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Rabbit Cingulate Cortex: Cytoarchitecture, Physiological Border With Visual Cortex, and Afferent Cortical Connections of Visual, Motor, Postsubicular, and Intracingulate Origin

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ABSTRACT

The connections of cingulate cortex with visual, motor, and parahippocampal cortices in the rabbit brain are evaluated by using a modified Brodmann cytoarchitectural scheme, electrophysiological mapping techniques, and the pathway tracers horseradish peroxidase (HRP) and tritiated amino acids. Rabbit cingulate cortex can be divided into areas 25, 24, and 29. Area 29 is of particular interest because area 29d has a lateral extension with a granular layer IV, area 29b has a caudal extension in which the connections differ from anterior area 29b, and there is a prominent area 29e. Cytoarchitectural delineation of the lateral border of area 29d with area 17 closely approximates the medial edge of the visual field representation in area 17 as determined electrophysiologically.

The main interconnections between visual and cingulate cortices occur between cingulate areas 24b and 29d and visual areas 18 and medial parts of area 17. Projections between areas 29d and 18 are organized in a loose topographic fashion with rostral parts of each and caudal parts of each being reciprocally connected. Neurons mainly in superficial layer II–III of areas 17 and 18 project to layer I of area 29d, while the reciprocal projection originates from neurons in layer V of area 29d and project mainly to layer I of areas 17 and 18.

The medial portion of motor area 8 projects to areas 18 and 29d and has a smaller projection to area 17.

Postsubicular area 48 is reciprocally connected with area 29d, and it also projects to areas 29b and c. The subiculum projects to areas 29a and 29c but only to the anterior two-thirds of area 29b not the posterior one-third.

Rostral area 29d receives the most extensive intrinsic cingulate projections including those from all major cytoarchitectural divisions. Interconnections between areas 29d and 29b appear to be topographically organized in the rostrocaudal plane. Area 29c projects more heavily to area 29b than vice versa. Finally area 29d projects mainly to area 24b in anterior cingulate cortex.

In conclusion, rostral area 29d has extensive connections with visual areas 17 and 18, motor area 8, and all subdivisions of cingulate cortex. In light of these connections, it may play a pivotal role in associative functions of the rabbit cerebral cortex including visuomotor integration.

Key words: corticocortical connections, limbic system, visuomotor integration, area 29d

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In primates primary sensory and cingulate cortices are connected via sequential corticocortical connections with parasensory and multimodal association areas in the frontal, parietal, and temporal lobes (Petras, '78; Künzle, '78; Vogt et al., '79; Baleydier and Mauguiere, '80; Pandya et al., '81; Goldman-Rakic et al., '84; Barbas and Mesulam, '85). Connections between sensory and cingulate cortices in rodents, however, are direct (Vogt and Miller, '83) and cingulate cortex is one of the principal intermediate association cortices between sensory and hippocampal areas. Thus, visual- and auditory-evoked single unit discharges have been recorded in cingulate and hippocampal cortices (Segal, '74; Vinogradova, '75), ablations in posterior cingulate cortex can abolish visual-evoked activity in hippocampus (Vastola, '82), and auditory-evoked activity can be conditioned in cingulate cortex (Segal, '73; Gabriel et al., '80).

A number of issues relating to visual and cingulate cortical connections in subprimate species are unresolved including the cytoarchitectural border between visual and cingulate cortices, the relationship of this border to the distribution of visual receptive fields, and the topographic organization of these connections. The border of visual and cingulate cortices has been located in a number of mediolateral positions in cytoarchitectural analyses (Brodmann, '09; Rose, '31; Rose and Woolsey, '48; Caviness, '75; Zilles et al., '80). We have modified the Brodmann map (Vogt and Peters, '81) and used it to describe corticocortical connections in rat. A number of functional and connectional studies in rabbit cortex also bear on these questions. Thompson et al. ('50) recorded visual-evoked field potentials considerably medial to the splenial sulcus, which implies that the physiological border betweeen visual and cingulate cortex may lie medial to the cytoarchitectural border. Variances of 1–2 mm in the location of this border were observed, however, and this finding was not correlated with the cytoarchitecture of this region. In contrast, geniculocortical studies of Holländer and Hälbig ('80) and Towns et al. ('82) suggest that the medial limit of visual thalamic afferents may closely approximate the cytoarchitectural border between visual and cingulate cortices.

The goal of the present study is to characterize the cytoarchitectonic areas of cingulate cortex in rabbit brain and their corticocortical connections with visual, motor, parahippocampal, and other cingulate areas. There are a number of reasons for pursuing a detailed analysis of cortical efferents to cingulate cortex in rabbit. First, cortical areas in the rabbit are large in relation to those in rat so injections of tracer substances can be more easily limited to parts of a single cytoarchitectural subdivision, allowing for topographical analysis. Second, little information is available regarding the corticocortical connections of cingulate cortex in rabbit (Montero, '81; Bassett and Berger, '82).

METHODS

Young adult, Dutch belted, male and female rabbits were used in most of these studies, although a few New Zealand animals were also employed. Each surgical procedure was initiated with either pentobarbital or chloropent anesthesia administered I.P. and with supplemental I.V. administrations as needed. Following the experimental procedures and an appropriate survival period, the subjects were deeply anesthetized with chloropent and perfused transcardially as noted below.

Cytoarchitectural procedures

Six normal, unoperated animals were perfused with a physiological saline wash and then 1 liter of 10% formalin. The brains were then celloidin embedded, cut in the coronal or horizontal plane at a 30-μm thickness, and stained with cresyl violet.

Electrophysiological mapping procedures

The purpose of our mapping experiments was not to determine precise topographical relations within rabbit visual cortex but to delineate the border of area 17 with cingulate cortex. Preliminary to mapping studies the following surgical procedures were performed under deep anesthesia. These procedures have been described by Swadlow and Weyand ('81). A stainless-steel bar was attached to one side of the cranium with screws and dental acrylic. A cranial trench (approximately 4 mm by 7–8 mm in a rostrocaudal direction) was opened to expose the splenial sulcus and surrounding cortex and it was then filled with a removable plug of dental acrylic. The subject was allowed to recover for 2–3 days postoperatively. Mapping was done over a period of three to six recording sessions per rabbit. During each session a single AP coordinate was explored in the lateromedial direction. Each session lasted 3–4 hours and recordings were done in the awake state to insure that visual responses were not depressed, particularly in limbic cortex. All surgical procedures were done previously under pentobarbital anesthesia. On recording days the only procedures necessary were removal of the acrylic plug, gentle cleaning of the dura, and insertion of the microelectrode through the intact dura. Rabbits did not appear to be disturbed in any way by these procedures. On subsequent recording days, coordinates were reestablished by means of marks on the steel bar attached to the animal's head. Experiments were terminated and continued on another day if the rabbit showed any signs of displeasure with the experimental situation (e.g., excessive fidgeting) but this was rarely necessary.

During a mapping experiment the animal was placed in a stocking on a foam pad and the bar was fastened to a stereotaxic instrument in a position that minimized tension on the neck. Eye movements were not controlled in these experiments. In a lighted environment, however, the range of eye movements in the rabbit is limited (Collewijn, '71). In our experimental situation, in individual penetrations, we observed a range of horizontal and vertical eye movements of less than 12°. Thus, our measure of eye position in the awake rabbit is accurate $\pm 6°$. The horizontal reference was the horizon that was defined as the plane that bisected the two eyes and was parallel to the floor. In awake rabbits the visual streak (the horizontal band of increased ganglion cell density) remains approximately horizontal and is centered about 5° above the horizon (Hughes, '71). Our vertical reference plane bisected the midline of the body axis and was perpendicular to the floor. Once the animal was positioned comfortably, the acrylic cement plug was removed, and glass-coated tungsten electrodes with 10–20-μm tip exposures were used for extracellular recording of multiple and single unit activity. In each animal a series of microelectrode penetrations were made over several rostrocaudal planes. At each plane the microelectrode was inserted into area 17 and the corresponding position of the visual field was mapped by displaying moving spots and

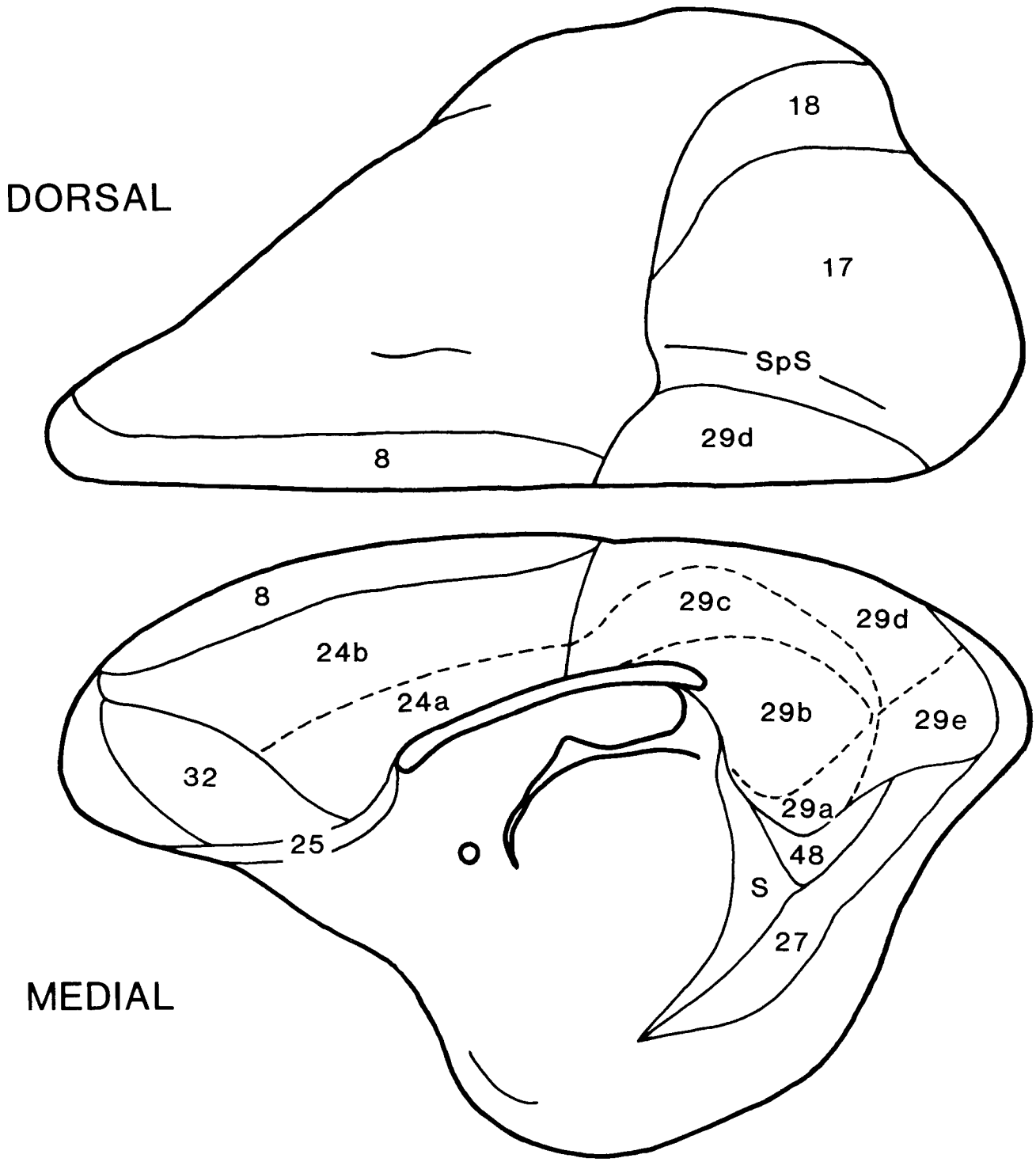

Fig. 1. Cytoarchitectural map of areas in the cerebral cortex of the rabbit brain. Since much of area 29b is oriented horizontally above the superior colliculus, it has been flattened on the view of the medial surface so that its full extent is represented. As a consequence, area 29a and parahippocampal areas 48 and 27 have been shifted ventrally and slightly caudal. SpS, splenial sulcus; S, subiculum.

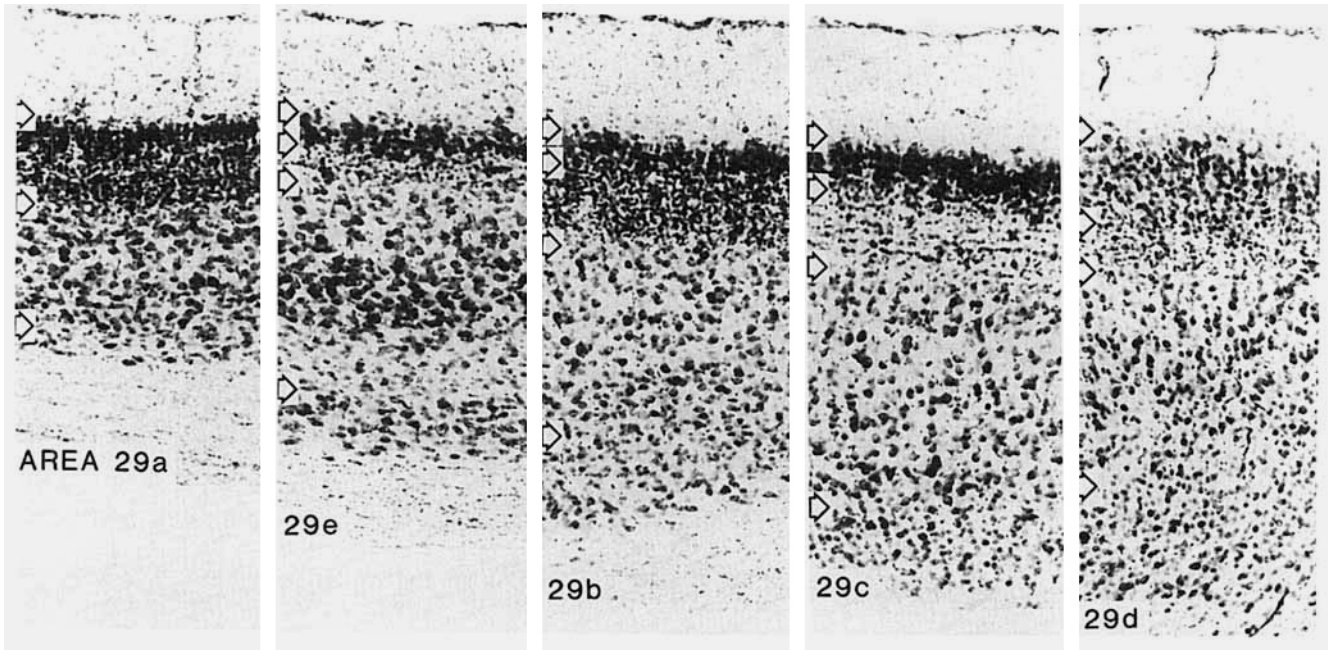

Fig. 2. Cytoarchitectural areas of posterior cingulate cortex. In area 29a the arrows designate the borders between layers I and II–IV, IV and V, and V and VI, while in the remaining areas they refer to the borders between layers I and II–III, II–III and IV, IV and V, and V and VI. Cresyl violet, 80×.

bars of various sizes and contrasts on a tangent screen positioned 1 m from the eye. The size, location, and orientation of receptive fields were marked on the screen. The microelectrode was then moved medially in steps that were usually less than 1 mm and avoided blood vessels. This procedure was repeated until visually driven unit activity was no longer evoked. This location was then marked with a lesion (+10 μA, 10 seconds). The electrode was then moved 1 mm posterior and the mapping procedure was repeated. In any particular animal, three to seven rostrocaudal planes were analyzed. The brains of these animals were prepared as noted above with celloidin embedding and Nissl staining. In addition, pins were placed in the brains before embedding to identify the position of bregma and all sections were saved through the lesion sites so that most penetrations in area 17 and all lesions could be identified and reconstructed with respect to bregma.

Retrograde transport techniques

A standard HRP technique was employed as described by Vogt et al. ('81). Briefly, this involved the injection of 0.02–0.05 μl of 5% wheat germ agglutinin–conjugated HRP (Sigma) into cingulate or visual cortices of 32 animals followed by a 2-day postoperative survival period. Injections were placed in cingulate cortex with respect to bregma and the splenial sulcus while those in visual area 17 or 18 (i.e., V-I or V-II, respectively) were made following visual field mapping as described by Swadlow and Weyand ('81). The animals were perfused with 100 ml of 0.9% saline followed by 1 liter of quarter-strength Karnovsky fixative for 30 minutes and then 1 liter of 10% sucrose phosphate buffer (pH 7.4) at 10°C for 30 minutes. Subsequently the brains were removed, stored overnight in 10% sucrose buffer in a refrigerator, and cut into 40-μm-thick sections. The sections were incubated in tetramethyl benzidene and counterstained with Neutral Red as previously reported.

Anterograde transport technique

Injections of ^{3}H-amino acids were made into dorsal cortical areas around the splenial sulcus according to the methods of Cowan et al. ('72). Injections of 0.02–0.03 μl of a ^{3}H-amino acid cocktail in 0.9% saline with a final specific activity of 100–125 μCi/μl were made as noted by Vogt and Miller ('83). Following a 4-day postoperative survival period, the animals were perfused with 200 ml of 0.9% saline followed by 1 liter 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 in NTB-2 Kodak Nuclear Track Emulsion, and exposed in light-tight containers in a freezer for 3–3.5 months. These autoradiographs were developed with Kodak D-19, fixed in Kodak Rapid Fixer without hardener, and counterstained with thionin.

RESULTS

Cytoarchitecture

The topography of the cingulate (areas 25, 24, and 29), visual (areas 17 and 18), motor (area 8), and parahippocampal (areas 48 and 27) cortical regions is presented in Figure 1. Area 32 is no longer considered part of the cingulate region because it does not receive anterior thalamic afferents as do cingulate areas and because of its distinct pattern of corticocortical connections (Vogt, '85). The structure of areas 25, 24a, 24b, and 8 are very similar to the rat (Vogt and Peters, '81) except that the density of the most superficial neurons in layer II–III are more clumped in the rabbit. The major differences between the rat and rabbit cortices occur in posterior cingulate and visual cortices. Of particular note in cingulate cortex is the expansion of areas 29b and 29d and the addition of area 29e.

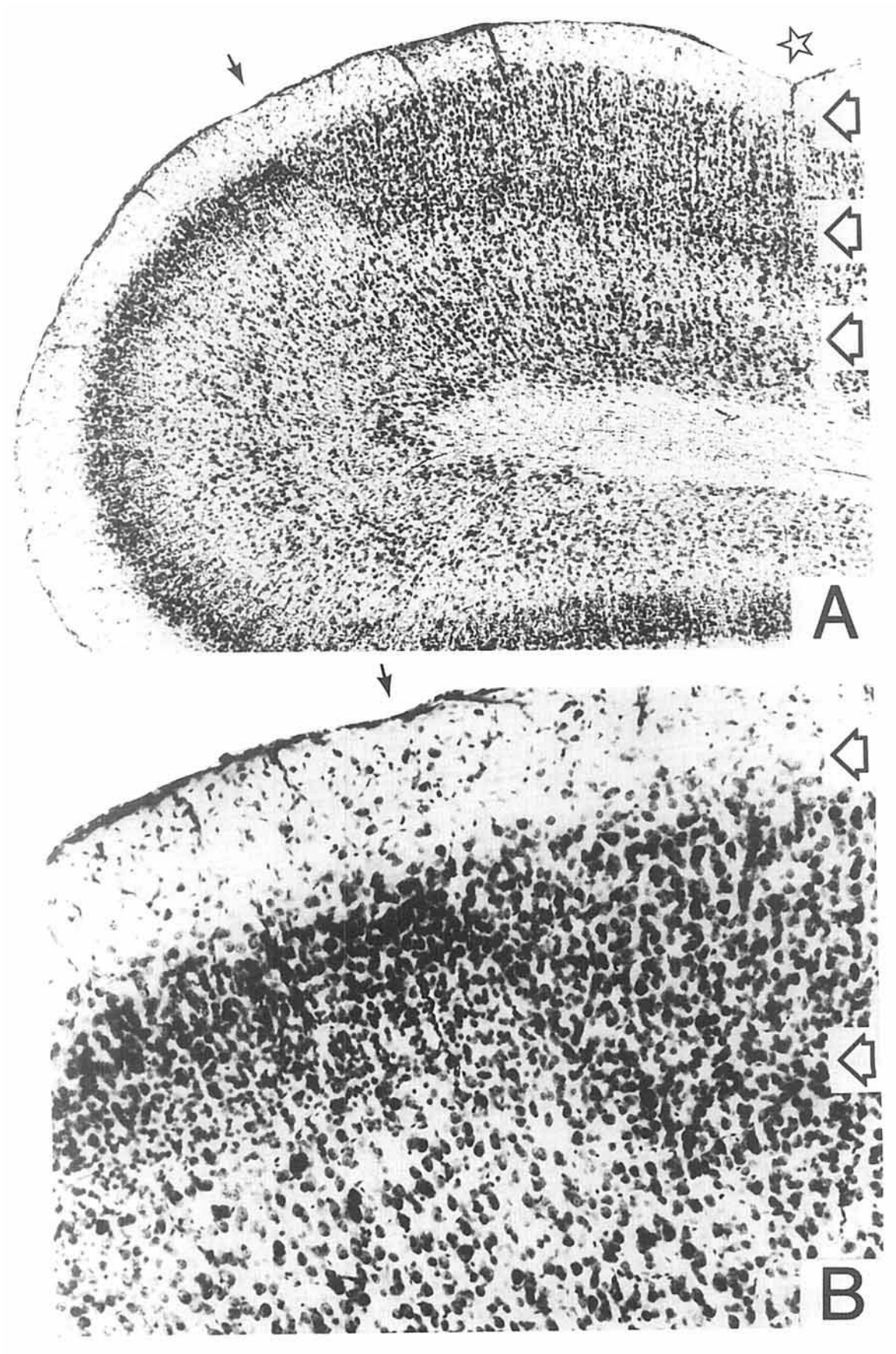

Fig. 3. Cytoarchitectural border between areas 29d (left of arrow) and 17 (right of arrow). In A the star is in the splenial sulcus and the open arrows indicate the following laminar borders: I/II–III, IV/V and V/VI. B. Higher magnification of the same border zone. Cresyl violet, A. 75×, B. 210×.

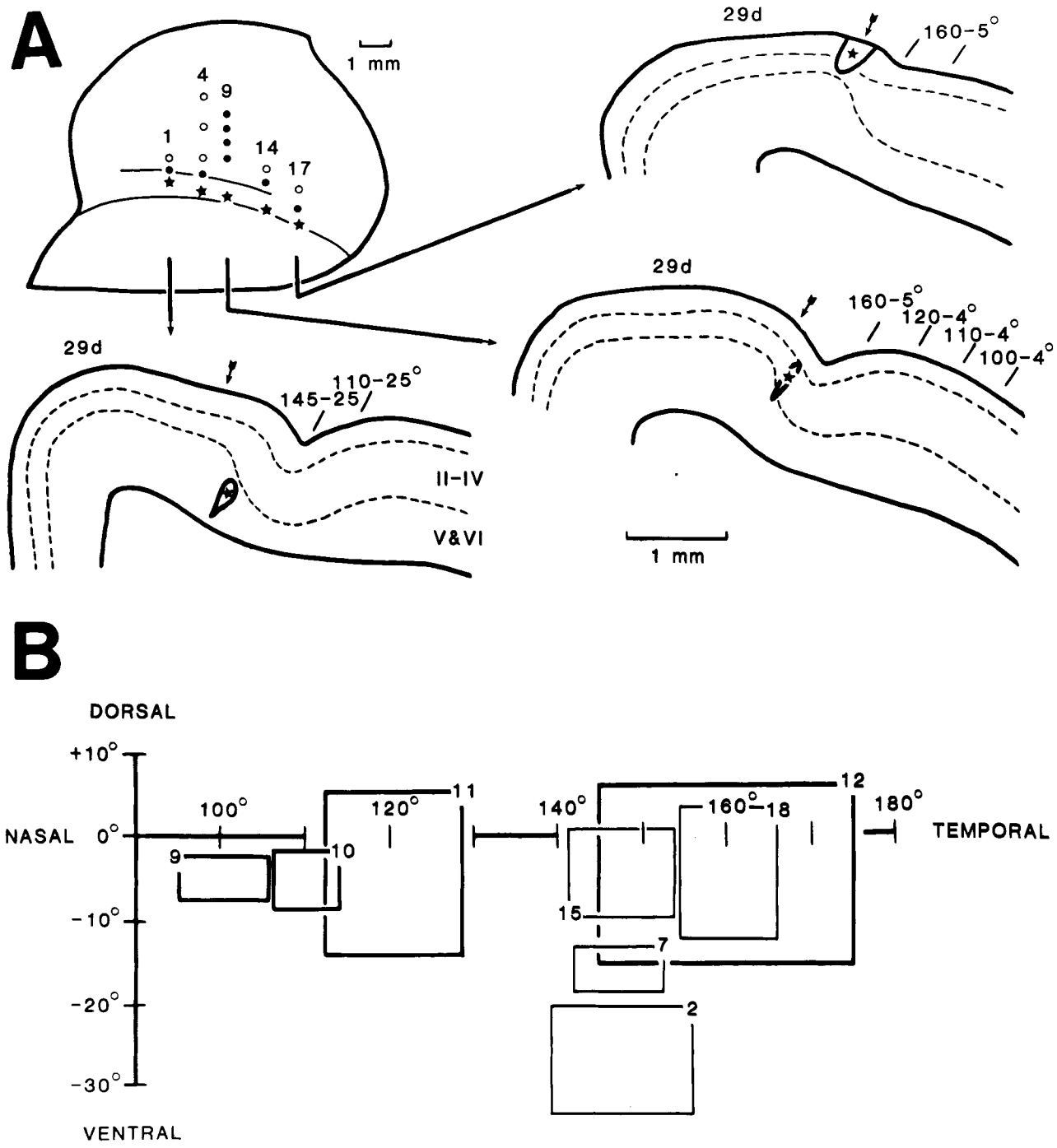

Fig. 4. Receptive field mapping of single units or unit clusters in area 17. A. dorsal view of one brain (anterior to left and medial surface facing down) showing five lateromedial series of three to five penetrations each. Both filled and open circles indicate positions of visually responsive units. At each AP level, a lesion was placed at the first lateromedial penetration in which no visual response was detected (stars). Coronal sections (midline to left) show the borders between areas 29d and 17 (arrows), lesion placements (stars), and position and coordinates of visual fields in degrees from the nasovertical and horizontal meridians. B. Examples of the position and size of receptive fields from this case (filled circles in A). The final receptive field of each lateromedial series and the complete series 9–12 are plotted.

Area 29d in the rat is dysgranular in the sense that layer IV is not well developed and is almost nonexistent at one mediolateral level. Although the medial part of area 29d in the rabbit also has a poorly developed layer IV, lateral area 29d has a clearly defined layer IV, as can be seen in Figure 2. Area 29e has not been observed on the medial surface of the rat brain, but it is a prominent cellular entity in the rabbit. This area has a thick layer II–III of large neurons and a very thin layer IV. This contrasts with area 29a where layers II–IV are not differentiated and areas 29b and

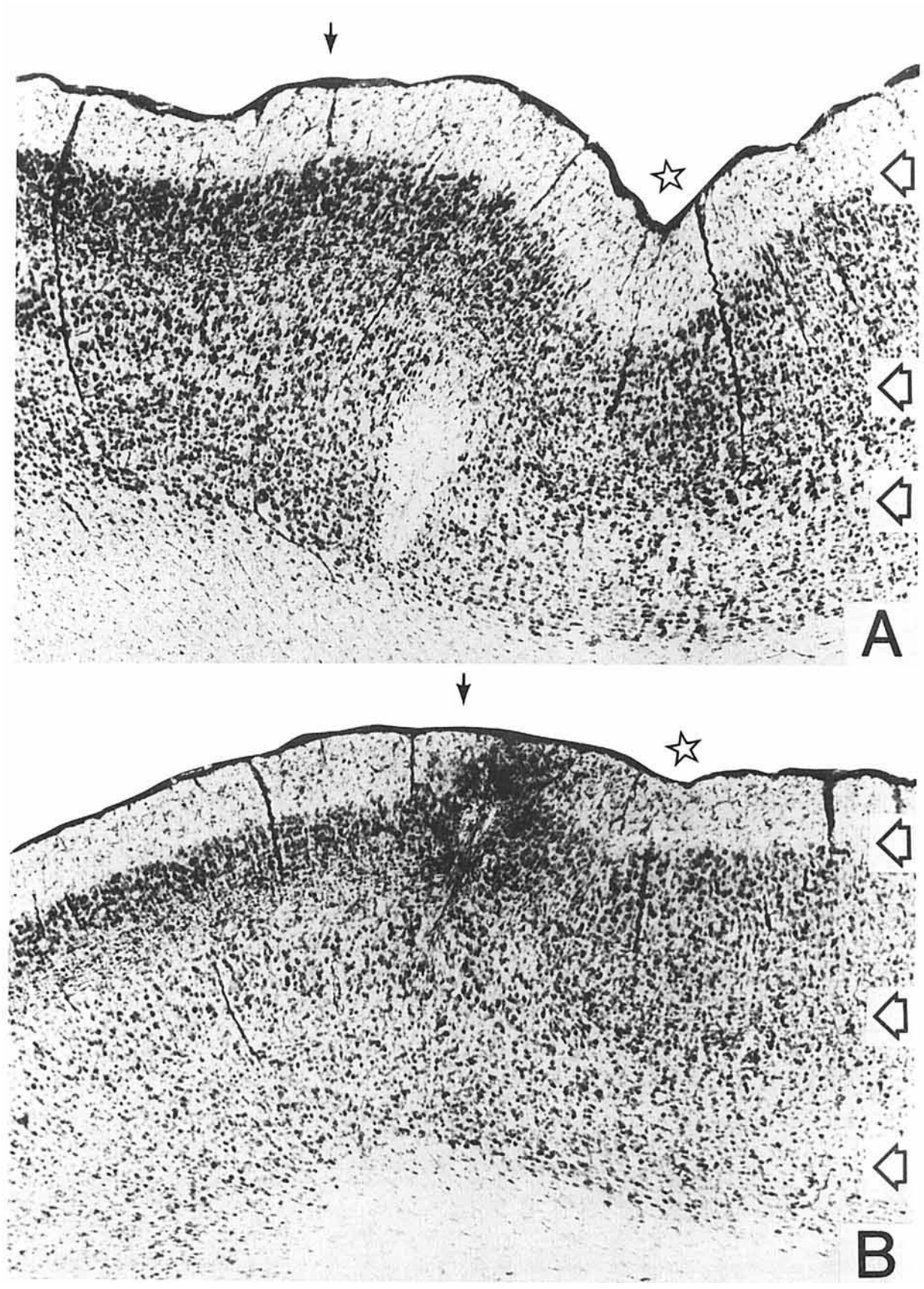

Fig. 5. Lesions in nonresponsive cortex in the case presented in Figure 4. Area 29d is right of the arrows and the stars mark the splenial sulcus. A. Penetration 3. B. Penetration 19. Cresyl violet, 85×.

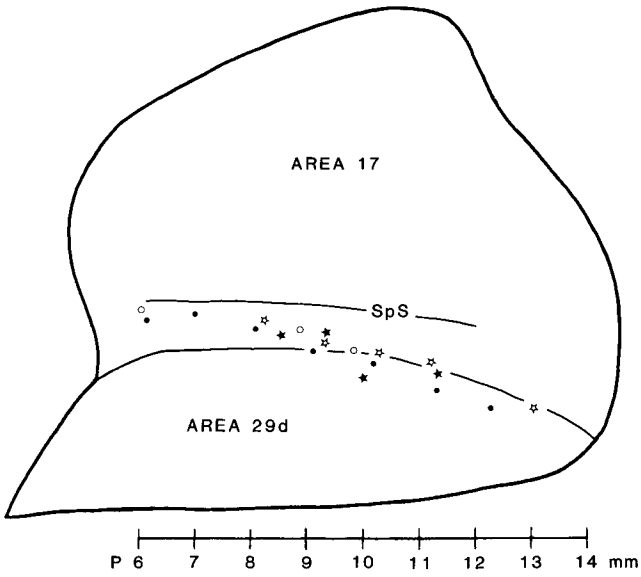

Fig. 6. Positions of lesions in nonresponsive cortex in four additional cases. Separate symbols represent lesions in each case. SpS, splenial sulcus; P, mm posterior from bregma.

29c where layer IV reaches its maximal thickness in the posterior cingulate region. In addition, area 29b is quite expanded topographically in the rabbit in the region that overlies the superior colliculus. Finally, an area 23 cannot be distinguished in the rabbit medial surface. As in the rat there is a short transitional region between areas 24 and 29, but no striking differences in cytoarchitecture are apparent that would warrant a separate designation.

Understanding of the visual areas 17 and 18 has undergone substantial revision since the time of Brodmann ('09), and Figure 1 reflects some of these changes. Of particular note is the lack of a dorsal area 18 adjacent to cingulate area 29d. Although Rose ('31) identifed an equivalent area (area peristriata) in the rabbit (see also Towns et al., '77), we have not used such a designation because there is no structural entity between areas 17 and 29d and there is a continuous map of the primary visual field across area 17 to the medial border of the splenial sulcus (Thompson et al., '50; see below). In comparison to area 18, area 17 has a distinct layer IV, and cells in layers II–IV are more dense and evenly distributed. In area 18 layer V is much broader such that the superficial layers I–IV and deep layers V and VI are approximately equal in thickness.

Border between areas 17 and 29d

The cytoarchitectural border between areas 17 (V–I) and 29d is distinct (Fig. 3). The transition from area 17 to area 29d is marked by an increased density of cells in layer II–III and a dramatic reduction in the thickness of layers II–IV. The relationship between this border and the medial edge of the visual receptive field map, however, has never been determined. This relationship was assessed by recording the activity of neurons in response to visual stimuli at 1-mm intervals in a grid along the area 17/29d border (Fig. 4).

The topographical relations between the cortical surface and the visual field were similar to those reported by Thompson et al. ('50). Receptive fields of neurons adjacent to the splenial sulcus were large and temporally placed in the visual field when compared to the receptive fields of more lateral units (Fig. 4B). Lesions were placed at the first point at which units could not be driven with visual stimuli. Figure 5 presents examples of two lesions that were made in visually nonresponsive cortex. In this case these lesions closely approximated the cytoarchitectural border of areas 17 and 29d at the more caudal levels sampled. This was true for all cases, as summarized in Figure 6. In general, these borders were closely correlated along the caudal two-thirds of their extent on the dorsal surface. In the rostral one-third of the dorsal extent of these borders, there was a skewing of the physiological border medial to the cytoarchitectural border. Finally, cells in area 29d were never found to have visual receptive fields with these mapping techniques.

Visual and cingulate connections

Visual efferents to cingulate cortex. The most extensive retrograde labeling of visual cortical neurons occurs following injections of HRP into area 29d. In one such case (Figs. 7, 8) most labeled neurons are in areas 17 and 18, with most cells in area 17 just lateral to the splenial sulcus. These cells are primarily located most superficially in layer II–III of both areas 17 and 18. In area 18, HRP-labeled neurons extend deeper into layer III but not layer IV. Labeled neurons are also present in layer V of area 18, though in limited numbers, and are rarely seen in layer V of area 17. It is generally the case that labeling in layer V neurons occurs below the most densely labeled parts of layer II–III.

There is evidence for a loosely organized topography in visual projections to area 29d. Thus, in an HRP case in which the most rostral part of area 29d is involved, labeled neurons are located primarily in rostral portions of areas 17 and 18 (Fig. 9A), including parts of area 17 just lateral to the splenial sulcus. A more caudal but much smaller injection of HRP (Fig. 9B), labels neurons throughout the full rostrocaudal extent of area 18. Finally, a caudal and ventral injection that also includes area 29b and the small caudal extension of area 29c labels neurons primarily in caudal area 18 (Fig. 9C). In this latter case, HRP-positive neurons in area 17 are also caudal and ventral to those in the most rostral case (Fig. 9A).

In most HRP cases retrograde labeling of neurons is often associated with anterograde labeling in layer I. Injections of ^{3}H-amino acids into dorsal visual and cingulate areas indicate that much of the anterograde HRP labeling in previous cases is probably associated with afferent axon terminals. Following ^{3}H-amino acid injections of dorsal area 17, lateral to the splenial sulcus, grains are distributed mainly over layer I of area 29d with less in the deeper layers. Many of these latter grains could be associated with axons of passage (Fig. 10A).

An HRP injection that involves the granular retrosplenial areas 29b and 29c also labels neurons in areas 17 and 18 (Fig. 11). In both areas these neurons extend as caudally and ventrally as those labeled in the area 29d cases. Like injections into area 29d, almost all neurons labeled following injections into areas 29b and 29c are in layer II–III of area 18 while in area 17 they are only in the superficial part of layer II–III next to the molecular layer.

Each HRP injection into area 24 labels neurons in areas 17 and 18. In a case with a small injection primarily into area 24b (Fig. 12A), the HRP-positive neurons are mainly in rostral parts of the visual areas. A larger injection into

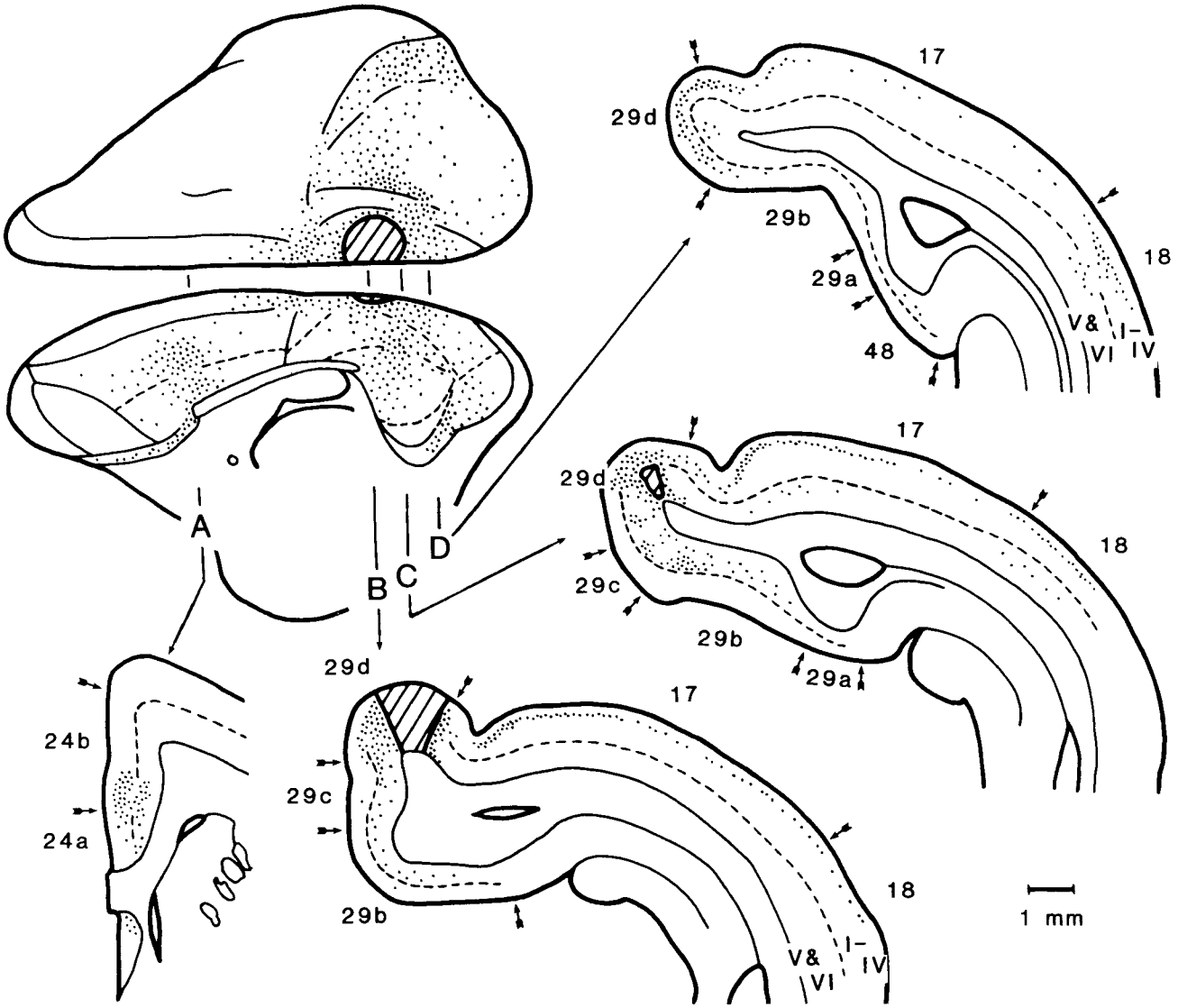

Fig. 7. Distribution of HRP-labeled neurons (dots) in the cerebral cortex following an injection into area 29d (hatched).

area 24b that also infringes upon areas 24a and 8 (Fig. 12B) labels more neurons at caudal levels of both visual areas. In both of these cases most of the area 17 labeling occurs medial to the splenial sulcus in continuity with labeled neurons in area 29d. It is interesting to note that the laminar organization of these projection cells differs from that following posterior cingulate injections. Although there is still clear labeling of neurons in layer II–III of area 18 (Fig. 12B), there are more HRP-positive cells in deep layer II–III and layer V with the proportion in layers II–III and V almost reaching unity. Throughout area 17 there are some labeled neurons in superficial layer II–III and very few in layer V; however, the density of labeling in layer II–III medial to the splenial sulcus reaches the maximum seen in any cases.

In conclusion, all parts of areas 17 and 18 contain cingulate projection neurons. The principal projections are from medial area 17 and area 18 to cingulate areas 29d and 24b. The location of cingulate projection neurons in area 18 appears to be organized topographically with neurons in rostral and caudal parts of area 18 projecting to rostral and caudal parts of cingulate cortex, respectively. However, this topography is considered loose because midlevels of area 18 appear to have a wide termination area within cingulate cortex and one which overlaps with those of projections originating in rostral and caudal cortices. Labeling of neurons in area 17 following cingulate injections is usually most pronounced around the splenial sulcus. This labeling is greatest lateral to the sulcus when area 29d is injected while it is more dense medial to the sulcus when area 24b

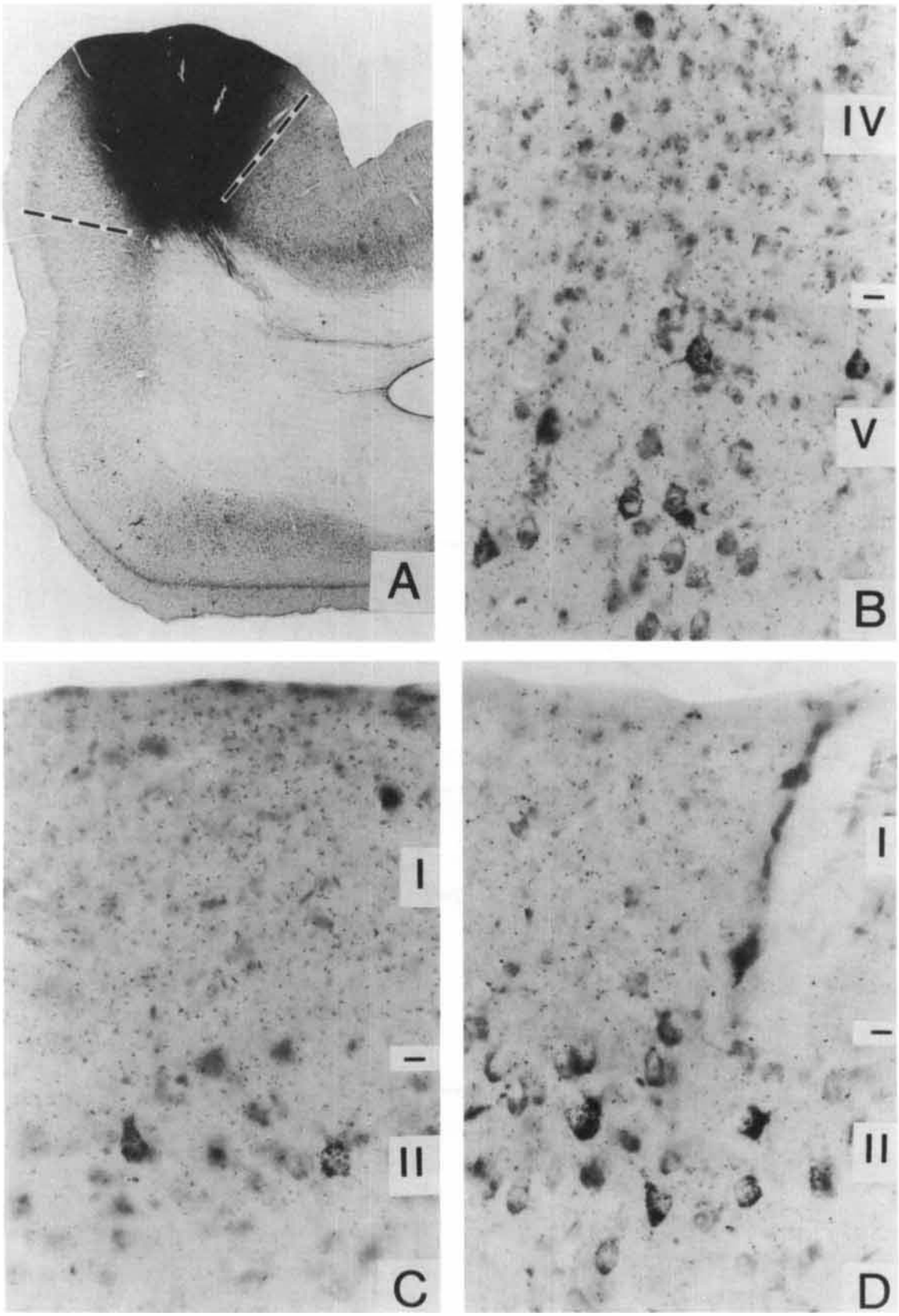

Fig. 8. Photomicrographs of case presented in Figure 7. The injection site is within the borders of area 29d (dashed lines). Examples of HRP-positive cells in layer V of area 29b (B), in superficial layer II–III of area 17 (C), and in superficial layer II–III of area 18 (D). A. 25×, B–D. 170×.

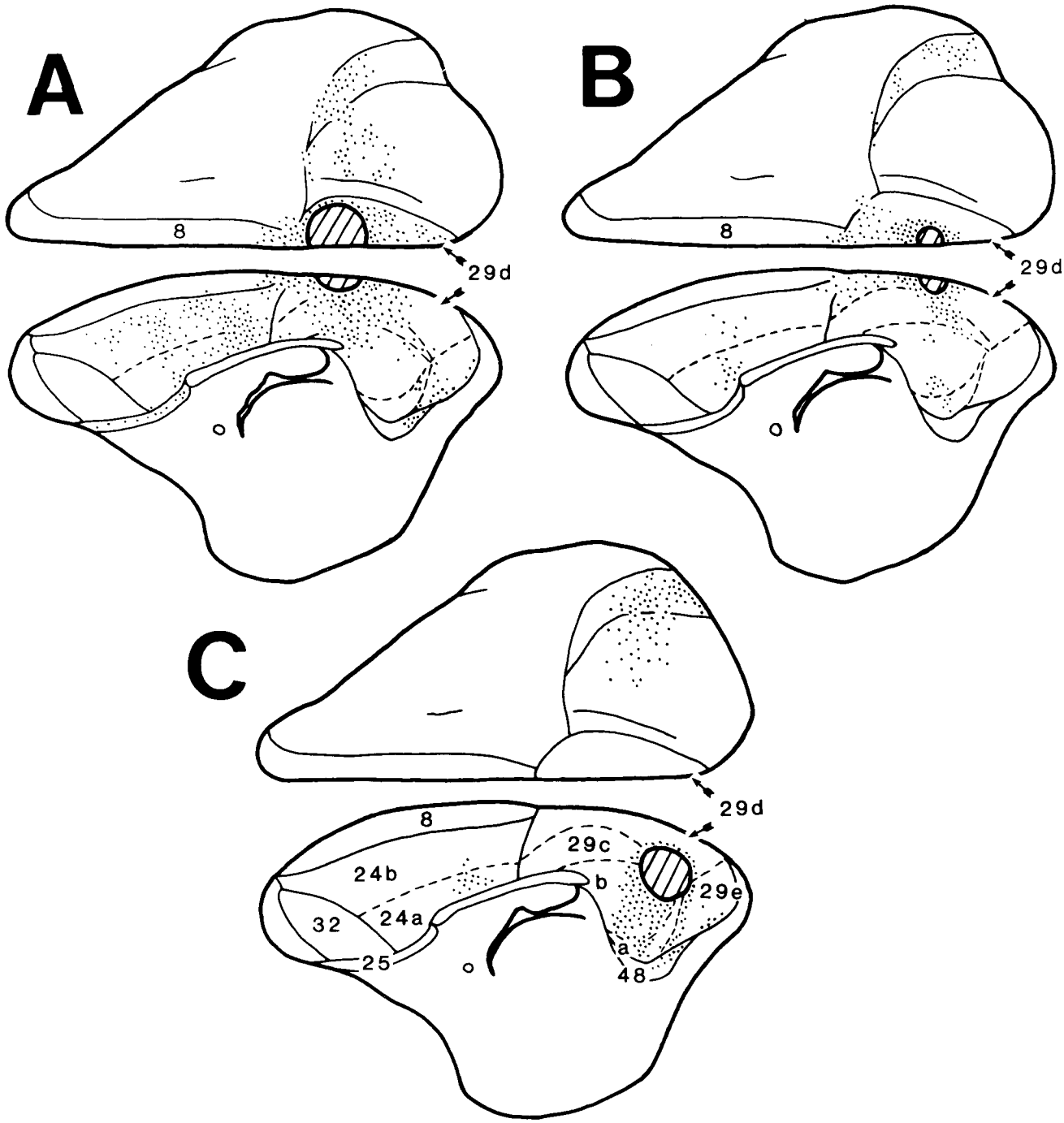

Fig. 9. Distribution of HRP-positive cells in three cases that involve progressively more caudal and ventral parts of area 29d.

is injected. Finally, most of the area 29d projection neurons of area 18 are located in the superficial part of layer II–III with fewer in deep layer II–III and less in layer V while those to area 24b are more evenly distributed across these layers. Cingulate projection cells in area 17 are almost entirely in superficial layer II–III with a few in layer V, and these cells terminate primarily in layer I of area 29d.

Cingulate efferents to visual cortex. Retrograde labeling of cingulate cortex neurons following HRP injections into areas 17 and 18 suggests a topographical organization and indicates that cingulate projections to area 18 and caudal area 17 are the most extensive. The purest injection of area 18, i.e., in which no involvement of surrounding cortex occurred, is presented in Figure 13A. This injection

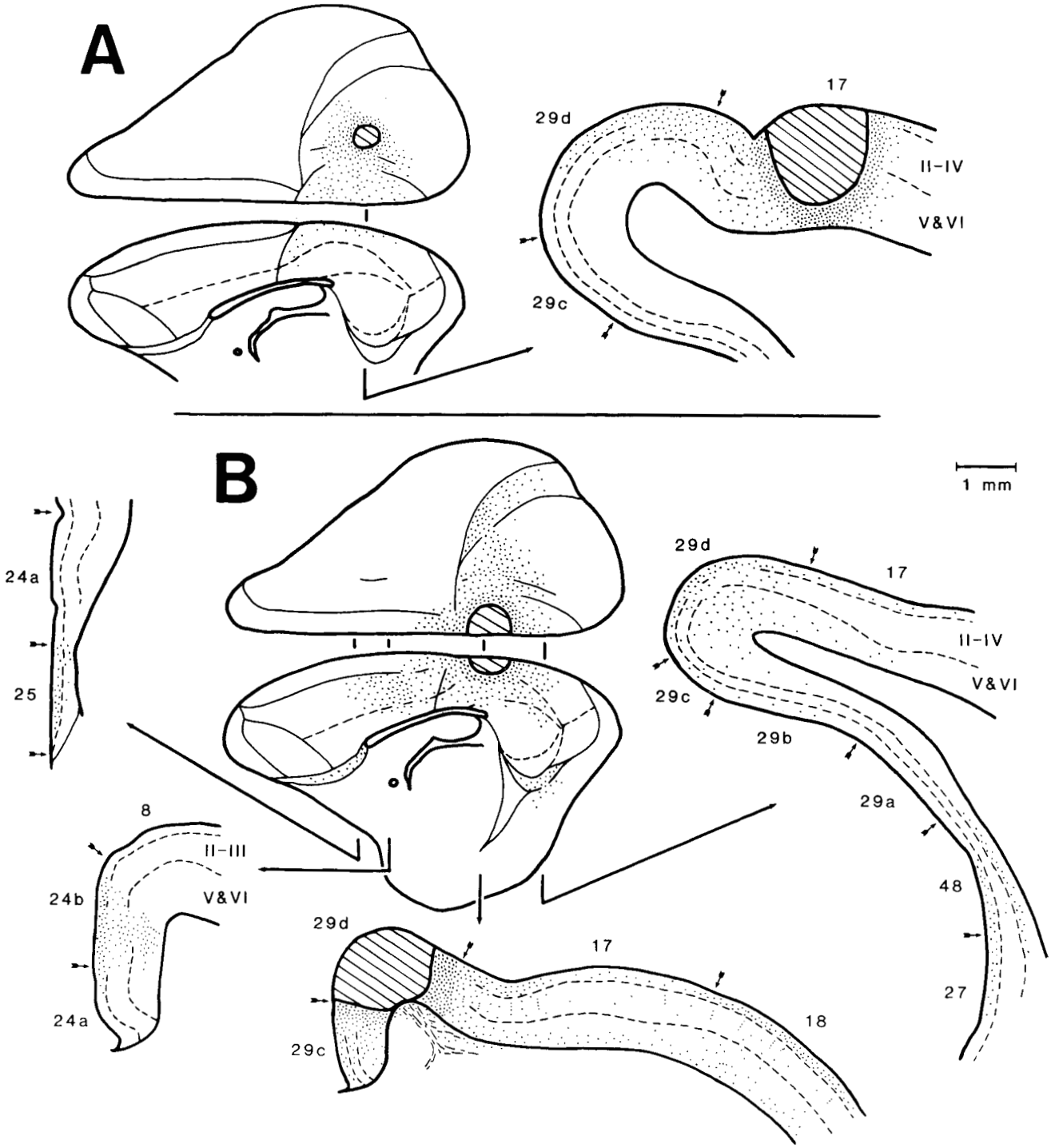

Fig. 10. Autoradiographic cases in which ^{3}H-amino acid injections (hatched) were made in areas 17 (A, distribution of label in area 18 not presented) and 29d (B).

is extremely small and only two small patches of layer V neurons are labeled in a caudal part of area 29d. Although a more rostral injection labels a few neurons in caudal area 29d (Fig. 13B), labeled neurons are generally more rostrally placed in area 29d. In the case with a large injection into area 18 (Fig. 13C), the labeling of neurons in area 29d occurs throughout its full rostrocaudal extent. In the two larger injections of area 18 (Fig. 13B,C), labeling also occurs in area 24b, and the laminar position of these neurons is different than that in area 29d. Thus, only neurons in layer

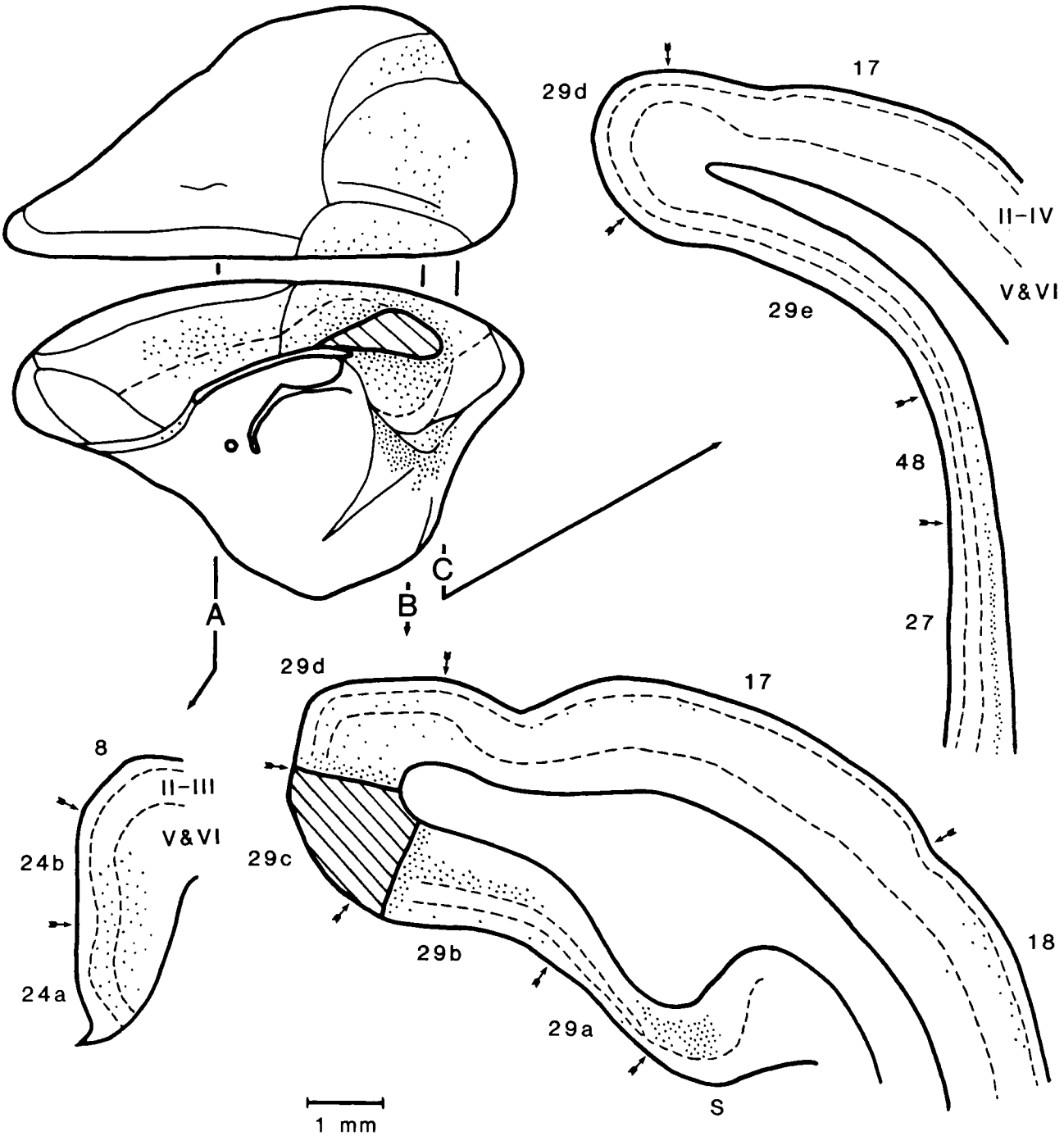

Fig. 11. HRP case in which the injection involved areas 29c and 29b.

V of area 29d label after area 18 injections (Fig. 14) while the HRP-positive neurons in area 24b are mostly in layer III with a few in layer V.

Labeled neurons following area 17 HRP injections are found only in posterior cingulate cortex, and there is a topographic organization of connections between these regions. A large and rostral injection of HRP into area 17 (Fig. 15A) labels neurons mainly in layer V of area 29d. More caudal cases (Fig. 15B,C) label neurons in caudal parts of area 29d but also in parts of areas 29b and 29a and

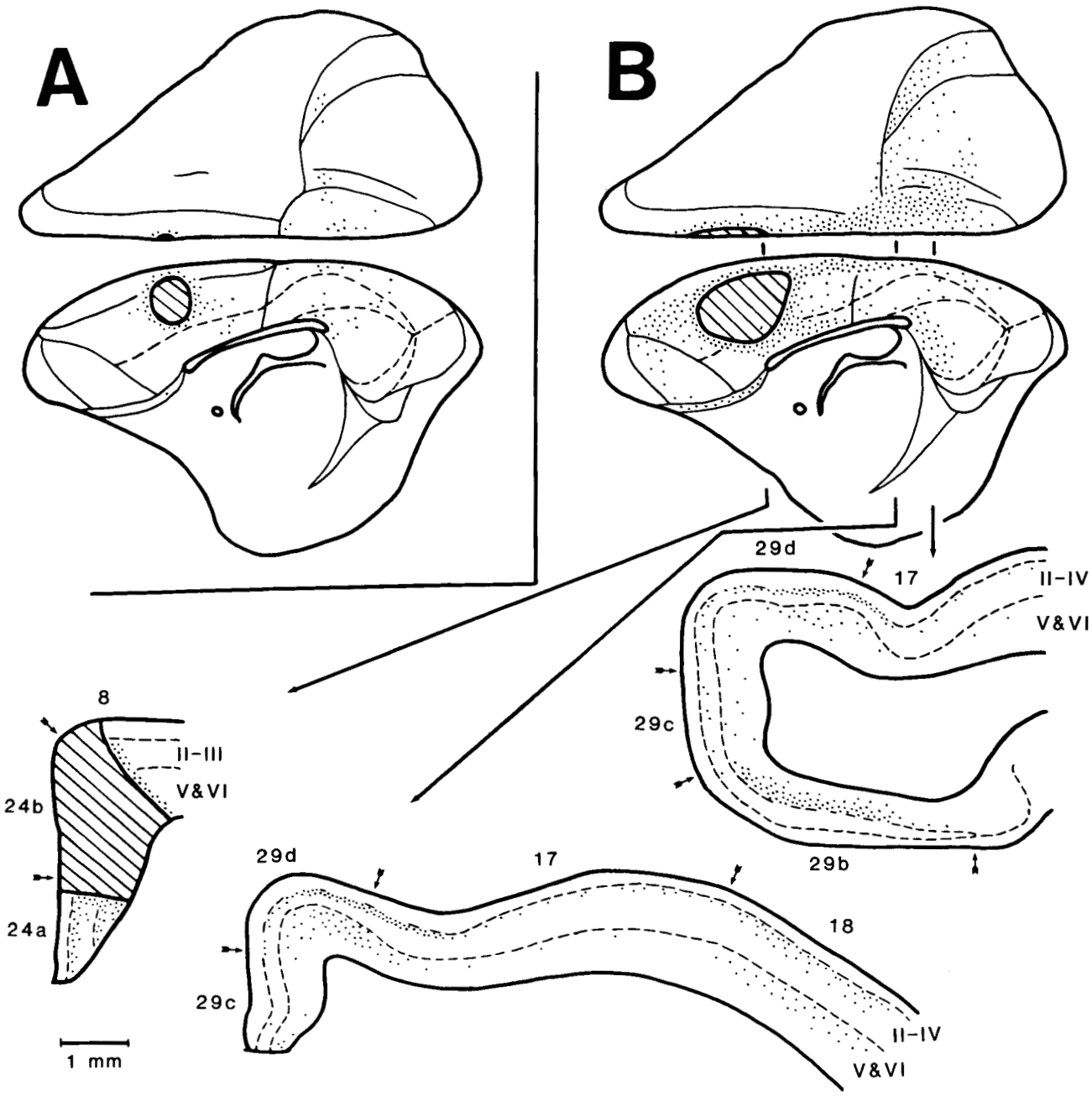

Fig. 12. Distribution of HRP-positive neurons following small (A) and large (B) injections into anterior cingulate cortex.

in much of area 29e. Virtually no labeling of neurons in any of these latter three areas occurs following area 18 injections.

Injections of ^{3}H-amino acids into area 29d label terminals throughout layer I of areas 17 and 18 (Fig. 10B). Linear arrays of grains are present in layers II–IV indicating axons of passage while some horizontally oriented axons are seen in layer VI and deep layer V. Figure 16 presents photographs of a ^{3}H-amino acid injection site in area 29d and the area 17 termination pattern. It is unclear in this material whether there is actually termination in the deeper layers of areas 17 and 18.

Area 8 efferents to visual and cingulate cortices

Injections of HRP into cingulate and visual regions indicate that areas 29d and 18 are the main target areas in these regions to receive motor area 8 input. Thus, rostral injections of HRP into area 29d label neurons in layer III of

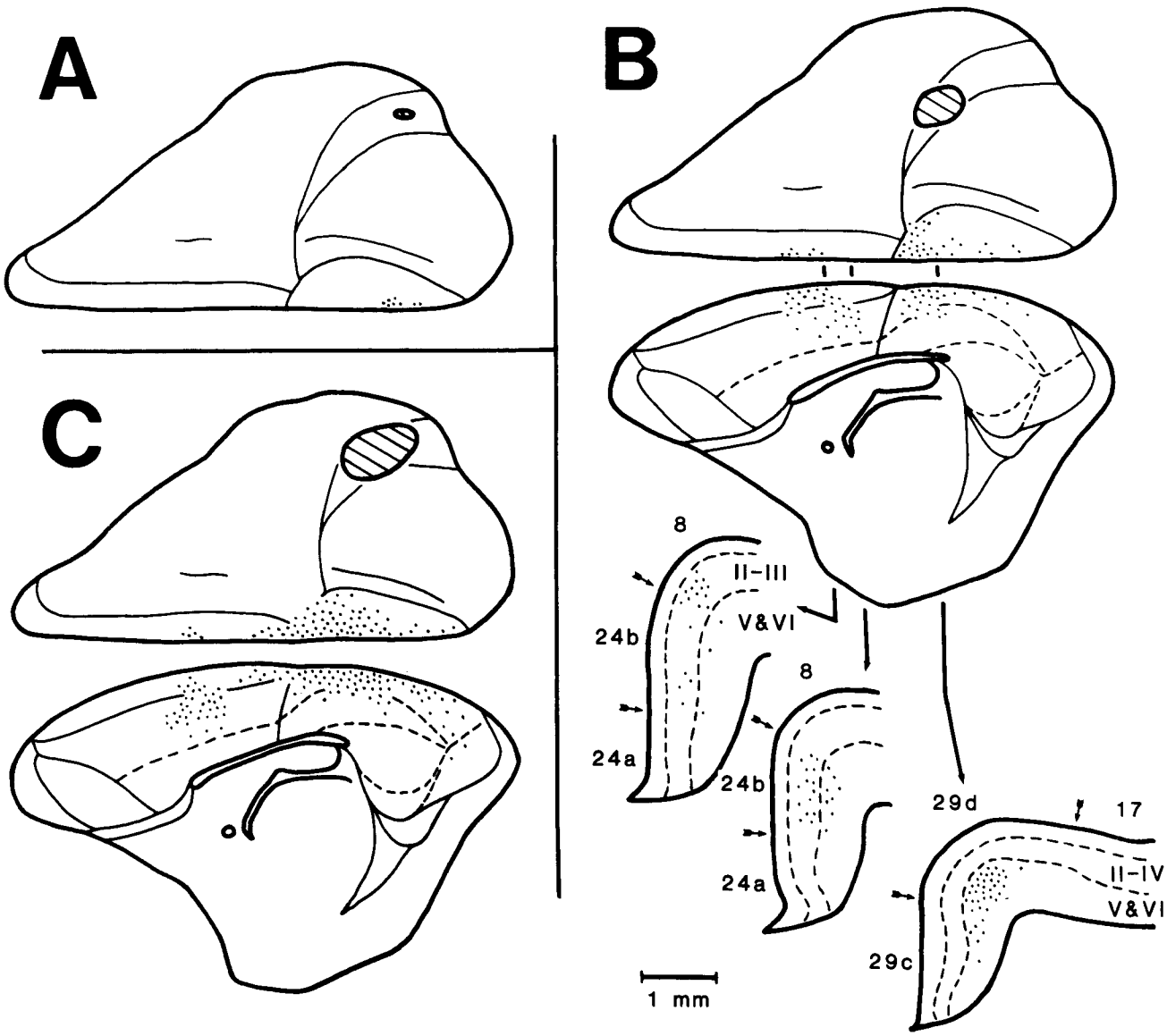

Fig. 13. Three HRP cases with different size injections into area 18. In A there were no labeled neurons in the medial cortex other than the two small patches noted in area 29d on the dorsal surface view.

the caudal part of area 8 (Figs. 7, 9A,B). Caudal and ventral injections into area 29d or areas 29b and c do not produce labeling of neurons in area 8 (Figs. 9, 11). Although no labeling of area 8 neurons occurs following area 17 HRP injections, medium and large HRP injections into area 18 result in labeling of layer III neurons in the medial part of area 8 (Fig. 13). These labeled cells are located more rostrally in area 8 than are labeled cells following any of the area 29d injections.

Postsubicular and subicular connections with cingulate cortex

Interconnections between areas 29d and the postsubiculum (area 48) do not appear to be topographically organized. Injections of each rostrocaudal part of area 29d label neurons in layer V of only the caudal one-third of area 48 (Figs. 7, 9A,C). Only in the smallest injection of area 29d is there no labeling in this part of the postsubiculum (Fig. 9B). Tritiated amino acid injections into area 29d (e.g., Fig. 10B) produce grains over layer I and, to a lesser extent, layer V throughout area 48 and the dorsal part of area 27.

Although there is no labeling of neurons in the subiculum following area 29d HRP injections, there is extensive labeling in the subiculum following the area 29b–c injection presented in Figure 11. In addition, neurons in layer V are labeled throughout most of area 48. Note, however, that in Figure 9C there is a case in which the HRP injection involved the caudal but not rostral part of area 29b. In this

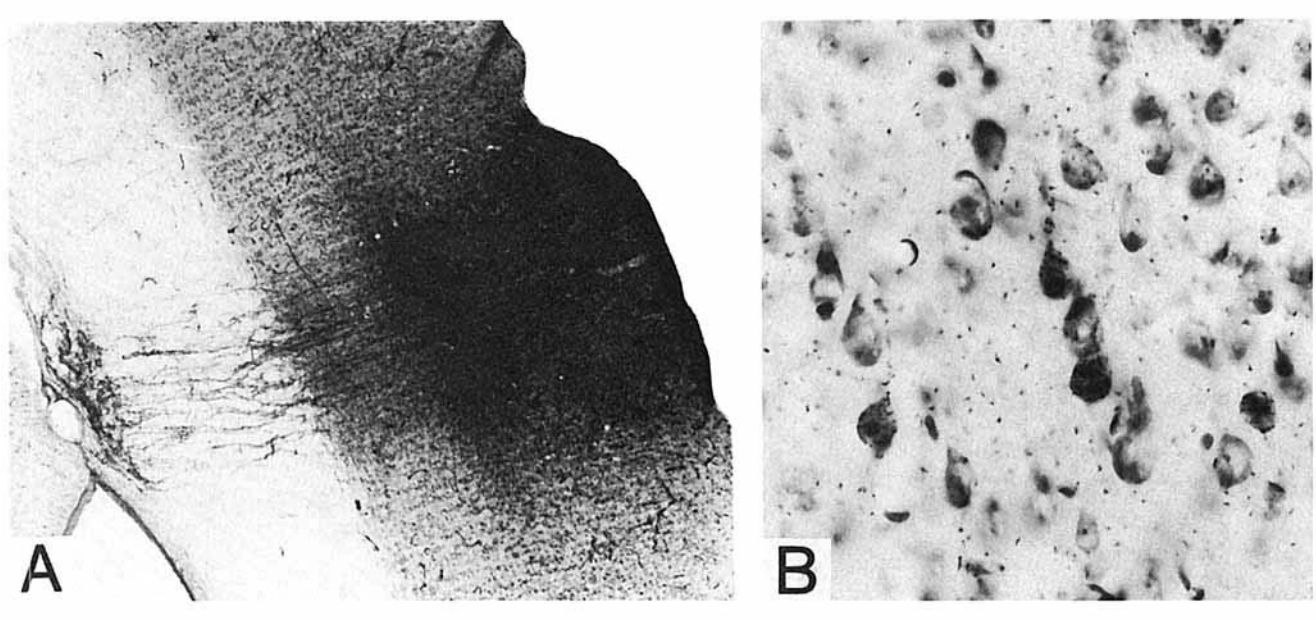

Fig. 14. An example of an area 18 HRP injection (see also Fig. 13B). B. HRP-positive neurons in layer V of area 29d. A. 40×, B. 200×.

case there is no labeling of cells in the subiculum. There is also no labeling in the subiculum or postsubiculum following HRP injections into area 24, 17, or 18.

Intracingulate connections

Injections of HRP into rostral parts of area 29d result in the most extensive labeling of neurons throughout cingulate cortex (Figs. 7, 9A). Thus, labeled neurons are present in almost all of area 29c and rostral area 29b. These cells are in layer V of both areas and are aggregated into clumps (Figs. 7, 8B). Labeled neurons are also scattered throughout ventral area 29e. All rostral cingulate areas contain HRP-labeled cells including areas 24a, 24b, and 25. In the case of area 24 most of these cells are located in layer III with fewer present in layer V. In each case in which injections of ^{3}H-amino acids are in area 29d (Fig. 10B), labeled terminals are mainly in area 24b with less in areas 24a and 25. In the posterior cingulate region grains are over most parts of area 29c and a little in area 29b. In both anterior and posterior cingulate cortices most grains are over layer I but increased densities in layer V suggest that, in addition to fibers of passage in the deep layers, there may also be some termination.

Injection of HRP into granular retrosplenial areas 29b and c labels neurons mainly in area 24b with reduced amounts in area 24a and only lightly in area 25 (Fig. 11). Moderate numbers of labeled neurons occur in area 29d, and when ventral parts of areas 29d, b, and c are involved in an injection site (Fig. 9C), there is virtually no labeling of neurons in dorsal and rostral parts of area 29d. Finally, neither area 29a nor area 29e contains labeled neurons following an area 29b–c injection.

Injections of HRP into area 24b (Fig. 12) label neurons mainly in layers II–III and V of area 29d and layer V of rostral area 29b. In addition, limited numbers of cells are labeled in layers II–III and V of area 29c and layer V of area 25; however, no labeled neurons are in areas 29a or 29e.

DISCUSSION

Of all the divisions of cingulate cortex area 29d stands out in terms of the density and extent of its corticocortical connections (Fig. 17). Its connections with visual, motor, and other cingulate areas suggest that particularly the rostral part of this area may be pivotal in many of the associative functions of the cerebral cortex—especially those related to sensorimotor function, as previously suggested for the rat (Vogt and Miller, '82, '83). Area 29d is also strategically placed to mediate feedback from other parts of the limbic system to visual areas. This latter conclusion is supported by the finding that neurons in area 29c label heavily with HRP following injections into area 29d, while little parahippocampal labeling occurs in these same cases. However, following injections of HRP into areas 29b and c heavy labeling occurs in the postsubiculum, presubiculum, and subiculum. Thus, area 29d is in a pivotal position to mediate limbic system (i.e., hippocampal) feedback into sensory cortices. Bidirectional connections between limbic and sensory cortices in the rat and rabbit provide a unique system in which to address specific sensorilimbic and sensorimotor functions.

Rose and Woolsey ('48) included areas 29c and 29d of Brodmann ('09) in their area cingularis, Cg. Extensive interconnections between area 29d and visual cortices but only weak connections between area 29c and the visual areas strongly support continued use of the Brodmann distinction between these areas. This parcellation is further supported by the preferential termination of the lateroposterior thalamic nucleus in area 29d but not area 29c (Fig. 2 in Towns et al., '82).

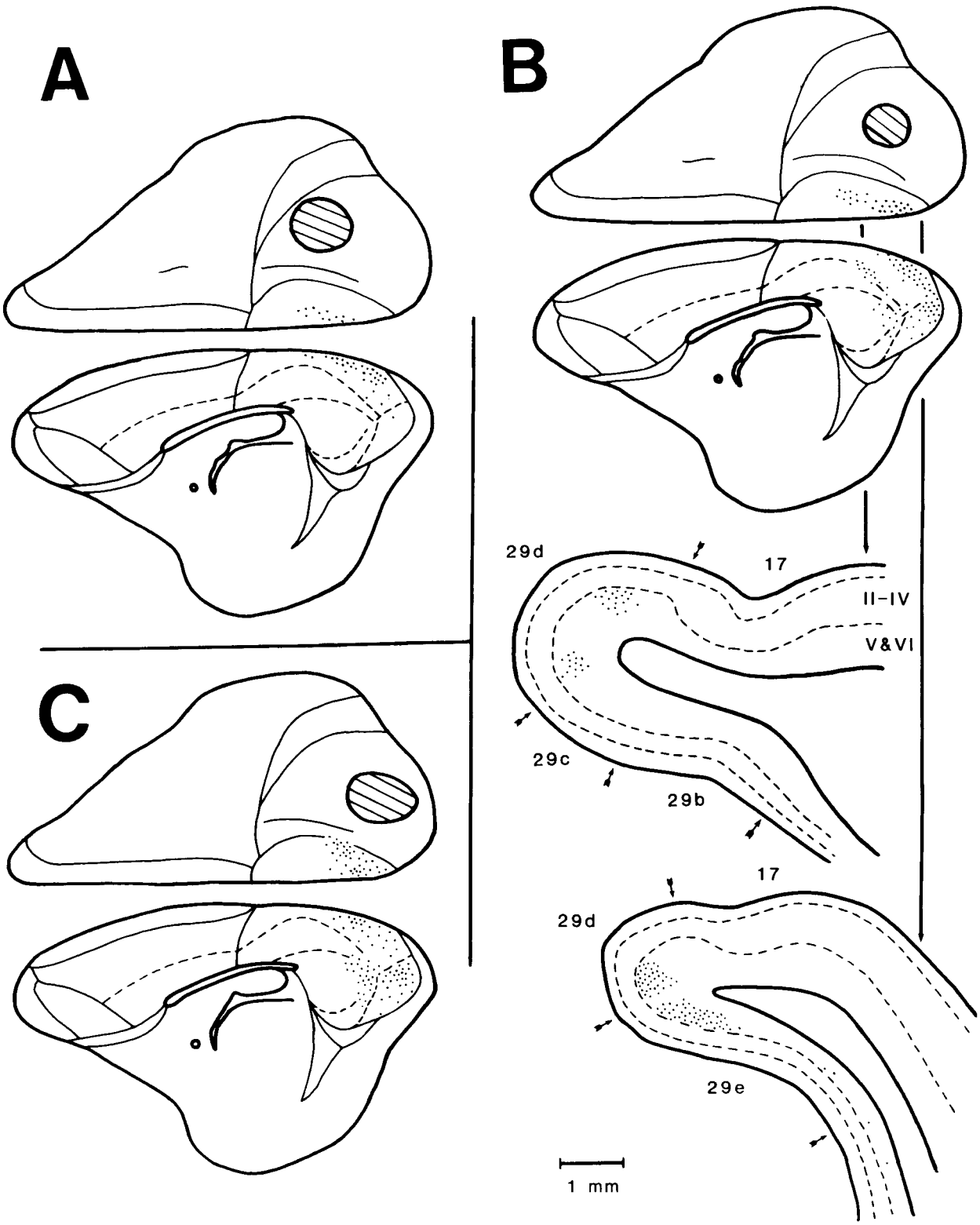

Fig. 15. Location of HRP-labeled cells in posterior cingulate cortex following injections into area 17.

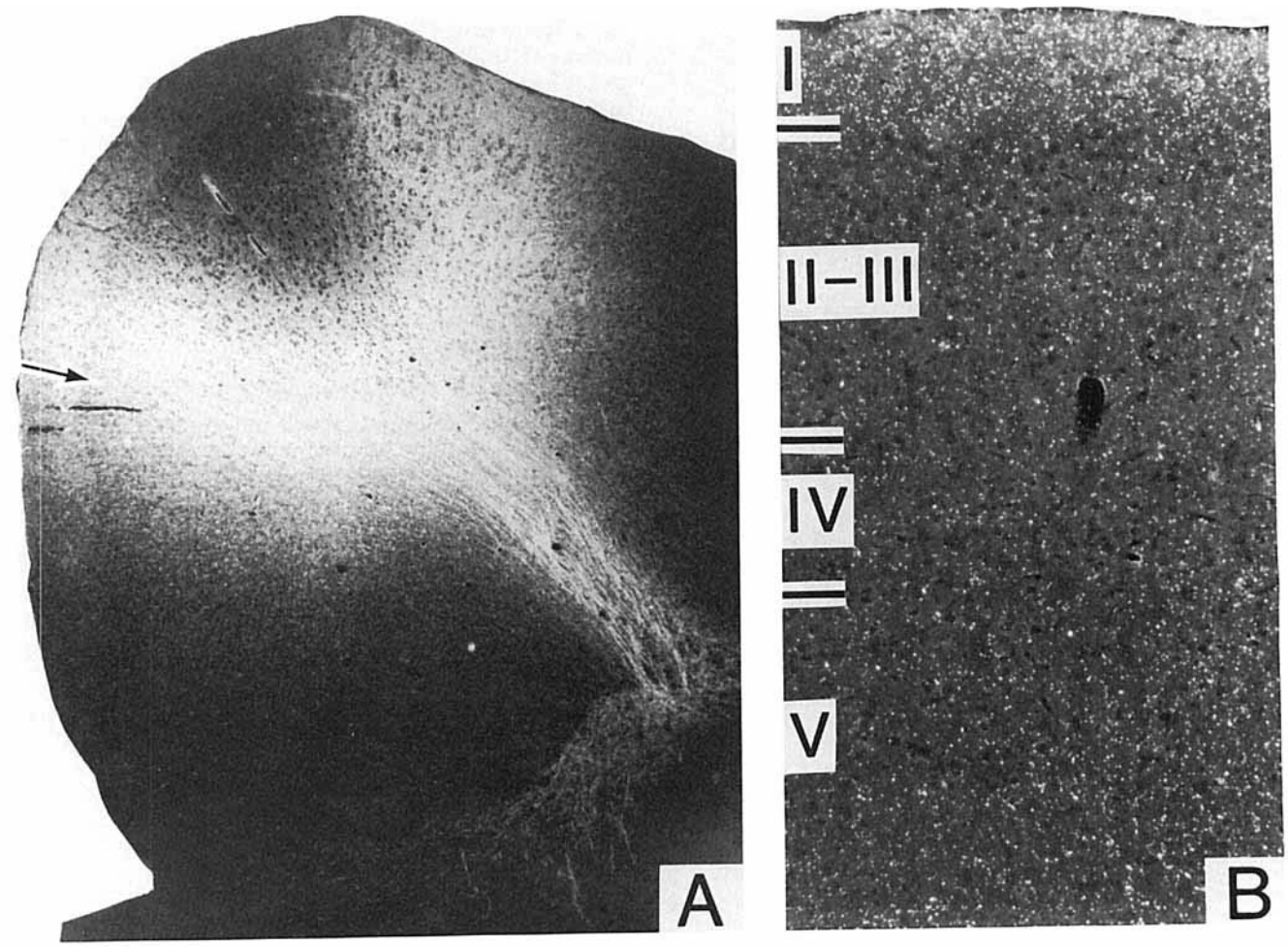

Fig. 16. Photomicrographs of an injection of ^{3}H-amino acids (A, arrow marks the area 29c/29d border) into area 29d (see also Fig. 10B) and a terminal field in area 17 (B). A. 50×, B. 120×.

Cytoarchitecture

Brodmann ('09) made one of the first comprehensive parcellations of the cerebral cortex in a number of species including that of cingulate cortex in the rabbit brain. Since then Krieg ('46) transposed this version with little alteration to the rat, while Rose and Woolsey ('48) devised a somewhat more simplified scheme for the rabbit. These latter authors state, however, that further cytoarchitectural divisions are possible, but their analysis of thalamocortical connections did not support such a detailed approach. We have since modified the Brodmann scheme to incorporate more recent cytological and experimental observations in the rat (Vogt and Peters, '81; Vogt and Miller, '83) and now the rabbit and compared this approach with that of Rose and Woolsey (Vogt, '85). The modified Brodmann scheme is a useful framework within which to analyze the cytology and connections of rabbit cingulate cortex.

Border of areas 17 and 29d

As noted in the beginning of this article, cytoarchitectural, functional and connectional analyses have produced conflicting evidence as to the exact position of the border between cingulate and visual cortices. In the present study, the medial border of the visual field was mapped physiologically and it correlates well with the cytoarchitectural border between areas 17 and 29d.

The portion of the visual receptive field map adjacent to area 29d differs in the rat and rabbit. In the rat an area 18 or 18b is adjacent to area 29d. In area 18b the visual field is rerepresented and the receptive fields of these neurons are larger than those in area 17 (Montero et al., '73; Espinoza and Thomas, '83). Although the receptive fields of neurons around the splenial sulcus in the rabbit are quite large, they represent part of a continuous map of area 17 as observed in the present study and by Thompson et al. ('50). Even though there may not be a separate representation of the visual field around the splenial sulcus, there is a region, part of which Rose ('31) termed area peristriata, that forms reciprocal connections with cingulate cortex. These connections with medial area 17 are more pronounced than are those with lateral area 17. In addition, thalamic afferents to the medial portion of V–I, which relays information about temporal visual fields, differs from those to the lateral part

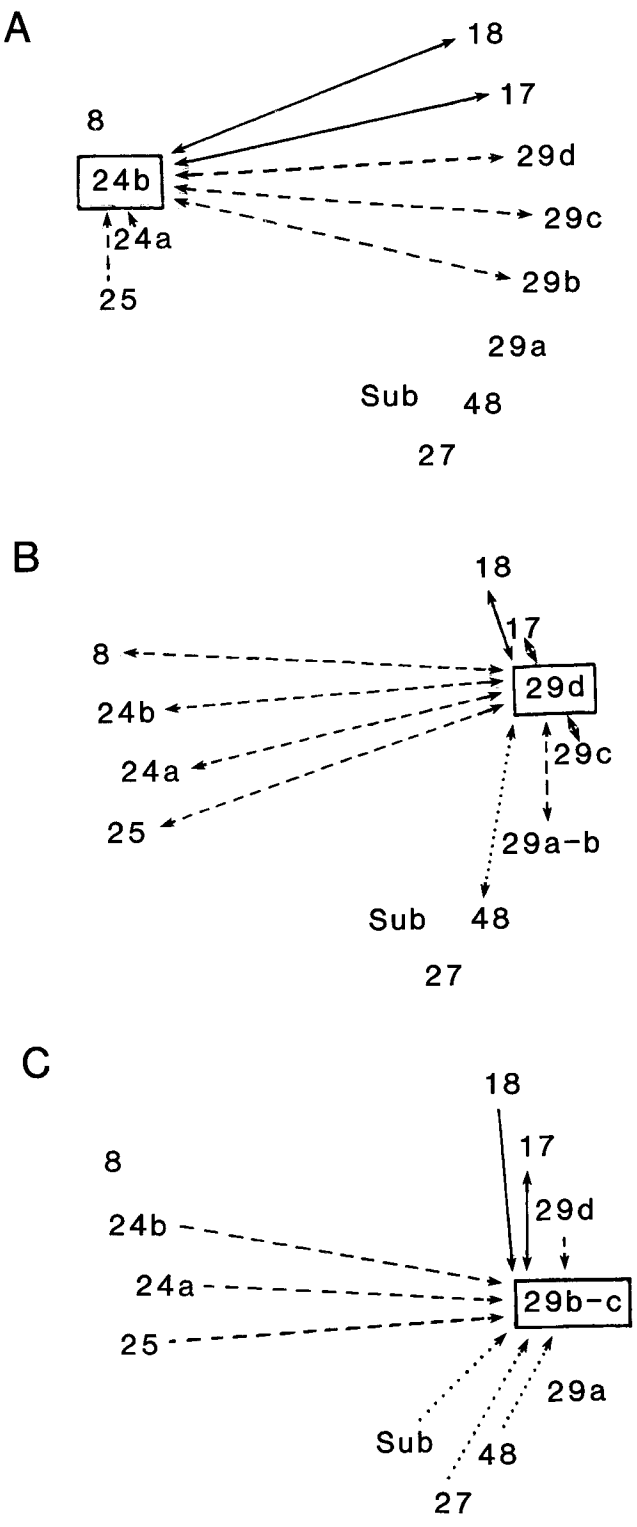

Fig. 17. Summary of some of the major cortical connections of rabbit cingulate cortex including areas 24b (A), 29d (B), and 29b–c (C). Areas are positioned to approximate topographical relationships in rabbit cortex. Visual, intracingulate and motor, and parahippocampal connections are indicated with solid, dashed, and dotted lines, respectively.

of V–I, which relays information about nasal visual fields. Thus, Towns et al. ('82) have shown that the lateroposterior nucleus of thalamus terminates more heavily in medial areas 17 and 29d (their area RS in Fig. 2) than in the lateral part of area 17. These differences in the afferent and efferent connections of the medial and lateral parts of area 17 suggest that these regions of visual cortex may have different response properties and/or that their connections reflect different magnification factors. In the latter case, for example, an increased density of reciprocal connections between medial area 17 and area 29d may counterbalance the decrease in volume of medial visual cortex associated with a constant unit of the visual field in comparison with that of lateral visual cortex.

Visual and cingulate cortical connections

Interconnections between visual and cingulate cortices in the rabbit are similar in many ways to those reported in the rat. Thus, it is primarily areas 24b and 29d that are connected with area 18. It is also true in both species that the predominant layer of termination in each of these cortices is layer I although the projection of areas 17 and 18 to area 29d may involve additional termination in layers III and IV. Layer I termination in sensory regions of primates is often related to reciprocal projections terminating in primary sensory areas. Projections of visual areas 19 to 18 and areas 18 to 17 terminate in layer I (Rockland and Pandya, '79), somatosensory connections of area 1 terminate in layer I of area 3 (Vogt and Pandya, '78), and parakoniocortical auditory areas terminate in layer I of koniocortical auditory cortex (Pandya and Sanides, '73). It is possible that layer I terminations activate mainly the apical dendrites of pyramidal projection neurons while those in deeper layers are more likely to activate local circuit neurons.

One of the major differences between visual and cingulate connections in rat and rabbit is the laminar origin of these projections. Thus, the projection from area 18 in rat originates mainly from layer V cells while in the rabbit it originates mainly from the most superficial part of layer II–III with a much smaller contribution from neurons deeper in layer II–III and from layer V. Note, however, that the reciprocal projection from area 29d originates from neurons in layer V of both species. Although cells in layer II–III of rat and rabbit visual cortex have visual receptive fields (Chow et al., '71; Murphy and Berman, '79; Parnavelas et al., '83), this is not true of neurons in cingulate cortex since no visually driven units were observed in this region in the present analysis. It is possible, therefore, that if visual receptive field properties are transmitted directly to cingulate cortex, they will require conditioning to uncover responses related to neuronal activity in layer II–III of visual cortex.

It is generally agreed that layers II and III of visual cortex are cytoarchitecturally indistinct in both rabbit and cat so most authors recognize a single layer II–III (e.g., Rose, '31; O'Leary, '41; Rose and Malis, '65; Lund et al., '79). Previous work on the laminar origin of efferent projections of area 17 has supported this notion because the cells of origin of both the callosal and V–II projections are found throughout the thickness of layer II–III (Swadlow and Weyand, '81). The present work demonstrating a projection from the superficial portion of layer II–III, i.e., just beneath the molecular layer, to cingulate cortex may be evidence for a further division of this layer into superficial and deep parts.

Connections between visual cortices and area 29d are organized topographically but this organization is not as refined as other connections of visual cortex. Rostral parts of area 29d are reciprocally connected with rostral parts of visual areas 17 and 18, and caudal parts of each of these areas are reciprocally connected. Whatever transformations occur via visual efferent projections to cingulate cortex, these transformations still bear a relationship to the retinotopic map in visual cortex. Since neurons in cingulate cortex can be conditioned to differentiate among pairs of different-frequency tones, one of which is associated with aversive footshock (Gabriel et al., '80), one possible transformation is learning the significance of a visual stimulus in a particular part of the visual field. This conditioning might involve avoidance of a visual stimulus such as a looming object in the peripheral visual field. Another possible function of topographically organized visual/cingulate connections might be to relate head position to visual space, as has been observed for neurons in the postsubiculum (Ranck, '84).

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