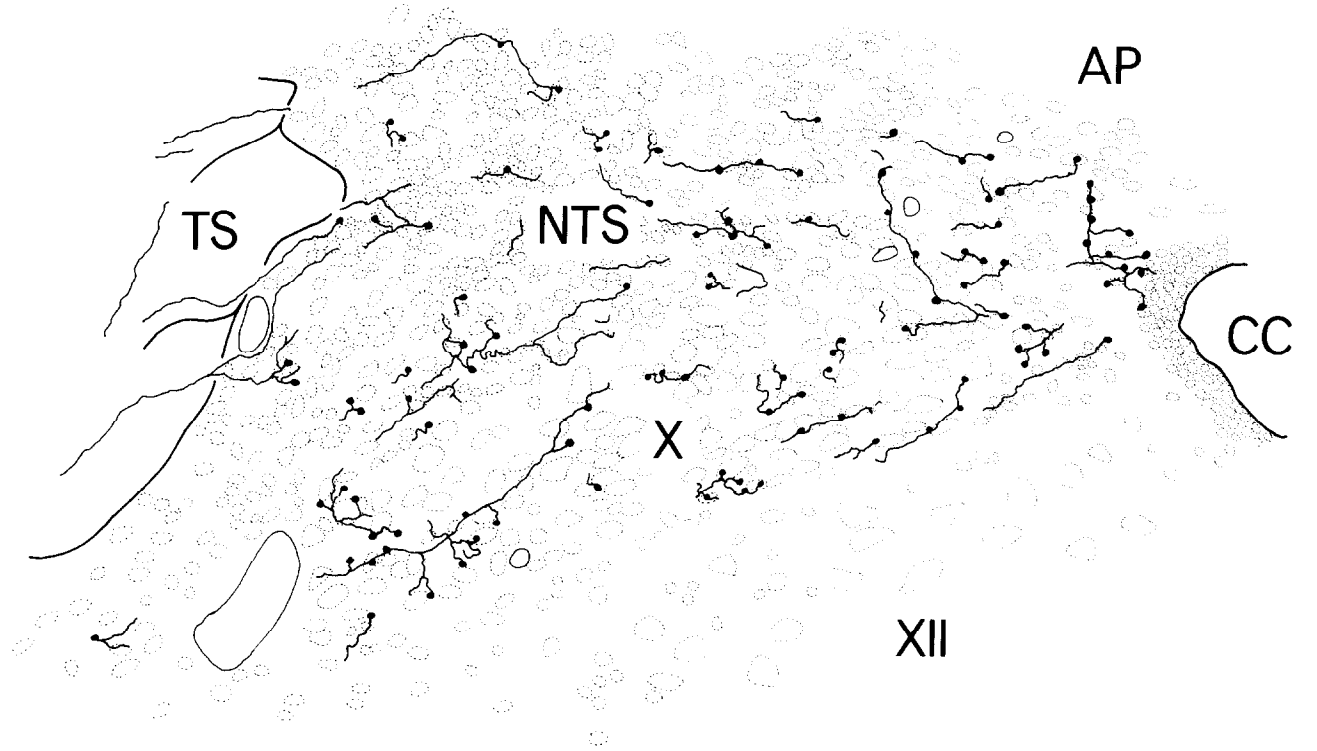


FIG. 7. Camera lucida drawing of DMH projections in the dorsal motor vagus (X) and solitary tract nucleus (NTS) as they appear in PHA-L sections.

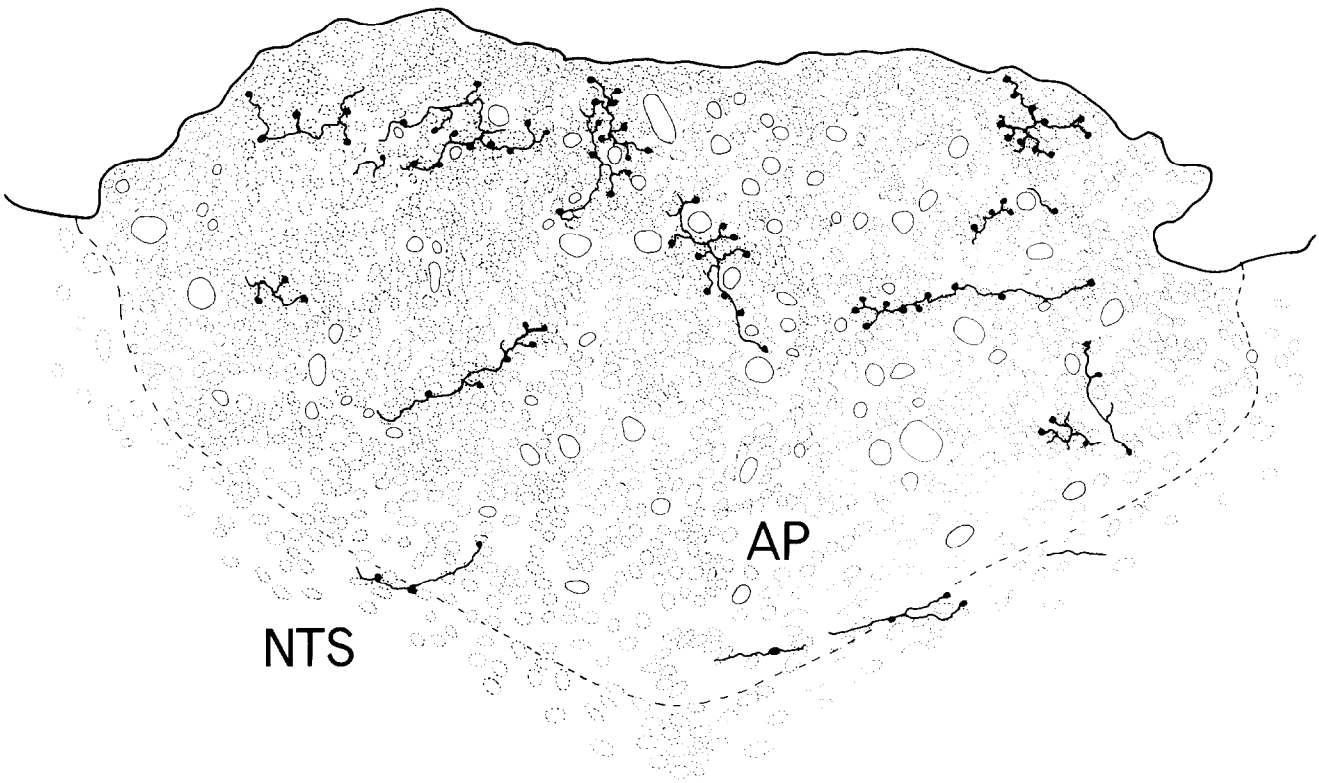


FIG. 8. Camera lucida drawing of PHA-L immunoreactive varicosities and terminal boutons in the area postrema (AP).

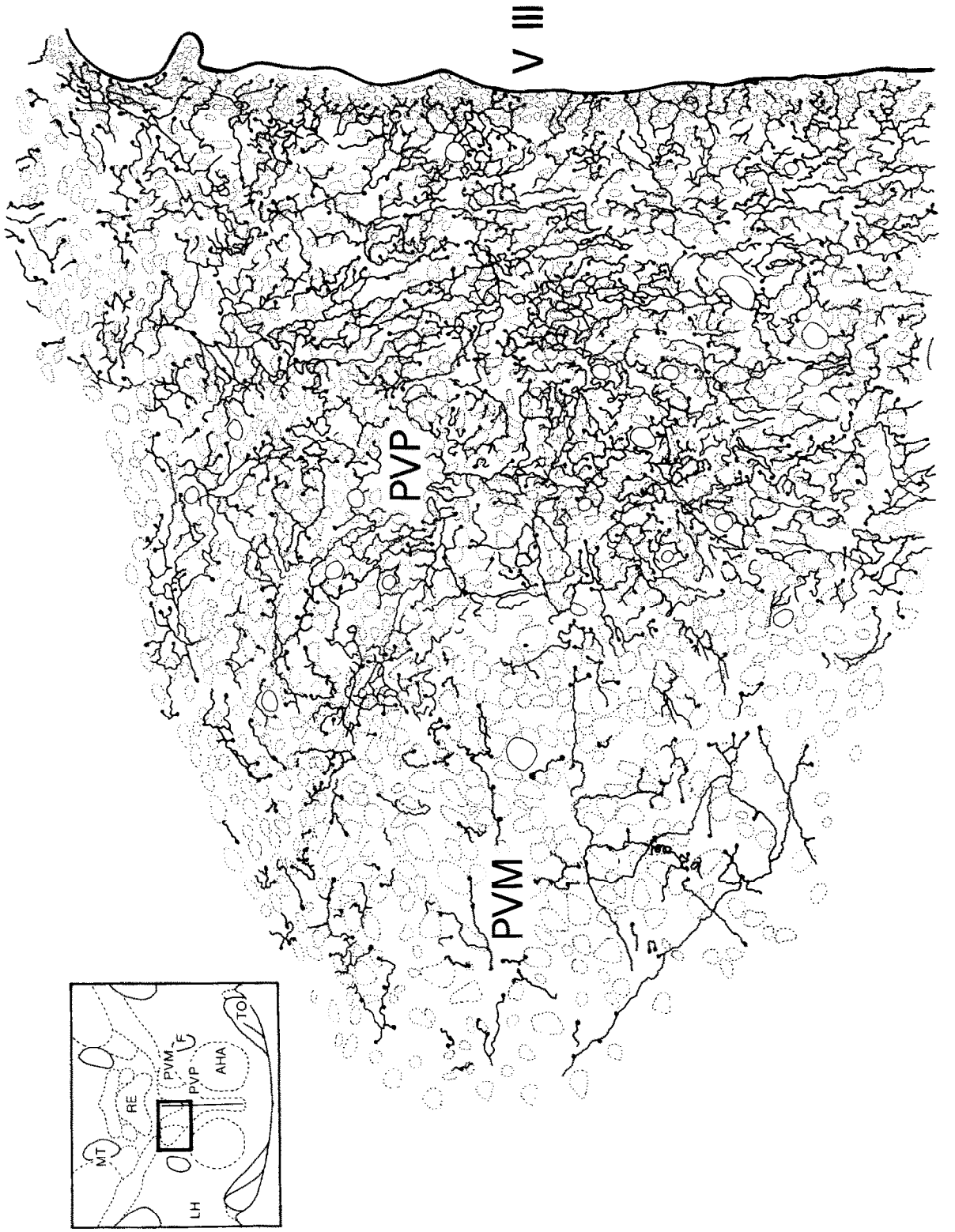


FIG. 9. Camera lucida drawing of the PHA-L positive fibers and terminal boutons in the paraventricular nucleus following a DMH injection.

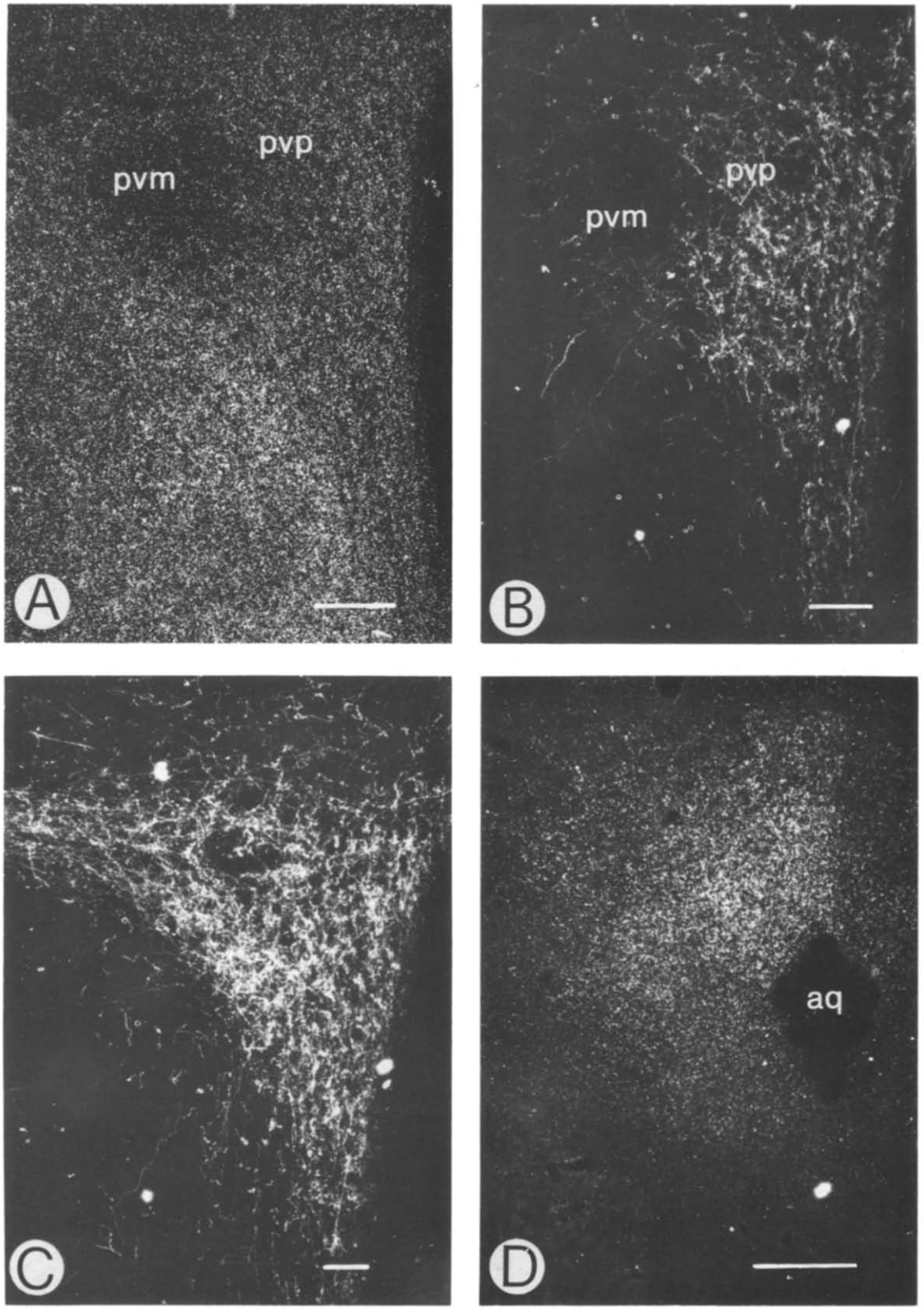


FIG. 10. (A) DMH projection to the parvocellular paraventricular nucleus (PVP) and dorsomedial AHA following a ^{3}H-Leu injection (dark-field photomicrograph). (B) Identical PVP projections after a small PHA-L deposit in the DMH. Note that AHA terminals are absent in this case with a small PHA-L deposit in the DMH (dark-field photomicrograph). (C) Photomicrograph revealing PHA-L positive fibers and terminal boutons in the caudal paraventricular nucleus (dark-field photomicrograph). (D) Periaqueductal gray projection following an iontophoretic ^{3}H-Leu deposit in the DMH (dark-field photomicrograph). Bar=100 μm.

the anterior DMH tracer deposits give rise to only sparse terminal labeling in the motor vagus nucleus and NTS.

Spinal projections. In case 8315, with a ^{3}H-Leu injection in the medial aspects of the DMH, a small increase in grain density occurs in the intermediolateral cell-column (IML) at the thoracic levels of the spinal cord (T4–T5). Such a projection, however, could not be demonstrated in any of the injections of PHA-L that are restricted to the dorsomedial hypothalamic nucleus. Moreover, Phaseolus lectin deposits in perifornical areas, as part of an extensive study of the hypothalamic efferent connections, give rise to terminal labeling in the IML of the thoracic cord (Ter Horst and Luiten, in prep.). This finding and the spreading of tracer into these medial perifornical areas in case 8315 (Figs. 2A, 3D) are indicative of a perifornical origin of the identified spinal projection.

Intrahypothalamic Projections (Fig. 4 C to F)

The most significant DMH projections in the hypothalamus are found in the perifornical area, the perinuclear shell of the ventromedial nucleus and in the peripheral zone of the lateral hypothalamic area. Furthermore, efferent fibers end in the arcuate nucleus (ARC), the median eminence (ME), in the periventricular area of the third ventricle and most conspicuously very strongly in the parvocellular paraventricular nucleus (PVP) (Figs. 9, 10 A, B, C). In contrast to PVP the magnocellular paraventricular division (PVM) is only a minor recipient of DMH input. The remaining hypothalamic terminal labeling is identified in the ventral anterior hypothalamic area (AHA), the retrochiasmatic area, the supra-optic nucleus (SO) and particularly intense in the organum vasculosum of the lamina terminalis of the third ventricle (OVLT) (Fig. 11, A, B, C). The efferent fibers to the OVLT end upon the neural elements and not upon the blood vessels within this circumventricular organ. The contralateral projections are found in the same nuclei and these are reached via efferent fibers that travel either dorsally over the third ventricle or ventrally crossing in the median eminence.

The extensive projections to the PVP and to the OVLT originate predominantly from the posterior parts of the DMH. The anterior DMH more richly provides efferents to the VMH and to the ventral premammillary nucleus (PMV). A topographic arrangement is also apparent for DMH connections to the anterior hypothalamic area. It appears that the posterior DMH is more related to the ventral AHA, whereas the anterior DMH projects more heavily to the lateral aspects of the AHA.

Finally, in cases 22 and 27 we have revealed a direct efferent connection with the posterior pituitary lobe. The terminal boutons appear in the lateral region of the neural tissue, close to the intermediate lobe (Fig. 12).

Thalamic and Forebrain Projections (Fig. 4 A to F)

In the dorsal diencephalon the DMH terminal labeling appears in the lateral habenular nucleus (LHB) and in the adjacent paraventricular thalamic nucleus. The forebrain projections are found in the ventral bed nucleus of the stria terminalis (BNST), the ventral and lateral aspects of the preoptic area (POA) and in the lateral and dorsal septum. A small number of labeled varicosities occurs in the frontoparietal cortex. In the basal forebrain we have observed terminal boutons in a third circumventricular structure—apart from the AP and OVLT—the subfornical organ (SFO) (Fig. 11 D′, D).

A topographic arrangement is apparent for the DMH projections to the POA. It appears that the posterior DMH maintains more extensive connections with the ventral POA, whereas the anterior DMH is more related to the lateral POA divisions.

Limbic Connections (Fig. 4 C to F)

Amygdala. Projections of the DMH to the amygdaloid body are identified by the occurrence of labeled terminal boutons in the medial parts of the central (CE), the basolateral (BL) and basomedial amygdaloid (BM) nuclei. The projections to the medial amygdaloid nucleus (ME) are confined to its dorsolateral aspects. All DMH efferents that are aimed at the amygdaloid body reach this structure running via the ventral amygdalofugal pathway.

Hippocampal formation. The caudal DMH injections give rise to some positively reacting fibers in the hippocampus, both ipsi- and contra-laterally. Terminal boutons are detected in the pyramidal layers of the Cornu Ammonis, divisions 3 (Ca 3) and 4 (Ca 4). Occasionally, some terminal labeling appears in the fascia dentata granular layer.

DISCUSSION

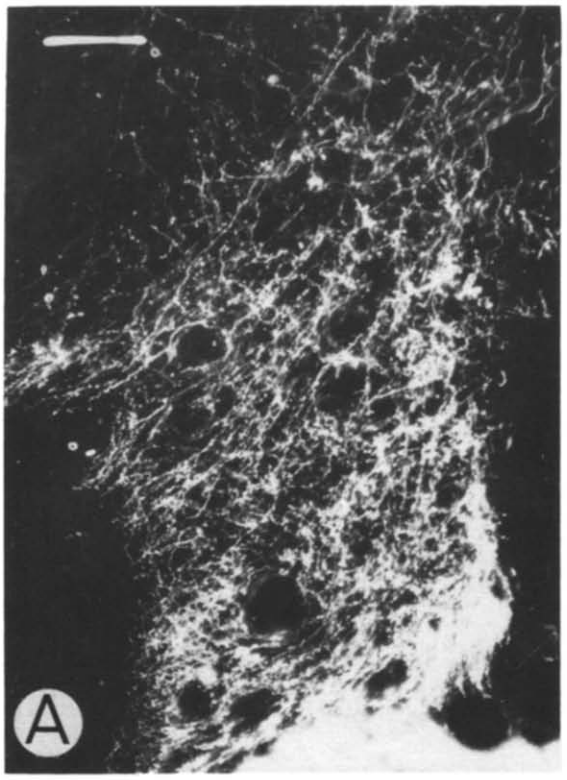
In this study we have investigated the efferent connections of the DMH, with particular attention to the descending pathways to the autonomic cell-groups of the lower medulla and to intrahypothalamic neuroendocrine connections (Fig. 13). These projections are presumed to be part of the anatomical substrate for the autonomic control of the hormone release by the endocrine pancreas. Furthermore, we have compared autoradiographic and PHA-L immunocytochemical tracing and demonstrated again the great morphological detail yielded by the lectin procedure [12,49].

Descending Pathways

With respect to the descending connections of the DMH, terminal labeling appears in the periaqueductal gray, the parvocellular reticular formation and in the DMV and AMB nuclei of the lower medulla oblongata. These two latter medullary structures reportedly contain the preganglionic parasympathetic cell-groups that innervate the endocrine pancreatic B-cells [26,52]. It is obvious, however, that only a limited amount of DMH efferent fibers project directly within the dorsal motor vagus and ambiguus nuclei. The parvocellular reticular formation, adjacent to the AMB, is also a recipient of DMH input. This reticular division is maintaining efferent connections not only with the DMV and the AMB nuclei [29, 35, 50] but also with the intermediolateral cell-groups of the thoracic spinal cord [29,36]. The thoracic spinal cord reportedly contains the preganglionic sympathetic pancreas [26] and adrenal [15,40] innervating cells which for the greater part are located in the IML column.

The DMH input to the CG at the level of the oculomotor nucleus appears predominantly in the medial divisions, lateral to the aqueduct. In preceding HRP investigations it was shown that neurons in this part of the periaqueductal gray project to the DMV, AMB and RPC [27, 35, 50]. The DMH projection to the CG, therefore, may constitute an additional route for the dorsomedial nucleus towards the autonomic centers in the lower medulla and spinal cord.

In summary, the DMH maintains direct and indirect efferent connections with the preganglionic para-sympathetic and sympathetic cellgroups of the lower medulla oblongata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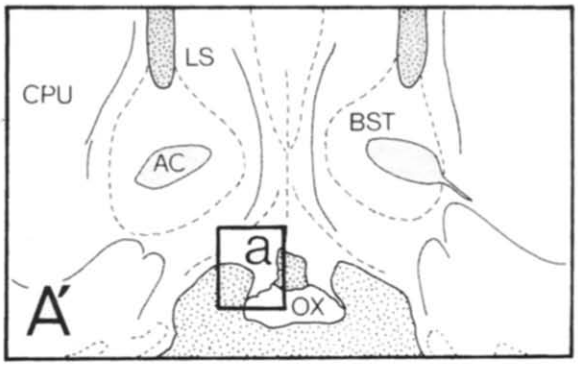
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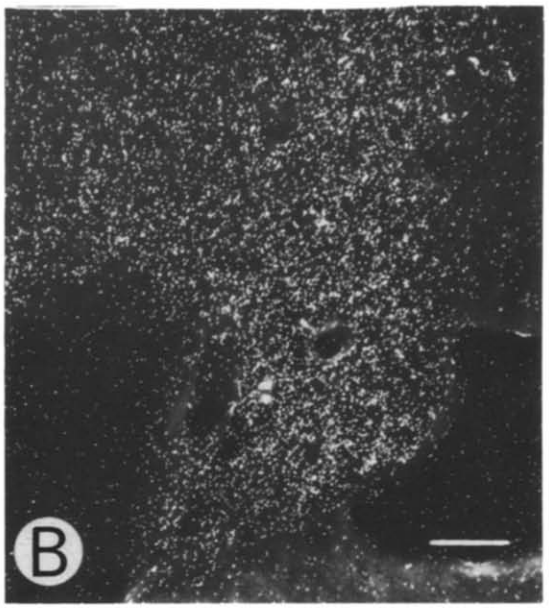
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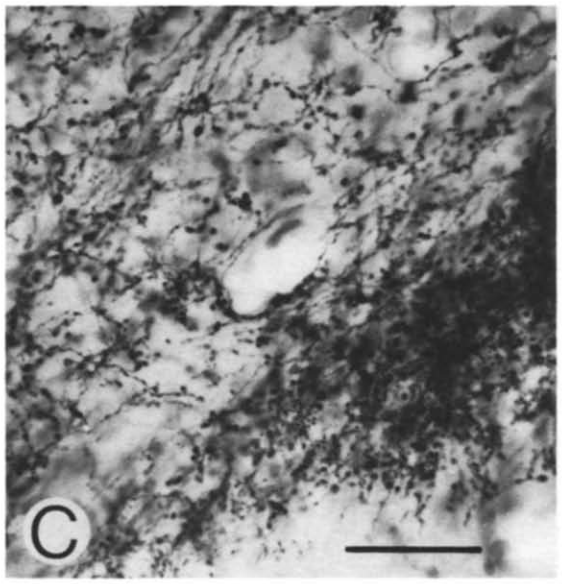
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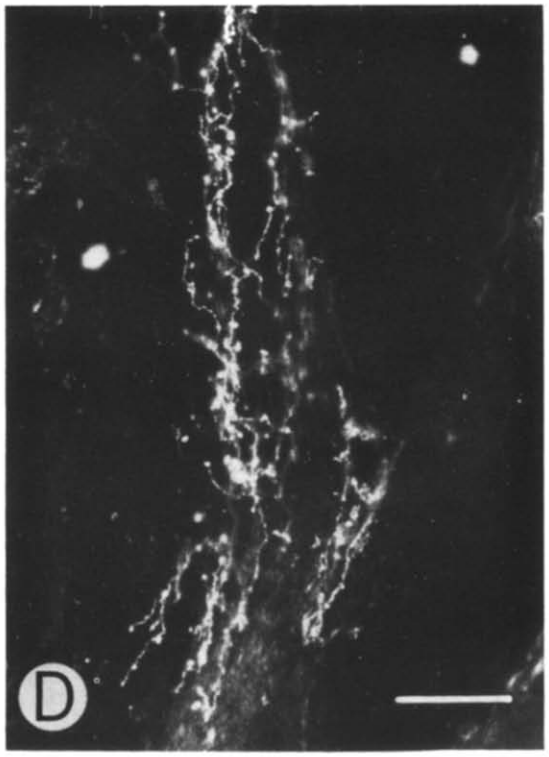
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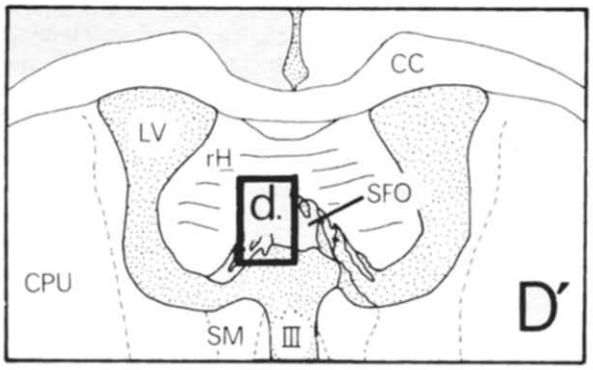
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and spinal cord, respectively. The direct projection is formed by the terminal labeling in the DMV and AMB nuclei, whereas the periaqueductal gray and the parvocellular reticular formation are intermediates for the indirect pathway.

Although the efferent connections of the DMH have not been studied before with anterograde tracing techniques, several HRP investigations have revealed DMH output channels to mesencephalic [14, 28, 31, 50], medullary [17, 37, 50] and spinal structures [37,41]. The latter connections, however, are absent in cases with PHA-L injections restricted to the DMH. The thoracic spinal cord projections originate from the perifornical area adjacent to the dorsomedial nucleus and not from DMH cells proper, which is in agreement with several retrograde transport studies [1, 16, 32, 47]. The lack of agreement with two other retrograde tracing studies [37,41], however, may arise from a topographical problem. In one paper the boundaries of the DMH are not indicated [41], in the other the fornix is used as the lateral limit for this hypothalamic nucleus. In this latter case perifornical area labeling is erroneously believed to represent dorsomedial nucleus connections with the spinal cord [37].

Apart from the anatomical observations indicating DMV involvement in the pancreatic modulation, it was recently shown that electrical stimulation of this nucleus produces an increase of the plasma insulin levels in the rat [18]. The involvement of the CG and RPC in the modulation of pancreatic hormone release has not been assessed in detail. The RPC participation in this modulation can, however, be determined from the effects of electrical stimulation of the ambiguus nucleus on simultaneously monitored plasma insulin levels [7]. Such a stimulation produced a significant increase of the hormone levels especially in cases where electrodes were localized in the RPC dorsolateral to the AMB. These physiological findings together with the presented anatomical data strongly indicate that this part of the RPC is an important relay structure in the brain circuitry involved in the control of the endocrine pancreas.

Intrahypothalamic Connections

With respect to the intrahypothalamic connections of the DMH, terminal boutons appeared in the LHA, VMH and PVP. The projections are found in the peripheral zone of the LHA and in the dendritic shell [30] of the VMH. By using relatively large HRP injections others [22, 23, 24] were able to demonstrate DMH input to LHA and VMH but did not notice the topographical distribution.

The LHA and VMH involvement in the autonomic control of the endocrine pancreas hormone release has been convincingly demonstrated by various authors [11, 19, 42, 43, 44, 45, 54], whereas we have revealed their descending nervous pathways to the preganglionic pancreas innervating cellgroups [50]. In general, the lateral hypothalamic area modulates insulin and the ventromedial nucleus glucagon release. This modulation is obtained through a shift in the balance between ortho- and para-sympathetic activity in the pancreas, which was elegantly demonstrated in a neurophysiological study of Yoshimatsu *et al.* [54]. These authors have shown simultaneous increases and decreases of neural activity in the vagal and splanchnic pancreatic nerve branches, following a destruction of the lateral or ventromedial hypothalamic nuclei.

The LHA and VMH presumably do not maintain direct connections with each other but are reciprocally linked with the dorsomedial nucleus [24]. In that anatomical position the DMH is believed to contribute, at the hypothalamic level, in the control of balance of the LHA and VMH output. The neuronal activity in the pancreatic branches of the vagal and splanchnic nerves is increased and decreased, respectively, following DMH lesions [54] which demonstrates a role of this nucleus in the control of the pancreatic secretion mechanisms. Furthermore, the PHA-L tracing revealed a dominant DMH projection to the PVP which was previously demonstrated with combined anterograde and retrograde tracing methods [38]. This finding strongly suggests that a DMH influence on pancreas activity might not exclusively be produced via the LHA and VMH descending pathways. The dense terminal labeling found in the PVP is most conspicuous in the lateral part of this paraventricular subnucleus, adjacent to the magnocellular division (PVM). The PVP, in turn, maintains circumscript, direct efferent connections with the autonomic cellgroups in the lower medulla and spinal cord [25, 32, 37, 41, 46, 48, 50] that contain the preganglionic ortho- and para-sympathetic pancreas innervating neurons. Furthermore, corticotropin-releasing factor (CRF) containing cells are identified in the PVP [21,39] and their position overlaps remarkably well with the termination area of DMH input. This overlap places the DMH in a position to modulate the release of CRF in the anterior pituitary and hence may add to the control of the plasma ACTH levels, which was physiologically demonstrated in the cat [13]. The electrical stimulations of the DMH produced a decrease of blood ACTH levels in this animal. Moreover, adrenal corticosteroid hormones are known to be involved in modulation of the pancreatic islet cell sensitivity to glucose [9,20].

Circumventricular Organ Projections

It was a striking observation that the DMH richly supplies efferents to the OVLT, SFO and area postrema. The input to the latter structure has already been identified with autoradiographic tracing methods [17]. The OVLT and SFO connections, however, have not been identified before.

A hypothalamic control system for the pancreatic hormone release requires a feedback loop between blood-borne parameters and the CNS. The circumventricular organs are lacking a blood brain barrier and are therefore considered as

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FIG. 11. Photomicrographs of PHA-L terminal labeling in the OVLT, with dark- (A, bar=100 μm) and light-field (C, bar=50 μm) illumination, and with ^{3}H-Leu (B, bar=100 μm) autoradiographic tracing (dark-field). Line drawings refer to the position of the labeled varicosities and terminal boutons in OVLT (A') and SFO (D') illustrated in photomicrographs (A) and (D). (D) Dark-field photomicrograph of PHA-L immunoreactive fibers and terminal boutons in the SFO. Bar=50 μm.

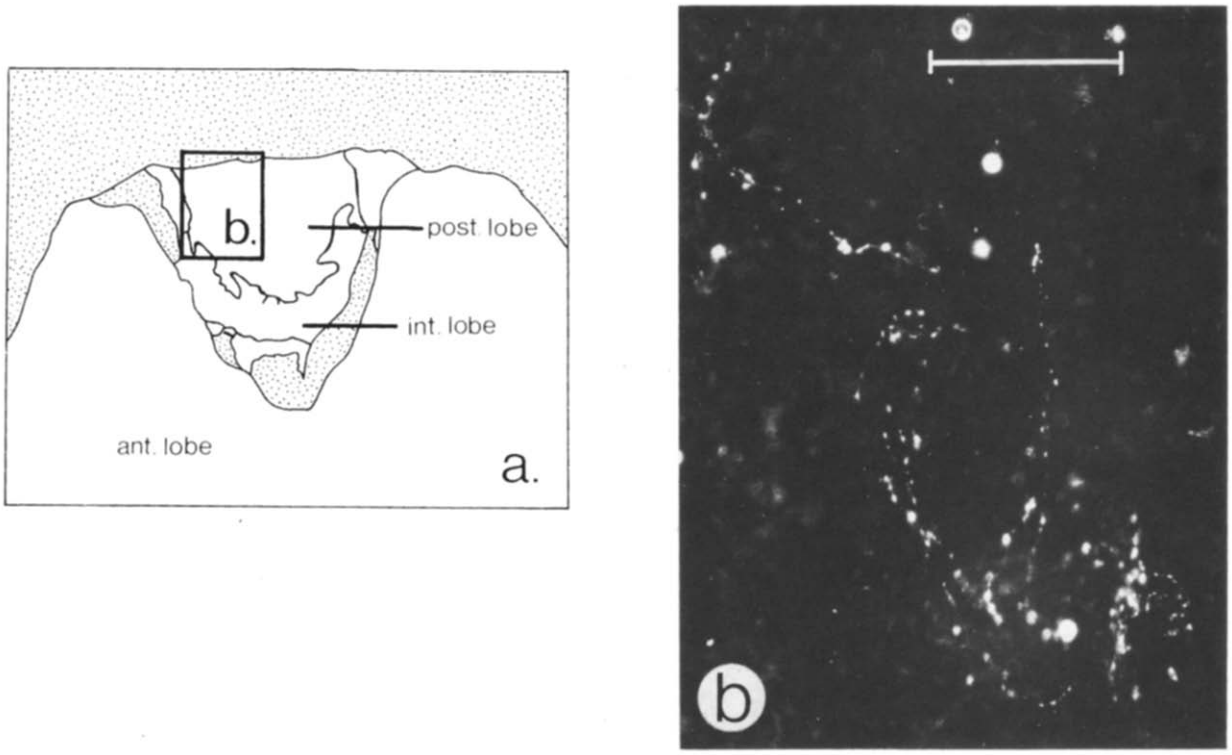

FIG. 12. (a) Horizontal section of the rat pituitary indicating the location (b) of the DMH projections in the posterior lobe. (b) Dark-field photomicrograph of PHA-L stained fibers and terminal boutons in the posterior pituitary lobe arising from the DMH. Bar=50 μm.

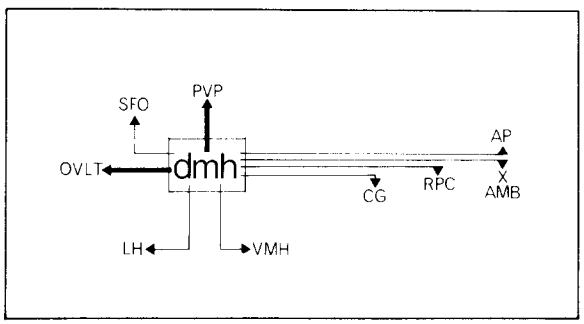

FIG. 13. Diagram presenting dorsomedial hypothalamic nucleus projections discussed in this paper. Abbreviations: AMB: ambiguus nucleus, AP: area postrema, CG: periaqueductal gray, DMH: dorsomedial hypothalamic nucleus, LH: lateral hypothalamic area, OVLT: organum vasculosum of the lamina terminalis, PVP: parvocellular paraventricular nucleus, RPC: parvocellular reticular formation, SFO: subfornical organ, VMH: ventromedial hypothalamic nucleus, X: dorsal motor vagus nucleus.

chemosensitive trigger zones for humoral factors [34,51]. Blood glucose and pancreatic hormone levels may be measured within the organum vasculosum of the lamina terminalis and relayed to hypothalamic areas controlling this hormone release. High binding of various peptide hormones, including insulin, is found in all circumventricular organs [51]. The OVLT, moreover, maintains also efferent connections with the DMH and paraventricular nucleus (unpublished observation). The extensive DMH input to the OVLT, in turn, could be modulating chemoreceptor sensitivity and hence control the hormone feedback mechanism.

Conclusions

This study presents anatomical evidence for a DMH participation in pancreatic islet cell secretory modulation via the autonomic innervation and through neuroendocrine mechanisms. The central autonomic pathways consist of direct and indirect efferent projections to the pancreas innervating cell-groups of the lower medulla and thoracic spinal cord. A neuroendocrine modulation may be obtained via DMH projections upon CRF positive cells in the paraventricular nucleus and the release of this peptide in the pituitary-portal vessels. In the anterior pituitary lobe CRF induces the secretion of ACTH in the blood, which again stimulates adrenal corticosteroid hormone release. The latter hormone is known to modulate the pancreatic B-cell sensitivity to glucose.

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