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EFFERENT CONNECTIONS OF DORSAL AND VENTRAL AGRANULAR INSULAR CORTEX IN THE HAMSTER, *MESOCRICETUS AURATUS*

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Abstract—The anterior portion of rodent agranular insular cortex consists of a ventral periallocortical region (AIv) and a dorsal proisocortical region (AId). Each of these two cortical areas has distinct efferent connections, but in certain brain areas their projection fields are partially or wholly overlapping. Bilateral projections to layers I, III and VI of medial frontal cortex originate in the dorsal agranular insular cortex and terminate in the prelimbic, anterior cingulate and medial precentral areas; those originating in ventral agranular insular cortex terminate in the medial orbital, infralimbic and prelimbic areas. The dorsal and ventral regions of the agranular insular cortex project topographically to the ipsilateral cortex bordering the rhinal fissure, which includes the posterior primary olfactory, posterior agranular insular, perirhinal and lateral entorhinal areas. Fibers to these lateral cortical areas were found to travel in a cell-free zone, between cortical layer VI and the claustrum, which corresponds to the extreme capsule. The dorsal and ventral regions send commissural projections to layer I, lamina dissecans and outer layer V, and layer VI of the contralateral homotopical cortex, *via* the corpus callosum. Projections from the ventral and dorsal regions of the agranular insular cortex to the caudatoputamen are topographically arranged and terminate in finger-like patches. The ventral, but not the dorsal region, projects to the ventral striatum and ventral pallidum. The thalamic projections of the ventral and dorsal regions are largely overlapping, with projections from both to the ipsilateral reticular nucleus and bilaterally to the rhomboid, mediodorsal, gelatinosus and ventromedial nuclei. The heaviest projection is that to the full anteroposterior extent of the medial segment of the mediodorsal nucleus. Brainstem areas receiving projections from the ventral and dorsal regions include the lateral hypothalamus, substantia nigra pars compacta, ventral tegmental area and dorsal raphe nucleus. In addition, the ventral region projects to the periaqueductal gray and the dorsal region projects to the parabrachial and ventral pontine nuclei.

These efferent connections largely reciprocate the afferent connections of the ventral and dorsal agranular insular cortex, and provide further support for the concept that these regions are portions of an outer ring of limbic cortex which plays a critical role in the expression of motivated, species-typical behaviors.

The rodent agranular insular cortex, first described by M. Rose,[56] is one portion of a complete ring of limbic cortex which is transitional in location, cytoarchitectural structure and pattern of afferent connections, between the ventrally-adjacent allocortex primitivus (terminology of Stephan & Andy[63]) and dorsally-adjacent isocortex.[55] Agranular insular cortex, like many other areas of limbic cortex, plays a significant role in arousal and a variety of motivated, species-typical social behaviors.[17,32,33,34,45,60,62] It also supports intracranial self stimulation, a response considered to involve monoaminergic-mediated motivation and reward.[7,8,35,46,57]

The studies of Rose,[56] Krettek & Price[36,37] and Reep & Winans[55] have shown that on the basis of location, cytoarchitectural structure and pattern of afferent connections, the anterior portion of rodent agranular insular cortex consists of two subdivisions, a ventral periallocortical zone (AIv) adjacent to primary olfactory cortex, and a dorsal proisocortical zone (AId) adjacent to sensory-motor isocortex. However, in two previous studies of the efferent connections of this region in the rat, areas AIv and AId were not differentiated but instead were treated together as 'sulcal cortex'[40] and the 'insular portion of the sulcal MD-projection cortex'.[3]

Because our previous horseradish peroxide (HRP) study showed the afferent connections of AIv and AId to originate in different brain areas and in topographically separate portions of common brain areas,[55] it seemed reasonable to consider the efferent connections of AIv and AId separately to see if a comparably distinct pattern emerged, and thus the present study was undertaken.

In this report comparisons are made with the results of other studies which have been done on rats, while a forthcoming paper deals with the comparative aspects of limbic cortex organization among rodents, carnivores and primates.

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Abbreviations: AId/AIv, dorsal/ventral agranular insular cortex; HRP, horseradish peroxidase; MDm, medial segment, mediodorsal thalamic nucleus.

EXPERIMENTAL PROCEDURES

Thirty adult male golden hamsters (*Mesocricetus auratus*) ranging in weight from 90–130 g were used. Pressure injections or iontophoretic deposits of tritiated amino acids were made under anesthesia with sodium pentobarbital (75 mg/kg) in the agranular insular cortex or in surrounding areas. L-[4,5-^{3}H(N)]-leucine (40–60 Ci/mmol) or L-[2,3-^{3}H]-proline (20–40 Ci/mmol) (both obtained from New England Nuclear), or an equal mixture of both, was evaporated and reconstituted to a concentration of 20–65 μCi/μl in 0.9% saline for pressure injections and in 0.01 M acetic acid for iontophohresis.

Pressure injections of 0.05–0.10 μl volumes were made using Hamilton syringes with flat or bevelled needle tips of 26s or 33 gauge, over a 10–30 min period using an Edwards microdrive apparatus. Iontophoretic deposits were made using single-barreled glass monofilament micropipettes (A-M Systems, Inc., Everett, WA) with tip diameters of 10–25 μm, over a 5–20 min period using positive DC pulses of 1.0–2.0 μA applied in a 7 s on, 7 s off pattern. Straight and angled (20° posterior from vertical) approaches were used.

After survival times of 2–10 days the animals were anesthetized and perfused through the heart with warm (37°C) phosphate-buffered 0.9% saline followed by 10% phosphate-buffered formalin at pH 7.2. The brains were removed and embedded in an egg yolk-gelatin mixture, and frozen coronal sections were cut at 25 μm. These were mounted on gelatin-coated glass slides, dried, coated with Kodak NTB-2 emulsion, then placed in light-tight boxes in a coldroom (4°C). Test slides were examined (see development procedure below) about every 14 days and the optimum exposure time (maximum signal/background ratio) determined for each brain. This was usually 3–4 weeks for brains with pressure injections and 8–10 weeks for those with iontophoretic deposits. The slides were developed in Kodak D-19 at 17°C for 3–4 min then stained with Cresyl Violet and studied microscopically using bright-field and dark-field illumination. The distribution of label above background was examined using dark-field illumination and plotted by eye on tracings of brain sections which had been made using a Bausch and Lomb microprojector, and upon which relevant cytoarchitectural boundaries had been delinated.

Anterograde transport of horseradish peroxidase (HRP) (Miles Laboratories, Inc., Elkhart, Indiana) from AId and AIv was also used to examine the efferent connections of these areas. Procedures were as described previously.[55]

RESULTS

Technical note

As discussed in detail by Jones & Hartman,[30] two of the primary difficulties encountered in using the autoradiographic technique are identification of the effective deposit site (the dimensions of the region from which transport occurs) and distinguishing labelled terminal fields from labelled axons.

The deposit site can be parcelled into three zones (see Fig. 2): (1) a central area in which cell bodies and the extracellular space are densely labelled; (2) a middle zone in which some cells are lightly labelled and much of the label is concentrated over the extracellular space; (3) an outer halo of scattered label. Based on the different projections of AId and AIv and the topographic pattern of labelling seen in our material, transport appears to occur only from zones 1 and 2, which contain labelled cells, and to a significantly greater extent from zone 1 than zone 2.

In the absence of combined electron-microscopic/autoradiographic analysis, to distinguish label associated with terminal fields from that associated with axons is not possible except in cases where linearly-oriented strands of grains are present over well defined fiber bundles such as the corpus callosum or inferior thalamic peduncle. Otherwise, we have relied on several criteria to distinguish these two possibilities: (1) correlation between the patterns of anterograde transport seen in autoradiographic and HRP material; (2) correlation with results obtained by others using anterograde degeneration techniques in rats; (3) identification of terminal fields by localization of grains over cytoarchitectonically-defined groups of cells such as thalamic and amygdaloid nuclei; (4) localization of grains over cell-free or cell sparse zones which probably correspond to fiber bundles; (5) the three dimensional trajectory of grain distributions, as determined by examination of successive sections. Thus, such terms as 'terminals' and terminal fields' are used when criterion (3) is met, while 'fibers' or 'axons' are used in case of criterion (4). Similarly, the description of label as being 'in' a given brain area should be understood to mean that silver grains are visible in the emulsion lying over that area because of the presence of labelled proteins in the tissue directly below.

The delineation of the effective uptake site in the case of HRP deposits has been discussed previously.[55] The procedure for distinguishing terminal fields from axons was identical to that above when fibers were cut in cross section. Often, however, HRP-filled axons were clearly visible in their longitudinal plane of travel.

Cytoarchitectural considerations

A detailed description of the location and structure of hamster agranular insular cortex was given in a previous report, and its role as a transition zone between the ventrally-adjacent primary olfactory cortex (allocortex primitivus) and dorsally-adjacent sensory-motor isocortex discussed.[55] As shown in Fig. 1, the anterior portion of agranular insular cortex consists of dorsal (AId) and ventral (AIv) fields. Based on its location adjacent to primary olfactory cortex, laminar structure and pattern of connections, AIv is a periallocortical zone. By similar criteria, AId is a proisocortical region (terminology of Sanides[58]).

Two cytoarchitectural features of agranular insular cortex which are of particular importance for the present report are illustrated in Figs 1B and C. First, a cell-sparse lamina dissecans lies between ('dissects') layer V and layers II/III. In the lateral precentral isocortex dorsal to AId the lamina dissecans is replaced by a granular layer IV. Second, a layer of

densely packed medium-size cells surrounds the forceps minor of the corpus callosum and is apparently continuous with the cells of the claustrum. Superficial to these cells and to the claustrum is a cell-sparse zone which lies deep to layer VI of the overlying cortex and apparently consists of cortical association fibers, as discussed below.

Experimental results: location of deposit sites

Figure 2 illustrates the deposit sites in twelve representative hamsters, including four located wholly or largely in AId, four similarly situated in AIv, and four which involve AId and AIv. In each of these three groups, anterior deposits are shown in the top row and posterior deposits in the bottom row. Also, within each group are deposits which selectively involve either superficial or deep layers as well as deposits which involve all layers. Thus, considering these brains as a whole, all layers of AId and AIv were affected throughout the entire a–p extent of these two areas. There was no labelling of cells along the needle or pipette track in any of the brains shown here, and results from straight and angled approaches were indistinguishable.

Experimental results: dorsal agranular insular cortex

Experiments 79006 and 80028 represent amino acid deposits into anterior portions of AId, with somewhat heavier involvement of layers II and III in 79006, and of layer VI in 80028 (Fig. 2). Brains 79081 and 80032 represent deposits into posterior portions of AId, with layers III and V most affected in the former and layer VI in the latter (Fig. 2).

Brain 80028. In animal 80028, a pressure injection of 0.1 μl of ^{3}H-leucine (30 μCi/μl) was made into AId. The survival time was four days and the exposure time was three weeks. The deposit site (Fig. 2) encroaches slightly upon the dorsally-adjacent lateral precentral isocortex. Layers V and VI of AId contain the greatest number of labelled cell bodies, with fewer seen in layer III and none in layer II.

In contralateral AId, label is concentrated in three bands located in layer I, lamina dissecans and outer layer V, and layer VI (Fig. 3, A–D). Grains are distributed continuously between the anterior and posterior borders of AId but are present in greatest quantity at the same a–p levels as the deposit site. Labelled fibers projecting to contralateral AId are visible in the corpus callosum (Figs 3 C–E).

In medial frontal cortex, label is found bilaterally in the prelimbic, anterior cingulate and medial precentral areas, concentrated in inner layer I, layer III and layer VI (Figs 3 A–F). The density of labelling is much greater ipsilaterally than contralaterally. Label in the anterior cingulate area is heaviest at the same a–p levels as the deposit site, and extends to its posterior boundary, where area 29c of retrosplenial cortex begins to appear and is characterized by combined layers II and III, a granular layer IV, and a cell-free lamina dissecans between layers IV and V.[67] According to these authors there is no discernable posterior cingulate cortex between the anterior cingulate and retrosplenial areas in the rat, and this corresponds to our own observations in the hamster. The transition from anterior cingulate to retrosplenial cortex occurs at the level shown in Fig. 3G. Anterior to the forceps minor of the corpus callosum, fibers from AId pass directly to ipsilateral medial frontal cortex, deep to layer VI of orbital cortex (Figs 3A–B), whereas more posteriorly they appear to travel deep to layer VI of the lateral sensory-motor isocortex in order to reach the medial precentral and anterior cingulate areas (Figs 3C–F). Fibers projecting to contralateral medial frontal cortex apparently do so via the corpus callosum.

Lateral cortical areas in which label is seen include the ipsilateral posterior agranular insular, perirhinal and entorhinal cortices (Figs 3E–L). Fibers from AId to these areas travel together caudally, appearing as a band of label within the cell-free zone lying deep to layer VI of posterior agranular insular cortex and superficial to the claustrum/endopiriform nucleus (Figs 3E–J and Fig. 9). Lighter accumulations of label are found in layers I, III and VI of the overlying posterior agranular insular cortex throughout its length. More caudally, label is concentrated in the deep layers of perirhinal cortex and area 28L (terminology of Haug[25]) of entorhinal cortex.

The dorsal striatum (caudatoputamen) receives a bilateral projection from AId, with fibers reaching the contralateral side *via* the corpus callosum. On both sides of the brain, label is seen in the ventrolateral quadrant of the caudatoputamen and is heaviest in its anterior regions (Figs 3D–G). Fibers destined for more caudal brain areas gather within the caudatoputamen just dorsal to the posterior limb of the anterior commissure and proceed posteromedially, contributing to the inferior thalamic peduncle and internal capsule (Figs 3F–H).

Fibers in the inferior thalamic peduncle terminate in the thalamic reticular, mediodorsal, central medial, rhomboid, paracentral, gelatinosus and ventromedial nuclei (Figs 3G–I). Labelling is heaviest in the mediodorsal nucleus and extends throughout its length within the medial segment (see Fig. 11). Bilateral terminal fields, heavier ipsilaterally, are seen in the mediodorsal, paracentral, gelatinosus and ventromedial nuclei, while labelling of the reticular nucleus is exclusively ipsilateral. The midline central medial and rhomboid nuclei contain evently distributed label.

In the amygdala, label is present ipsilaterally in the lateral and basolateral nuclei, being heaviest in the anterior portion of the basolateral nucleus (Figs 3H–I and Fig. 12).

Fibers in the most ventral portion of the internal capsule/cerebral peduncle give rise to labelling in the lateral hypothalamus (Fig. 3I) and, more posteriorly, in substantia nigra and the ventral tegmental area (Figs 3J–K and Fig. 13). Grains are most dense over the pars compacta region of substantia nigra. As

shown in Figs 3K–M, some fibers continue dorsally through the ventral tegmental area to reach the dorsal raphe nucleus, and others leave the lateral boundary of substantia nigra pars compacta, producing label in the midbrain reticular formation, cuneiform nucleus, parabrachial nucleus (see Fig. 14) and locus coeruleus. Finally, a moderate amount of label can be followed caudally within the cerebral peduncle and longitudinal fibers of the pons, terminating in the ventral pontine nucleus (Fig. 3L) and magnus portion of the raphe nucleus (Fig. 3M).

Additional AId deposits. In other brains in which the amino acid or HRP deposit site was wholly or largely confined to AId, the pattern of anterograde labelling was similar to that described above for experiment 80028. However, certain findings warrant further mention and are presented below.

In experiment 79006 (Fig. 2), there was markedly heavier labelling of cells at the deposit site in anterior AId than in brain 80028, probably because the concentration of ^{3}H-leucine used was 65 μCi/μl in the former and 30 μCi/μl in the latter. The only notable difference in the pattern of transported label between these two brains was that the lateral and basolateral amygdaloid nuclei were labelled bilaterally in 79006 and only ipsilaterally in 80028. A similar bilateral distribution of label in the amygdala was also seen in brain 79005, in which the concentration of ^{3}H-leucine used was also 65 μCi/μl. The deposit site in this brain included posterior portions of AId and AIv (Fig. 2).

In experiment 80032, the deposit site was centered in posterior AId and affected layer VI with little involvement of layer V (Fig. 2). Transported label in medial frontal cortex and the caudatoputamen was located more caudally than in brain 80028. There was very sparse labelling of contralateral AId and no label visible in the amygdala. However, the ipsilateral posterior agranular insular, perirhinal and entorhinal cortices were heavily labelled (see Fig. 7). In contrast, in experiment 79081, where layer V of posterior AId is predominantly effected (Fig. 2), the labelling pattern was identical to that seen in brain 80028 except that the medial frontal cortex and caudatoputamen were labelled slightly more caudally in 79081.

Abbreviations used in Figures

A	nucleus accumbens
AA	anterior amygdaloid area
ACd	dorsal portion, anterior cingulate cortex
ACv	ventral portion, anterior cingulate cortex
acp	posterior limb of anterior commissure
AId	dorsal agranular insular cortex
AIp	posterior agranular insular cortex
AIv	ventral agranular insular cortex
AM	anteromedial thalamic nucleus
BLa	anterior portion, basolateral amygdaloid nucleus
BLp	posterior portion, basolateral amygdaloid nucleus
CeL	central lateral thalamic nucleus
CL	Claustrum
CM	central medial thalamic nucleus
cp	cerebral peduncle
CU	cuneiform nucleus
C1	anterior cortical amygdaloid nucleus
C2	posterolateral cortical amygdaloid nucleus
C3	posteromedial cortical amygdaloid nucleus
DPC	dorsal peduncular cortex
DR	dorsal raphe nucleus
E	endopiriform nucleus
flp	longitudinal fibers of the pons
fm	forceps minor of the corpus callosum
fr	fasciculus retroflexus
fx	fornix
G	gelatinosus (submedial) thalamic nucleus
GP	globus pallidus
HL	lateral hypothalamus
ic	internal capsule
IL	infralimbic cortex
itp	inferior thalamic peduncle
L	lateral amygdaloid nucleus
LC	locus coeruleus
LD	lamina dissecans
LH	lateral habenula
MDm	medial segment, mediodorsal thalamic nucleus
MH	medial habenula
ml	medial lemniscus
MO	medial orbital cortex
MR	magnus portion of raphe nucleus
MRF	midbrain reticular formation
mt	mammillothalamic tract
NAOT	nucleus of the accessory olfactory tract
NLOT	nucleus of the lateral olfactory tract
OTu	olfactory tubercle
P	ventral pontine nucleus
PaC	paracentral thalamic nucleus
PAG	periaqueductal gray
PBl	lateral parabrachial nucleus
PBm	medial parabrachial nucleus
PC	medial precentral cortex
PF	parafascicular thalamic nucleus
PL	prelimbic cortex
POCp	posterior primary olfactory cortex
PR	perirhinal cortex
PT	parataenial thalamic nucleus
PVa	anterior portion, paraventricular thalamic nucleus
RE	reuniens thalamic nucleus
RH	rhomboid thalamic nucleus
RT	reticular thalamic nucleus
SG	substriatal gray
sm	stria medullaris
SNc	substantia nigra pars compacta
SNr	substantia nigra pars reticulata
ST	subthalamic nucleus
TR	entorhinal cortical area TR
VM	ventromedial thalamic nucleus
VP	ventral pallidum
VTA	ventral tegmental area
ZI	zona incerta
28L	lateral entorhinal cortical area 28L
28L'	lateral entorhinal cortical area 28L'
V	nucleus of mesencephalic tract of trigeminal nerve

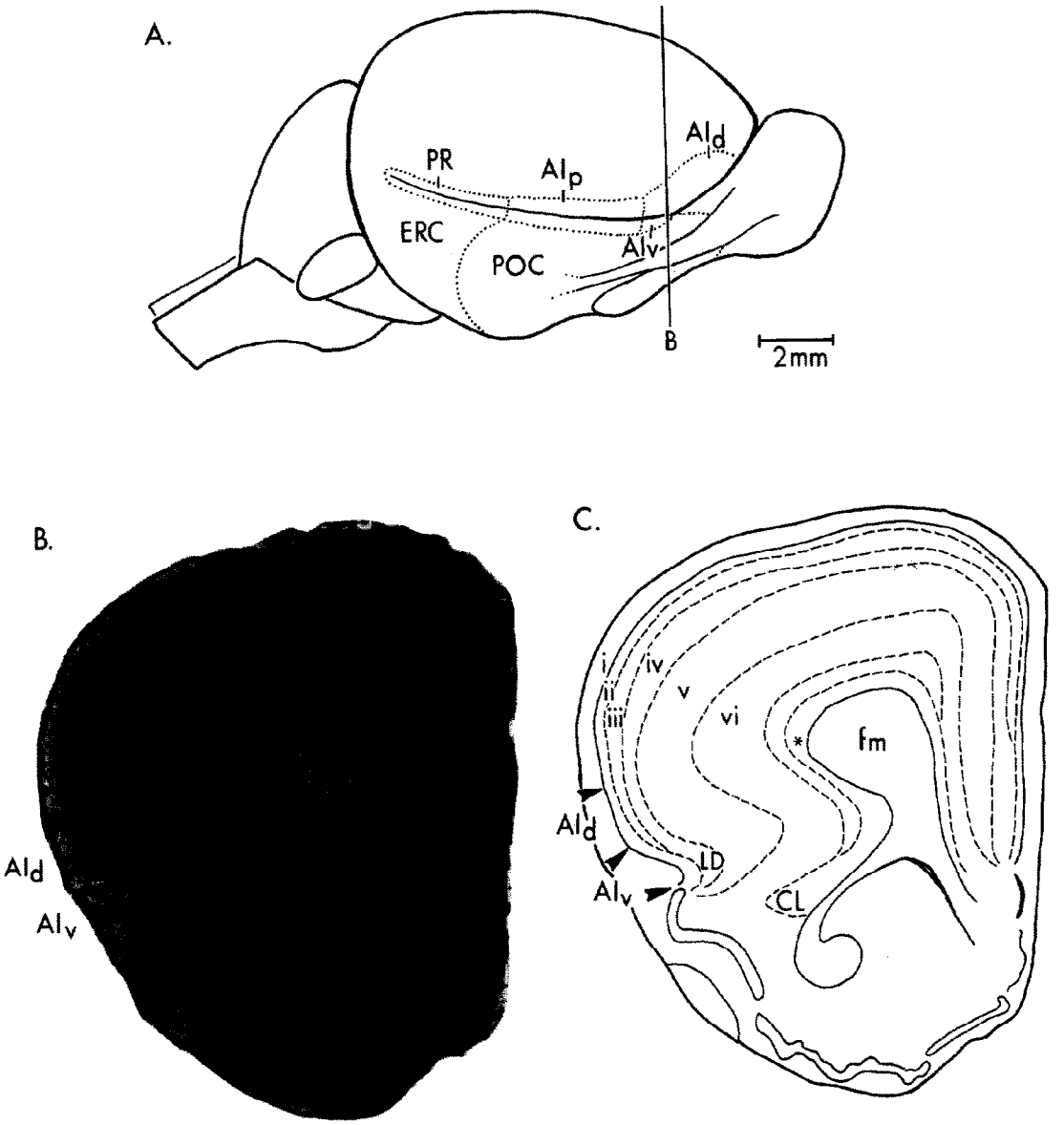
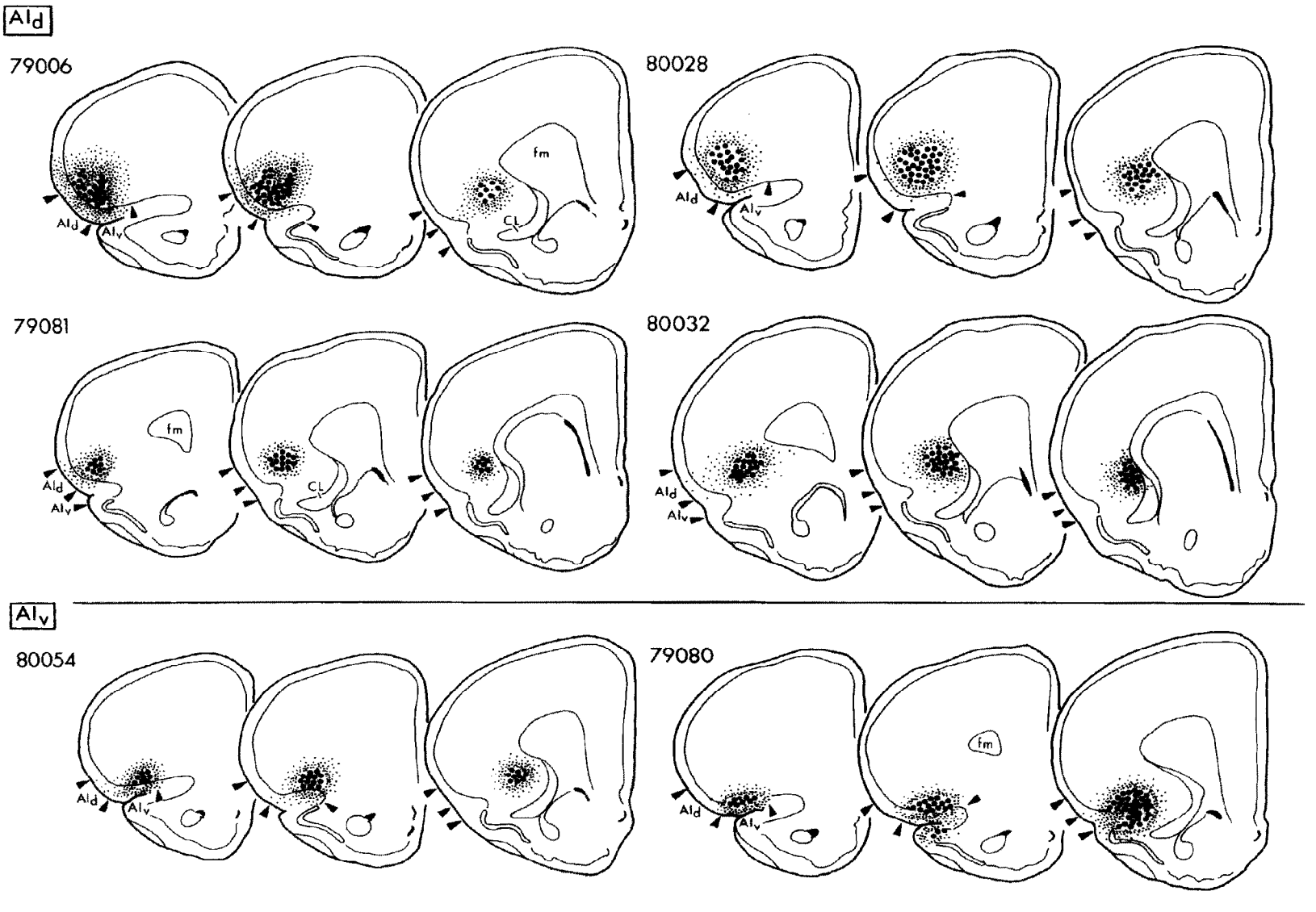


Fig. 1. A. Lateral view of the right side of a hamster brain, showing the boundaries of cortical areas adjacent to the rhinal fissure. B. Coronal section at the level shown in A. Cresyl Violet stain. C. Delineation of cortical layers in B. Note cell-free lamina dissecans between layer V and layers II/III of AId and AIv, and layer of cells (asterisk) surrounding the forceps minor of the corpus callosum.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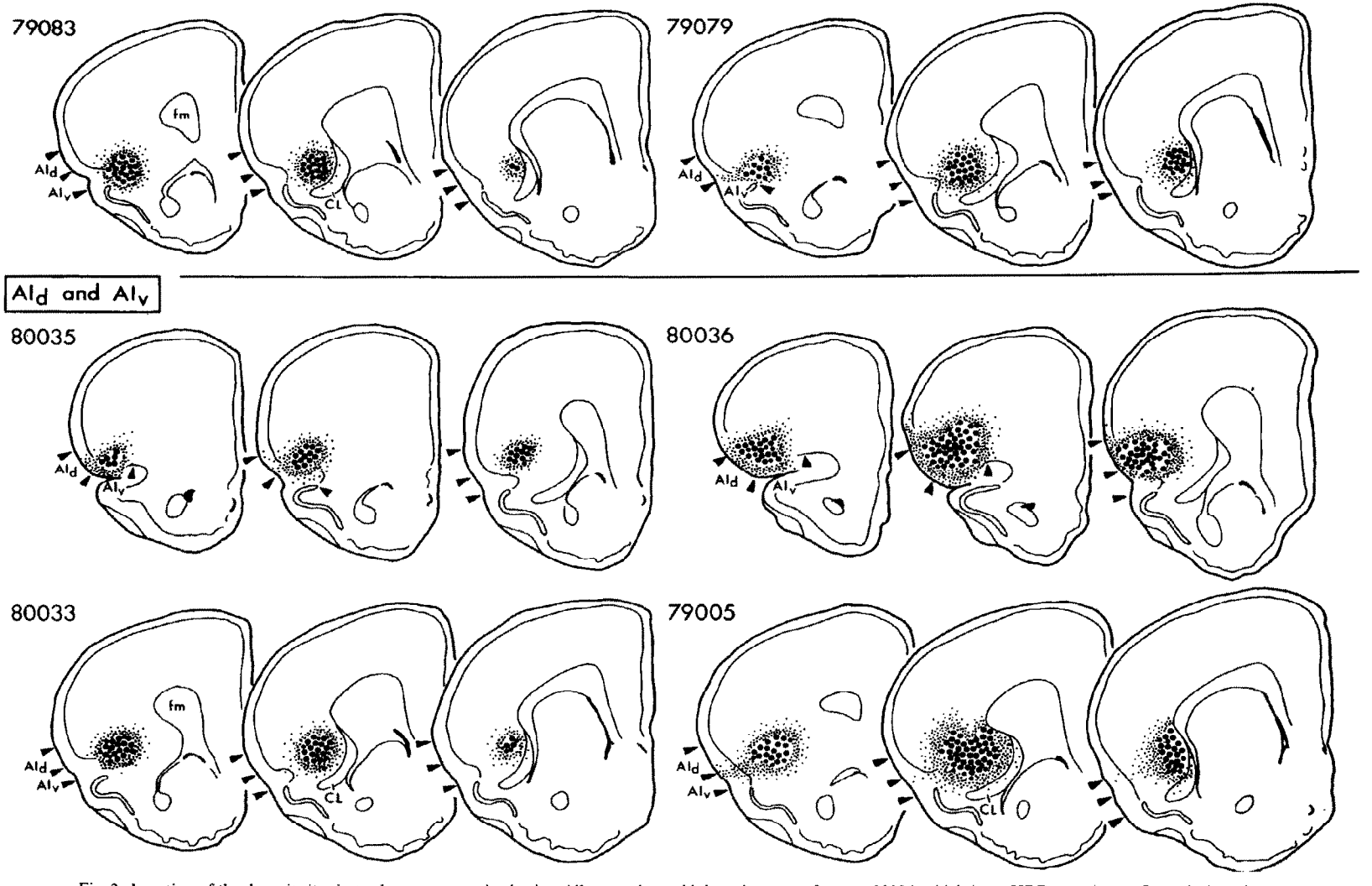
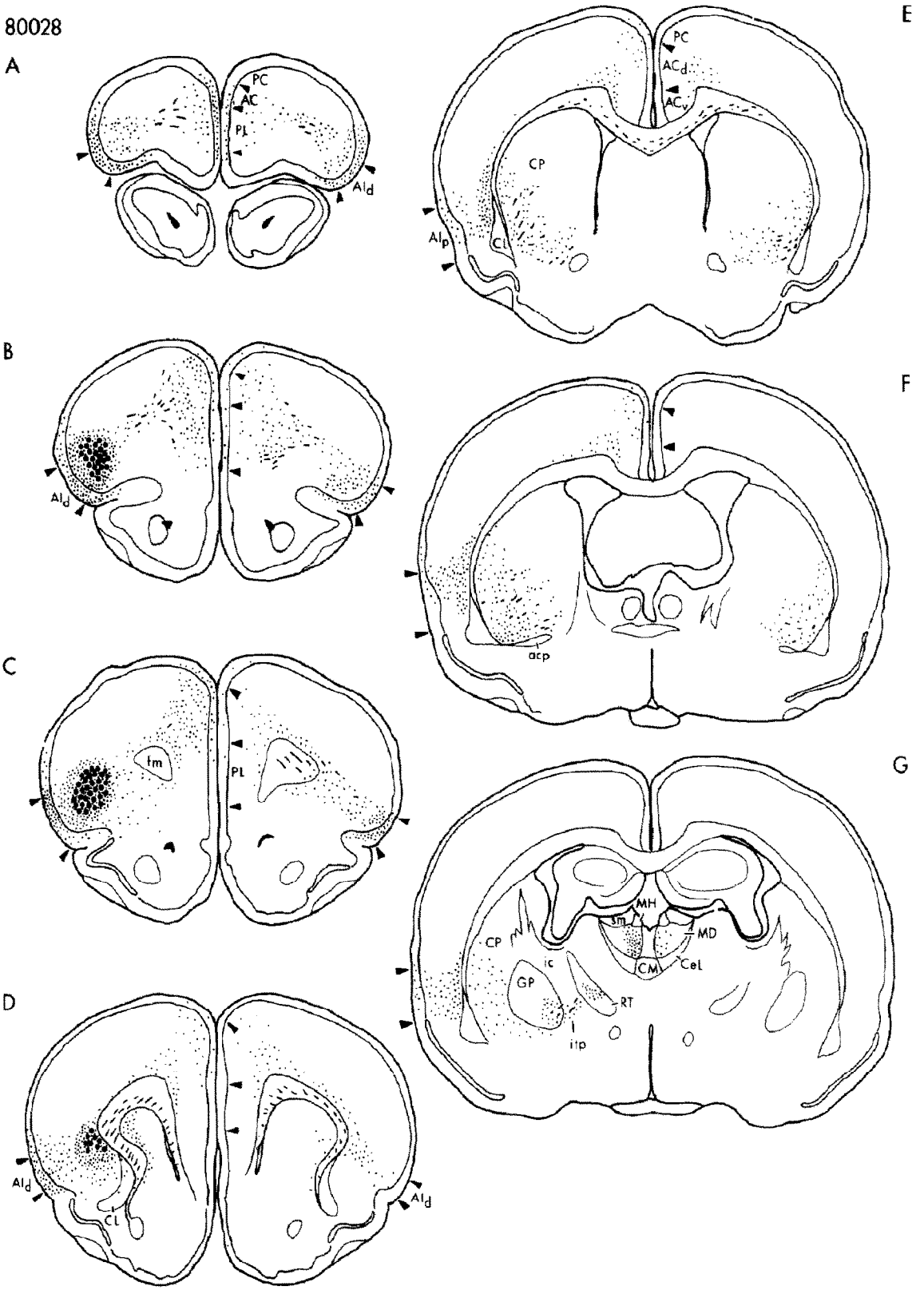


Fig. 2. Location of the deposit sites in twelve representative brains. All are amino acid deposits except for case 80036, which is an HRP experiment. In each deposit site three zones are illustrated (see text): an inner zone of heavily labelled cells represented by large dots, and two outer zones represented by dense and light stippling, respectively.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PC
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E
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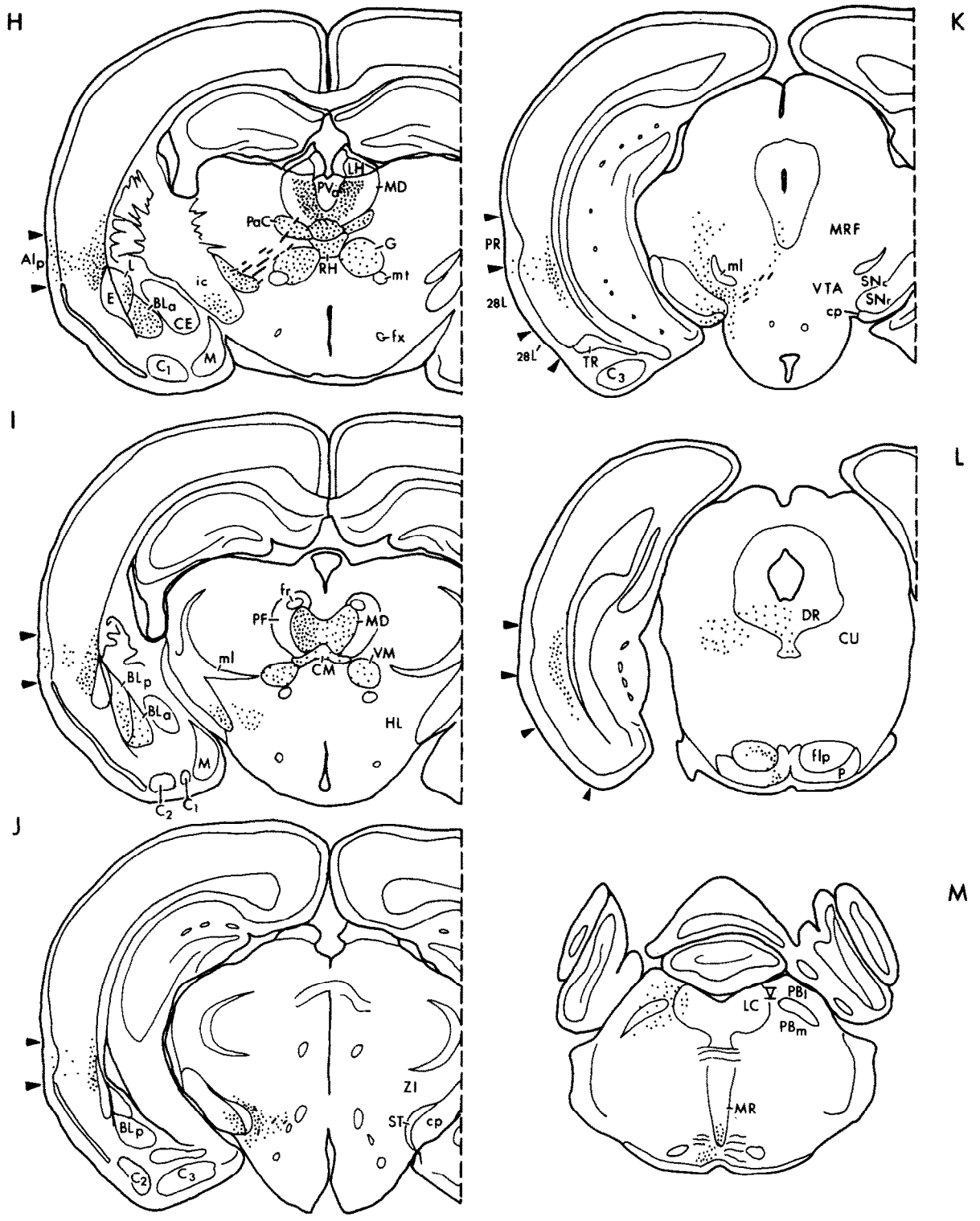


Fig. 3. A series of representative coronal sections illustrating the distribution of label in brain 80028.

Analysis of all autoradiographic and HRP experiments involving deposits in AId revealed that the projections to medial frontal cortex and the caudatoputamen are topographically organized such that more anterior deposits in AId result in more anterior labelling of these areas. There was no apparent topography in the labelling pattern of subcortical structures. For example, the full extent of the medial segment of the mediodorsal thalamic nucleus was labelled regardless of the a–p location of the deposit site within AId.

Experimental results: ventral agranular insular cortex

Experiments 80054 and 79080 represent amino acid deposits into anterior portions of AIv, with layer VI largely unaffected in the latter case (Fig. 2). In case 79080 the portion of AIv which curves around the fundus of the rhinal fissure is more involved and there is also some encroachment on the primary olfactory cortex. Brains 79083 and 79079 represent deposits into middle and posterior portions of AIv (Fig. 2). Layers II–VI are affected in both cases.

Brain 79079. In animal 79079, an iontophoretic deposit of ^{3}H-leucine (30 μCi/μl) was made into AIv, through a micropipette having tip diameter of 24 μm using a 1 μA current for five min. The survival time was two days and the exposure time was eight weeks. At the deposit site layers V and VI contain the greatest number of labelled cells, with fewer seen in layers II and III (Fig. 2).

Labelling in contralateral agranular insular cortex is similar to brain 80028, being located in layer I, lamina dissecans and outer layer V, and layer VI (Figs 4B–D), but in AIv rather than in AId. Labelling is heaviest at the same a–p levels as the deposit site, and can be followed in the corpus callosum from the deposit site to contralateral AIv.

In medial frontal cortex, bilateral labelling more ventrally than in 80028, in the medial orbital, infralimbic and prelimbic areas, concentrated in inner layer I, layer III and layer VI, and is heavier on the ipsilateral side (Figs 4A–D). Fibers from AIv to medial cortex travel in the manner described above for AId, as shown in Figs 4B and D.

Laterally, label is seen in the posterior agranular insular area and claustrum deep to it, posterior primary olfactory cortex and the endopiriform nucleus deep to it, and the perirhinal and entorhinal cortices (Figs 4E–M). Fibers from AIv to these areas pass caudally in the cell-free zone between layer VI of posterior agranular insular cortex and the claustrum/endopiriform nucleus (Figs 4E–J and Fig. 9). Scattered label in the overlying posterior agranular insular cortex is found primarily in layers I, III and VI throughout its length. More caudally, labelling is heaviest in the deep layers of areas 28L and 28L′ of lateral entorhinal cortex and in perirhinal cortex.

The dorsal striatum receives a bilateral topographically-organized projection from AIv similar to that from AId, with fibers reaching the contralateral side *via* the corpus callosum. Label is heaviest in the middle ventral portion of the anterior caudatoputamen on both sides and its largely arranged in finger-like patches (Figs 4D–F). Fibers destined for the thalamus and brainstem are organized as described above (Figs 4F–I).

The ventral striatum, composed of the substriatal gray, nucleus accumbens and olfactory tubercle, receives a bilateral projection from AIv (Figs 4C–G). Fibers travel to the contralateral ventral striatum in the anterior commissure (Fig. 4F). Within the olfactory tubercle, label is heaviest over the cell bridges which extend from the deep to superficial layers and no label is seen in the fascicles of the medial forebrain bundle which occupy the spaces between these cell bridges (Fig. 4E and Fig. 10). A moderate amount of label is seen medial to the substriatal gray, in the ventral pallidum (Figs 4F–G). More caudally this label splits into a relatively sparse lateral component which can be followed into the anterior amygdaloid area and a more substantial medial component which joins the most ventral portion of the internal capsule/cerebral peduncle (Figs 4H–I).

In the thalamus, fibers distribute to the parataenial, reticular, rhomboid, mediodorsal, gelatinosus and ventromedial nuclei (Figs 4G–J). The projection to the reticular nucleus is ipsilateral but those to the parataenial, mediodorsal, gelatinosus and ventromedial nuclei are bilateral and heavier ipsilaterally than contralaterally. Labelling is greatest in the mediodorsal nucleus and extends throughout its length within the medial segment (see Fig. 1).

As in case 80028, there is light labelling of the lateral hypothalamus, substantia nigra and ventral tegmental area (Figs 4J–L and Fig. 13). More caudally, label is seen in the dorsal raphe and periaqueductal gray regions of the brainstem (Fig. 4M).

Additional AIv deposits. In other brains in which the amino acid or HRP deposit site was primarily situated in AIv, the anterograde labelling pattern was similar to that of experiment 79079 above. Results deserving further consideration are described below.

In experiment 79083 (Fig. 2) the deposit site in posterior AIv was nearly identical to that of brain 79079. Although the deposit site in 79079 encroaches upon the claustrum (Fig. 4D) whereas that in 79083 does not, the distribution of transported label was virtually the same in these two brains. Thus, involvement of the claustrum in the deposit site of 79079 was apparently of little consequence.

The deposit site in brain 80054 (Fig. 2) was situated more anteriorly than in 79079. Likewise, though the distribution of label was very similar in these two brains, the medial frontal cortex and caudatoputamen were labelled somewhat more anteriorly in 80054 than in 79079.

Experiment 79080 is notable in that the deposit site encroached upon the most dorsal portion of anterior primary olfactory cortex (Fig. 2). Labelling of the olfactory tubercle in this brain was much heavier than

in brains 79079, 79083 and 80054, all of which had deposit sites confined to AIv. Also, whereas the medial orbital, infralimbic and prelimbic areas of medial frontal cortex were labelled in all four of these brains, the dorsal peduncular cortex (terminology of Haberly & Price[23]) was heavily labelled in 79080, but not at all in the others.

Analysis of all cases involving deposits in AIv showed that while there was no apparent topographical organization in the subcortical projections of AIv, the projections to medial frontal cortex and the caudatoputamen are arranged so that more anterior regions of AIv project to more anterior portions of these areas.

Further considerations

In a number of experiments the deposit site was centered on the boundary between AId and AIv, and involved portions of each. Four such cases are illustrated in Fig. 2. In each of these brains and in others in which the deposit site was similarly situated, the areas to which label was transported included some specific to deposits in AId alone and some specific to deposits in AIv alone. Thus, for example, in brain 80036 there was labelling of the anterior cingulate cortex and basolateral amygdala, structures labelled by deposits in AId alone, as well as label in the medial orbital cortex and olfactory tubercle, structures labelled by deposits into AIv alone. Similarly, brain 80033 had AId-related label in the parabrachial nucleus and AIv-related label in the ventral striatum, among other areas.

Comparison between AId and AIv

AId and AIv each send commissural projections to the corresponding contralateral homotopical cortex, with labelling concentrated in layer I, lamina dissecans and outer layer V, and layer VI (Fig. 5). The posterior extent of this labelling is the same in AId and AIv, and lies at the boundary between these areas and the posterior agranular insular cortex (see Figs 3 and 4).

As shown in Fig. 6, the bilateral projections from AId and AIv to medial frontal cortex are topographically arranged such that the dorsally-situated anterior cingulate and medial precentral areas receive input from AId while the ventrally-located infralimbic and medial orbital areas receive input from AIv. Prelimbic cortex, which lies between these dorsal and ventral zones, receives projections from both AId and AIv. In all areas of medial frontal cortex label is heaviest over inner layer I, layer III and layer VI, and is greater ipsilaterally than contralaterally.

Input to ipsilateral cortical areas bordering the rhinal fissure is topographically organized in a dorsoventral fashion as shown in Fig. 7. Area AId projects most heavily to the dorsally-situated posterior agranular insular and perirhinal areas, while AIv projects mainly to the ventrally-located posterior primary olfactory and lateral entorhinal cortices. Fibers to all these areas travel caudally from AId and AIv in the cell-free zone between layer VI of posterior agranular insular cortex and the claustrum/endopiriform nucleus (Fig. 9). The endopiriform nucleus, which lies deep to primary olfactory cortex, receives input from AIv but not AId.

The dorsal striatum is projected upon bilaterally and in a topographic manner by AId and AIv. Input from AId is located in the lateral ventral portion of the caudatoputamen (Figs 3E–F) while that from AIv is located in the middle ventral portion (Figs 4E–F). In both cases much of the label is arranged in finger-like clusters.

The ventral striatum receives input from AIv but not AId, as does the ventral pallidum.

In the thalamus, the reticular nucleus is labelled ipsilaterally and the rhomboid, mediodorsal, gelatinosus and ventromedial nuclei are labelled bilaterally (heavier ipsilaterally than contralaterally) in all brains. The parataenial, central medial and paracentral nuclei were often lightly to moderately labelled but these projections did not appear to correspond systematically to the location of the injection site.

AIv projects lightly to the anterior amygdaloid area whereas AId has a substantial input to the anterior basolateral and lateral amygdaloid nuclei.

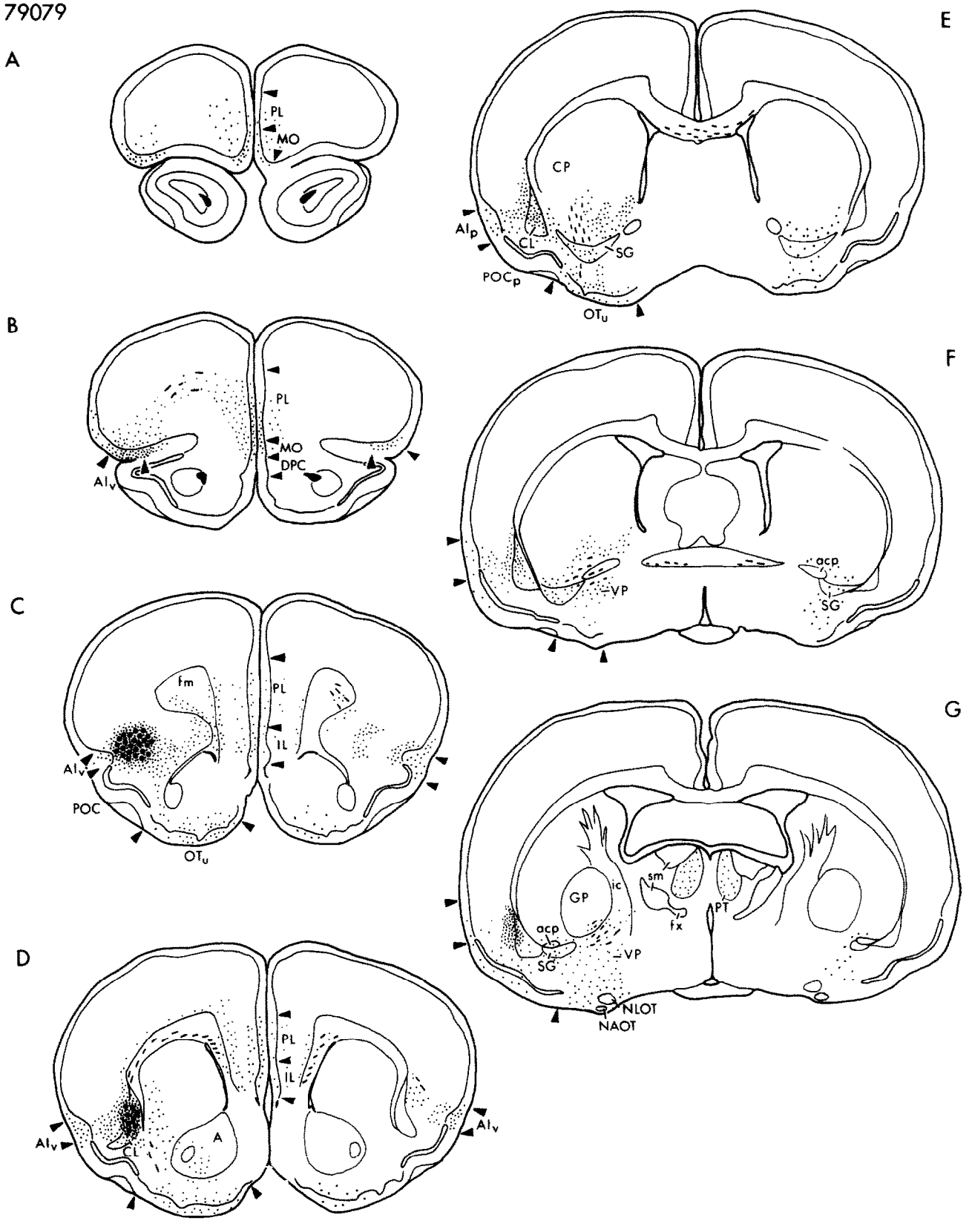
AId and AIv both project to the lateral hypothalamus, substantia nigra, ventral tegmental area and dorsal raphe nucleus. The parabrachial nucleus receives input from AId but not AIv, while the periaqueductal gray receives input from AIv but not AId.

The major subcortical projections of AId and AIv are presented schematically in Fig. 8.

DISCUSSION

The present study has shown that in the hamster, AId and AIv each have distinctive sets of efferent connections and that these projections are partially overlapping. Many of the projections reciprocate the afferent connections of AId and AIv, which were described in a previous report.[55] We have discovered previously undocumented projections from AId to the parabrachial nucleus and from AIv to the medial orbital and infralimbic cortices.

A number of efferent connections which were previously described only as originating in 'agranular insular' or 'sulcal' cortex[3,40] have been found to arise specifically from AId or AIv alone. Furthermore, we have shown that in at least four regions to which AId and AIv both project (contralateral agranular insular cortex, medial frontal cortex, caudatoputamen and lateral cortex) there is a well defined topographic organization to the projections such that AId and AIv innervate separate but partially overlapping portions of these regions. In certain subcortical areas such as the thalamus the efferent projections of AId and AIv appear to be completely overlapping.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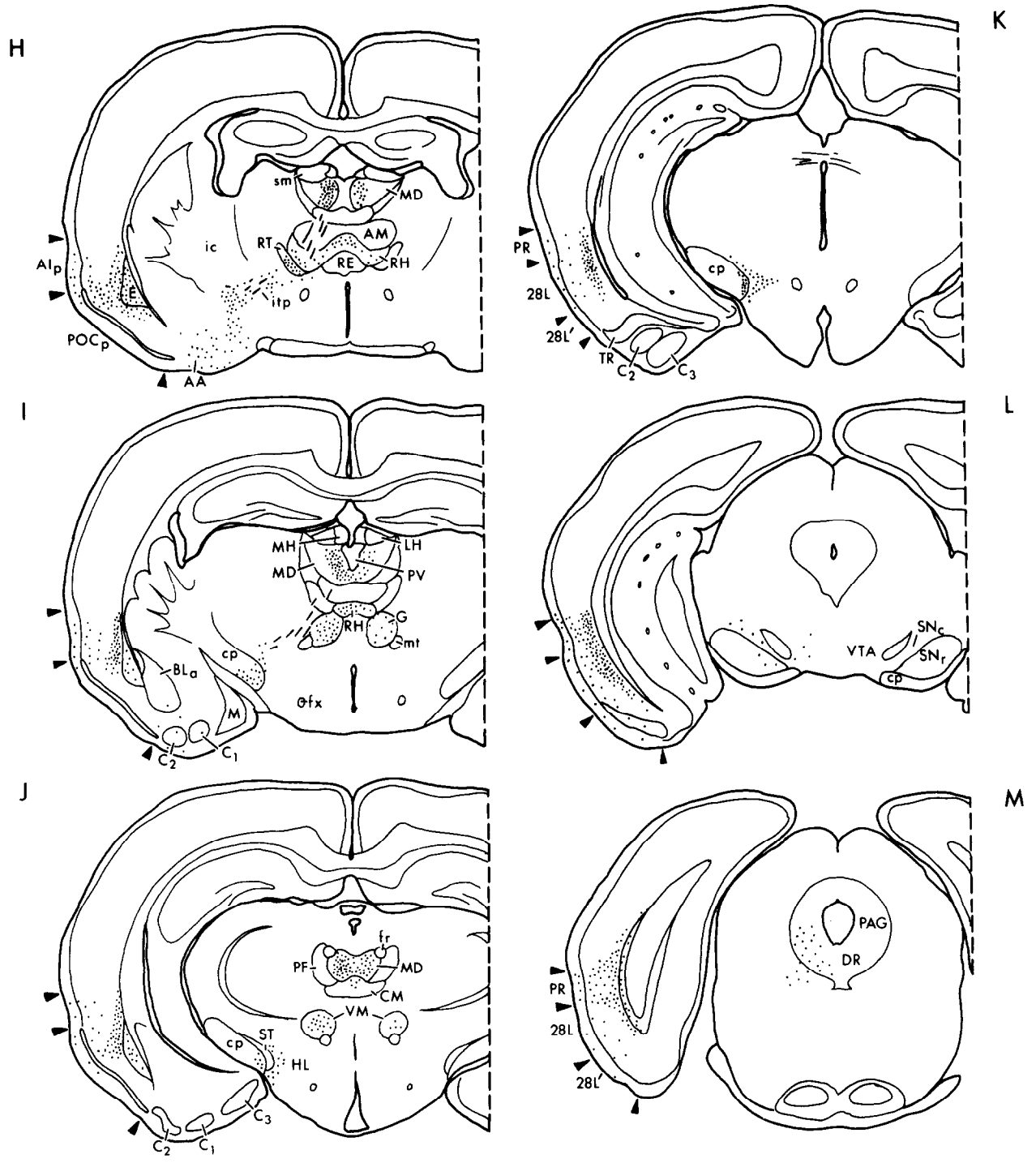


Fig. 4. A series of representative coronal sections illustrating the distribution of label in brain 79079.

Cortico-cortical connections

Commissural projections. Labelling in contralateral agranular insular cortex was heaviest when the deposit site involved layers III and V of AId or AIv, and this agrees with our previous finding, using retrograde transport of HRP, that the commissural projections of AId and AIv originate 90% from pyramidal cells in superficial layer V and 10% from pyramidal cells in layer III.[55] The distribution of label in layer I, lamina dissecans and outer layer V, and layer VI (Fig. 5) seems to agree with the results of Beckstead[3] (see his Fig. 7A), although this projection was not explicitly discussed by him.

Medial frontal cortex. The topographic organization of the efferents from AId and AIv to medial

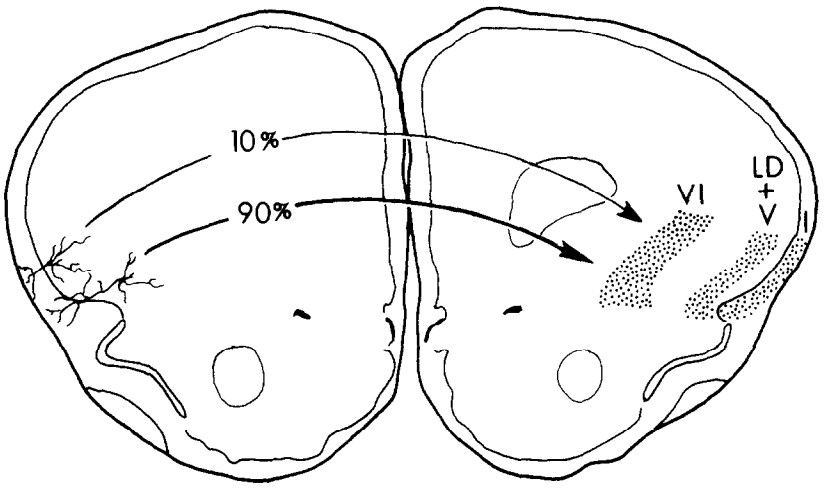


Fig. 5. Schematic diagram showing that the commissural projections from AId and AIv originate 90% from pyramidal cells in outer layer V and 10% from pyramidal cells in layer III,[55] and that they terminate in layer I, lamina dissecans and outer layer V, and layer VI of the contralateral homotopical cortex.

frontal cortex (Fig. 6) matches that of the reciprocal projections from medial frontal cortex to AId and AIv (see Fig. 7B of Reep & Winans[55]). Thus, in both instances the dorsally-located AId, anterior cingulate and medial precentral areas are interconnected, while the ventrally-located AIv, infralimbic and medial orbital areas are interconnected, and the prelimbic area has reciprocal connections with both AId and AIv. Furthermore, the projections from AId and AIv to medial frontal cortex and those from medial frontal cortex to AId and AIv are all bilateral (heavier ipsilaterally) and terminate in layers I, III and VI (this report; ref. 3).

Lateral cortical areas. The topographic organization of the ipsilateral projections from AId and AIv to cortical areas bordering the rhinal fissure (see Fig. 7) is readily understood not only on the basis of dorsoventral position but also within the context of certain afferent relationships, as described below.

AId projects most heavily to the dorsally-situated posterior agranular insular and perirhinal areas, and all three of these dorsal cortical regions receive substantial input from the anterior portion of the basolateral amygdaloid nucleus.[37,55] In rats, it has been shown that the basolateral nucleus also provides input to that portion of the mediodorsal nucleus which projects to AId.[37]

AIv projects most heavily to the ventrally-located posterior primary olfactory cortex and lateral entorhinal area, both of which receive direct input from the main olfactory bulb.[11,61] Furthermore, AIv receives input from both of these areas and from another tertiary olfactory area, the posterolateral cortical amygdaloid nucleus.[31,55] AIv also projects to the

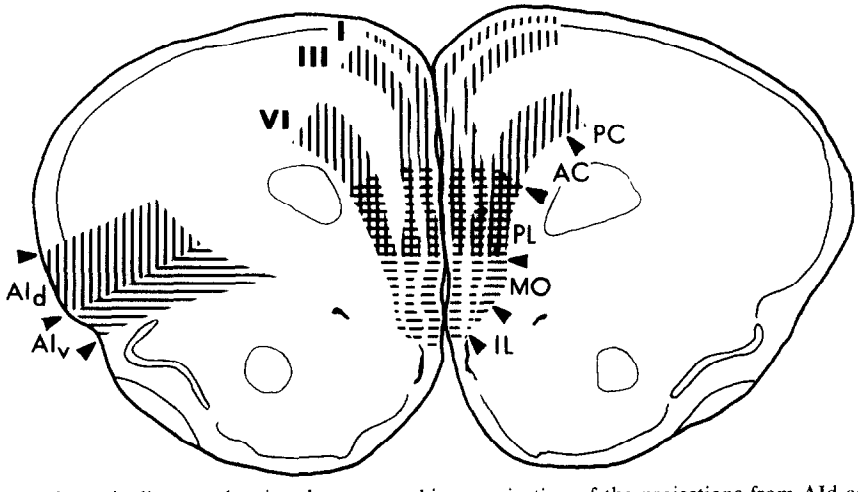


Fig. 6. Schematic diagram showing the topographic organization of the projections from AId and AIv to medial frontal cortex. Areas to which AId projects are indicated by vertical stripes, while those to which AIv projects are marked by horizontal stripes. Note that the projection fields are all bilateral to layers, I, III and VI, and overlap in the prelimbic area.

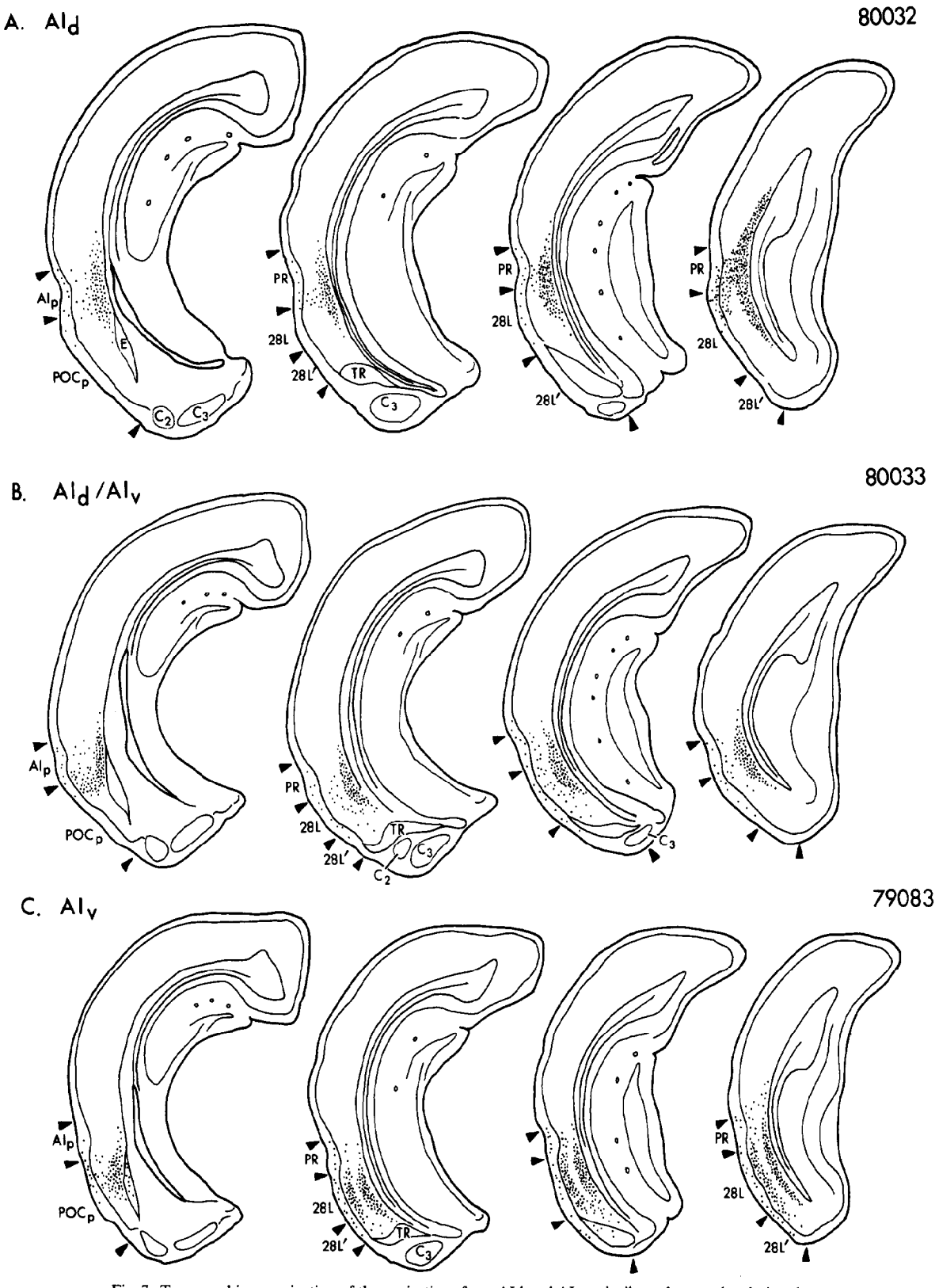


Fig. 7. Topographic organization of the projections from AId and AIv to ipsilateral cortex bordering the rhinal fissure. Similarly spaced coronal sections with the distribution of transported label are shown from: A. Brain 80032, in which the deposit site is located in AId (see Fig. 2). B. Brain 80033, in which the deposit site includes portions of AId and AIv (see Fig. 2). C. Brain 79083, in which the deposit site is located in AIv (see Fig. 2). Note that with more dorsal deposit sites, the distribution of label in lateral cortex in heavier over more dorsally-located structures.

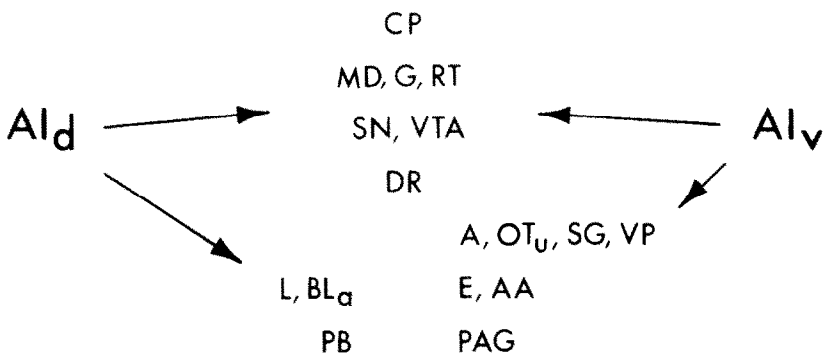


Fig. 8. Schematic summary of the major subcortical projections of AId and AIv.

endopiriform nucleus, which may be considered an olfactory area by virtue of its position deep to primary olfactory cortex and the fact that it receives input from the anterior olfactory nucleus and primary olfactory cortex,[22] and from the posterolateral cortical amygdaloid nucleus,[31] all of which are tertiary olfactory areas. In rats (and perhaps in hamsters), the portion of the mediodorsal thalamic nucleus which projects to AIv receives input from the primary olfactory cortex and the endopiriform nucleus.[26,37,40]

Our finding that in the hamster both AId and AIv project to lateral entorhinal cortex is at variance with two studies done in rats in which retrograde transport of HRP from entorhinal cortex was used to assess its afferent connections. Beckstead[2] (see his Figs 3A–E) and Haberly & Price[22] (see their Fig. 9) found labelled cells in dorsal portions of anterior and posterior primary olfactory cortex, and in the claustrum/endopiriform nucleus, but not in agranular insular cortex. Since many of our amino acid deposits which produced labelling in entorhinal cortex did not involve cells in either of these areas, but were instead restricted to AId or AIv, we conclude that this discrepancy reflects either a differential sensitivity of the anterograde autoradiographic and retrograde HRP techniques, or a species difference in the organization of inputs to lateral entorhinal cortex.

The cell-free zone between later VI of posterior agranular insular cortex and the claustrum/endopiriform nucleus, in which fibers travel from AId and AIv to lateral cortical areas, corresponds in location to the extreme capsule. Furthermore, at least some of these fibers may comprise an uncinate fasciculus, which in primates interconnects orbitofrontal and temporal pole cortical areas.[39,48,69] An analogous organization is found in the medial cortex of the rat, where corticocortical fibers interconnecting the anterior cingulate and retrosplenial areas travel "in the deepest cortical layer where they form an indistinct fiber stratum superficial to the fasciculus cinguli proper".[14]

Dorsal and ventral striatum

Our finding of topographically-organized projections from AId and AIv to the caudatoputamen is consistent with the fact that this area, which constitutes the dorsal striatum, receives spatially-ordered projections from the entire isocortex[6,16,68] and from at least some periallocortical and proisocortial regions.[3,40] Leonard[40] and Beckstead[3] both reported projections from agranular insular cortex to the caudatoputamen but the former's lesion (see her Fig. 7) and latter's amino acid deposit site (see his Fig. 7) included both AId and AIv. In agreement with the results of Domesick[16] and Goldman & Nauta,[21] we found much of the corticostriate label to be arranged in finger-like patches. Similarly, other neuronal systems involving the dorsal striatum have been shown to exhibit a mosaic pattern of organization.[28]

The ventral striatum receives major cortical input from allocortical and periallocortical regions which are usually thought of as components of the olfactory and/or limbic systems.[3,49,50,70] At least some of the cortical projections to nucleus accumbens terminate in finger-like patches similar to those noted above in the corticostriate projections to the caudatoputamen.[51] Our finding that AIv projects to the ventral striatum is in agreement with the finding that layer V pyramidal cells in AIv are retrogradely labelled following HRP deposits in the olfactory tubercle.[50] Projections from agranular insular cortex to the ventral striatum were reported by Leonard[40] and Beckstead[3] but, as noted above, the affected cortical regions included both AId and AIv. The finding that AIv projects to the ventral striatum, but AId does not, further emphasizes the distinction between AIv as a periallocortical zone and AId as a proisocortical zone. Furthermore, AIv has extensive afferent and efferent connections with allocortical and periallocortical regions of medial frontal and lateral cortex which also project to the ventral striatum, whereas AId is more strongly associated with proisocortical and isocortical regions which apparently do not (this report; ref.[55]).

Thalamus

Our finding that AId and AIv each project bilaterally to the entire a–p extent of the medial segment of the mediodorsal nucleus (MDm) contrasts with the fact that the thalamocortical projections from MD are ipsilateral[12,13,20,36,55] and with our previous finding that in the hamster, AIv receives input only from

the anterior portion of MDm while AId does so only from its posterior portion.[55] In rats, AIv has a similar reciprocal relationship with the central segment of MD, in that thalamocortical input arises ipsilaterally[30,36] while the corticothalamic projection is bilateral (Beckstead;[3] see his Fig. 7). Since hamsters have no well defined central segment it may be that in these animals an incipient central segment, which is reciprocally connected with AIv, is contained within the medial segment.[55] The relationship between AId and MD in the rat is less clear, since the studies of Krettek & Price[36] and Gerfen & Clavier[20] disagree concerning the source of thalamic input to AId, and since the autoradiographic study of Beckstead[3] apparently included no amino acid deposits into AId alone.

An ipsilateral thalamocortical/bilateral corticothalamic pattern is also seen in the connections of AId and AIv with the gelatinosus and ventromedial nuclei, in hamsters and rats (this report; see also refs 3, 20, 27, 40, 55).

The projections from AId and AIv to midline and intralaminar thalamic nuclei are similar to those reported in the rat,[3,40] except that in the hamster we find no labelling of nucleus reuniens. Sparse to moderate labelling of the rhomboid and central medial nuclei reciprocates the similarly modest projections from these nuclei to AId and AIv.[55]

The significance of the finding that both in hamsters (this report) and rats[40] the projections of AId and AIv to the thalamic reticular nucleus are ipsilateral, whereas those to other thalamic nuclei are bilateral, is unknown.

Amygdala

Our present description of a projection from AId to the anterior basolateral and lateral amygdaloid nuclei complements previously reported input to AId from these two areas in the hamster.[55] Rats appear to have a similar organization, as indicated in the studies of Beckstead[3] and Krettek & Price.[37] While AIv is excluded from these connections and instead projects lightly to the anterior amygdaloid area, the anterior portion of the basolateral nucleus appears to represent a point of overlap for neuronal circuits participated in by AId or AIv alone. Thus, while its cortical associations are largely with AId, many of the subcortical relationships of the anterior basolateral nucleus involve brain areas with which AIv, but not AId, is associated. These include input from the ventral pallidum,[52] and outputs to the ventral striatum[38,49,50] and ventral pallidum.[38]

It should be noted that in addition to its projection to AId, the basolateral nucleus also projects to the posterior agranular insular and perirhinal cortices laterally, and the infralimbic and prelimbic areas medially,[37] and that these are all portions of a continuous belt of transitional limbic cortex (*vide infra*). While only the prelimbic area and AId have been shown to have reciprocal efferent connections with the basolateral nucleus, it is probable that at least some of the other cortical areas mentioned do so as well.

A comparison of the results obtained in brains 80028, 80032 and 79081 (see section on Additional AId deposits) suggests that the projection from AId to the lateral and baolateral amygdaloid nucei originates from layer V pyramidal cells.

Brainstem

From both AId and AIv, caudally-directed fibers in the cerebral peduncle were found to project to several areas which form a continuum from the diencephalic midbrain junction through the pons. By comparing Figs 3I–M and 4J-M of the present report with Fig. 7 of Leonard[40] and Fig. 7 of Beckstead,[3] one can see that the pattern of efferent projections from AId and AIv to these areas is similar in the hamster and rat, and consists of fibers which accumulate in the caudal portion of the lateral hypothalamus, then split into a laterally-directed bundle traversing the substantia nigra pars compacta and midbrain reticular formation, and a medial bundle which enters the ventral tegmental area and dorsal raphe nucleus. As mentioned earlier, in cases of deposits into AId, the lateral bundle continues dorsally from the reticular formation into the parabrachial nucleus (which in turn projects to AId[55] and in cases of deposits into AIv the medial bundle continues dorsally from the dorsal raphe nucleus into the periaqueductal gray. Following deposits of HRP in the periaqueductal gray of the rat, Hardy & Leichnetz[24] found labelled pyramidal cells in layer V of AIv.

Because in many of these areas there is extensive intermingling of fiber tracts and nucleus groups, it is often quite difficult to estimate how much of the label seen is associated with fibers and how much with terminals. In her degeneration study, Leonard[40] was unable to find dense terminal fields in these same areas, but since the density of degenerating fibers decreased caudally, she suggested that the fibers were terminating as they proceeded posteriorly. In our autoradiographic material and in that of Beckstead[3] a similar decrease in density of labelling is seen as one proceeds caudally, suggesting that fibers may be terminating in substantia nigra pars compacta and the midbrain reticular formation laterally, and in the ventral tegmental area and dorsal raphe medially.

It seems significant that most of these brainstem areas have been shown to contain monoaminergic cell bodies[5,9,18,29,42,53,66] and that agranular insular cortex, particularly AId, has been shown to receive monoaminergic innervation from the ventral tegmental area, dorsal raphe and locus coeruleus.[4,19,20,41,43,44,47,54,65,66] Furthermore, several functional studies have emphasized the arousal and reinforcement role played by these monoaminergic systems in agranular insular cortex.[7,8,35,46,57]

The trajectory of the fibers projecting from AId and AIv to brainstem areas appears to be quite similar to

that of the medial forebrain bundle, and it may be that some of the label identified above as being in the most ventral portion of the internal capsule/cerebral peduncle and lateral hypothalamus actually represents fibers associated with the medial forebrain bundle. The resolution of this point depends on the histofluorescent delineation of the medial forebrain bundle in hamsters.

Comparison between anterior agranular insular cortex (AId and AIv) and medial frontal cortex

There are several major anatomical features common to the anterior agranular insular and medial frontal cortical fields. To begin with, each is transitional in location and cytoarchitectural structure between ventrally-adjacent allocortex primitivus (primary olfactory cortex laterally, taenia tecta medially) and dorsally-adjacent isocortex[55,58] and are components of a complete ring of such transitional cortex which includes the orbital fields at the frontal pole,[36] agranular insular and perirhinal cortex laterally, and medial frontal and retrosplenial cortex medially. The perirhinal and retrosplenial areas are contiguous at the caudal pole. These cortical areas are considered by us to constitute an outer ring of limbic cortex, and Yakovlev[71] referred to this outer ring as the mesopallium. As described by Krettek & Price,[36] the agranular insular, orbital and medial frontal cortical areas are all agranular, lacking a layer IV, and in many of their subdivisions a cell-free lamina dissecans is present between the inner and outer cell strata.[58]

Another way in which the anterior agranular insular and medial frontal cortices are fundamentally alike is in their pattern of afferent and efferent connections. As shown in this report and in our previous HRP study,[55] there are topographically-organized reciprocal bilateral connections between these two areas. Furthermore, there are reciprocal connections between portions of both these areas and the mediodorsal thalamic nucleus, lateral and basolateral amygdaloid nuclei, and dopaminergic nuclei of the midbrain.[1,3,4,10,12,13,14,15,31,36,37,40,43,59,64] Perhaps most striking is the fact that AId and the prelimbic cortex are the only areas which have reciprocal connections with all three of these subcortical regions. Since the efferent connections of the prelimbic area with the ventral striatum, periaqueductal gray and entorhinal cortex[3] overlap projections from AIv to these areas (present report), and since the prelimbic area receives thalamic input from MDma, the same part of the mediodorsal nucleus which projects to AIv,[36,55] it appears to be a medial counterpart to both AId and AIv. It is interesting in this regard that the prelimbic area is the only portion of medial frontal cortex to have reciprocal connections with both AId and AIv (this report and ref. 55).

Finally, the anterior agranular insular and medial frontal cortical areas both influence arousal, reinforcement and a variety of motivated species typical social behaviors (for references, see introductory section of this report and ref. 55). Future behavioral

Fig. 9. Posterior agranular insular cortex in brain 80032. A. Bright-field photomicrograph of a Cresyl Violet stained section. Box denotes the area of part B in this and subsequent Figures. B. Dark-field photomicrograph of the same section. Grains are heaviest over the cell sparse zone corresponding to the extreme capsule, marked by an asterisk in A.

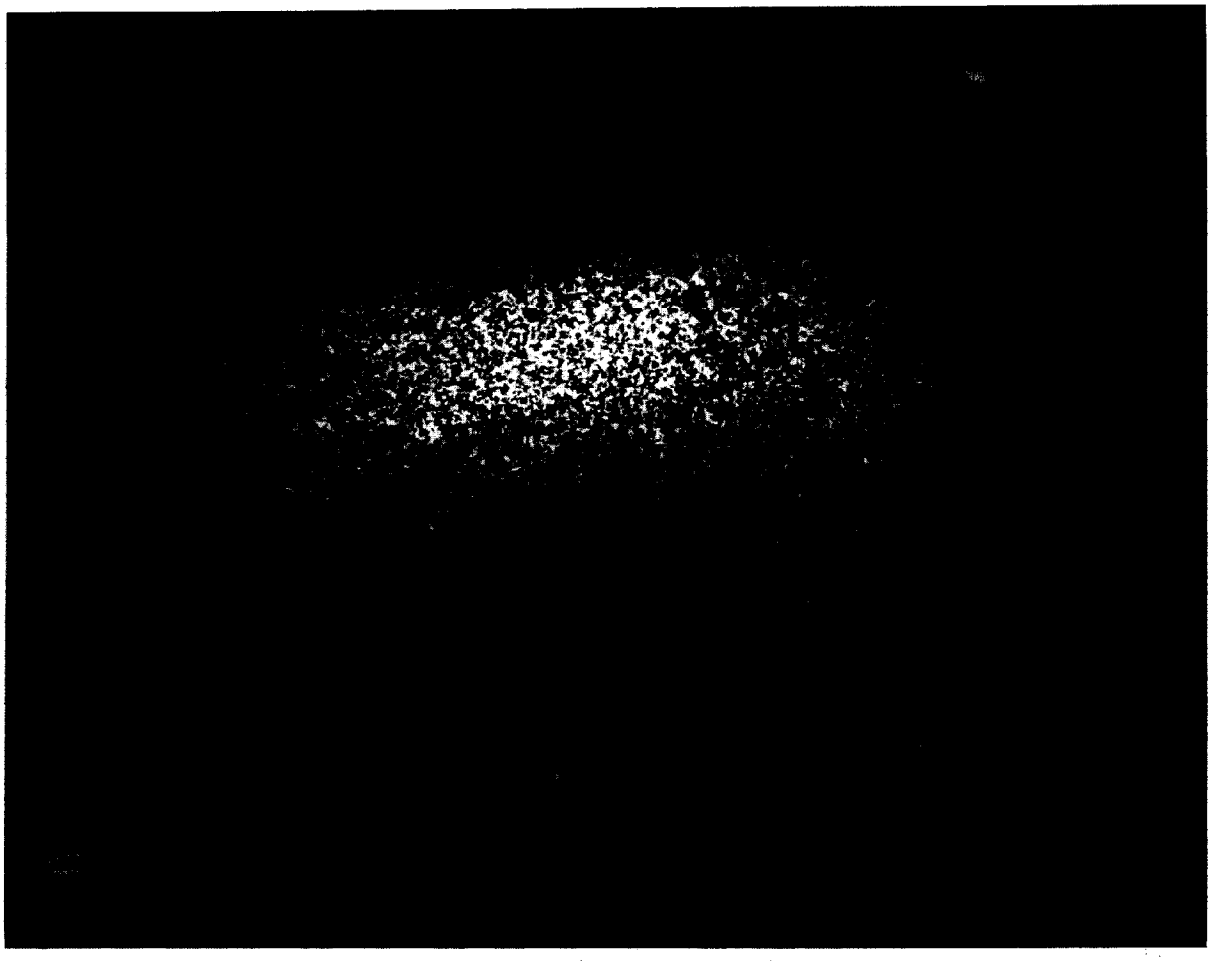
Fig. 10. Ventral striatum in brain 80033. A. Bright-field photomicrograph of a Cresyl Violet stained section at the same level as in B, but from another brain. Asterisk marks a cell bridge between the substriatal gray and olfactory tubercle, and the arrow points to a fascicle of the medial forebrain bundle. B. Dark-field photomicrograph of a section from brain 80033. Arrow points to labelled bundles of corticofugal fibers in the caudatoputamen. Note heavy concentration of grains over the substriatal gray and cell bridges of the olfactory tubercle.

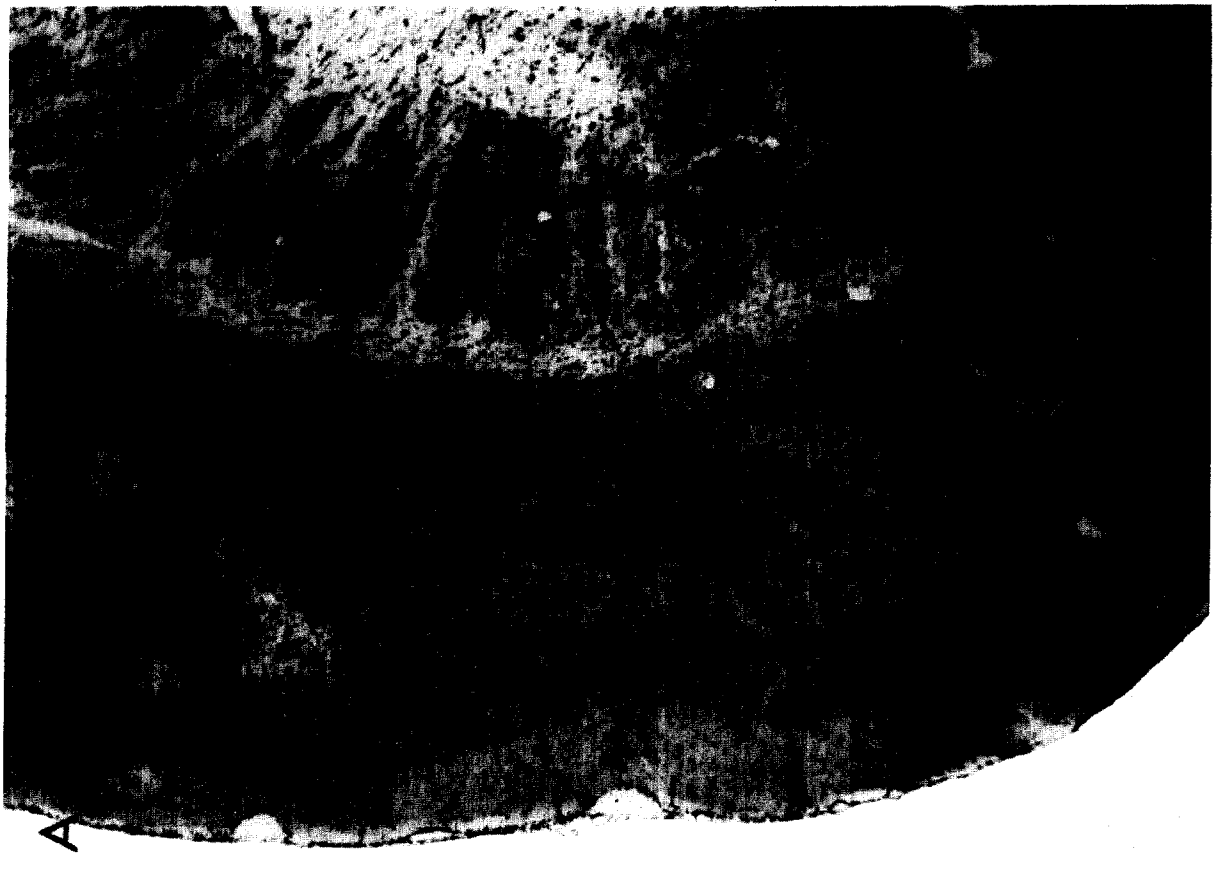
Fig. 11. Mediodorsal thalamic nucleus in brain 80028. A. Bright-field photomicrograph of a Cresyl Violet stained section. B. Dark-field photomicrograph of the same section. Labelling is greatest in the ipsilateral medial segment of MD, and is substantial in the midline and contralateral portions of MDm as well.

Fig. 12. Basolateral amygdala in brain 80028. A. Bright-field photomicrograph of a Cresyl Violet stained section. CE = central amygdaloid nucleus; M = medial amygdaloid nucleus. B. Dark-field photomicrograph of the same section. A moderately dense terminal field is present in the anterior portion of the basolateral nucleus.

Fig. 13. Substantia nigra and the ventral tegmental area in brain 80028. A. Bright-field photomicrograph of a Cresyl Violet stained section. B. Dark-field photomicrograph of an adjacent section. Labelling is heaviest over the ventral-most portion of the cerebral peduncle and the pars compacta of substantia nigra. Dorsomedially, label extends into the ventral tegmental area. Moderate accumulations of label are found in the lateral hypothalamus and pars reticulara of substantia nigra.

Fig. 14. Parabrachial nucleus in brain 80028. A. Bright-field photomicrograph of a Cresyl Violet stained section. B. Dark-field photomicrograph of the same section. Label is seen over the medial and lateral portions of the parabrachial nucleus.

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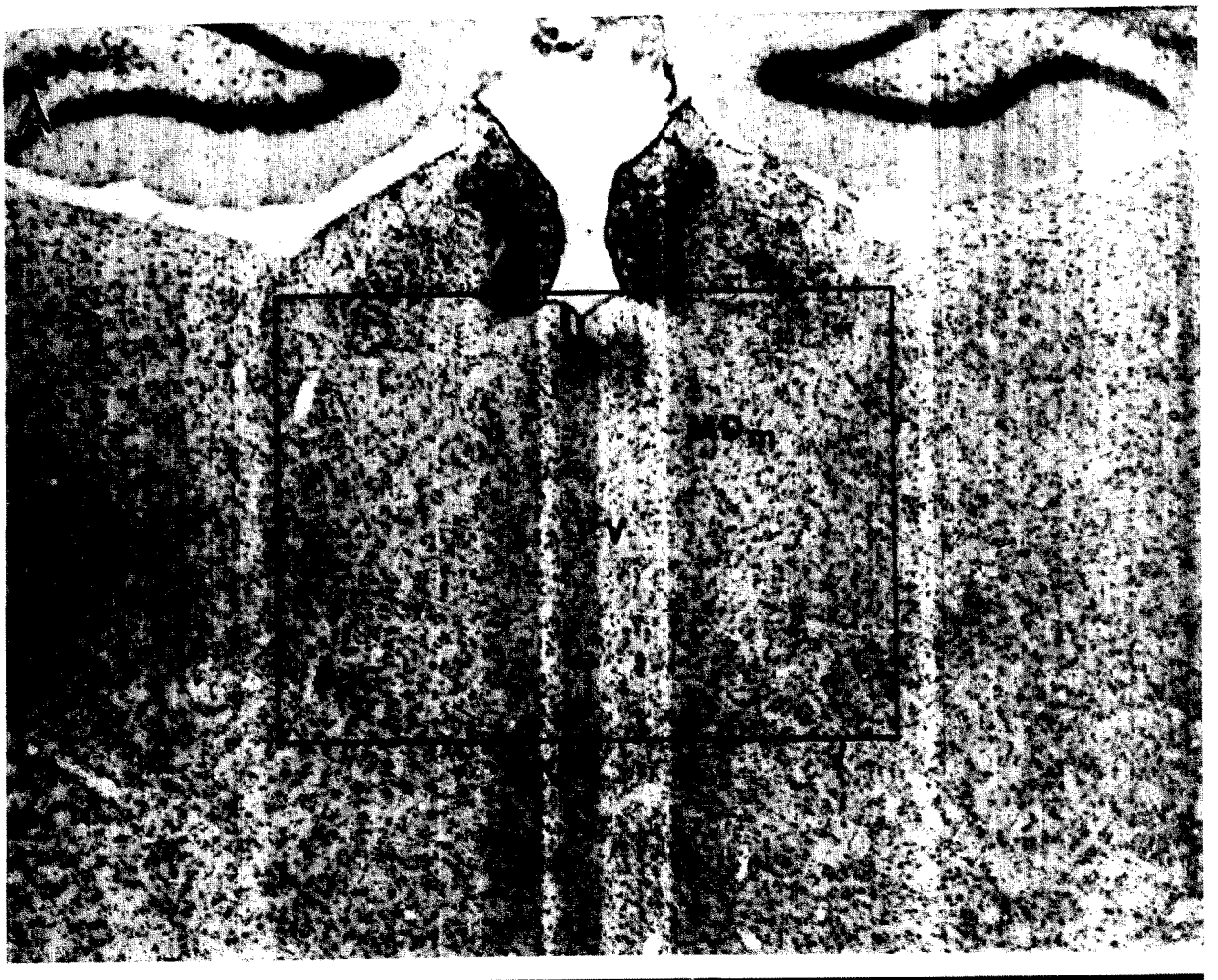
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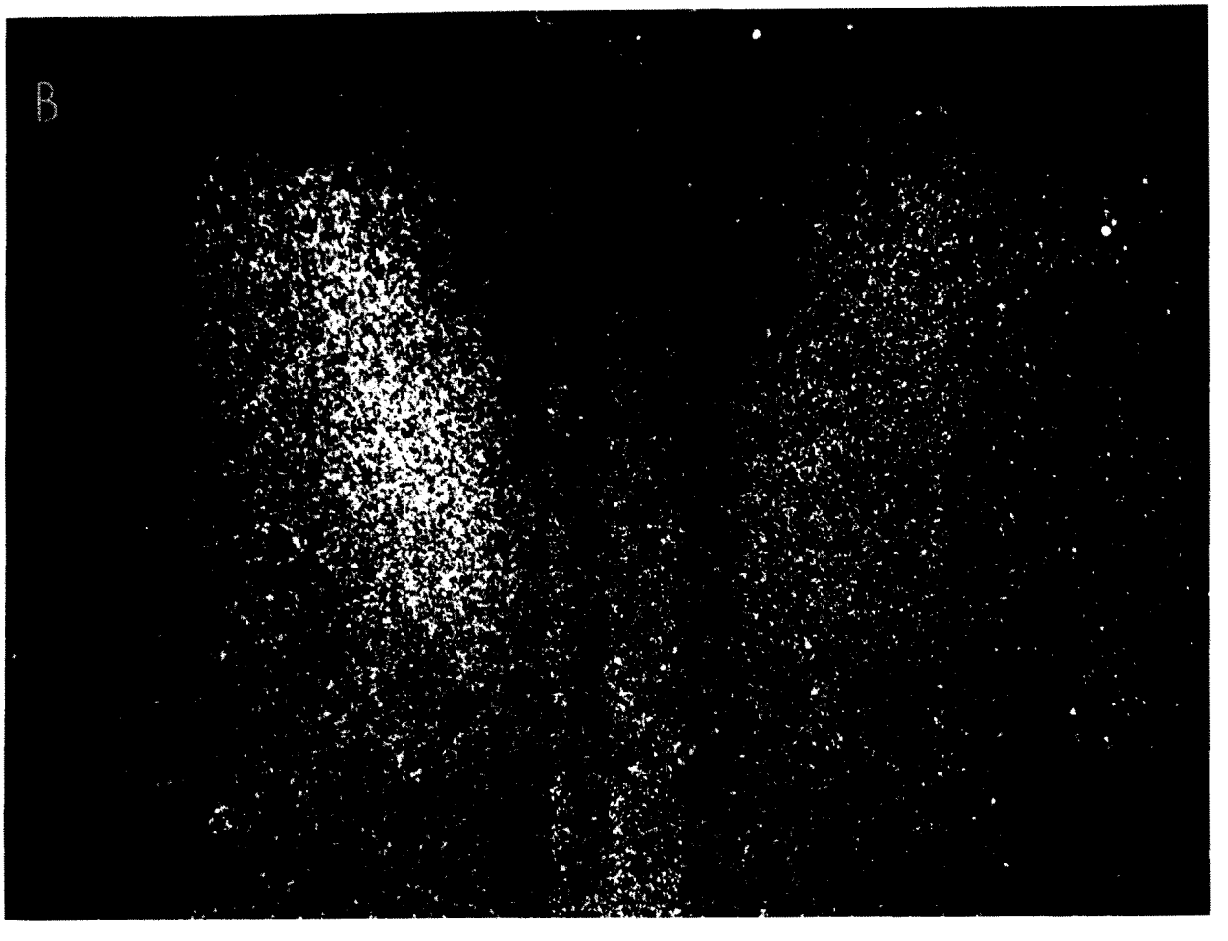
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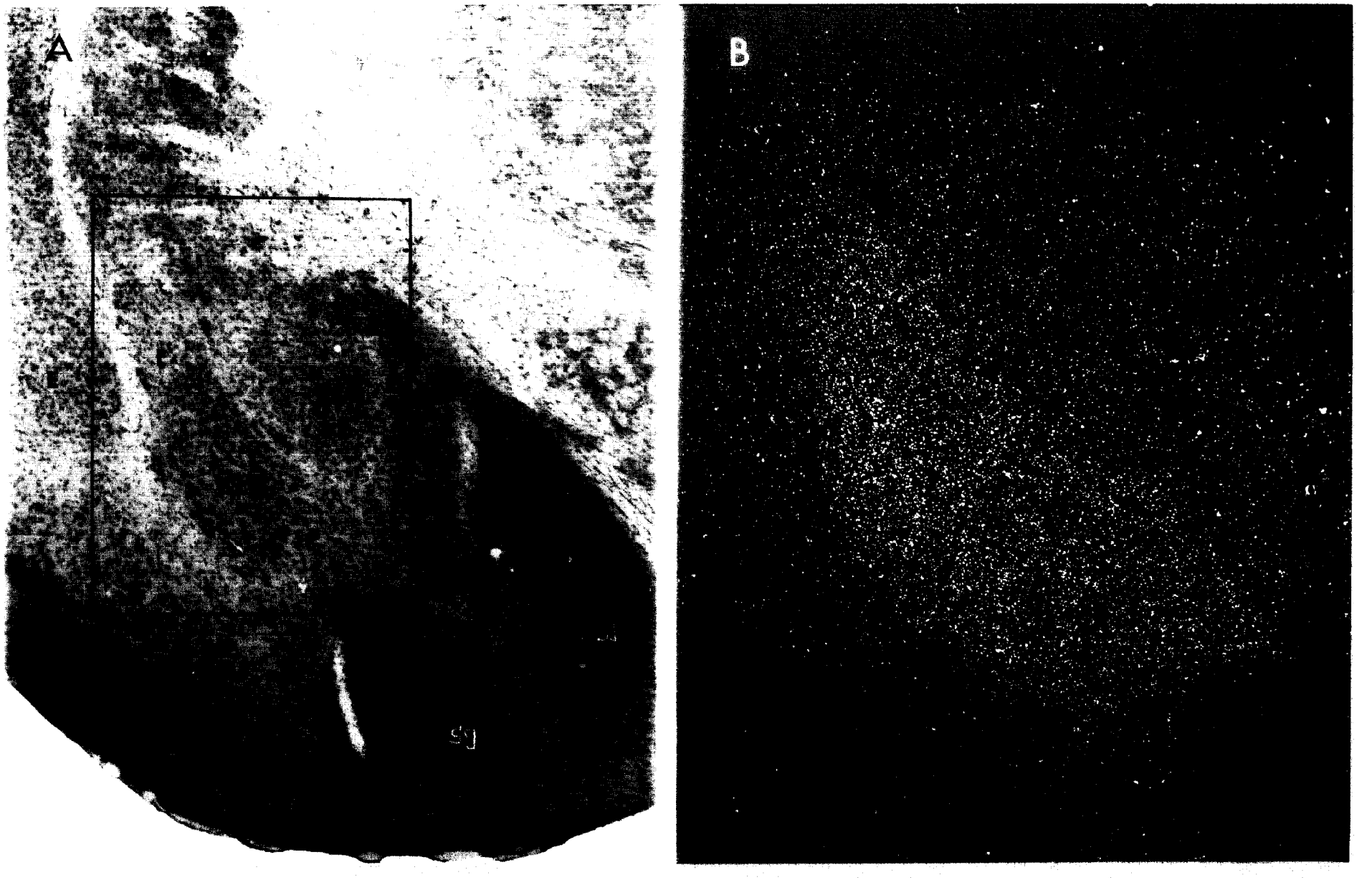
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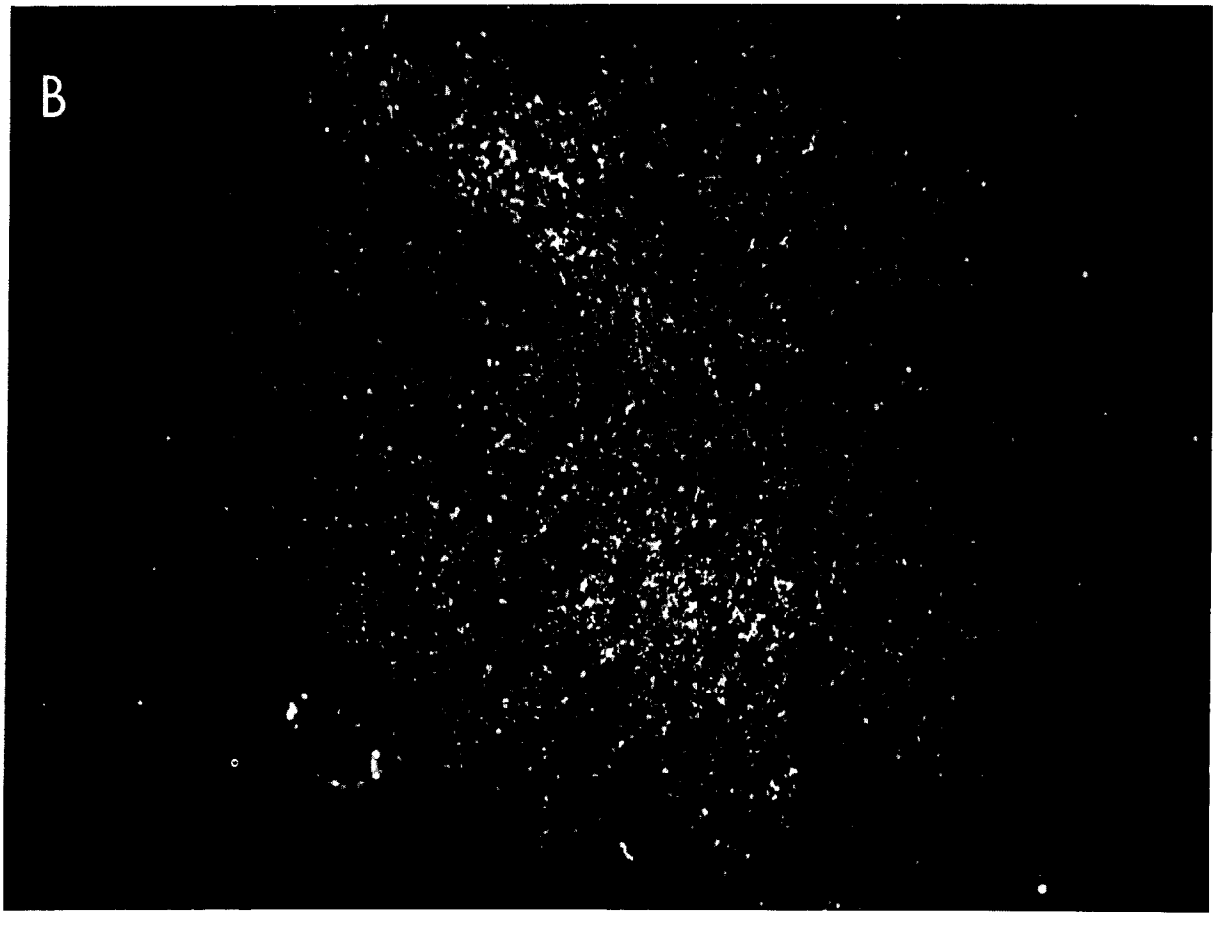
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studies can clarify the particular roles played by AId and AIv, and by the various divisions of medial frontal cortex. Further anatomical studies are needed in order to determine fully the afferent and efferent connections of other portions of limbic cortex, specifically the posterior agranular insular and perirhinal areas laterally, the orbital areas frontally, and the infralimbic and retrosplenial areas medially.

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