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Cortical Connections Between Rat Cingulate Cortex and Visual, Motor, and Postsubicular Cortices

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ABSTRACT

The connections of rat cingulate cortex with visual, motor, and postsubicular cortices were investigated with retrograde and anterograde tracing techniques. In addition, connections between visual and the postsubicular (area 48) and parasubicular (area 49) cortices were evaluated with the same techniques. The following conclusions were drawn.

Area 29 connections: Afferents to area 29 originate mainly from cingulate areas 24 and 25, visual cortex (primarily area 18b), motor cortex area 8, area 11 of frontal cortex, areas 48 and 49, and the subiculum. Efferent connections of area 29 within cingulate cortex and to visual areas differ for each cytoarchitectural subdivision of area 29. Thus, area 29c has limited projections both within cingulate cortex and to areas 48 and 49, while area 29d projects to these areas as well as to area 8, area 18b, and medial area 17. These visual cortex afferents originate mainly from layer V neurons of areas 29b and 29d, while areas 29a and 29c have virtually no projections to visual cortex.

Area 24 connections: Afferents to area 24 originate primarily from cingulate areas 25 and 29 and visual area 18b and medial area 17. Efferent projections of area 24a are distributed within cingulate cortex, while area 24b has more extensive projections to posterior cingulate and visual cortices. Area 24b is the cingulate subdivision which is both the primary recipient of visual cortex afferents as well as the source of most of the projections of anterior cingulate cortex to visual areas.

Visual cortex has reciprocal connections with parts of the postsubicular and parasubicular cortices. Neurons of the internal pyramidal cell layer of both areas 48 and 49 project to areas 17 and 18b, while layers I and III of these parahippocampal areas receive projections from areas 17 and 18b.

In conclusion, areas 29d and 24b have particularly extensive interconnections with visual cortex, while area 29d also maintains projections to area 8 of motor cortex. This connection scheme supports the view that cingulate cortex may have a role in feature extraction from the sensory environment, as well as in sensorimotor integration. Finally, the postsubiculum may be classified as a limbic association cortex in which extensive visual and cingulate efferents converge.

Key words: cingulate cortex, connections, visual cortex, sensorimotor

One stage in the circuitry of medial limbic structures proposed by Papez ('37) was a cortical connection between cingulate and hippocampal cortices. Although direct connections with the hippocampus (i.e., subsectors CA1-CA4) have not been observed in subsequent studies, pathways have been demonstrated between cingulate and parahippocampal cortices in the rat (Swanson and Cowan, '77; Meibach and Siegel, '77) and monkey (Rosene and Van Hoesen, '77; Vogt et al., '79; Baleydier and Mauguiere, '80; Pandya

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and Van Hoesen, '81). Primate studies have also shown that cingulate cortex has additional cortical connections with the frontal and parietal lobes (Petras, '71; Pandya et al., '71; Pandya and Van Hoesen, '81; Künzle, '78; Vogt et al., '79; Baleydier and Mauguiere, '80). However, little is known about these other neocortical connections in the rodent and it is possible that these connections are partially responsible for some of the sensory-evoked responses in cingulate cortex as noted below.

Responses to sensory stimuli have been evoked in mammalian limbic cortex. Cuénod et al. ('65) found that neurons in primate cingulate and parahippocampal cortices could be driven by photic stimulation and Bachman et al. ('77) provided evidence that electrical stimulation of the vagus nerve activated unit responses in anterior cingulate cortex of the monkey. Gross visual and auditory stimuli also drive neurons in rabbit cingulate, parahippocampal, and hippocampal cortices (Vinogradova, '75).

Sensory-evoked responses in cingulate cortex may play an important role in learning. Thus, short latency, unconditioned neuronal spiking in rat cingulate cortex produced by a tone was increased and spike latencies shortened when the tone was paired with a positive reward (Segal, '73). Furthermore, Gabriel et al. ('77, '82) and Foster et al. ('80) have shown that neuronal responses may be enhanced in rabbit cingulate cortex to a positive conditioning stimulus (a tone frequency paired with foot shock) in relation to that of a negative conditioning stimulus (another tone frequency not paired with foot shock) during the acquisition of an active avoidance behavior.

These findings suggest that pathways exist whereby specific sensory stimuli can activate cingulate cortical neurons. One possible source of sensory afferents may be visual and auditory cortices. Nauta and Bucher ('54) observed fiber degeneration in cingulate and presubicular cortices following visual cortex lesions, but discounted it because of white matter involvement by their lesions. The presence of a medial visual pathway to the hippocampus was also suggested by Vastola ('82), who was able to abolish visual-evoked responses in the hippocampus with lesions placed in posterior cingulate cortex.

In addition to the possibility of sensory afferents to limbic cortex, efferents to the motor system may arise from limbic cortex. Motor responses have been produced by electrical stimulation of cingulate cortex which included unilateral and/or bilateral limb movement and changes in the rate of respiration, heartbeat, and pupillary dilation (Smith, '45; Kaada, '51; Lende and Woolsey, '54; Woolsey, '58).

Within the context of rodent limbic cortex circuitry as presently understood, it is difficult to interpret the mechanisms which underly sensory and motor functions of cingulate cortex. Therefore, we undertook a reinvestigation of connections among visual, cingulate, motor, and postsubicular cortices to elucidate the morphological basis of sensory-limbic and limbic-motor functions. This analysis of corticocortical connections suggests that specific subdivisions of cingulate cortex may serve as sensorimotor association cortices.

MATERIALS AND METHODS

Hooded (Long-Evans) and albino (Sprague-Dawley) rats weighing 275-450 gm were used for these studies. Each animal was anesthetized with Chloropent (0.32 cc/100 gm body weight, Fort Dodge Labs, Fort Dodge, Iowa) and placed in a Kopf Small Animal Stereotaxic Instrument. All injections of horseradish peroxidase (HRP) or ^{3}H-amino acids were made with a 32-gauge, 5-μl Hamilton syringe. Injections of either compound into cingulate cortex were made from a contralateral approach with the cannula angled at 45°. No brains were included in this analysis in which the cingulum bundle was penetrated by the cannula tip.

Retrograde transport procedures

Standard HRP procedures were employed as described by Vogt et al. ('81). Briefly, these involved the injection of 0.01-0.05 μl of 5% wheat germ agglutinin-conjugated HRP (Sigma) into the cingulate or visual cortices of 23 animals with a 2-day postoperative survival period. In order to avoid white matter damage, many of these HRP injections were made with a syringe from which 1.0 mm of the tip was removed. In addition, to verify that the size of the injection site observed after a 2-day postoperative survival period reflected the entire area of uptake, some animals were sacrificed 12 or 24 hours following the injection. The animals were perfused with 50-100 ml of 0.9% saline followed by 200-300 ml of quarter-strength Karnovsky fixative for 30 minutes and then with 200-300 ml of 10% sucrose phosphate buffer (pH 7.4) at 10°C for 30 minutes. Subsequently the brains were removed, stored in 10% sucrose buffer overnight in a refrigerator, frozen onto a freezing microtome stage, and cut into 40-μm-thick sections. Every sixth section was reacted and when the cannula tract was observed during sectioning, all sections through the injection site were reacted to confirm that the white matter was not damaged. The sections were first incubated in 5% tetramethyl benzidine in acetate buffer for 20 minutes and then 0.4 ml of 0.3% H_2O_2 was added to this solution for another 20 minutes. The reacted sections then were mounted on chrome-alum-subbed slides and counterstained with neutral red.

Anterograde transport procedures

The autoradiographic methods of Cowan et al. ('72) were employed to verify the observations made with the HRP procedure and to assess the distribution of afferent axonal terminals within each cortical area. This involved desiccating a solution of 25 μl of ^{3}H-leucine, ^{3}H-proline, ^{3}H-lysine, and a mixture of ^{3}H-amino acids (New England Nuclear) and reconstituting them in 0.8-1.0 μl of 0.9% saline for a final solution of 100-125 μCi/μl. Injections of 0.02-0.1 μl each were made in the brains of 38 animals that had a postoperative survival time of 4 days. These animals then were perfused with 50-100 ml of 0.9% saline followed by 200-300 ml of 10% formalin for 20 minutes. The brains were removed, postfixed in 10% formalin for 1 week, embedded in paraffin, and cut into 10-μm-thick sections. Every tenth section was deparaffinized, dipped into NTB-2 Kodak Nuclear Track Emulsion, and exposed in light-tight containers in a freezer for 3-3½ months. Following exposure, the autoradiographs were developed with Kodak D-19, fixed in Kodak Rapid Fixer, and counterstained with thionin.

Cytoarchitecture

Figure 1 presents the cytoarchitectural map employed throughout this study. The subdivisions of cingulate cortex are similar to those previously described by Vogt and Peters ('81). Brodmann ('09) and Rose ('12) identified an

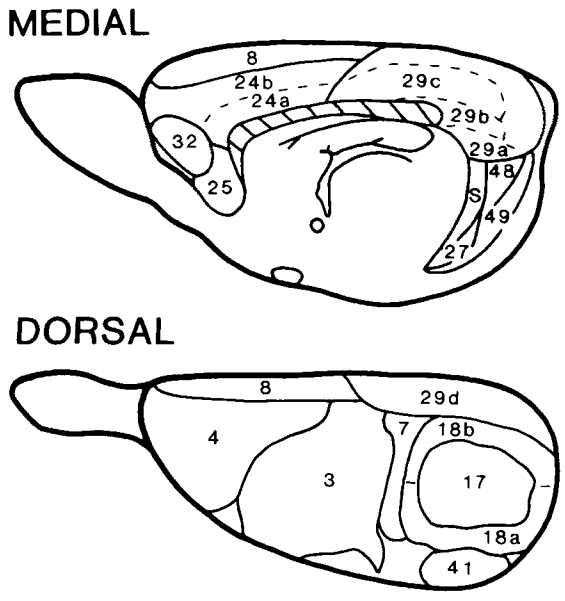


Fig. 1. Cytoarchitectural map of cingulate, subicular (S = subiculum, area 48 = postsubiculum, area 49 = parasubiculum), and dorsal neocortical areas.

area 48 ventral to area 29a of cingulate cortex. Area 48, also termed the postsubiculum, is composed of an external and an internal pyramidal cell layer that are separated by a very thin lamina dissecans (Fig. 3A). The composition of layers II-III helped to distinguish the postsubiculum (area 48) from the parasubiculum (area 49) and the presubiculum (area 27). The parasubiculum is located posterior to areas 48 and 27 and has larger and rounder neurons in layers II-III, while the presubiculum is anterior and ventral to the postsubiculum and has smaller and more densely packed neurons in layers II-III. It should be noted that coronal sections through the point of contact among areas 29d, 48, and 49 were often difficult to interpret because of cortical curvature. At this juncture Brodmann ('09) and Blackstad ('56) identified an area 29e, but we have not been able to resolve the composition of this area in coronal planes of section. Finally, neocortical areas 3, 7, 8, 17, 18a and 18b, 41, and 36 were delineated according to the criteria of Krieg ('46), Caviness ('75), and Schober and Winkelmann ('75). The bulk of primary motor cortex is contained within area 4, or the lateral agranular field of Donoghue and Wise ('82), while area 8 is a part of their medial agranular field.

RESULTS

Area 29 afferents

Injections of lectin-conjugated HRP into areas 29c-d resulted in retrograde filling of neurons throughout most cingulate, subicular, and many neocortical areas (Fig. 2). Within cingulate cortex neurons were labeled in area 25 below the rostrum of the corpus callosum and limited neuronal filling was present in layers V and VI of rostral areas 24a and 24b. In contrast, posterior area 24 had extensive neuronal labeling throughout all cellular layers. Area 29d anterior and posterior to the injection site was densely packed with HRP-positive neurons in layers II-IV with some also present in layer V, while area 29b contained a large number of labeled neurons in layers II-IV. Area 29c had limited filling of neurons mainly in layer V and only a few cells were labeled in area 29a.

The subicular cortices contained large numbers of HRP-labeled neurons including the subiculum, postsubiculum (area 48), and parasubiculum (area 49). The para- and postsubicular neurons were located in the internal pyramidal cell layer (layer V, Fig. 3A).

It is of interest to note that wherever retrogradely labeled neuronal somata were most dense, axonal HRP labeling was also present in the external layers of the same cortical area. Figure 3A presents an example of axonal labeling in area 48. This HRP labeling could be due to either backfilling of the axon collaterals of retrogradely filled layer V neurons in area 48 or anterograde labeling of the axons of cells located in areas 29c-d. An injection of ^{3}H-amino acids into area 29d (Fig. 9) resulted in anterograde labeling of axonal terminals in the same layers of area 48 which contained HRP-positive axons following HRP injections that involved area 29d (compare Fig. 3A with 3B). Thus, HRP labeling of axons represents anterograde transport from the injection site. Since anterograde axonal and retrograde somal HRP labeling was present in areas 48, 24a, and 24b following an area 29c-d injection (Fig. 2, large dots in layer I), areas 48 and 24 appeared to be reciprocally connected with areas 29c-d.

Many neocortical areas contained HRP-positive cells. Area 8, which is part of motor cortex (Lende and Woolsey, '54; Hall and Lindholm, '74), had HRP-labeled neurons mainly in layers III and V with some also in layer VI. Furthermore, each major subdivision of visual cortex contained HRP-labeled neurons. The most extensive retrograde transport was to neurons in layer V of area 18b with fewer cells labeled in layers III and VI (Figs. 2D,E, 4A). A limited number of neurons in layers V and VI of area 17 labeled, whereas area 18a contained a moderate number of HRP-positive neurons in layer V and a few in layer VI. Layers I and IV of both areas 18b and 18a also contained anterograde axonal HRP labeling, suggesting a reciprocal connection between these areas and area 29. Finally, neuronal labeling in layer VI continued ventral to area 18a to include auditory cortex (areas 41 and 36).

Although the HRP injected into areas 29c-d did not appear to spread into area 18b, it was always possible that, during early periods of uptake (i.e., the first 12 hours), the active injection site was larger than it appeared after a 2-day postoperative survival time. If this were true, the effective injection might have been much larger and, for example, HRP filling of cells in area 18b would then be due to local uptake and not transport from areas 29c-d. In order to verify the projection between areas 29 and 18b, very small injections of HRP (0.01 μl) were made into areas 29c-d and the animals were perfused 12 hours postoperatively. These injection sites were restricted to area 29 (Fig. 4B) and lightly labeled neurons were present in area 18b up to 2 mm from the injection site (Fig. 4C), thus confirming observations made with slightly larger injections and 2-day postoperative survival times.

The visual cortex efferents demonstrated to area 29 with HRP were confirmed with anterograde transport of tritiated amino acids. A large injection of ^{3}H-amino acids was made into area 18b (Fig. 5). At the edge of this particular injection site (as defined by dense extracellular grain deposits) there were some neurons which concentrated amino

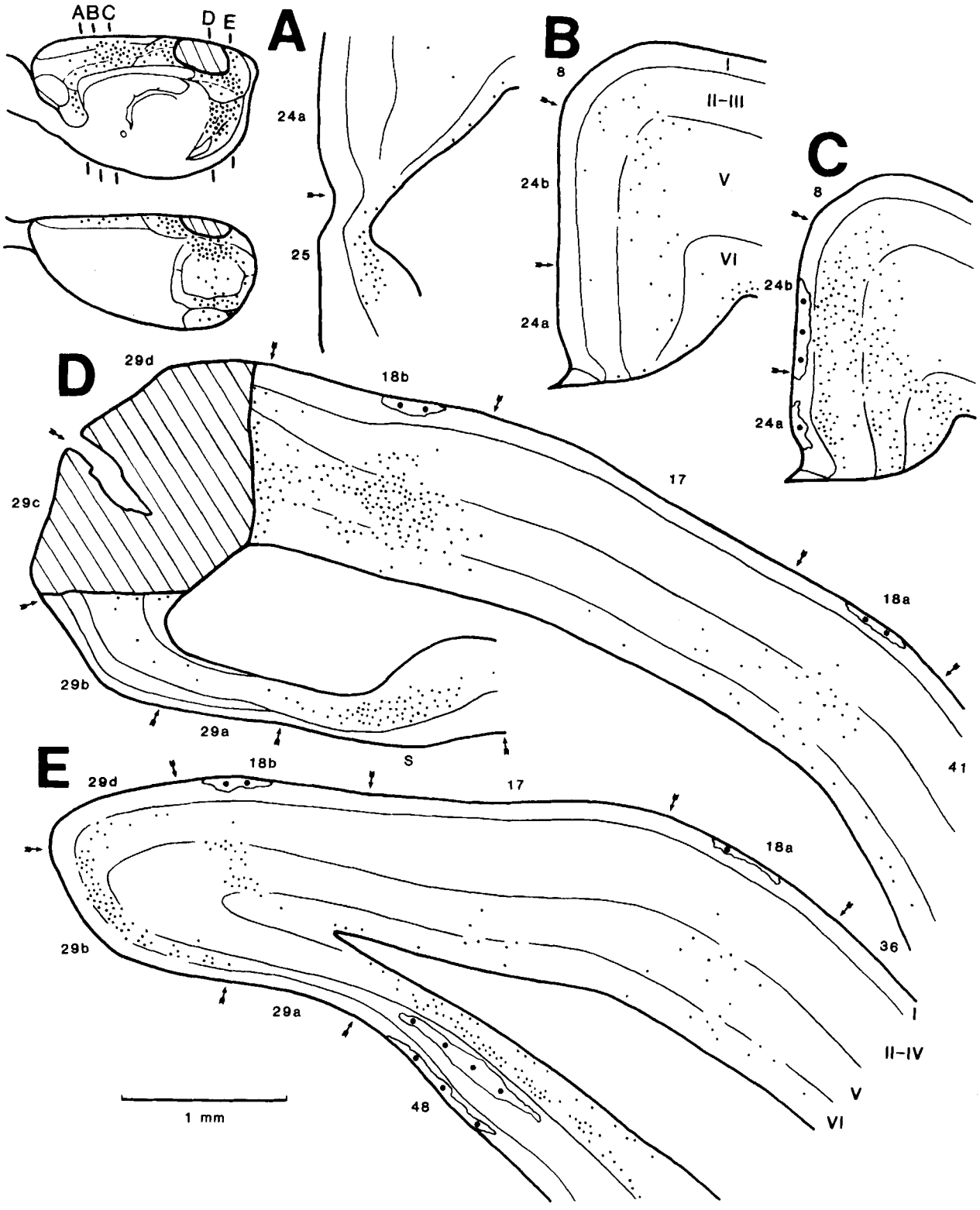


Fig. 2. Distribution of HRP-positive neurons following an injection of HRP into areas 29c–d (hatching). Each dot on the transverse sections represents approximately three HRP-positive neurons and the irregular outlines with large dots in layer I indicate regions of particularly dense HRP-positive axonal plexuses.

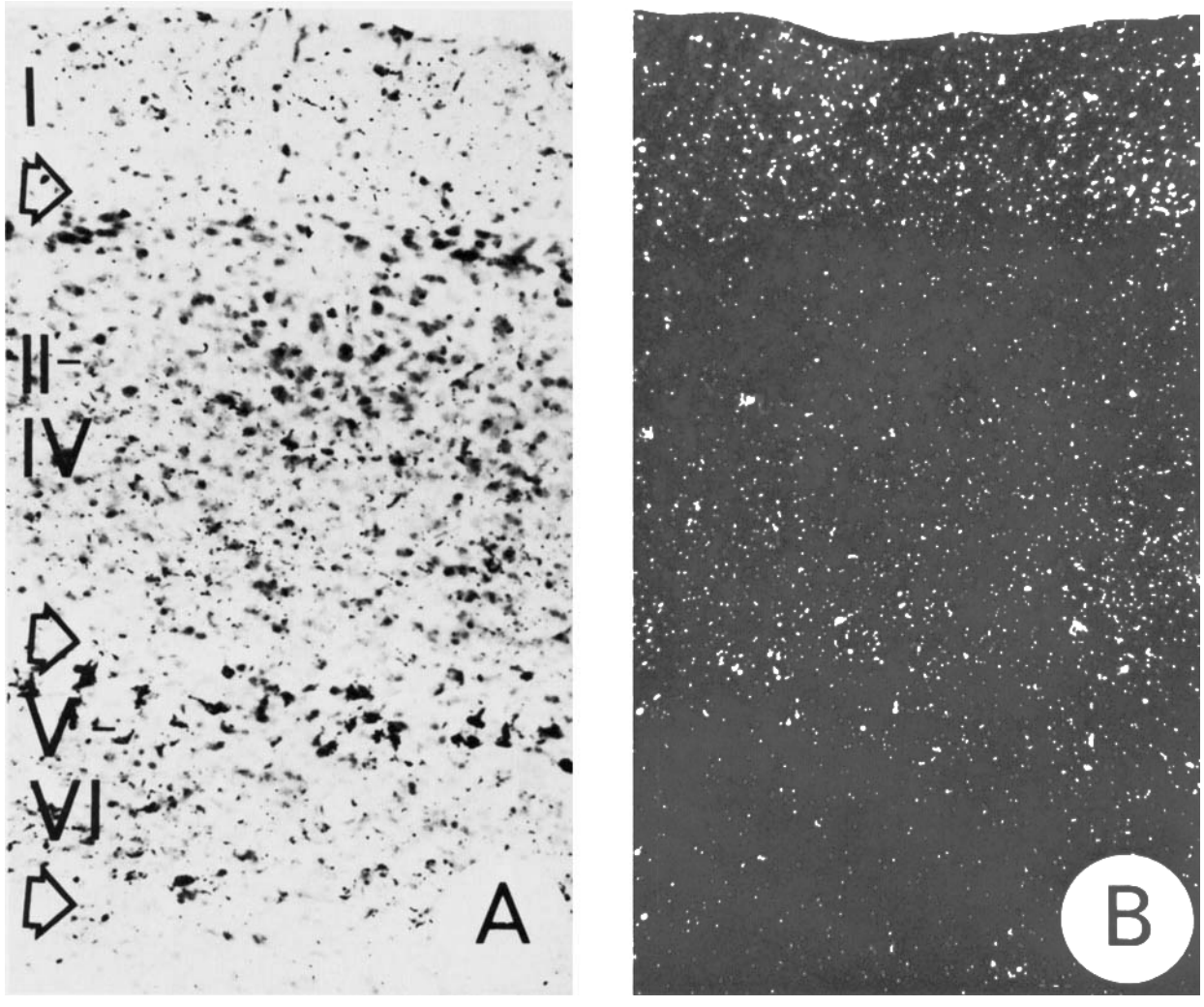


Fig. 3. Reciprocal connections between the postsubiculum (area 48) and areas 29c-d. A. Postsubiculum following an HRP injection into areas 29c-d (see also Fig. 2E) with HRP-labeled neurons in layer V and HRP-positive axons mainly in layers I and III. B. Darkfield microphotograph of the postsubiculum following an injection of ^{3}H-amino acids into area 29d (see also Fig. 9B,D). Anterogradely labeled axon terminals were present in layers I and III. ×120.

acids in their somata, suggesting uptake, but not necessarily transport of the amino acids. Therefore, the injection site may have extended beyond area 18b into area 29b and medial area 17, as conservatively presented in Figure 5. Terminal fields within cingulate cortex were present mainly in areas 24b, 29d, and 29b with fewer labeled terminals in areas 29a and 29c, least in area 24a, and none in areas 32 or 25. Termination in area 29 was most dense in layer I, with fewer terminals in layers V and VI and almost none in layers II-IV of the granular areas 29a-c. Although terminal fields of different densities were evenly distributed in layer I of areas 29d, c, and a, the inner two-thirds of layer I of area 29b received most termination with very little in layer Ia (outer one-third).

Injections of ^{3}H-amino acids into medial area 17 and area 18a produced axonal labeling only in area 29d of posterior cingulate cortex. Figure 6 presents a case in which area 18a was injected and demonstrated a light projection mainly to layer I of area 29d. Projections to area 29d from medial area 17 were very light to layer I and appeared to have been a continuation from the much more extensive projection of area 17 to area 18b (Fig. 6A). In conclusion, the relative density of visual cortex afferents to cingulate cortex demonstrated by both retrograde and anterograde transport techniques indicated that areas 18b, 24b, and 29d are interconnected extensively, while areas 18a and 17 have more limited connections with area 29d. It was also observed that retrograde labeling of cells in areas 17 and 18a after areas 29c-d were injected with HRP resulted from projections of areas 17 and 18a to area 29d, but not to area 29c.

In addition to direct visual cortex afferents to cingulate areas, it is possible that indirect routes exist whereby visual cortex may influence the activity of cingulate neurons. Two such possibilities were noted. An injection of HRP into posterior cingulate cortex retrogradely labeled neurons in layer II of area 11 in frontal cortex and in dorsal regions of the claustrum (Fig. 7). Similar levels of both of

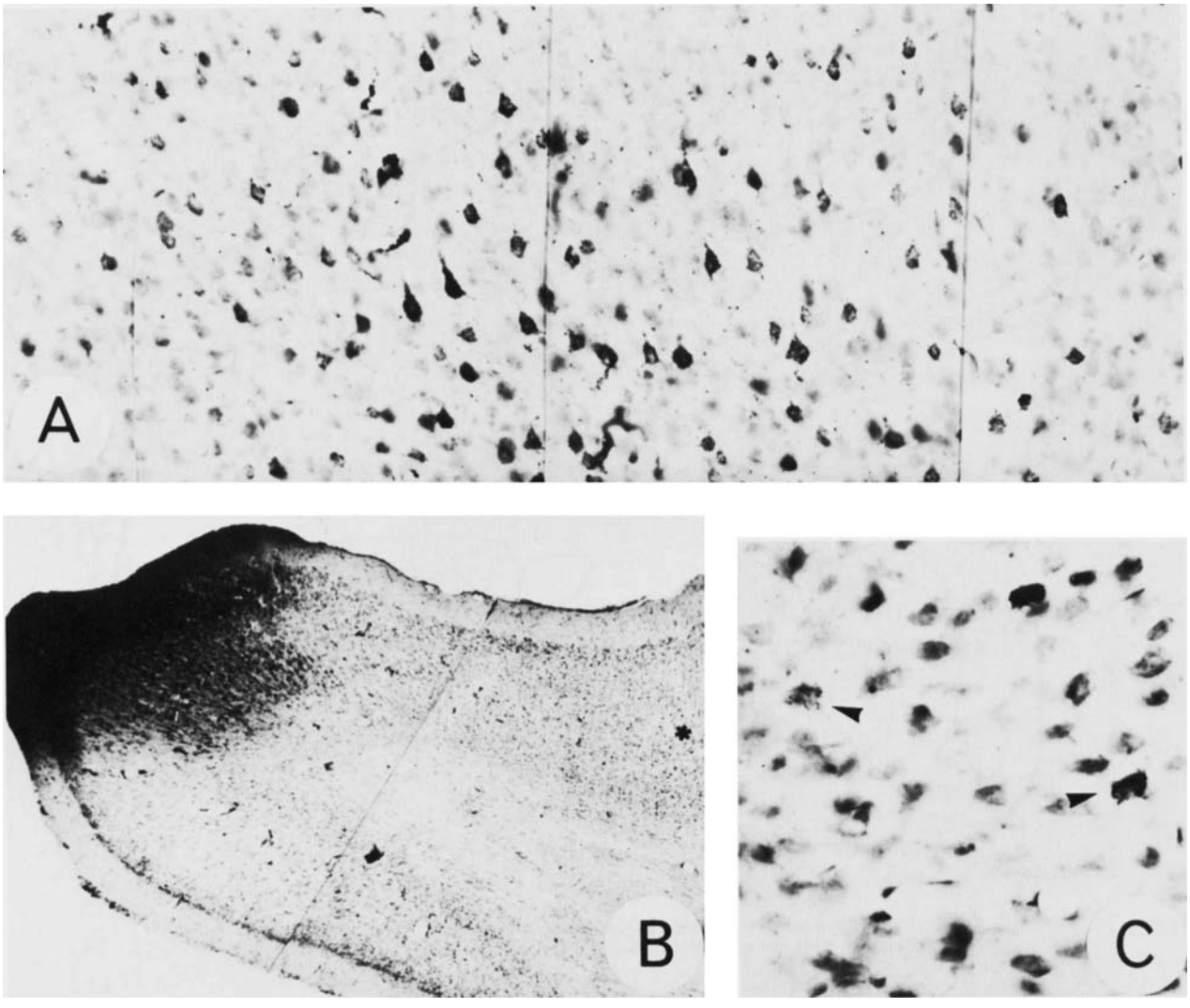


Fig. 4. Retrograde transport of HRP from areas 29c-d by neurons of area 18b. A. HRP-positive neurons in layer V of area 18b (see also Fig. 2D, 48-hour survival). ×300. B. Small HRP injection into areas 29c-d followed by a 12-hour survival period. ×47. Neurons at asterisk enlarged in C where lightly labeled layer V neurons (arrowheads) were present in area 18b. ×470.

these regions also appeared to receive direct visual afferents, since injection of ^{3}H-amino acids into area 18b labeled terminals in layer I of area 11 and in the dorsal claustrum at similar levels in which neurons were retrogradely labeled in HRP cases. It must be noted, however, that HRP-positive neurons in the claustrum extended rostral to level B in Figure 7 and that axonal labeling in the claustrum extended more caudal than level D in Figure 7, meaning that there were regions in these cases in which there was no overlap as well as intermediate levels of overlap within the claustrum.

Area 29 efferents

Areas 29c and 29d have differential connections both within cingulate cortex and in relation to neocortical areas. An injection that involved area 29c (Fig. 8) resulted in labeling of terminals mainly in areas 24b and 29d with less present in area 24a, while moderate termination was also evident in areas 48 and 49. In terms of the laminar distribution of these efferents, most terminals were in layers I and II of area 24b, while much of the labeling in layers II and V-VI probably represented axons of passage. Labeled terminals in area 29d were relatively evenly distributed throughout all layers, while labeling in areas 48 and 49 was present mainly in layer I and the inner part of the external pyramidal layer with some grains also in the inner pyramidal cell layer.

The projections of area 29d were more extensive both within cingulate and parasubicular cortices as well as in relation to neocortical structures. Figures 10 and 11 present a case in which an injection of ^{3}H-amino acids mainly involved area 29d. In this case most labeled terminals in cingulate cortex were in areas 24b and posterior parts of areas 29a-d. The terminals in area 24b and areas 48 and 49 were distributed in a bilaminar pattern as described previously for the area 18b connections. This injection also labeled

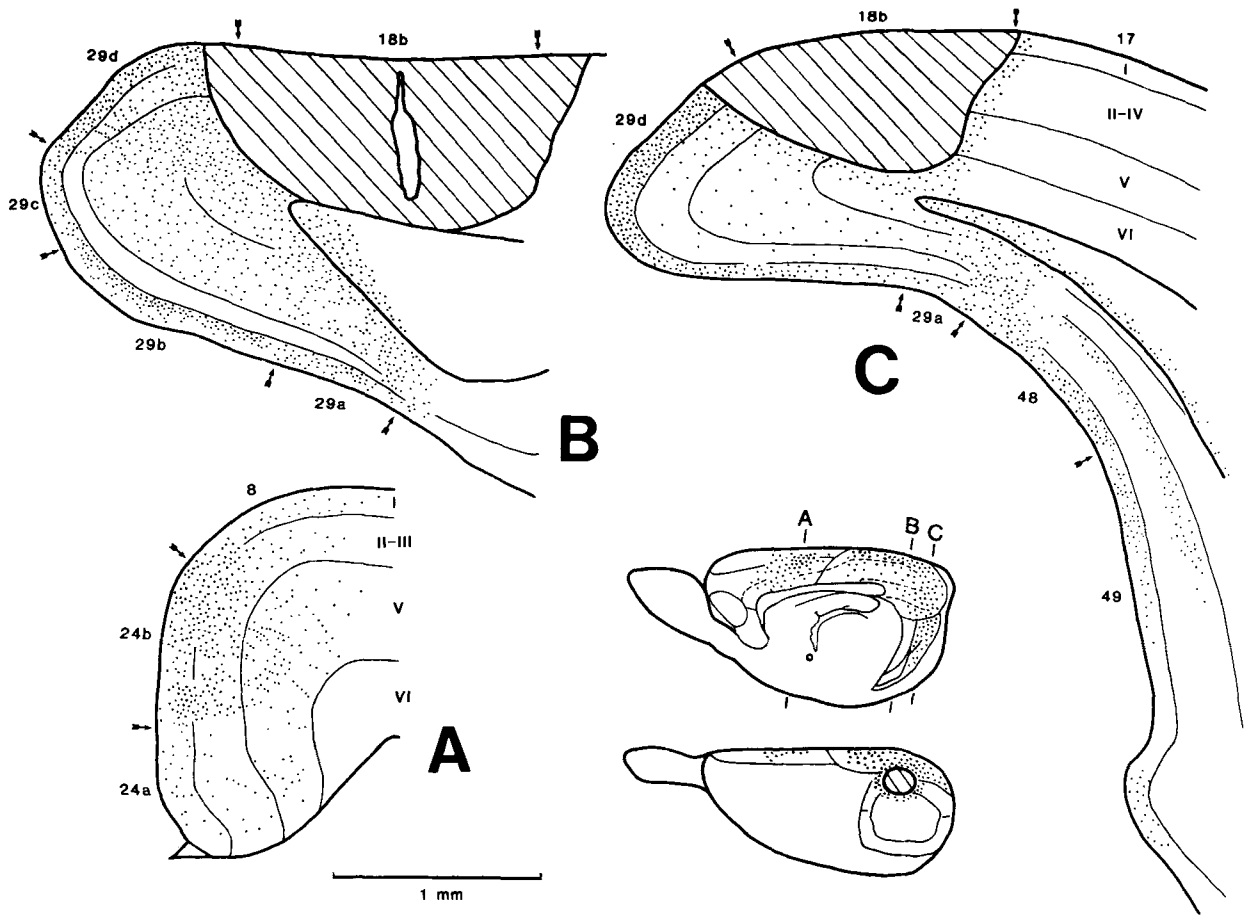


Fig. 5. Grain distribution in cingulate, postsubicular, and parasubicular cortices following an injection of ^{3}H-amino acids into area 18b.

projections to neocortex which were not present in the area 29c case, including projections to medial area 8, areas 7 and 18b, and medial parts of area 17.

The projections of area 29d to area 18b and medial area 17 were difficult to assess because of the close proximity of these areas to the injection site. However, four observations supported the notion that the grains in visual cortex represented a connection and not just diffusion from the injection site. First, there was a region in layers II-VI of area 18b in which there was a reduced grain density (Fig. 9B between points 2 and 3). Second, the grain density was not evenly distributed around the injection center, as would be expected from simple diffusion. Thus, when the number of grains were counted at two points equidistant from the injection center (i.e., at points 1 and 2 in Fig. 9B) there were more grains per unit area in all layers lateral to the injection site than there were medial to it (Fig. 11). Also, at point 3 in lateral area 18b, which was 1½ times as far from the injection center as point 1, there were more grains per unit area than there were at point 1. Third, most grains in lateral area 18b were in layers I-IV (Figs. 9B, 10B, and 11: point 3) and there was no evidence of termination in area 18a, as would have been produced by an injection into area 18b (Simmons et al., '82; Miller and Vogt, '83). Fourth, HRP injections into areas 18b and 17 produced retrograde transport of HRP to neurons in area 29d as described below.

Area 29 projections to visual cortex appear to originate primarily from layer V neurons in areas 29d and 29b. An HRP injection into area 18a (Fig. 12A) resulted in retrograde HRP filling of layer V neurons in a limited part of area 29d. In contrast, an injection of HRP into area 18b (Fig. 12B-E) produced more extensive retrograde filling of neurons in layer V of areas 29d (Fig. 13B) and 29b with very few HRP-positive neurons in areas 29a and 29c. A similar pattern of retrogradely labeled neurons appeared after a large injection of HRP into area 17 (Fig. 14). In this case, most HRP-positive neurons were found in layer V of areas 29d and 29b, and only very few in areas 29a and 29c.

Area 24 afferents

The location of cortical neurons projecting to subdivisions of area 24 was demonstrated by injections of HRP into anterior cingulate cortex. Since these injections also involved area 8, an analysis of ^{3}H-amino acid injections was made to determine which cingulate or neocortical subdivisions (areas 24a, 24b, and/or 8) received these projec-

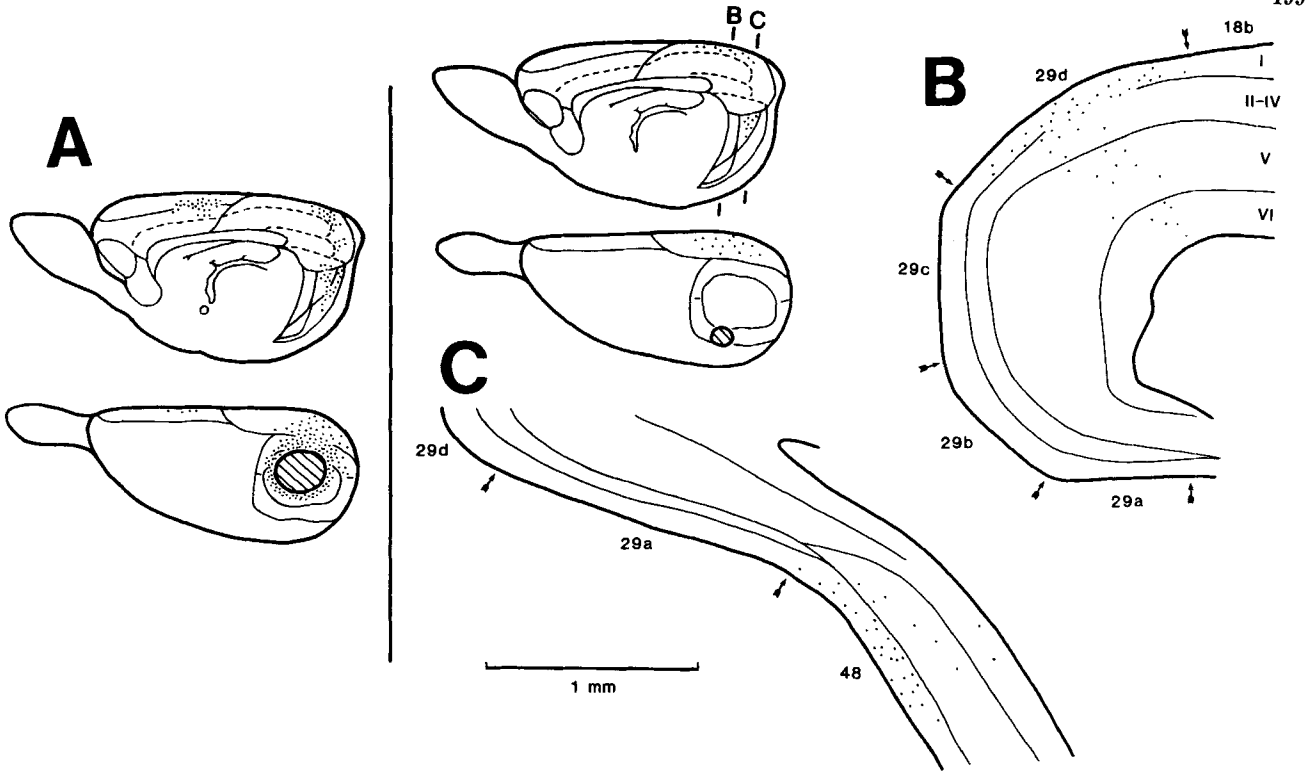


Fig. 6. Location of grains in cingulate and postsubicular cortices following ^{3}H-amino acid injections into area 17 (A) and area 18a (B,C).

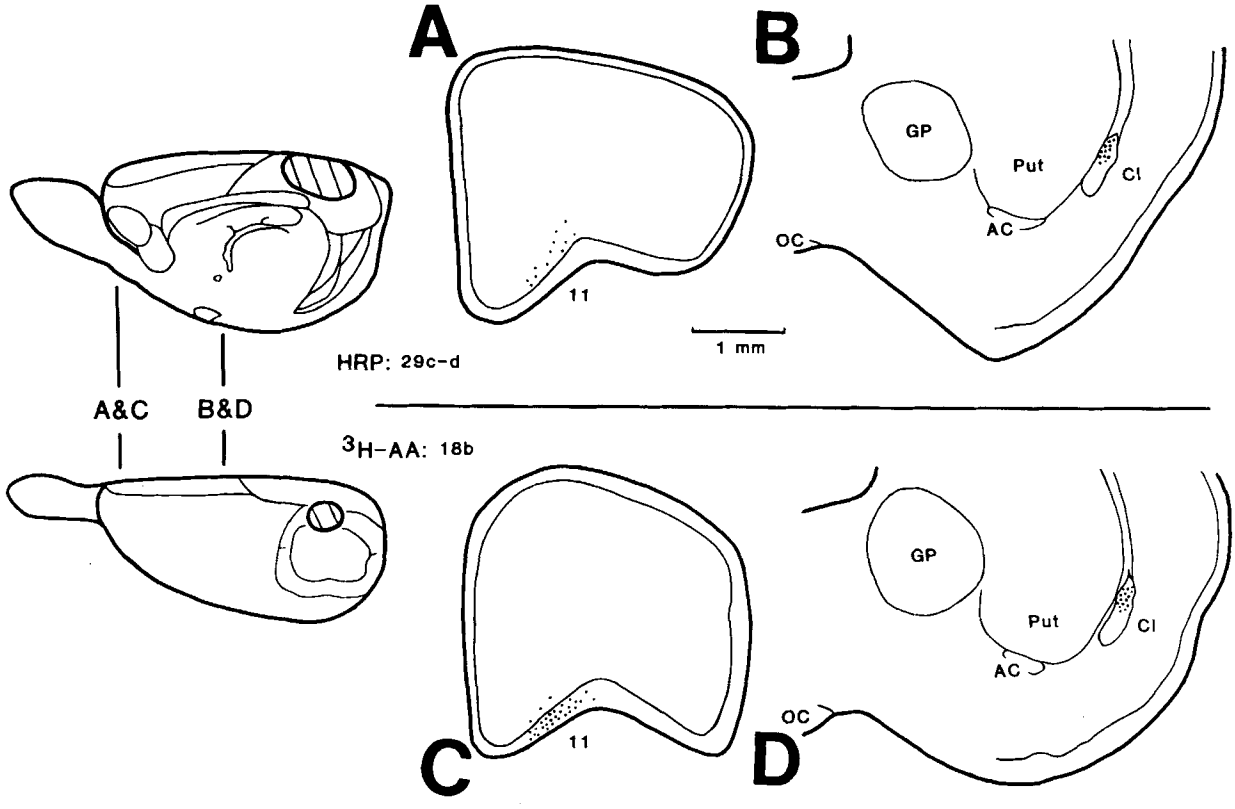


Fig. 7. Indirect pathways by which visual afferents could influence neurons in cingulate cortex. Both cortical area 11 (A) and the claustrum (B) contained HRP-positive neurons following an injection of HRP into posterior cingulate cortex. An injection of ^{3}H-amino acids into area 18b demonstrated that similar regions of area 11 (C) and the claustrum (D) received visual cortex afferents.

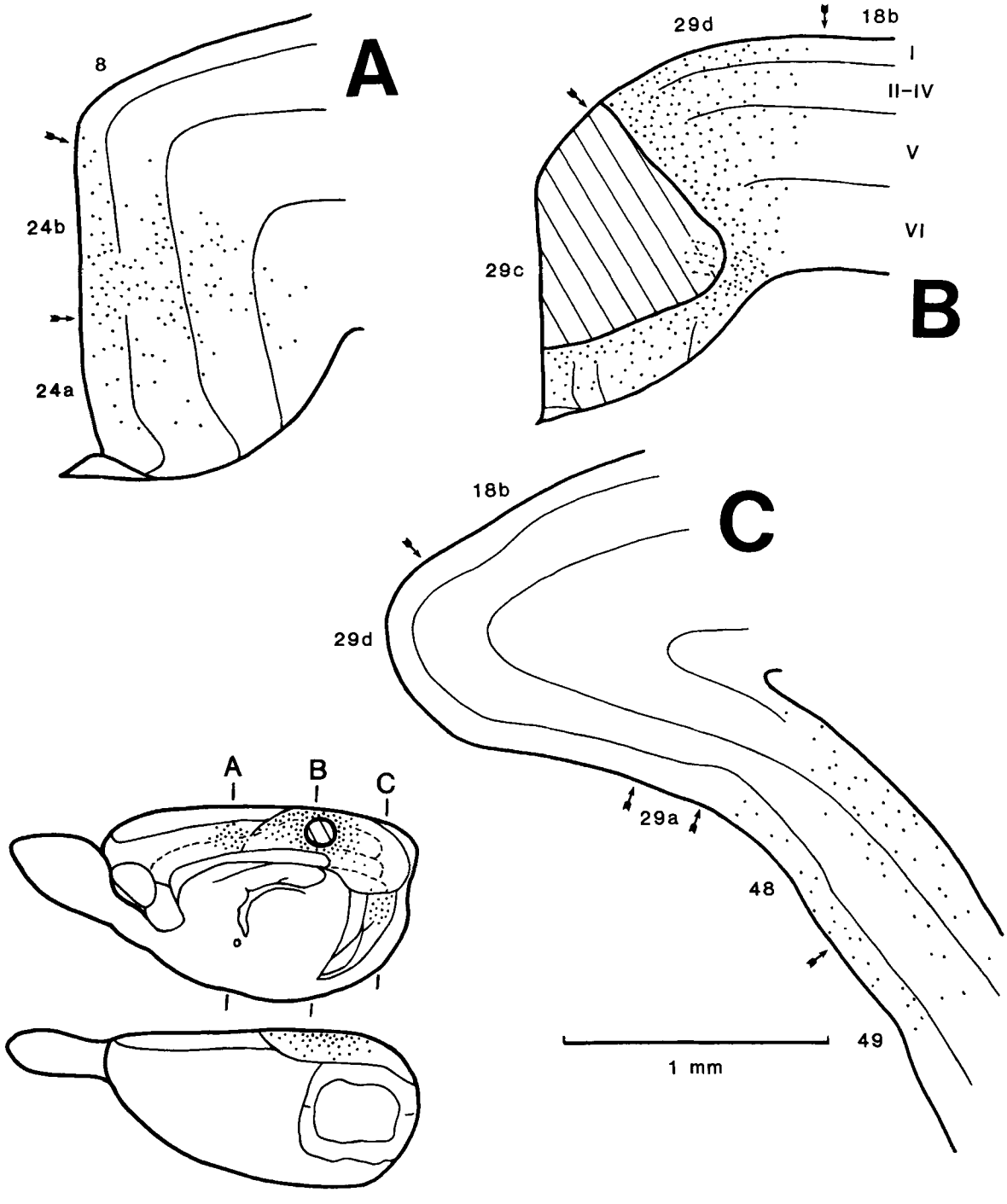


Fig. 8. Termination of area 29c efferents within cingulate and parahippocampal cortices.

tions. An injection of HRP into area 24b that also involved areas 24a and 8 (Fig. 15) labeled large numbers of cortical neurons in cingulate areas 25 and 29b and neocortical areas 11 and 18b. A more limited number of cells was also labeled in areas 29c and 29d and medial area 17. It is of interest to note that almost all neurons in posterior cingulate and visual cortices were located in layer V and that HRP-positive neurons were not present in areas 32, 48, 49, 29a or areas 18a and lateral area 17.

Injections of ^{3}H-amino acids into areas containing HRP-positive neurons in previous cases confirmed these projections and indicated that many projections into area 24b

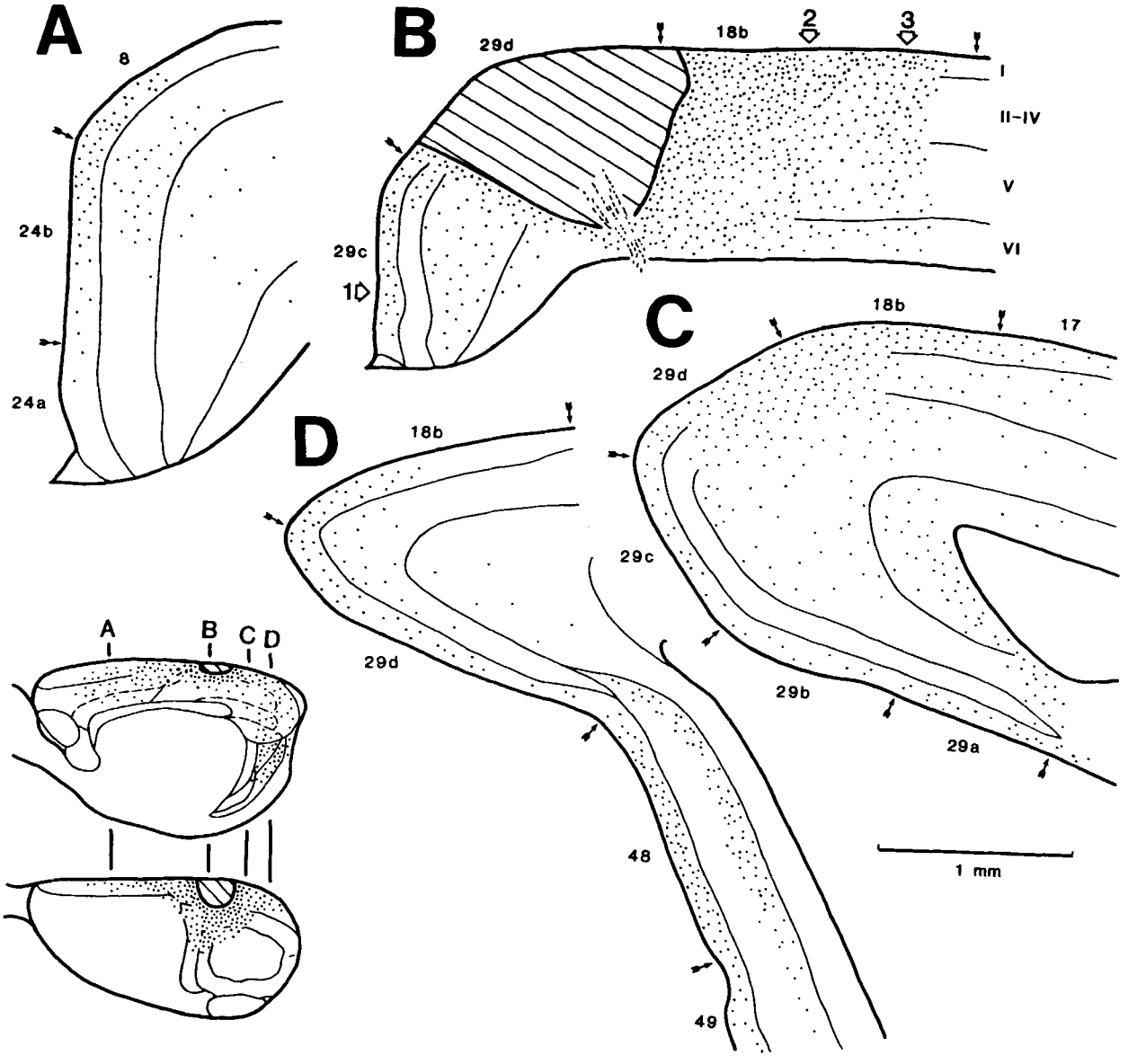


Fig. 9. Distribution of grains following an injection of ^{3}H-amino acids into area 29d. Empty arrows in B point to levels through which grain counts were made (Fig. 11). Points 1 and 2 were equivalent distances from the injection center. Grains at the base of the injection site reflect filling of efferent fibers.

were shared with either area 24a or area 8. An injection of ^{3}H-amino acids which involved mainly area 18b (as previously noted in Fig. 5) labeled numerous axonal terminals in layers I-III of area 24b, less in the superficial layers of area 8, and least in area 24a. A large ^{3}H-amino acid injection into area 17 (Fig. 6A) produced grains mainly in area 24b and medial area 8, but none in area 24a, while injections into area 18a (Fig. 6B,C) and lateral area 17 did not result in anterograde transport into anterior cingulate cortex. Thus, area 24b appears to be the primary recipient of the projection from the visual to the anterior cingulate cortex. Area 24a appears to receive no more than a sparse projection from area 18b and none from other parts of the visual cortex. No evidence of visual cortex projections to area 25 was found. Finally, as noted previously, injections of ^{3}H-amino acids into subdivisions of posterior cingulate cortex resulted in anterograde transport to area 24. In a case in which an injection was made in area 29c (Fig. 8) grains were distributed mainly in layers I-III of area 24b with less in area 24a, while none appeared in area 8. An injection into area 29d (Fig. 9) resulted in labeling of terminals mainly in areas 24b and 8 and more sparsely in area 24a.

Area 24 efferents

Efferent projections from anterior cingulate cortex had an underlying pattern which was similar to that of its afferents: area 24a had relatively restricted cortical connections, while area 24b had more extensive connections with cingulate and visual cortices. Thus, an injection of ^{3}H-

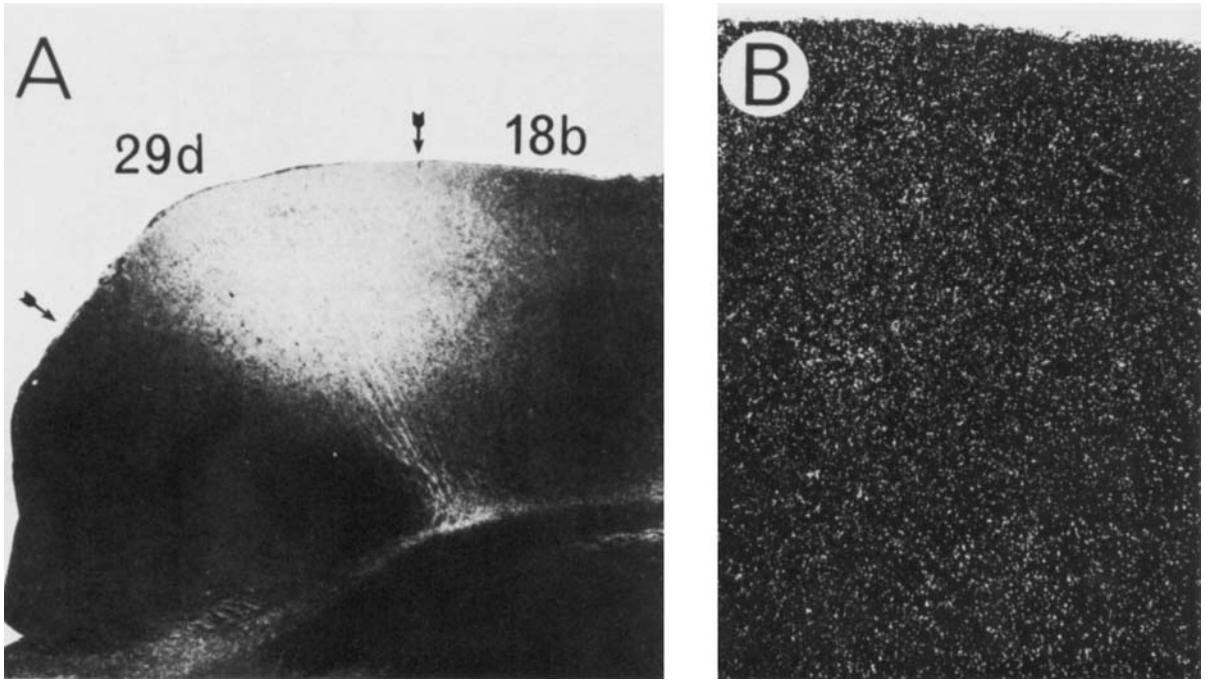


Fig. 10. Darkfield micrograph of the injection of ^{3}H-amino acids into area 29d (A, ×45) presented in Figure 9. B. Distribution of grains in layers I–V of area 18b following this injection. ×140. This photograph was taken at point 3 in Figure 9B.

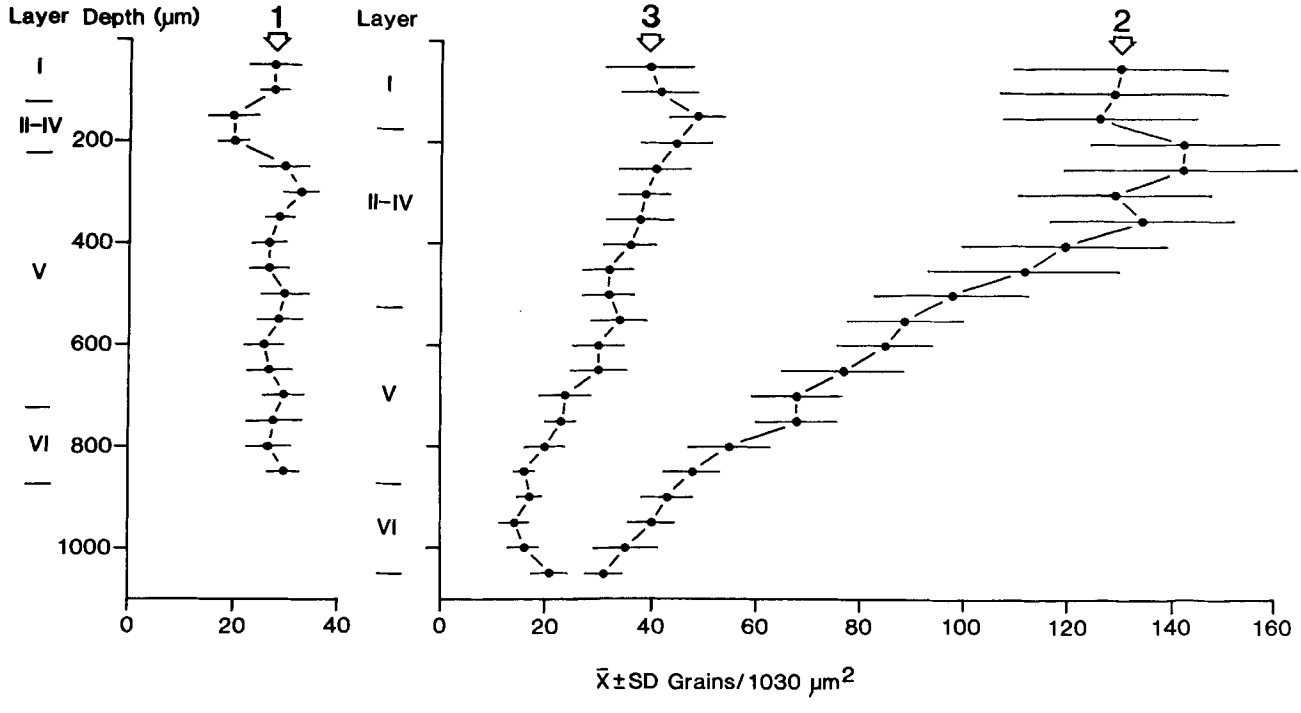


Fig. 11. Mean number of grains per unit area at different depths in three strips of cortex following the injection into area 29d. See also Figure 9B.

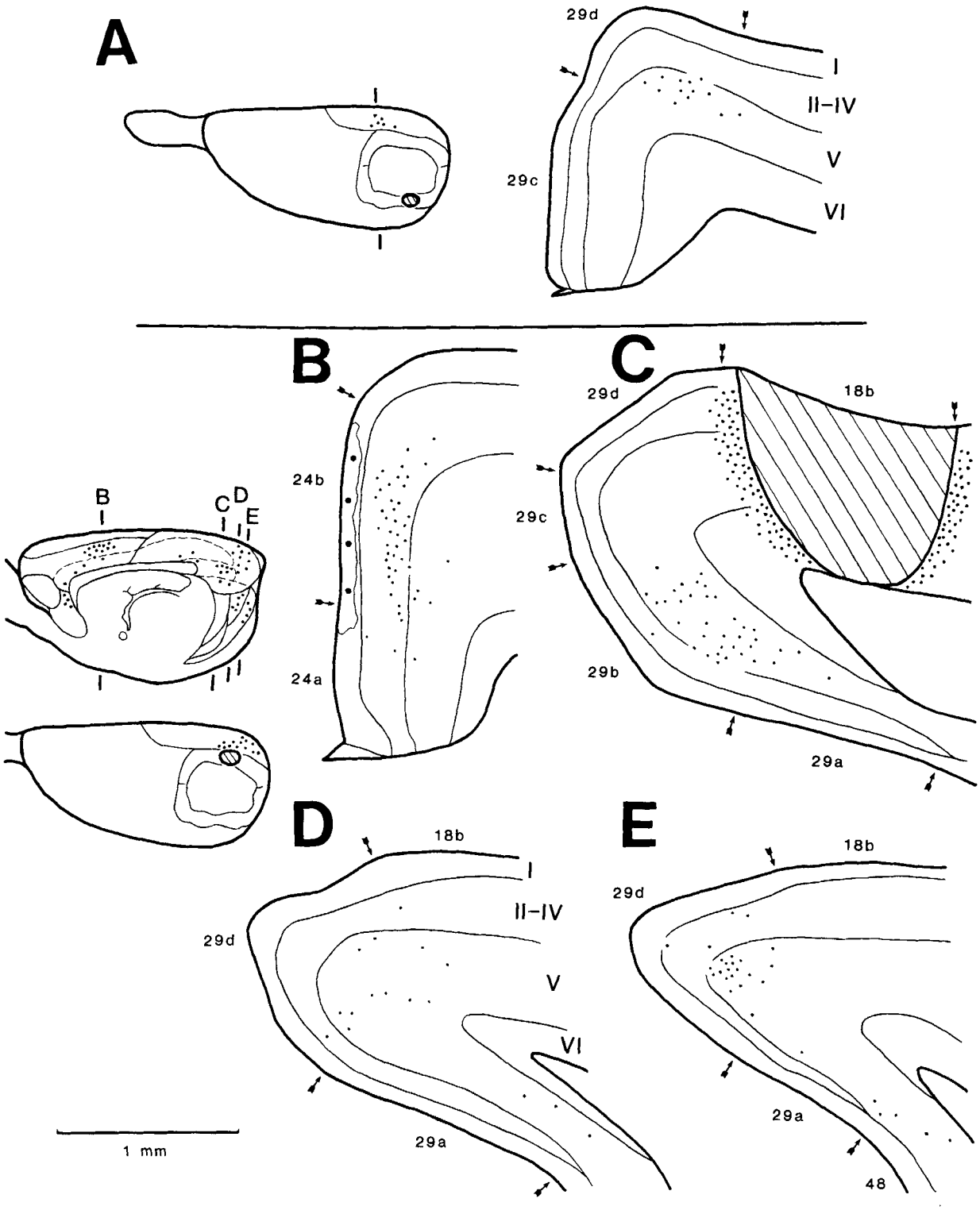


Fig. 12. Location of HRP-positive neurons following HRP injections into visual cortex. A. Area 18a injection. B–E. Area 18b injection.

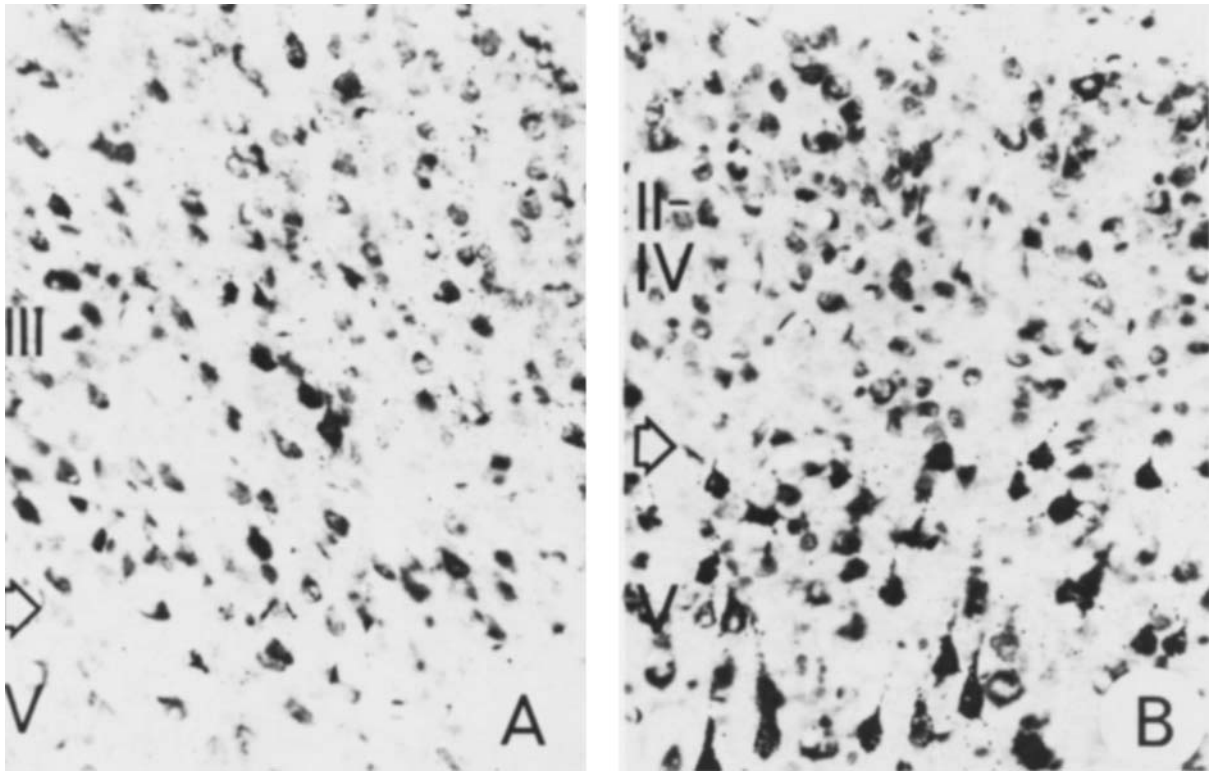


Fig. 13. Micrograph of HRP-labeled neurons in cingulate cortex following an injection of HRP into area 18b. A. Layer III of area 24b. B. Layer V of area 29d. ×375.

amino acids restricted to area 24a (Fig. 16A) labeled projections to the anterior cingulate cortex that were confined to area 24b, caudal area 32, and dorsal area 25. Terminal labeling within posterior cingulate cortex was light and restricted to the more rostral parts of areas 29a and 29b.

Tritiated amino acid injections that involved area 24b in addition to area 24a produced more extensive labeling of terminals in posterior cingulate cortex with further labeling in postsubicular and visual cortices. Thus, a large injection into area 24b that also partially involved areas 24a and 8 (Fig. 16C) labeled terminals in all subdivisions of area 29 and areas 18a and 18b. It was possible that the lateral neocortical (area 4) and area 11 labeling were due to involvement of area 8 in the injection. Also, since the area 18a HRP case presented in Figure 12A did not label neurons in area 24b, it seems likely that the axonal labeling in area 18a likewise was due to area 8 involvement. Finally, a much larger injection of areas 24b and 8 enhanced the termination pattern noted in the previous case with the addition of some terminal labeling in areas 17, 7, and 3. Although area 17 does receive input from both areas 24b and 8, as noted above, it was possible that the labeling of axons in areas 3 and 18a reflected the extensive involvement of area 8 in this case. The laminar distribution of terminal labeling in visual cortex was different from that observed after injections into posterior cingulate cortex (e.g., area 29d, Fig. 9). Area 24b and 8 projections appeared to terminate primarily in layers I and VI of areas 17 and 18 (Fig. 16E), while additional terminals were labeled in layers IV and V of areas 18a and 18b.

The projections from area 24 to cingulate and visual areas appear to originate in different layers. HRP injected into areas 29c–d labeled neurons in all layers of posterior areas 24a and 24b (Fig. 2C), while in rostral area 24 most of the labeled neurons were located in layer V of area 24b (Fig. 2B). Although HRP injections into area 17 of visual cortex retrogradely labeled many neurons in layer V of area 24b and fewer in layer III (Fig. 14A,B), an injection of HRP into area 18b labeled cells mainly in layer III of area 24b (Figs. 12B, 13A).

Area 32

Pandya and Van Hoesen ('81) suggested that based on efferent connections area 32 in the monkey seemed more closely related to prefrontal than to cingulate cortex. This view appeared to gain further support in the present analysis from the distribution of area 32 efferents. A large injection of ^{3}H-amino acids into area 32 that also involved limited parts of areas 24a and 25 (Fig. 16B) labeled terminals mainly in frontal area 11 and anterior cingulate cortices (area 24a with some in area 24b). All injections of ^{3}H-amino acids which involved area 24 but not area 32 caused axonal labeling in posterior cingulate cortex (Fig. 16A,C,D) that was absent following an injection predominantly involving area 32. Also, HRP injections into posterior (Fig. 2) and anterior (Fig. 15) cingulate cortices consistently labeled neurons in area 25, but failed to label neurons in area 32. In addition, HRP injections into visual cortex (Figs. 12B-E, 14) retrogradely labeled neurons in areas 25, 24, and 29, but not in area 32. Thus, on the basis

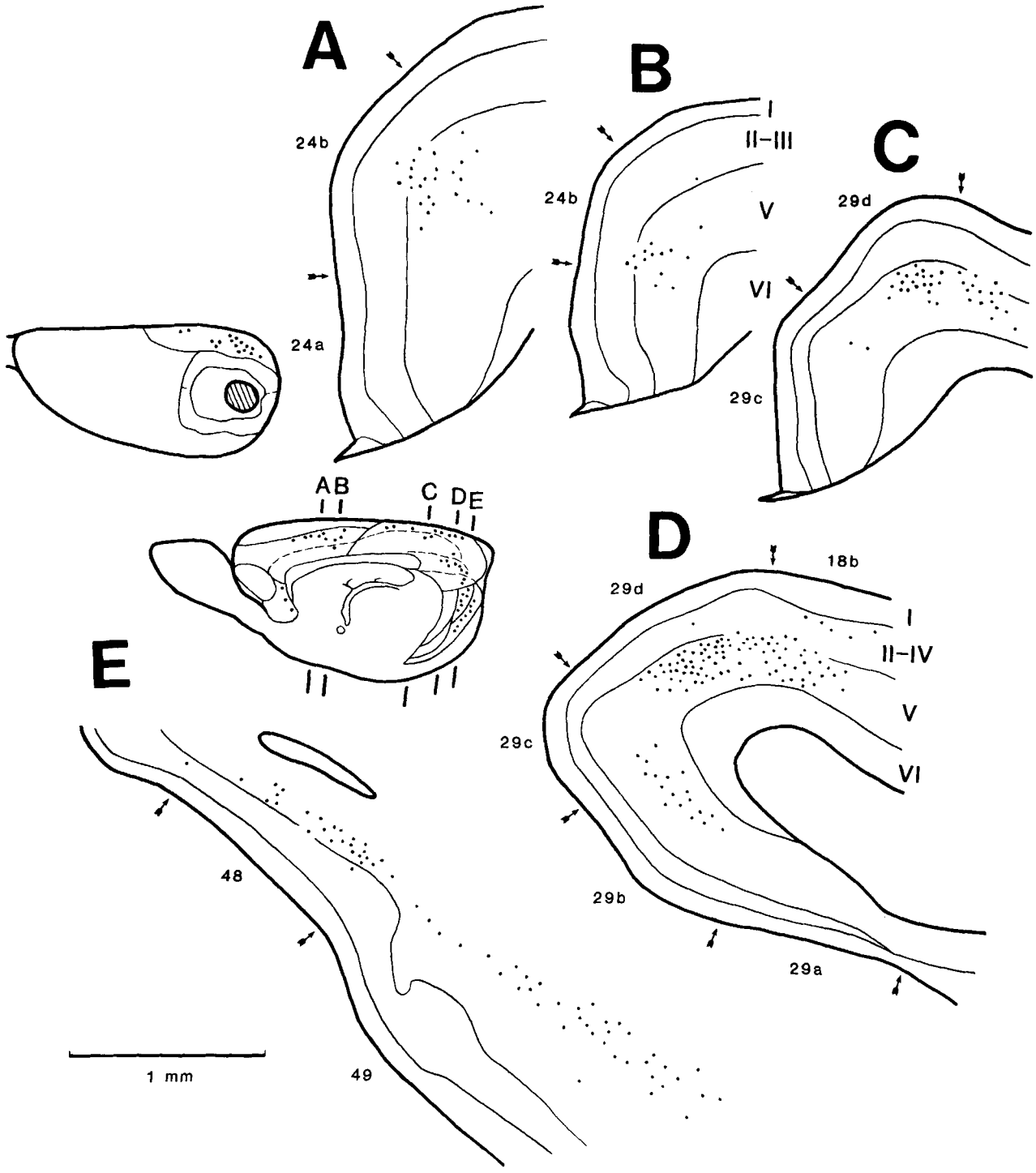


Fig. 14. Distribution of HRP-labeled neurons following an injection of HRP into area 17.

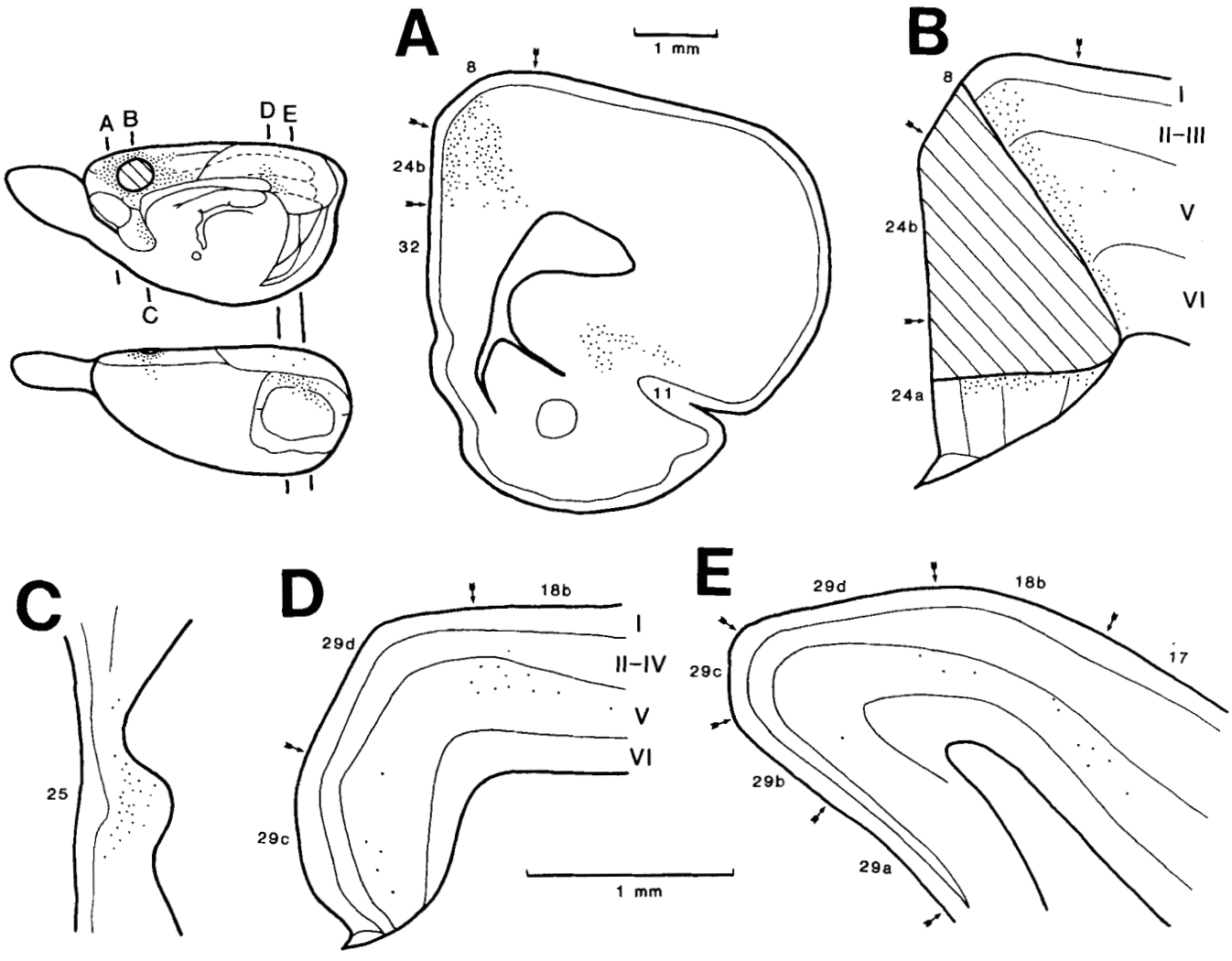


Fig. 15. Distribution of HRP-positive neurons in the cortex following an injection of HRP that was centered in area 24 and also involved areas 24a and 8. Note the reduced magnification of level A necessary to accommodate the labeled neurons in both areas 11 and 24. Levels B–E were all at higher magnification.

of both cingulate and visual cortical connections, area 32 should probably be considered a subdivision of frontal cortex.

Connections of the subicular cortices

Swanson and Cowan ('77) and Meibach and Siegel ('77) have shown that the subiculum and postsubiculum project to posterior cingulate cortex in the rat. As already noted in the present analysis, HRP injections into areas 29c–d resulted in retrograde filling of neurons in these areas as well as in the parasubiculum, while HRP injections into anterior cingulate cortex did not produce retrograde labeling in these areas.

Although the present experiments did not demonstrate projections from cingulate cortex to the subiculum, tritiated amino acid injections into area 29c (Fig. 8) and 29d (Fig. 9) produced labeling of terminals in areas 48 and 49. In contrast, projections to parahippocampal cortex from anterior area 24 appear to be more limited. Thus, an injection of ^{3}H-amino acids into area 24a (Fig. 16A) did not label terminals in areas 27, 48, or 49, but injections that involved areas 24b and 8 (Fig. 16C,D) labeled terminals in areas 48 and 49. The injection shown in Figure 16C involved areas 24b and 8 and labeled a limited projection into the postsubiculum, while the injection illustrated in Figure 16D involved area 24b and more of area 8 and demonstrated more extensive projections to parahippocampal cortex that included both areas 48 and 49.

Connections between visual and parahippocampal cortices were evident in many of the previously described cases. Projections of parahippocampal cortex appear distributed to areas 17 and 18b, since an HRP injection into area 17 (Fig. 14) labeled neurons in the inner pyramidal cell layers of areas 48 and 49, while an injection into area 18b

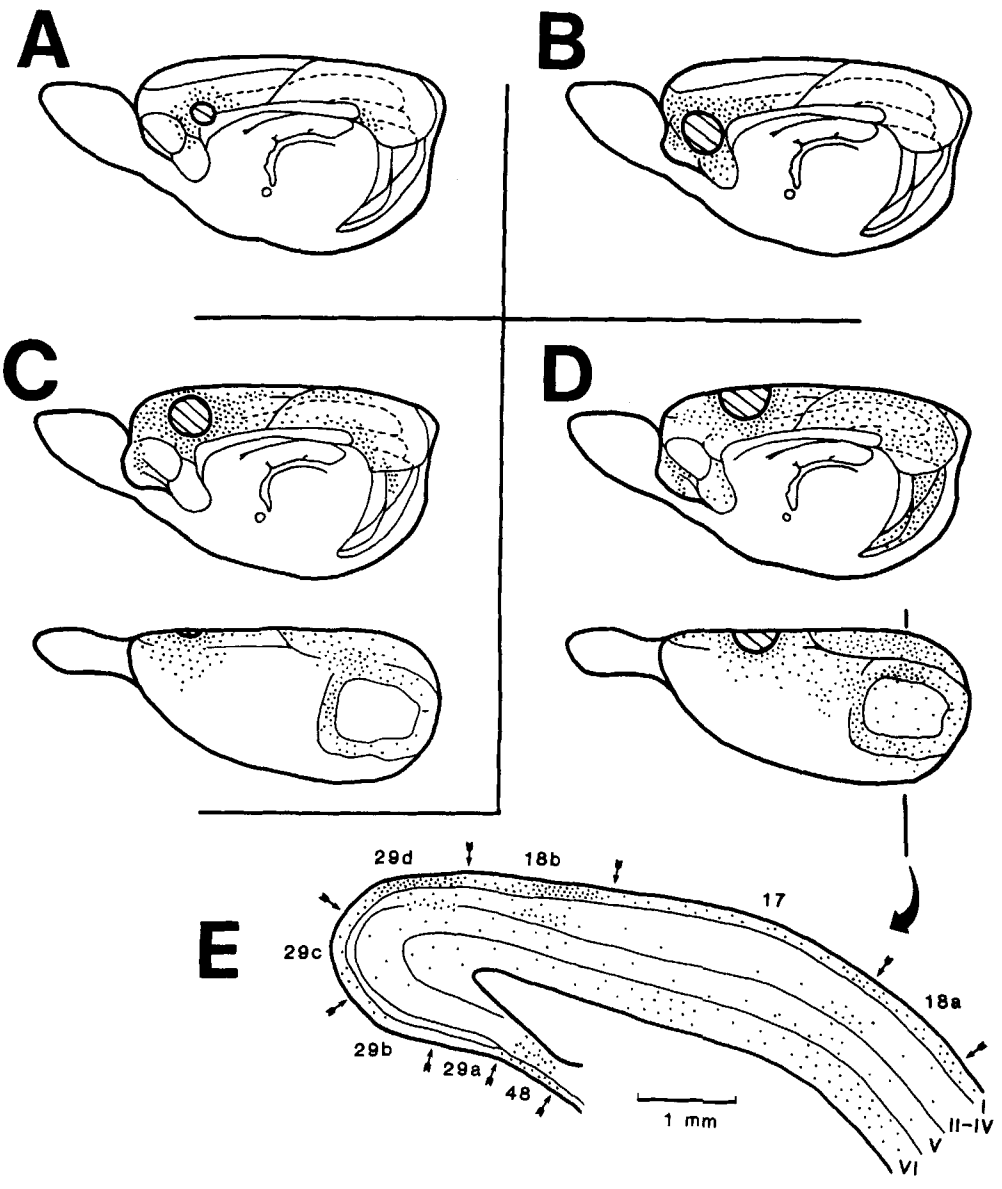
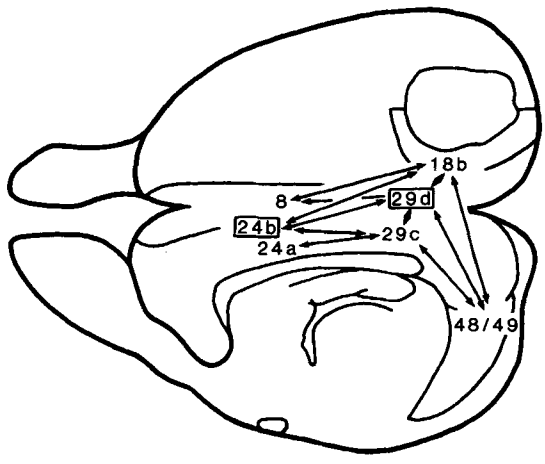


Fig. 16. Location of grains following injections of ^{3}H-amino acids into anterior cingulate cortex. A. Area 24a injection. B. Injection mainly into area 32. Note that area 11 is shown by opening the sulcus between the olfactory bulb and the cerebral cortex. C. Injection into area 24b with limited involvement of areas 24a and 8. D. Large injection into areas 24b and 8. E. Distribution of grains in visual, cingulate, and parahippocampal cortices.

(Fig. 12) also labeled neurons in the same layer of areas 48 and 49. These connections also appear to be reciprocal, as an injection of ^{3}H-amino acids into area 18b (Fig. 5) labeled fibers in layer I and the inner one-third of the external pyramidal cell layer of most of area 48, and part of area 49. Many axons appeared to reach layer I of area 48 by ascending from the white matter and passing through each cortical layer of the dorsal part of area 48. A smaller contingent of labeled axons passed ventrally along the white matter to enter ventral parts of area 48 and area 49. In a case in which the isotopes were injected into area 17 (Fig. 6A) terminal labeling was found in posterior parts of the postsubiculum and much of dorsal portions of area 49. It should also be noted that anterograde axonal transport of HRP to layer I of the postsubiculum was also evident in cases in which HRP was injected into area 17. Finally, projections of area 18a to parahippocampal cortex appeared to be distributed only to the postsubiculum (Fig. 6C).

DISCUSSION

An understanding of the mechanisms underlying mammalian learning will require a specification of the sensory connections, sensorimotor interconnections by which stimuli guide behavior, as well as the means by which sen-



AREAS 24b & 29d: LIMBIC CORTICES INVOLVED IN SENSORIMOTOR INTEGRATION

Fig. 17. Schematic representation of some of the most extensive connections observed among the cingulate, visual, motor, and postsubicular/parasubicular cortices.

sorimotor responses are paired with motivational or previously learned associations. The present analysis details a series of corticocortical connections in rodent cingulate cortex which suggest that cingulate cortex may be uniquely situated to mediate many of these functions.

Sensory connections

A summary of some of the major corticocortical connections of areas in the dorsal and medial cortex of the rat is presented in Figure 17. Areas 24b and 29d stand out as particularly important cingulate areas in terms of their connections with visual cortex, since both are reciprocally connected with area 18b, while area 29d also has more limited connections with areas 17 and 18a.

Visual cortex afferents may serve as the basis for flash-evoked responses of units in rabbit cingulate cortex (Vinogradova, '75). Furthermore, units in hippocampal and parahippocampal cortices also respond to flashes of light (Vinogradova, '75) and hippocampal responses have been abolished with posterior cingulate cortex lesions (Vastola, '82). It appears from the present observations that hippocampal/parahippocampal visual-evoked responses in rodents could be accounted for by direct visual cortex afferents to areas 48 and 49 and/or by a series of connections which pass from visual to cingulate cortices and from there to parahippocampal cortices.

These direct sensory-limbic connections of the rodent cortex may not be present in primate cortex. Although HRP injections into monkey cingulate cortex do not produce retrograde filling of neurons in primary sensory cortices (Vogt et al., '79), flash-evoked responses have been produced in neurons of cingulate, parahippocampal, and hippocampal cortices of the monkey (Cuénod et al., '65). Exactly which series of connections might be responsible for such activity is not known, since the outflow from primary sensory cortices may have to pass through a number of neocortical association cortices before entering the limbic system (Pandya and Kuypers, '69; Van Hoesen et al., '72).

Although stroboscopic responses of neurons in limbic cortex may be relayed through a series of corticocortical connections from visual cortex, it is unlikely that these are the only responses that are transmitted through these pathways. Extensive physiological studies indicate that the primary functions of neurons in rodent visual cortex involve detecting the edges, length, orientation, direction, and motion of objects (Chow et al., '71; Dräger, '75; Tiao and Blakemore, '76; Mangini and Pearlman, '80). Since visual cortical afferents terminate directly in cingulate cortex, the possibility must now be considered that neurons in cingulate cortex may have specific receptive field properties that have been overlooked in studies which employ gross flashes of light. Since layer V neurons of visual cortex (mainly in area 18b and to a lesser extent in areas 18a and 17) appear to be the main sources of projections into cingulate cortex, it may be the receptive field properties of these neurons which will have the greatest influence over visual-evoked responses in cingulate neurons. For example, layer V of area 17 contains directionally selective neurons (Chow et al., '71) and removal of visual cortex reduces direction selectivity in the superior colliculus (Graham et al., '82). It is possible that direction selectivity is also present in cingulate cortex neurons and is specified by visual cortex in a manner similar to that in the superior colliculus.

There are at least three routes in addition to these cortical pathways by which visual stimuli might influence neurons in cingulate cortex. First, a limited projection has been suggested between the retina and anterior thalamus which in turn projects to cingulate cortex (Conrad and Stumpf, '75; Itaya et al., '81). Second, the superior colliculus and pretectal area project to thalamic nuclei which may also project to cingulate cortex including the lateral and lateral posterior nuclei (Martin, '69; Robertson et al., '80; Mason and Groos, '81). Third, there are reciprocal connections between the claustrum and visual cortex in the tree shrew (Carey et al., '80), and we have observed that parts of the rat claustrum which receive input from area 18b also contain neurons which project to cingulate cortex; furthermore, the cat claustrum has neurons that respond to visual stimuli (Spector et al., '74; Sherk and LeVay, '81). Thus, it is possible that claustral afferents to cingulate cortex relay visual sensory input.

Finally, the pathways by which other sensory stimuli activate cingulate neurons are not well established. It is known that auditory stimuli activate cingulate neurons in rabbits (Vinogradova, '75) and that similar responses can be conditioned in rats (Segal, '73). There are at least two possible pathways which may mediate these responses. First, a limited number of layer VI cells in auditory cortex of the rat (areas 41 and 36) project to cingulate cortex. Second, claustral neurons respond to auditory stimuli (Spector et al., '74) and may provide another pathway by which cingulate neurons are influenced by auditory input. As is the case with visual afferents, the specific receptive field properties of acoustically sensitive neurons in cingulate cortex have not been addressed.

Limbic afferents

Although there are many possible sources of afferents to cingulate cortex which could be the basis for further representations of the sensory fields, there are at least two rea-

sons why strict, point-to-point, and separate representations of visual and auditory spaces would not be expected. First, individual neurons in cingulate cortex usually do not respond to only one sensory modality (Vinogradova, '75), suggesting multimodal convergence. Second, at some point during the signal detection process conditioning must occur whereby specific sensory features are selected as relevant to producing a motor output. This feature extraction activity may be one of the major functions of cingulate cortex and could result in a drastic reorganization of sensory representations.

One means of assessing feature extraction and conditioning in cingulate cortex is to compare neuronal spiking in auditory and cingulate cortices throughout the acquisition of a conditioned avoidance task (Gabriel et al., '77, '82). In this paradigm, neurons in cingulate cortex produce more spikes earlier in the behavioral task to the positive conditioning stimulus (one tone frequency paired with footshock) than to the negative conditioning stimulus (another tone frequency not paired with footshock). These differential neuronal responses do not occur in primary auditory cortex or the medial geniculate nucleus of the thalamus during early stages of behavioral learning (Gabriel et al., '82), suggesting that this conditioning may be a unique activity of cingulate cortex.

A question that follows naturally from these observations is: What connections does cingulate cortex have which might be responsible for conditioning that are not also present in sensory cortices? One possibility is anterior thalamic afferents, since Gabriel et al. ('81) have placed lesions in the anterior thalamic nuclei and were able to abolish the usual excitatory and associative neuronal responses to the positive conditioning auditory stimulus. Another afferent to cingulate cortex which does not enter sensory cortices is subicular in origin. Although subicular neurons are retrogradely labeled following HRP injections into area 29, these cells are not labeled after injections into visual cortex. Thus, anterior thalamic and possibly subicular afferents may be the limbic afferents which are responsible for conditioning in cingulate cortex.

Motor efferents

The wide range of somatic and autonomic motor responses produced by electrical stimulation of cingulate cortex in lower mammals (Lende and Woolsey, '54, '58) may be mediated by efferent connections of cingulate cortex to the caudate, midbrain tegmentum, central gray, superior colliculus, and/or pons (Beckstead, '79; Morrell et al., '81; Wyss, '81; Hardy and Leichnetz, '81; Wiesendanger and Wiesendanger, '82). The present report demonstrates that a corticocortical pathway also exists from area 29d to area 8. Since electrical stimulation of area 8 produced eye and eyelid movements (Hall and Lindholm, '74), area 29d afferents to area 8 provide another route into the motor system from cingulate cortex.

It may be concluded that the extensive sensory and motor functions of cingulate cortex are not the result of generalized, "nonspecific" activity, but rather the product of a series of well-defined interconnections which provide for specific sensory input, conditioning of certain features of sensory afferent activity, and pathways that can modify motor activity according to conditioned responses.

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