

# Topographically Specific Hippocampal Projections Target Functionally Distinct Prefrontal Areas in the Rhesus Monkey

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**ABSTRACT:** The sources of ipsilateral projections from the hippocampal formation, the presubiculum, area 29a-c, and parasubiculum to medial, orbital, and lateral prefrontal cortices were studied with retrograde tracers in 27 rhesus monkeys. Labeled neurons within the hippocampal formation (CA1, CA1', prosubiculum, and subiculum) were found rostrally, although some were noted throughout the entire rostrocaudal extent of the hippocampal formation. Most labeled neurons in the hippocampal formation projected to medial prefrontal cortices, followed by orbital areas. In addition, there were differences in the topography of afferent neurons projecting to medial when compared with orbital cortices. Labeled neurons innervating medial cortices were found mainly in the CA1' and CA1 fields rostrally, but originated in the subicular fields caudally. In contrast, labeled neurons which innervated orbital cortices were considerably more focal, emanating from the same relative position within a field throughout the rostrocaudal extent of the hippocampal formation.

In marked contrast to the pattern of projection to medial and orbital prefrontal cortices, lateral prefrontal areas received projections from only a few labeled neurons found mostly in the subicular fields. Lateral prefrontal cortices received the most robust projections from the presubiculum and the supracallosal area 29a-c. Orbital, and to a lesser extent medial, prefrontal areas received projections from a smaller but significant number of neurons from the presubiculum and area 29a-c. Only a few labeled neurons were found in the parasubiculum, and most projected to medial prefrontal areas.

The results suggest that functionally distinct prefrontal cortices receive projections from different components of the hippocampal region. Medial and orbital prefrontal cortices may have a role in long-term mnemonic processes similar to those associated with the hippocampal formation with which they are linked. Moreover, the preponderance of projection neurons from the hippocampal formation innervating medial when compared with orbital prefrontal areas followed the opposite trend from what we had observed previously for the amygdala (Barbas and De Olmos [1990] *J Comp Neurol* 301:1-23). Thus, the hippocampal formation, associated with mnemonic processes, targets predominantly medial prefrontal cortices, whereas the amygdala, associated with emotional aspects of memory, issues robust projections to orbital limbic cortices. Lateral prefrontal cortices receive robust projections from the presubiculum and area 29a-c and sparse projections from the hippocampal formation. These findings are consistent with the idea that the role of lateral prefrontal cortices in memory is distinct from that of either medial or orbital cortices. The results suggest that signals from functionally distinct limbic structures to some extent follow parallel pathways to functionally distinct prefrontal cortices. © 1995 Wiley-Liss, Inc.

**KEY WORDS:** medial prefrontal cortex, orbitofrontal cortex, memory, CA1, subiculum, presubiculum, area 29, lateral prefrontal cortex, working memory

## INTRODUCTION

The prefrontal cortex in the rhesus monkey is a large cortical expanse associated with complex cognitive, mnemonic, and emotional processes (for reviews, see Fuster, 1989; Barbas, 1995a). The functions of prefrontal areas are likely to depend on their connections with other cortical and subcortical structures. Classically known as polymodal, prefrontal cortices receive distributed projections from areas associated with each of the sensory modalities but do not appear to be committed to processing input from any single sensory modality (for reviews, see Barbas, 1992, 1995a). Rather, sensory input to prefrontal areas appears to be linked to action (for reviews, see Fuster, 1990, 1993), a hypothesis supported by the close connective association of prefrontal cortices with the premotor and supplementary motor cortices (Matelli et al., 1986; Barbas and Pandya, 1987; Arikuni et al., 1988). In addition, prefrontal areas are characterized by the robust projections they receive from limbic structures as noted in classic studies (Nauta, 1971, 1972, 1979). The limbic system has been implicated in emotional and mnemonic processes, and its input onto prefrontal areas is likely to have profound effects on their function and on behavior (for review, see Damasio, 1994).

Input from sensory and limbic structures to the various prefrontal areas is not uniform. The connective heterogeneity of prefrontal cortices is likely to underlie their functional specialization. Our previous studies suggest that the connective organization of prefrontal cortices appears to depend on two major factors. One is based on cortical type, and the other is based broadly on cortical region (for reviews, see Barbas, 1992, Barbas, 1995a). With regard to cortical type, prefrontal areas

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vary widely, ranging from those which have three or four layers, exemplified in transitional (limbic) cortices, to those which have six layers, which typify eulaminate areas. Although input to eulaminate prefrontal areas is distributed, by comparison with the limbic areas it is considerably more focal. Thus, sensory input to those eulaminate prefrontal areas with the best laminar definition originates from cortices associated with one or two modalities, intrinsic input is from neighboring cortices, thalamic projections emanate primarily from the mediodorsal nucleus, and input from limbic structures is sparse (for review, see Barbas, 1995a). In contrast, the structurally identified prefrontal limbic areas, situated on the caudal orbital and medial surfaces, have the most diverse connections among prefrontal areas. Thus, prefrontal limbic cortices receive robust projections from cortical and subcortical limbic structures, from several modality-specific and polymodal cortices, and from a diverse set of thalamic nuclei (for review, see Barbas, 1995a).

The connections of prefrontal areas also vary broadly on a regional basis. Thus, prefrontal cortices situated on the medial and dorsolateral aspect of the cerebral hemisphere receive input associated with spatial aspects of the sensory environment and spatial memory. In contrast, prefrontal areas found on the basal and ventrolateral aspect of the cerebral hemisphere process input related to the features of stimuli and their memory (Bauer and Fuster, 1976; Fuster et al., 1985; Barbas, 1988; Wilson et al., 1993).

There is less information on the regional organization of projections from limbic structures to functionally distinct prefrontal cortices. This information is important, because like sensory areas, different limbic structures have functionally distinct attributes. For example, the amygdala and the hippocampus have different roles in the emotional and mnemonic functions classically associated with the limbic system (Nishijo et al., 1988; Zola-Morgan et al., 1989b, 1991; Davis, 1992). Projections from limbic structures to prefrontal cortices are, to some extent, regionally specific. Thus, although the amygdala primarily targets the limbic areas of the prefrontal cortex, it projects most heavily to its caudal orbital component, and to a lesser extent to the medial limbic areas. The origins of input from limbic thalamic nuclei to orbital and medial parts of limbic prefrontal cortex are also distinct (Barbas et al., 1991; Dermon and Barbas, 1994).

The organization of specific projections from other limbic

structures to prefrontal cortices has not been clearly defined. The hippocampus projects to some prefrontal areas (Rosene and Van Hoesen, 1977; Goldman-Rakic et al., 1984; Morecraft et al., 1992), although the extent of its influence onto prefrontal cortices is not known. Situated in the temporal lobe behind the amygdala, the hippocampus has been implicated in specific aspects of mnemonic processing distinct from the role of the amygdala in emotive behavior (Zola-Morgan et al., 1989b, 1991; for review, see Zola-Morgan and Squire, 1993). In this study we addressed several questions about the interface of the hippocampus with prefrontal cortices within the context of the structural attributes of both the prefrontal cortex and the hippocampal region. Which prefrontal areas receive projections from the hippocampus? Does the hippocampus have a regionally specific projectional relationship with distinct prefrontal cortical sectors, as was noted for the amygdala? Are there differences in the topography or number of hippocampal neurons projecting to the prefrontal limbic cortices on the medial or orbital surfaces and to eulaminate areas on the lateral surface?

## MATERIALS AND METHODS

### Surgical Procedures

Experiments were conducted on 27 rhesus monkeys (*Macaca mulatta*) according to the NIH Guide for the Care and Use of Laboratory Animals (NIH publication 80-22, 1987). The animals were anesthetized with ketamine hydrochloride (10 mg/kg, intramuscularly) followed by sodium pentobarbital administered intravenously through a femoral catheter until a surgical level of anesthesia was achieved. Additional anesthetic was administered during surgery as needed. Surgery was performed under aseptic conditions. The monkey's head was firmly positioned in a holder which left the cranium unobstructed for surgical approach. A bone defect was made, the dura was retracted, and the cortex was exposed.

Injections of horseradish peroxidase conjugated to wheat germ agglutinin (HRP-WGA, Sigma, St. Louis, MO) were placed in prefrontal cortices in 21 animals, and fluorescent dyes (fast blue, diamidino yellow, or rhodamine-labeled latex microspheres) were injected in six animals. All injections were made with a microsyringe (Hamilton, 5  $\mu$ l, Reno, Nevada) mounted on a microdrive. The needle was lowered to the desired site under microscopic guidance. In each case small amounts (0.05  $\mu$ l, 8% HRP-WGA; 0.4  $\mu$ l, 3% diamidino yellow, fast blue, or rhodamine-labeled latex microspheres) of the injectate were delivered 1.5 mm below the pial surface at each of two adjacent sites separated by 1–2 mm over a 30-min period. In cases with fluorescent dyes two or three different sites can be injected in each animal. The total number of prefrontal sites examined was 33. Of these, eight were placed in medial prefrontal cortices, 13 in orbital areas, and 12 in lateral areas.

In the HRP experiments 40–48 h after injection the monkeys were anesthetized deeply and perfused through the heart with saline followed by 2 liters of fixative (1.25% glutaraldehyde, 1% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4), followed by 2 liters of cold (4°C) phosphate buffer (0.1 M, pH 7.4). The

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Abbreviations used: A = arcuate sulcus; CA1–CA4 = Cornu Ammonis CA1–CA4: hippocampal fields of Lorente de N6 (1934); CC = corpus callosum; Cg = cingulate sulcus; cs = calcarine sulcus; CTA = corticoamygdaloid transition area; DG = dentate gyrus; dy = diamidino yellow dye; EC = entorhinal cortex; Fm = fimbria; fb = fast blue dye; gc = granule cell layer of the dentate gyrus; HATA = hippocampal-amygdaloid transition area; hf = hippocampal fissure; IG = Indusium griseum; LO = lateral orbital sulcus; mf = mossy fiber layer (stratum lucidum); MO = medial orbital sulcus; OLF = olfactory: olfactory tubercle, anterior olfactory nucleus; P = principal sulcus; PAlI = periallocortex (agranular cortex); ParaS = parasubiculum; Pir = prepiriform cortex; PreS = presubiculum; ProS = prosubiculum; r = rhodamine-labeled latex microspheres; Ro = rostral sulcus; S = subiculum; TH = cortical area TH of Von Bonin and Bailey, (1947).

brain then was removed from the skull, photographed, placed in glycerol phosphate buffer (10% glycerol and 2% dimethylsulfoxide [DMSO] in 0.1 M phosphate buffer at pH 7.4) for 1 day and in 20% glycerol phosphate buffer for another 2 days. The brain then was frozen in  $-75^{\circ}\text{C}$  isopentane (Rosene et al., 1986), transferred to a freezing microtome, and cut in the coronal plane at  $40\ \mu\text{m}$  in ten series. One series of sections was treated to visualize HRP (Mesulam et al., 1980). The tissue was mounted, dried, and counterstained with neutral red.

In animals injected with fluorescent dyes the survival period was 10 days. The animals then were anesthetized deeply and perfused with 4% or 6% paraformaldehyde in 0.1 M cacodylate buffer (pH 7.4). The brain then was post-fixed in a solution of 4% or 6% paraformaldehyde with glycerol and 2% DMSO, frozen, and cut as described above. Three adjacent series of sections were saved for microscopic analysis. These series were mounted and dried onto gelatin-coated slides. The three series were stored in light tight boxes with Drierite at  $4^{\circ}\text{C}$ . The first series was coverslipped with Fluoromount 7 days later and was returned to dark storage at  $4^{\circ}\text{C}$ . The second series was left uncoverslipped and was used to chart the location of retrogradely labeled neurons. After the second series was charted it was stained with cresyl violet, coverslipped, and used to determine cytoarchitectonic boundaries. Because the cresyl violet obscured the fluorescence of series 2, further verification of the projection zones was made from the immediately adjacent sections from series 1 and 3.

In all experiments, series of sections adjacent to those prepared to visualize retrograde tracer labeling were stained for Nissl bodies, and acetylcholinesterase (AChE), or myelin (or both) to aid in delineating architectonic borders (Geneser-Jensen and Blackstad, 1971; Gallyas, 1979).

## Data Analysis

Brain sections prepared according to the methods described above were viewed microscopically under brightfield and dark-field illumination for HRP cases or under fluorescence illumination in experiments with dyes. Hippocampal drawings, the location of labeled neurons ipsilateral to the injection site, and the site of blood vessels used as landmarks were transferred from the slides onto paper by means of a digital plotter (Hewlett Packard, 7475A) electronically coupled to the stage of the microscope and to a computer (Austin 486). In this system the analog signals are converted to digital signals via an analog-to-digital converter (Data translation) in the computer. Software developed for this purpose ensured that each labeled neuron noted by the experimenter was recorded only once, as described previously (Barbas and De Olmos, 1990). Movement of the stage of the microscope was recorded via linear potentiometers (Vernitech) mounted on the X and Y axes of the stage of the microscope and coupled to a power supply. This procedure allows accurate topographic presentation of labeled neurons within the hippocampus.

All of the prepared slides through the hippocampus in one series were examined and charted. Labeled neurons were counted by outlining the area of interest (e.g., one field) by moving the X and Y axes of the stage of the microscope. The number of labeled

neurons within the enclosed areas was calculated by an algorithm written for this purpose.

## Reconstruction of Cortical Injection Sites and Hippocampal Projection Sites

The cortical regions containing the injection sites were reconstructed serially by using the sulci as landmarks, as described previously (Barbas, 1988), and are shown on diagrams of the surface of the cortex. The latter were drawn from photographs of each brain showing the external morphology of the experimental hemispheres. References to architectonic areas of the prefrontal cortex are according to a previous study (Barbas and Pandya, 1989).

Retrogradely labeled neurons throughout the hippocampal formation were represented on two-dimensional flattened maps of the hippocampus (Blatt and Rosene, 1988), according to a modification of a method described previously (Van Essen and Maunsell, 1980). To minimize distortion, measurements of areas were made through the middle of the pyramidal cell layer in coronal sections throughout the hippocampal formation. In the flattened map each contour represents the area of the hippocampus in one coronal section. The map represents ammonic fields CA1-CA3, the prosubiculum, subiculum, CA1', a rostral subfield in the uncus, and the anterior body of the hippocampal formation (Rosene and Van Hoesen, 1987). The dentate gyrus and the hilar region, which are not involved in prefrontal cortical projections, are not included in the two-dimensional map.

Comparison of the flattened hippocampal maps in three animals revealed that the overall shape of the flattened subfields was similar and the contours were relatively invariant as well. This consistency may reflect the simple architecture of the hippocampus and the consistent blocking and processing of each brain. Thus, one map was used as a template and retrogradely labeled neurons were plotted by identifying the corresponding rostro-caudal level for each case.

## RESULTS

### Hippocampal Terminology and Borders

The terminology for the hippocampus is according to the map of Lorente de N6 (1934), which has been adapted by other investigators (for review, see Rosene and Van Hoesen, 1987) or used in a slightly modified form (Amaral and Insausti, 1990). The term hippocampal formation in this report refers to the dentate gyrus, fields CA1-CA4, the prosubiculum, and subiculum (for review, see Rosene and Van Hoesen, 1987). The term hippocampal formation has also been employed more broadly to include all of the above regions as well as the presubiculum, parasubiculum, and the entorhinal cortex (Amaral and Insausti, 1990). Here we will present the projections of the hippocampal formation in its narrower sense and distinguish them from those emanating from the presubiculum and parasubiculum. The reason for the distinction is based on structural grounds (Rosene and Van Hoesen, 1987). The fields of the hippocampal formation belong to the allocorti-

cal architectonic type (Fig. 1), whereas the presubiculum and parasubiculum can be considered periallocortical (Saunders and Rosene, 1988). Projections from the entorhinal cortex, which were included in cortical studies previously (e.g., Barbas, 1993), will not be considered here.

Investigators generally agree on the borders of the various hippocampal fields, although there are some differences in the precise terminology used (for discussion, see Amaral, 1987; Rosene and Van Hoesen, 1987). The minor differences encountered commonly in the modern literature will be pointed out briefly so that comparisons can be made among studies that use one or the other convention. Investigators who have adhered to the parceling of Lorente de N6 (1934) have distinguished between an area called the prosubiculum and the subiculum (Rosene and Van Hoesen, 1987), whereas others consider the entire region to be part of the subiculum (Amaral and Insausti, 1990). In this report we have retained the designation prosubiculum because its borders are prominently defined by acetylcholinesterase (AChE; Fig. 2). Another difference centers on the term used for a rostral field, designated CA1' by Rosene and Van Hoesen (1987), but considered to be part of the subiculum by Amaral and Insausti (1990). Finally, the ammonic field which dips into the hilar region has been referred to as CA4 in the nomenclature of Lorente de N6 (1934), but is considered to be a continuation of CA3 by Amaral and Insausti (1990).

Architectonic boundaries of hippocampal fields containing labeled neurons were determined from series of matched sections stained with cresyl violet and myelin or AChE using criteria described by other investigators (Bakst and Amaral, 1984; Rosene and Van Hoesen, 1987; Figs. 1, 2). In cases with injections of fluorescent dyes, hippocampal borders were placed in the same brain sections used for recording labeled neurons which were stained with cresyl violet after charting.

## Injection Sites

Most of the cases with labeled neurons in the hippocampal formation have been described in detail in previous studies in connection with their amygdaloid (Barbas and De Olmos, 1990), thalamic (Barbas et al., 1991; Dermon and Barbas, 1994), or cortical projections (Barbas, 1988, 1993, 1995b). In recent studies these cases have been identified by the same codes (Barbas, 1993, 1995b; Dermon and Barbas, 1994), which also included the designations used in older studies. Two cases which have not been described in recent studies were denoted previously (Barbas and Mesulam, 1985) as case v (case SF here) and, in Barbas and De Olmos (1990), as case 6 (case MAV here).

Intact axons in the white matter are not thought to take up HRP, although there is less information about fluorescent dyes in this regard (LaVail, 1975; Mesulam, 1982). The injections in this study were relatively small, and in all but one case, the needle mark was restricted to the cortical mantle with no apparent damage to the underlying white matter. In case A1b the dye spread to the adjacent tip of the corpus callosum. At that level the corpus callosum carries fibers which interconnect prefrontal areas of the two hemispheres (Barbas and Pandya, 1984), and it is unlikely that the pattern of hippocampal projections was affected in

this case. At the level of the injection in case A1y the olfactory tubercle is continuous with the ventral striatum, and there is no white matter border between the two structures (for review, see Alheid et al., 1990). In this animal the injection extended 1.8 mm deep from the pial surface.

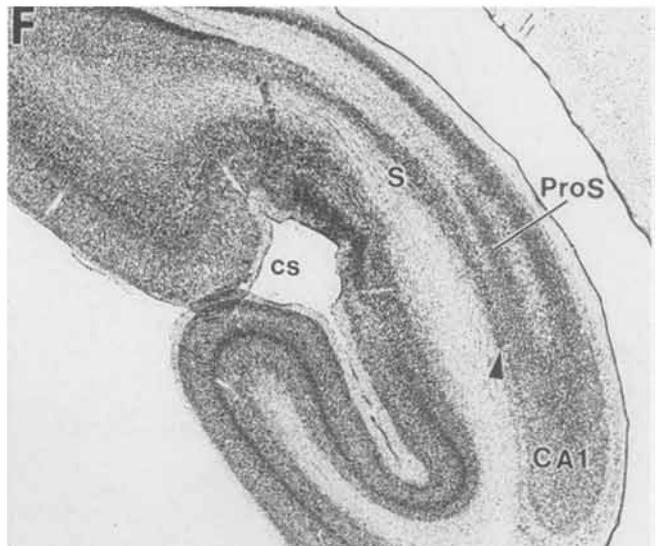
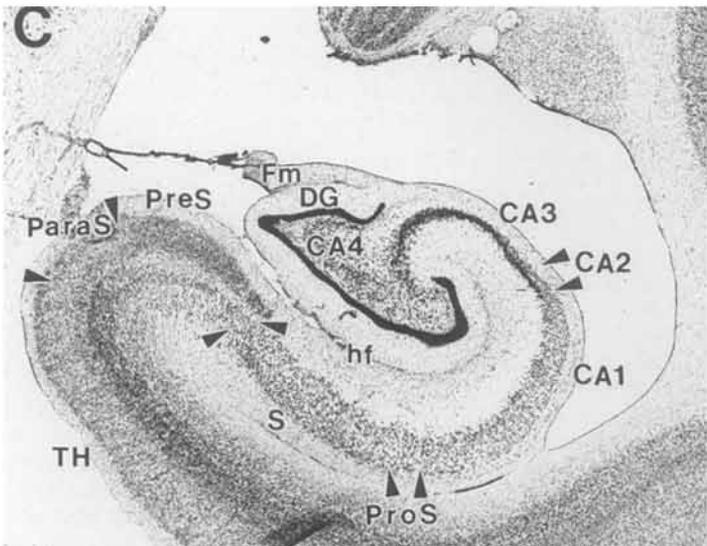
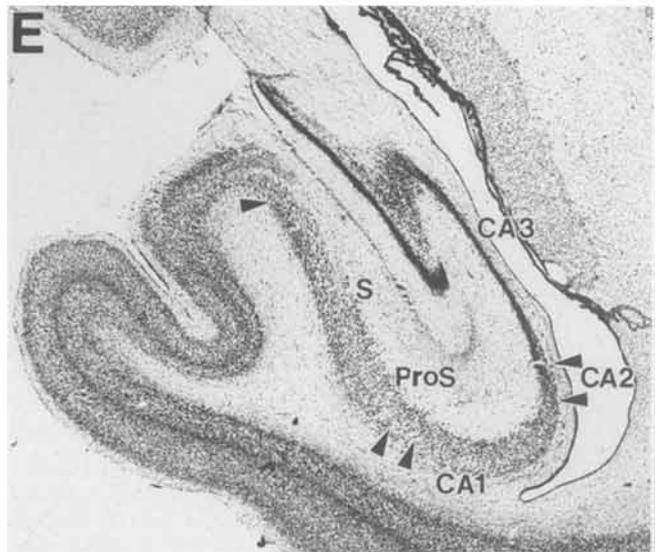
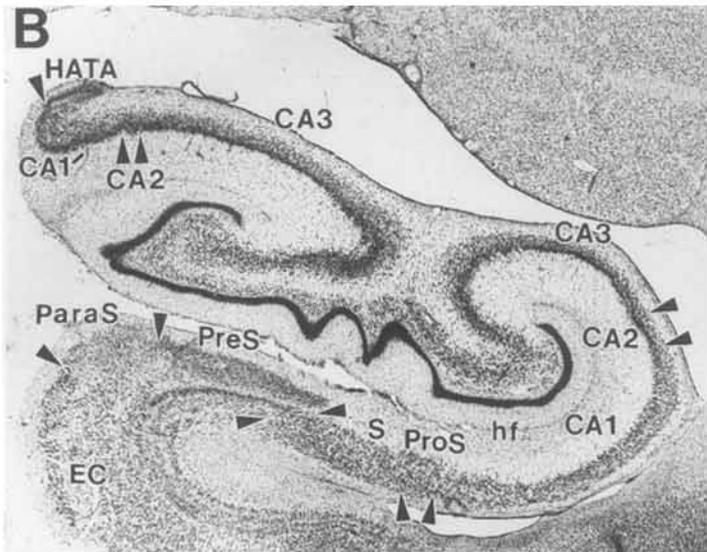
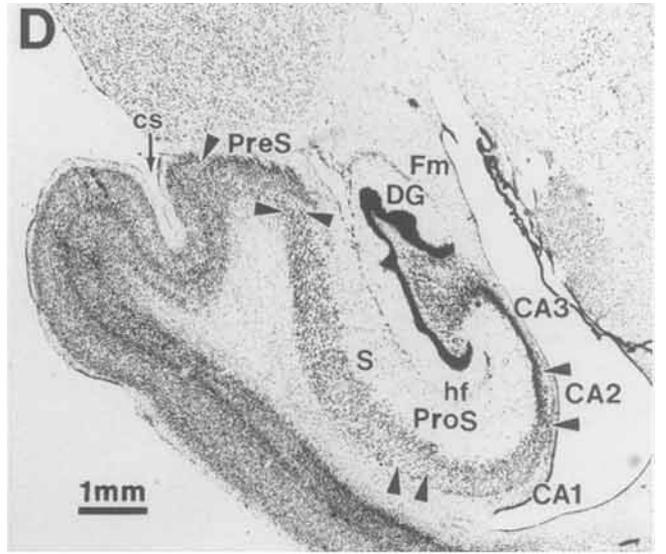
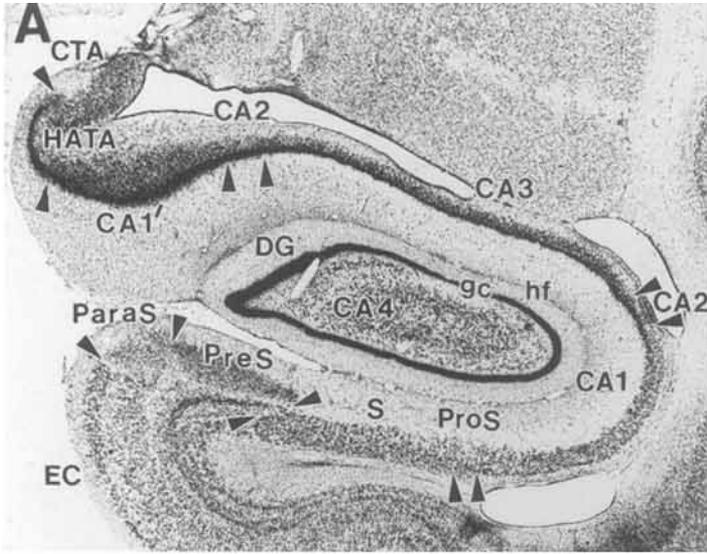
## Density Estimates of Labeled Neurons

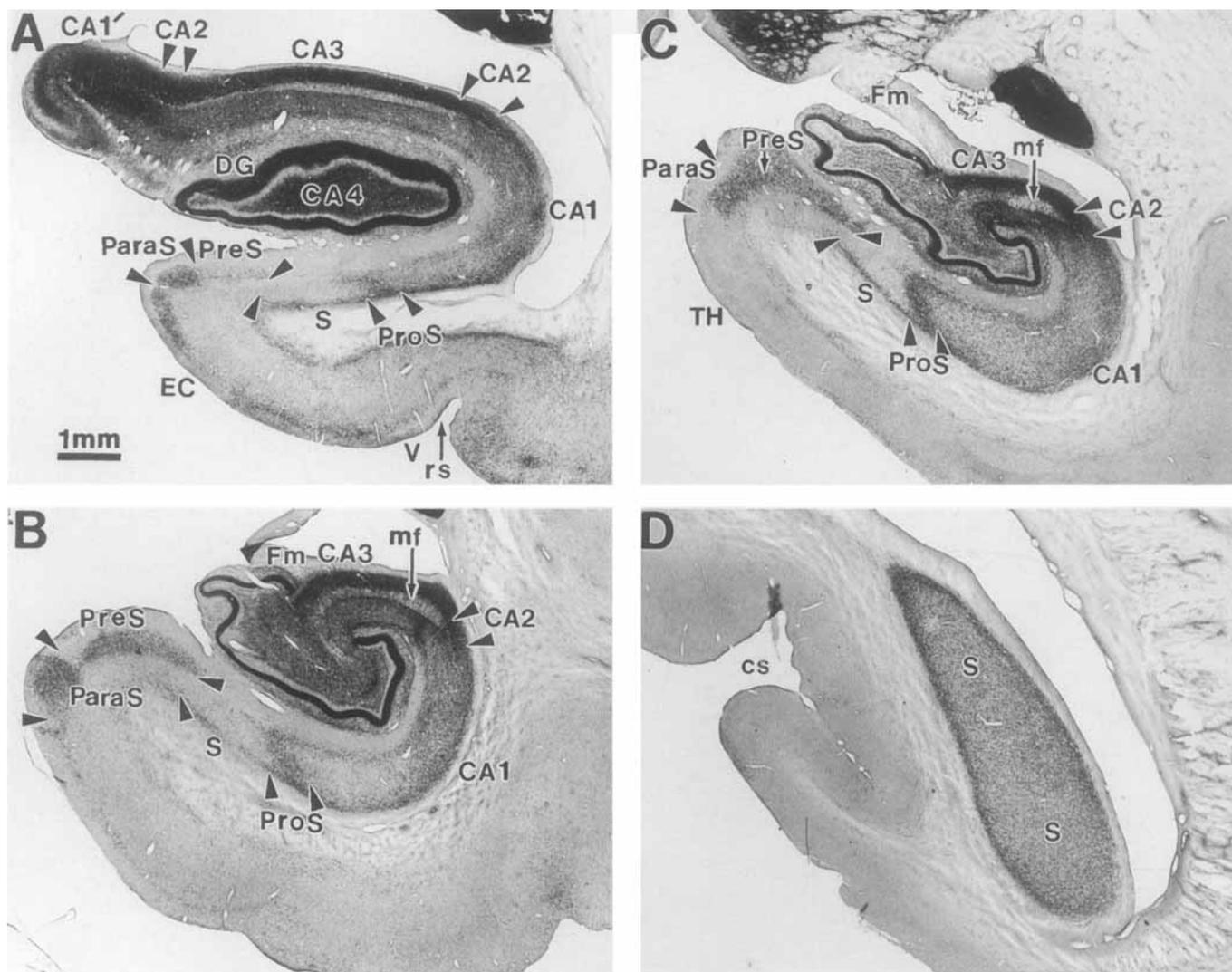
The data described below are based on counts of labeled neurons throughout the hippocampal formation, the presubiculum, and parasubiculum. The size of the injection sites and the density of labeled neurons varied among cases (Fig. 3). However, there was no significant correlation between the size of the injection site and the number of labeled neurons in the hippocampal formation ( $r_{rbo} = 0.22$ ,  $P > 0.1$ ) or in the presubiculum and parasubiculum ( $r_{rbo} = 0.14$ ,  $P > 0.1$ ). These findings suggest that, with regard to the present group of injection sites, differences in the density of labeled neurons are related to the topography of the injections and not to their absolute size. In addition, it does not appear that some tracers were more sensitive than others based on the number of neurons labeled across cases. For example, as shown in Figure 3, the number of labeled neurons after injection of fast blue in different animals was high in cases A1b and AKb, moderate in cases DLb and ALb, low in case AJb, and there were none in case ANb (not shown). The same range in the density of labeled neurons was found after injection of HRP-WGA (Fig. 3). Nevertheless, because it was not possible to determine from our data whether the number of labeled neurons would have varied if different tracers were injected in the same area in different animals, the numerical results should be viewed with these caveats in mind.

## Afferent Input From the Hippocampal Formation to Prefrontal Areas

Figure 3A is a composite of the injection sites on the medial (1), lateral (2), and basal (3) surfaces of the prefrontal cortex in all cases where positive neurons were noted in the hippocampal formation. The injection sites are superimposed on a map showing the architectonic subdivisions of the prefrontal cortex (Barbas and Pandya, 1989). Labeled neurons were noted in the hippocampal formation (CA1, CA1', prosubiculum, subiculum, and to a minor extent in CA4), after injection of retrograde tracers in 19 of the 33 sites investigated. Labeled neurons in the hippocampal formation were found in seven of eight medial sites studied, in ten of 13 orbital sites, and in two of 12 lateral sites. Medial cortical sites that received projections from the hippocampal formation included area 32/24 (case A1b; Fig. 4), area 14 (cases AKb and DLb; Fig. 5), area 25 (case AH; Fig. 6), area

**FIGURE 1.** Brightfield photomicrographs of coronal sections stained with cresyl violet taken from rostral (A) through caudal (F) sectors of the hippocampal formation showing the cytoarchitecture of the various fields. A: Shows a section taken through the genu. B: Uncus and anterior body. C,D: Mid-body. E,F: Posterior body of the hippocampal formation. The presubiculum (PreS) and parasubiculum (ParaS) are indicated in rostral sections (A–D).





**FIGURE 2.** Brightfield photomicrographs of coronal sections from rostral (A) through caudal (D) sectors of the hippocampal formation. The sections were treated to show the distribution of AChE which delineates several hippocampal fields. Note that the prosubiculum (ProS) and parasubiculum (ParaS) are especially well delineated by the AChE stain.

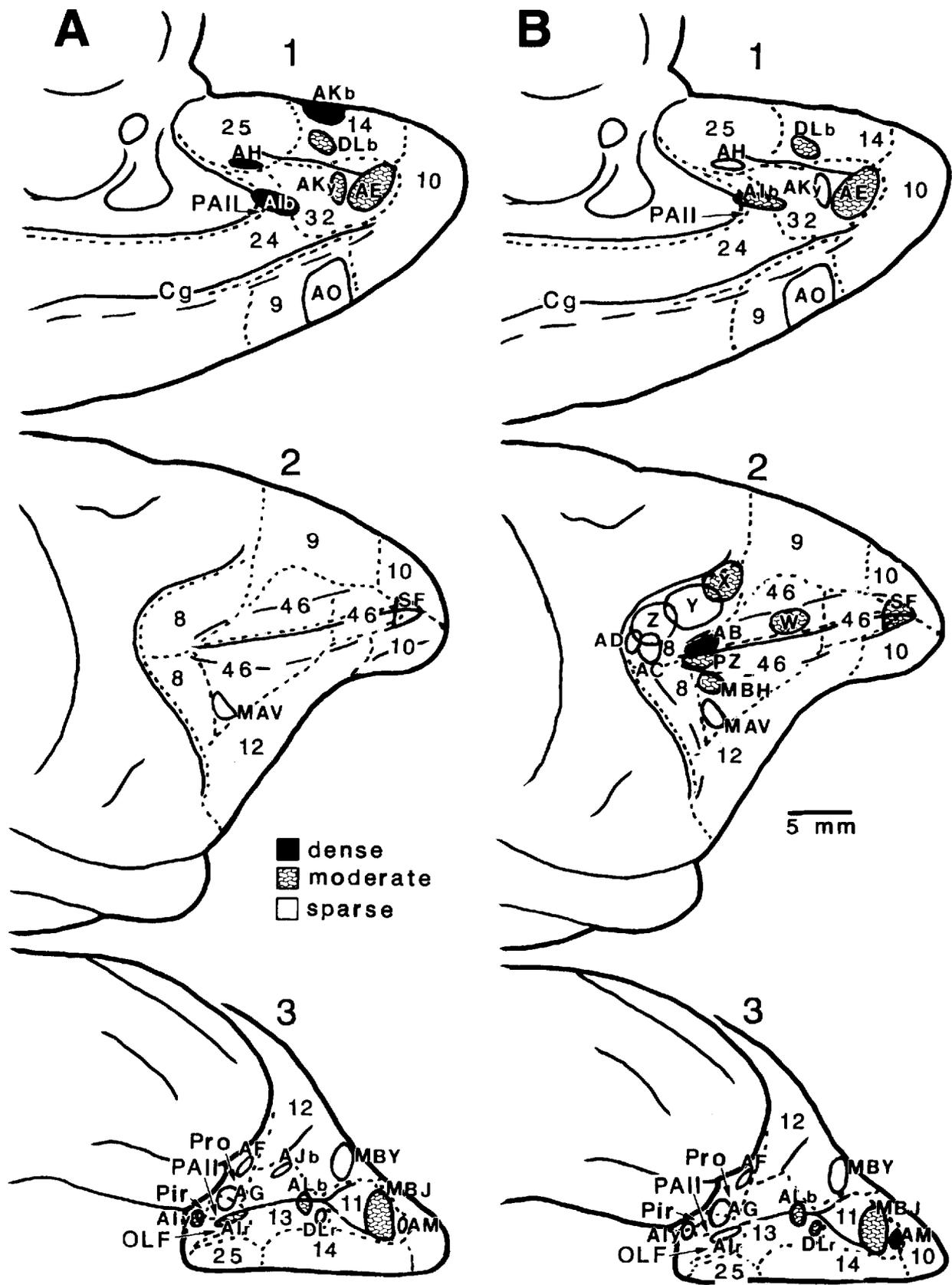
32 (cases AKy, AE; Figs. 5, 7), and, to a minor extent, medial area 9 (case AO; Fig. 3). Orbital sites included the prepiriform area/olfactory tubercle, or OLF/PAll (cases AIy; AIr; Fig. 4), area 11 (cases MBJ and AM; Figs. 3, 8), the rostral part of orbital area 12 (MBY; Fig. 9), area 13 (cases ALb, DLr and AJb; Figs. 3, 10), and to a minor extent, areas PAll/Pro (case AG) and area Pro (case AF; Fig. 3). Lateral sites whose injection resulted in labeling of a few neurons in the hippocampal formation included the rostral frontal pole (areas 10/46, case SF; Fig. 3) and ventral area 46 (case MAV; Fig. 3).

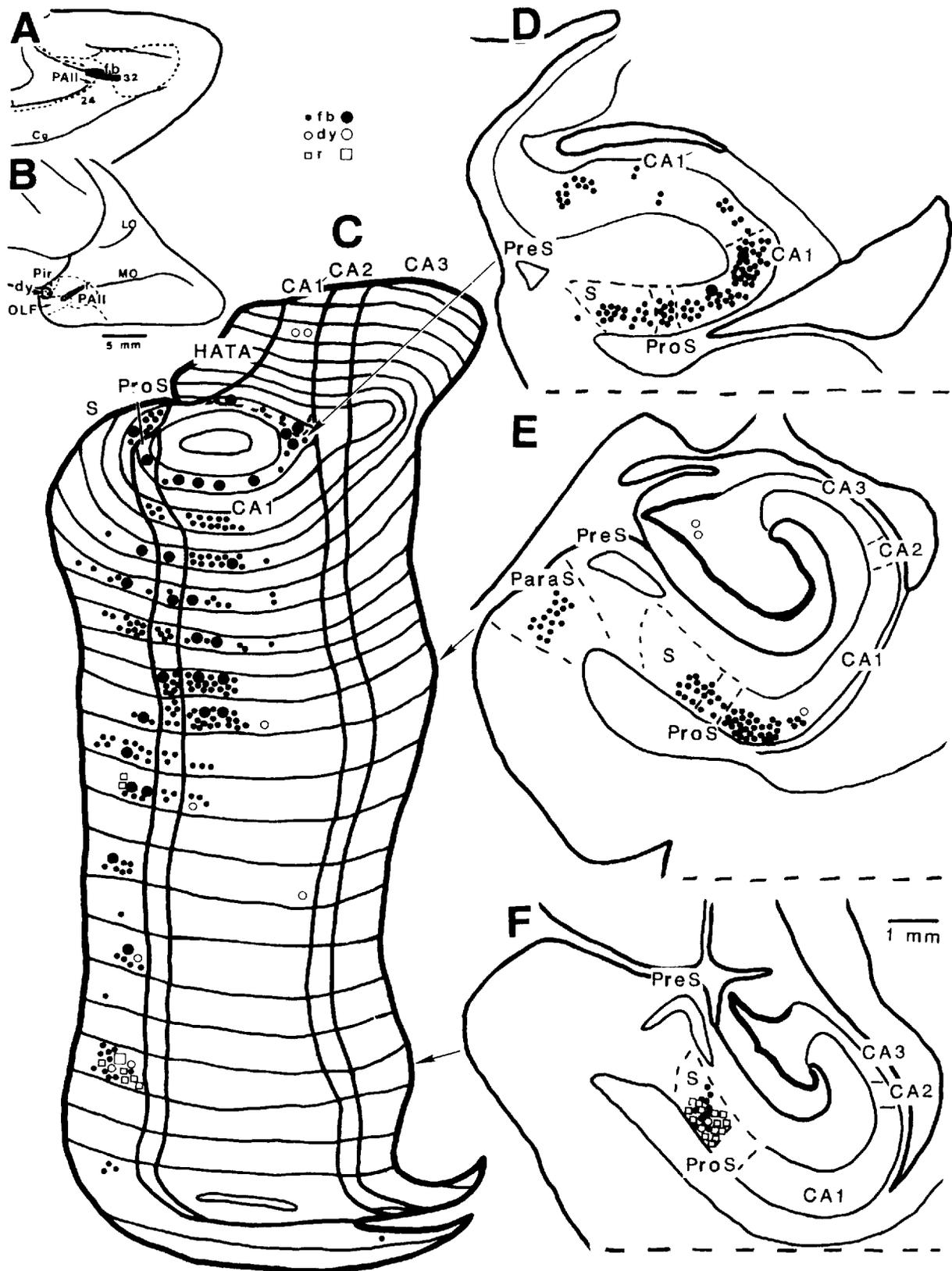
### Relative Density of Labeled Neurons in the Hippocampal Formation

The relative density of labeled neurons in the hippocampal formation in each case is depicted in Figure 3A. In the majority of cases with medial injections seen in Figure 3A (six of seven sites: AH, AIb, AKb, DLb, AKy, AE), the total number of la-

beled neurons was within the high or moderate range, and in one (case AO) it was in the sparse range. In contrast, in the majority of cases with orbital injections see in Figure 3A (six of ten sites), labeled neurons were sparsely distributed (cases AM, MBY, DLr,

**FIGURE 3.** Composite of injection sites shown on the medial (1), lateral (2), and orbital (3) surfaces of the cerebral hemisphere. The relative density of labeled neurons found in each case is depicted in black (dense), cuboidal pattern (moderate), and in outline (sparse) forms. A: Cases with labeled neurons in the hippocampal formation. B: Cases with labeled neurons in the presubiculum, area 29a-c, and parasubiculum. Cases with no labeled neurons are not shown on the map but are described in Results. The injection sites are superimposed on an architectonic map of the prefrontal cortex (Barbas and Pandya, 1989). Dotted lines demarcate architectonic areas indicated by numbers. PAll, Pro, Pir, and OLF also indicate architectonic areas. All other letter combinations refer to cases. Key: dense = >190 neurons; moderate = 20-150 neurons; sparse = <20 neurons.





AJb, AF, AG), and in the rest ( $n = 4$ ) they were within the moderate range (cases MBJ, ALb, AIr, AIf). Only two cases with lateral injections included labeled neurons in the hippocampal formation, and in both the number was low (cases SF and MAV; Fig. 3A).

Medial cases included the highest number of labeled neurons in the hippocampal formation ( $n = 1,555$ , 87%; Fig. 11A) followed by cases with orbitofrontal injections ( $n = 218$ , 12%; Fig. 11B). In contrast, only a few labeled neurons were found in cases with lateral injections ( $n = 14$ , 1%). Among medial cases, an in-

**FIGURE 4.** In case AI, labeled neurons (black dots) seen after injection of fast blue (fb) in medial area 32/24 (A, black area) are depicted on a two-dimensional map where the hippocampal formation was unfolded (C) and in coronal sections (D–F) taken at the level indicated in C. Labeled neurons are seen in CA1', CA1, prosubiculum, and subiculum. An injection of diamidino yellow in olfactory areas (Pir, OLF) on the basal surface (B, dy) resulted in a few labeled neurons (circle outlines) in the hippocampal formation. An injection of rhodamine-labeled latex microspheres in area PAll/OLF (B, r) resulted in a few labeled neurons restricted to the subiculum (squares). The fast blue injection also resulted in a cluster of labeled neurons in the parasubiculum (E). In the map showing the reconstructed hippocampal formation (C) each contour represents one unfolded coronal section. The most rostral section is represented by the small circular contour (top), and successive sections are seen around it. Caudal sections are at the bottom of the map. Note that in rostral sections, because of the folding pattern of the hippocampal formation single coronal sections pass through some fields more than once. These conventions apply for Figures 4–11. Unless otherwise indicated, each symbol represents one labeled neuron. In this figure each large symbol represents ten neurons.

jection of fast blue in the caudalmost part of area 32 and adjoining area 24 (case AIy; Fig. 4) labeled the highest number of neurons ( $n = 918$ , 51% of the total), followed by a case with an injection in the lower part of medial area 14 (case AKb,  $n = 320$ , 18%; Fig. 5) and area 25 (case AH,  $n = 215$ , 12%; Fig. 6). Cases with injections in the rostral part of area 32 and the upper part of medial area 14 combined accounted for 7% of all labeled neurons ( $n = 121$ ; cases AKy, AE and DLb; Figs. 5, 7).

In contrast to medial areas, only 12% of the total number of labeled neurons in the hippocampal formation were found in orbital cases. Among these, most labeled neurons projected to area 11 (case MBJ,  $n = 72$ , 4%; Fig. 8), followed by central area 13 (ALb,  $n = 58$ , 3%; Fig. 10) and the olfactory/PAll region (cases Aly, Alr, two sites, 3% combined; Fig. 4). Only scattered labeled neurons ( $<10$ ) were noted in cases with injections in areas PAll, Pro (cases AG, AF), caudal area 13 (case AJb), or rostral area 11 (case AM; Fig. 3). Among cases with injections in lateral prefrontal cortices, only two (case SF, area 10/46; and case MAV, caudal ventral area 46), included a few positive neurons, and these accounted for only 1% ( $n = 14$ ) of the total among those projecting to the prefrontal areas examined.

### Topography of Labeled Neurons in the Hippocampal Formation

Most labeled neurons were found in the rostral half of the hippocampal formation. However, a few labeled neurons were noted along the entire rostrocaudal extent of the hippocampal formation including the caudalmost part of the subiculum and CA1 (Fig. 11). Among hippocampal fields, CA1 included the highest number of labeled neurons (41%), followed by the subiculum (25%) and a rostral field (20%) designated CA1' by Rosene and Van Hoesen (1987) or the rostral subiculum by Amaral and Insausti (1990). The prosubiculum included 13% of all labeled neurons, and fewer than 0.5% were noted in the CA4 region. The ranking of the relative density of labeled neurons in the different hippocampal fields did not differ in cases with injections

in medial or orbital cortices. In contrast, the few labeled neurons noted in cases with lateral prefrontal injections were restricted to the subicular fields.

The most notable difference in the topography of labeled neurons innervating medial, when compared with orbital, areas was in their distribution along the rostrocaudal extent of the hippocampus. In medial cases labeled neurons were noted in CA1 rostrally and shifted to the subicular fields caudally (Figs. 4, 6, 7). In contrast, in orbital cases labeled neurons retained a remarkably consistent distribution within the hippocampal formation, and the same relative position within a given field (Figs. 8–10). Consequently, in medial cases labeled neurons were noted in three to four hippocampal fields, whereas the band of labeled neurons projecting to orbital sites was more narrow and often involved only one hippocampal field. The projection pattern did not appear to be related to the size of the injection.

### Depth of Labeled Neurons Within the Hippocampal Formation

In all cases the labeled neurons were found in the intermediate or deep parts of the pyramidal cell layer and showed a consistent regional variation within the different hippocampal fields. Most labeled neurons in CA1 (94%) were found in the deepest part of the field close to the white matter. In contrast, in the prosubiculum and subiculum the majority of labeled neurons (80% and 91%, respectively) were found in an intermediate zone, which was also the case for neurons taking origin in CA1' (96%).

### Negative Sites

There was no evidence of labeled neurons in the hippocampal formation after injection of tracers in the caudal part of medial area 9, the caudal part of orbital area 12 (two cases), or an adjacent sector of area Pro (cases not shown). As noted above, a few scattered labeled neurons were noted after injection of HRP in the rostral part of medial area 9 (case AO), the rostral part of area 12 (case MBY), or another sector of area Pro (case AF). Taken together, the above findings suggest that only a few, if any, afferent neurons from the hippocampal formation project to the laterally situated orbitofrontal cortices, including orbital area 12, area Pro and area PAll, or to medial area 9. In addition, there was no evidence of labeled neurons in the hippocampal formation following injection of HRP or fluorescent dyes in most lateral prefrontal sites including dorsal area 8 (five cases), the caudal part of area 46 (three cases), the central part of dorsal area 46 (one case), or lateral area 12 (one case).

### The Presubiculum and Area 29a–c

The presubiculum (area 27 of Brodmann, 1909) and parasubiculum (area 49 of Brodmann, 1909) differ from the fields included in the hippocampal formation on structural grounds. As shown in Figure 1, the presubiculum has a clearly delineated dense upper layer, and a less well-defined deep cellular layer invaded partly by the medial extension of the subiculum (see also Amaral and Insausti, 1990). The presubiculum gives way to the para-

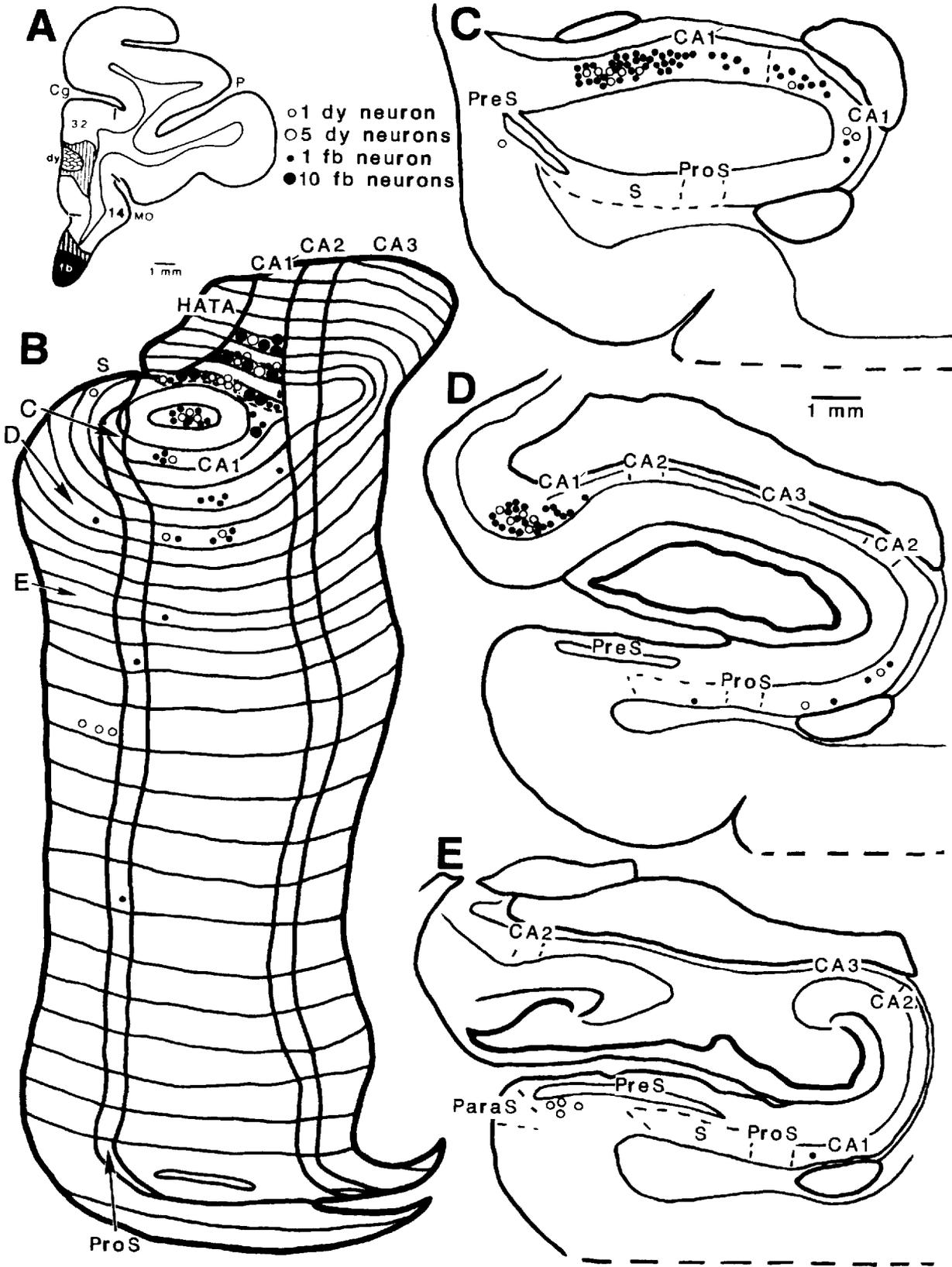


FIGURE 5. An injection of fast blue in the lower part of area 14 in case AK (A, black area) labeled neurons (black dots) in CA1', CA1, prosubiculum, and subiculum; a similar distribution of labeled neurons (circle outlines) was seen after injection of diamidino yellow in rostral area 32 (A, dy, cuboidal pattern). The distribution of

labeled neurons is shown on a two-dimensional map of the hippocampal formation (B) and on coronal sections (C-E) taken from the levels shown in B. The halo of the injection sites is represented by vertical stripes.

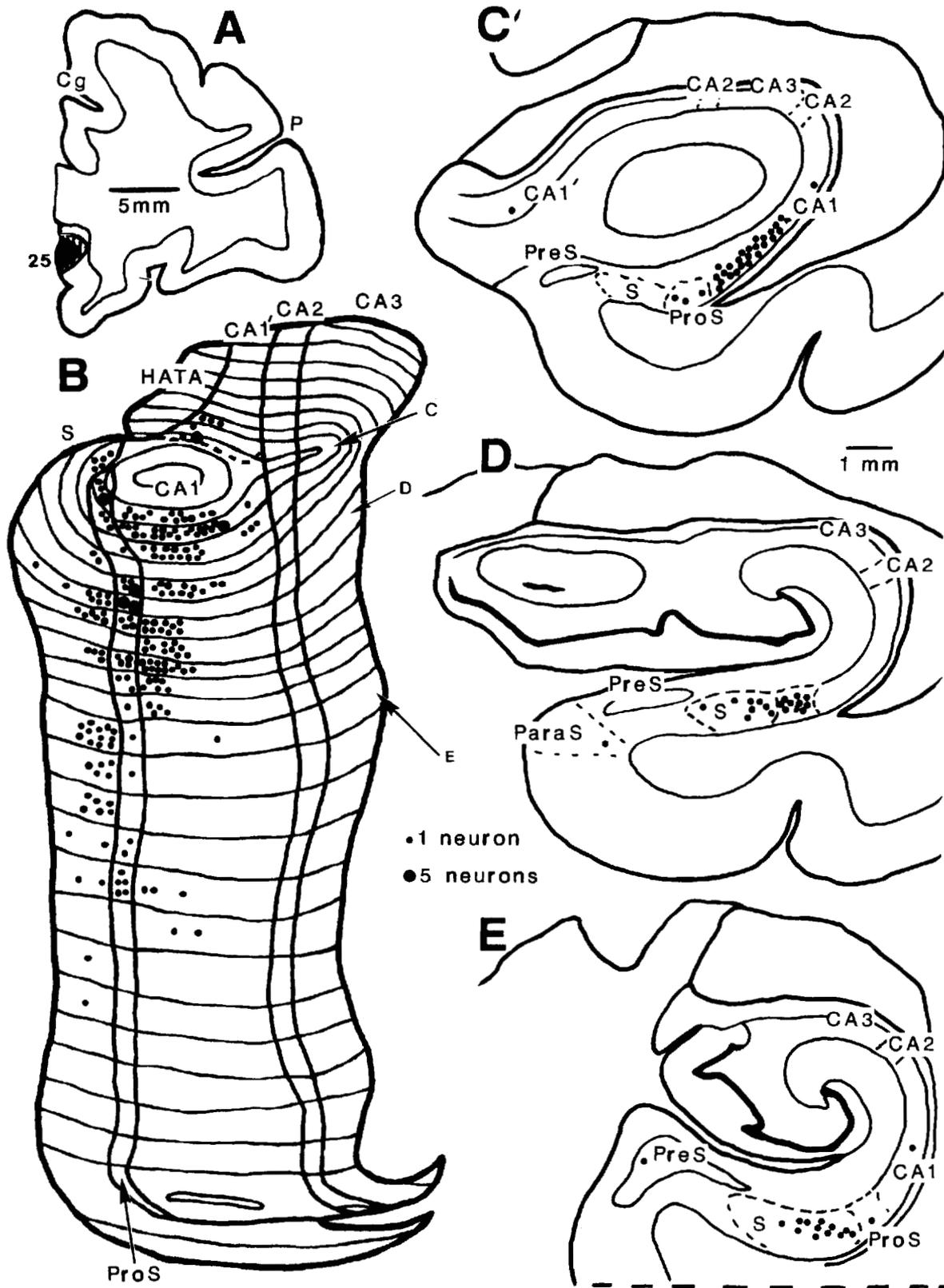


FIGURE 6. An injection of HRP in medial area 25 in case AH shown on a coronal section (A, black area) labeled neurons (black dots) in CA1', CA1, prosubiculum, and subiculum, as seen on a map where the hippocampal formation was unfolded (B) and in coronal

sections (C-E) taken at the levels indicated in B. Note the shift of labeled neurons from CA1 in rostral hippocampal levels to the subiculum at caudal levels. The halo of the injection site is represented by the vertical stripes.

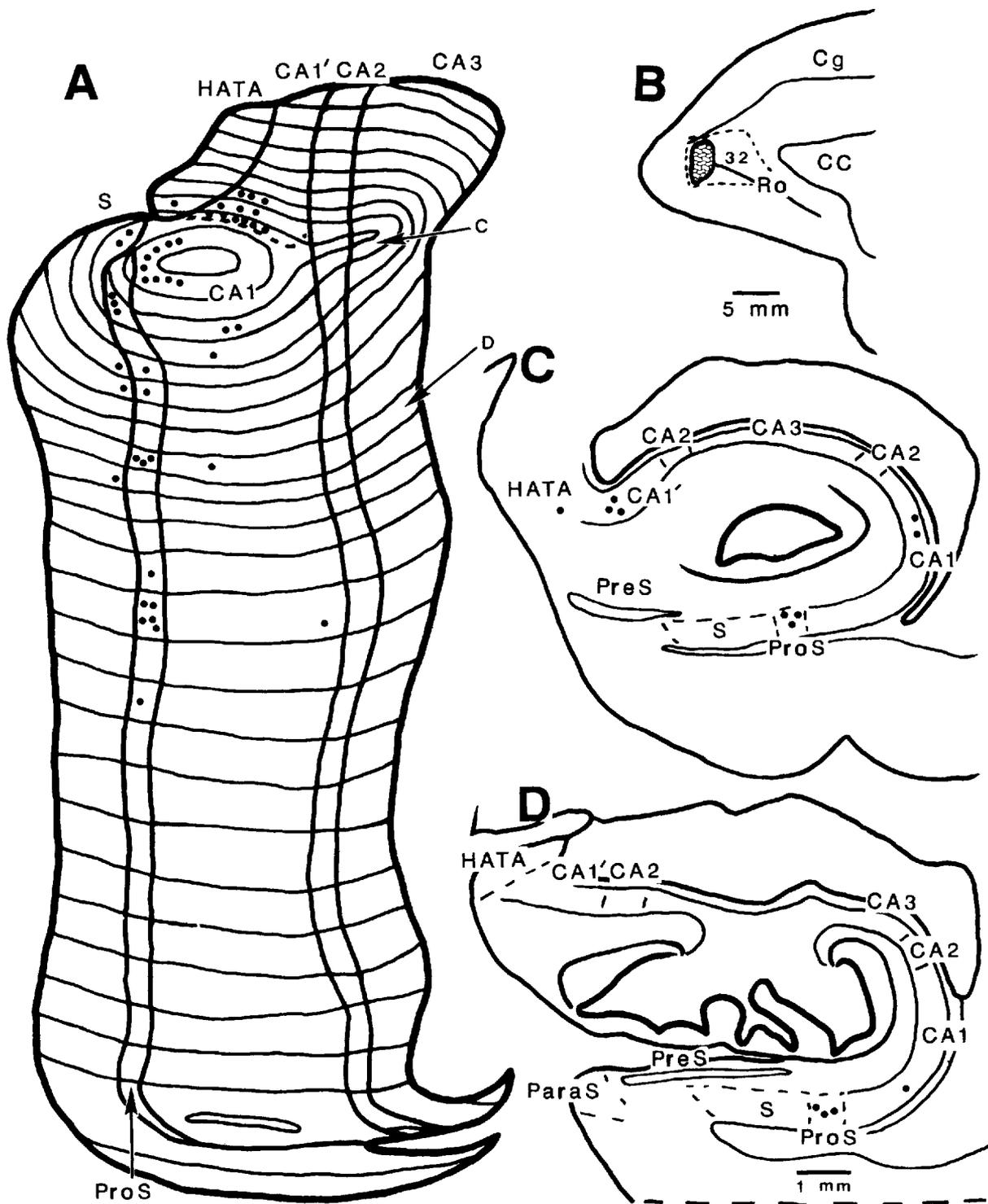


FIGURE 7. Labeled neurons in CA1', CA1, prosubiculum, and subiculum (black dots) seen on an unfolded map of the hippocampus (A) and in coronal sections (C-D) taken at the level indicated in A, after an HRP injection in rostral area 32 in case AE (B, cuboidal pattern).

subiculum medially, which has a less densely packed external cellular layer than the presubiculum (Fig. 1A-C) and has a dense plexus of AChE in the upper layers (Fig. 2A,B). The presubiculum is situated next to the subiculum and is readily recognizable

near the subcallosal part of the subiculum. Structurally the presubiculum resembles the dorsally situated area 29a-c. However, even though several investigators have recognized that a small part of the subiculum extends dorsally above the corpus callosum, the

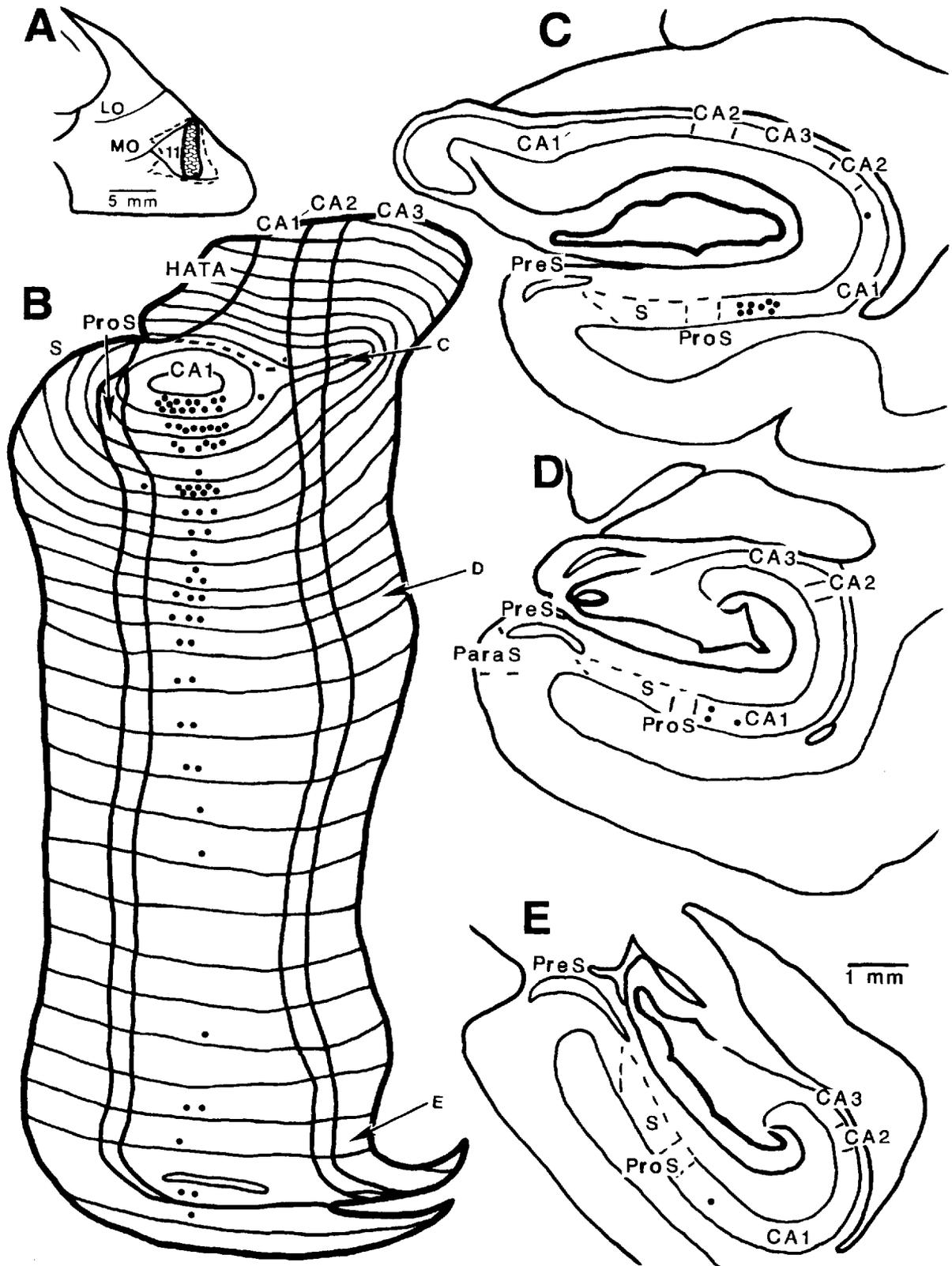


FIGURE 8. An HRP injection in orbital area 11 in case MBJ (A, cuboidal pattern) labeled neurons (black dots) mainly in CA1 seen on an unfolded map of the hippocampal formation (B) and in coronal sections (C-E) taken at the levels indicated in B. Note the consistent topography of labeled neurons within CA1 from rostral (top) to caudal (bottom) levels of the hippocampal formation.

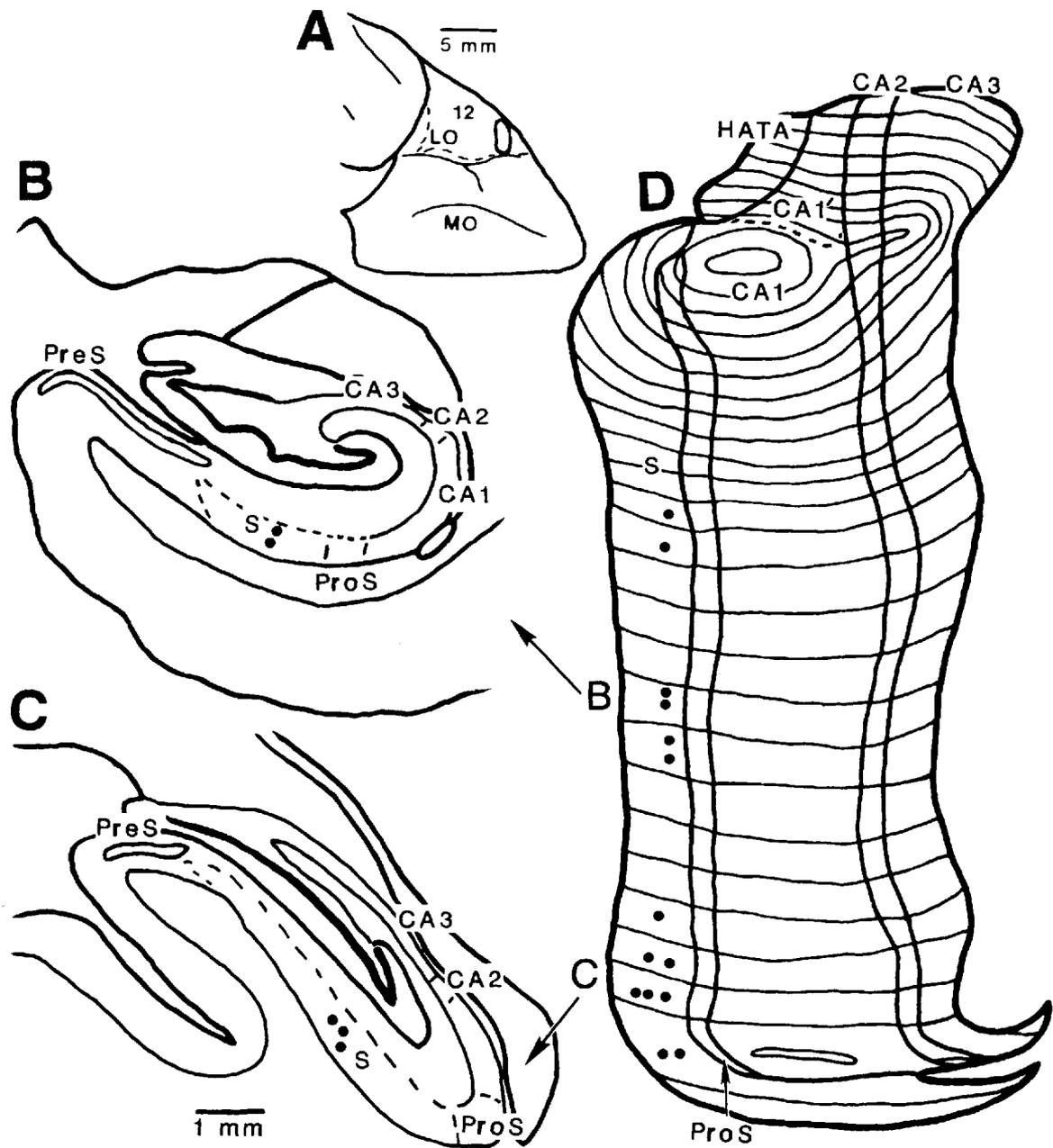


FIGURE 9. An injection of HRP in orbital area 12 in case MBY (A, outline) labeled neurons (black dots) in the subiculum as seen on coronal sections (B,C), and on a map where the hippocampal formation was unfolded (D).

adjacent region has been designated area 29a-c (Vogt et al., 1987) and has been differentiated from the presubiculum situated below the corpus callosum. Our observations indicate that the presubiculum is topographically continuous with area 29a-c. In addition, we have noted a close structural relationship between the presubiculum and area 29a-c, as seen in Nissl sections taken from a level just before and immediately caudal to the splenium of the corpus callosum (Fig. 12). We entertain the possibility that the presubiculum and area 29a-c may be the same area. In this section we will continue to distinguish the ventrally situated pre-

subiculum from the dorsally situated area 29a-c according to convention, but with the above idea in mind.

#### Projections From the Presubiculum and Parasubiculum to Prefrontal Cortices

In most medial cases where labeled neurons were noted in the hippocampal formation, there were labeled neurons in the presubiculum and/or the parasubiculum as well (six of seven sites; Fig. 3). A similar observation was made for orbital cases, where

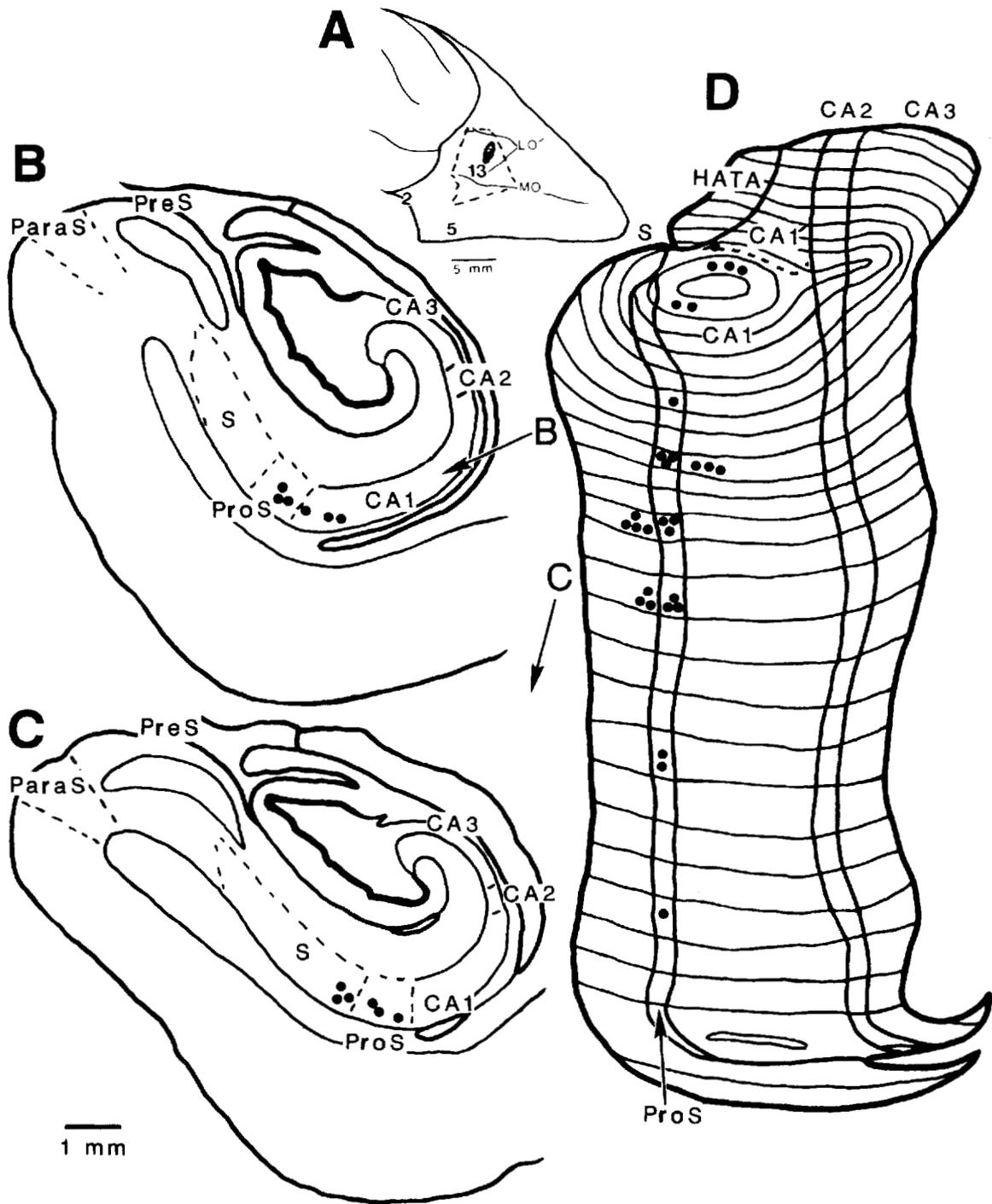


FIGURE 10. An injection of fast blue in area 13 on the orbital surface in case AL (A) retrogradely labeled neurons in CA1, ProS, and the proximal part of the subiculum, as seen in coronal sections (B,C), and on an unfolded map of the hippocampal formation (D).

nine of the ten cases with positive neurons in the hippocampal formation also had labeled neurons in the presubiculum and/or presubiculum (Figs. 3, 13A–C). In contrast, labeled neurons were noted in the presubiculum and/or parasubiculum in 11 lateral cases, of which only two included a few labeled neurons in the hippocampal formation (Figs. 3, 13D–F; 14). We compared the densities of labeled neurons in the hippocampal formation and

the presubiculum/parasubiculum in cases that had labeled neurons in both structures. As shown in Figure 3, in eight of 17 such cases, the relative density of labeled neurons differed between the hippocampal formation and the presubiculum/parasubiculum. For example, in case AH (area 25) the number of labeled neurons in the hippocampal formation was high, whereas in the presubiculum and parasubiculum it was low (Fig. 3, top). The re-

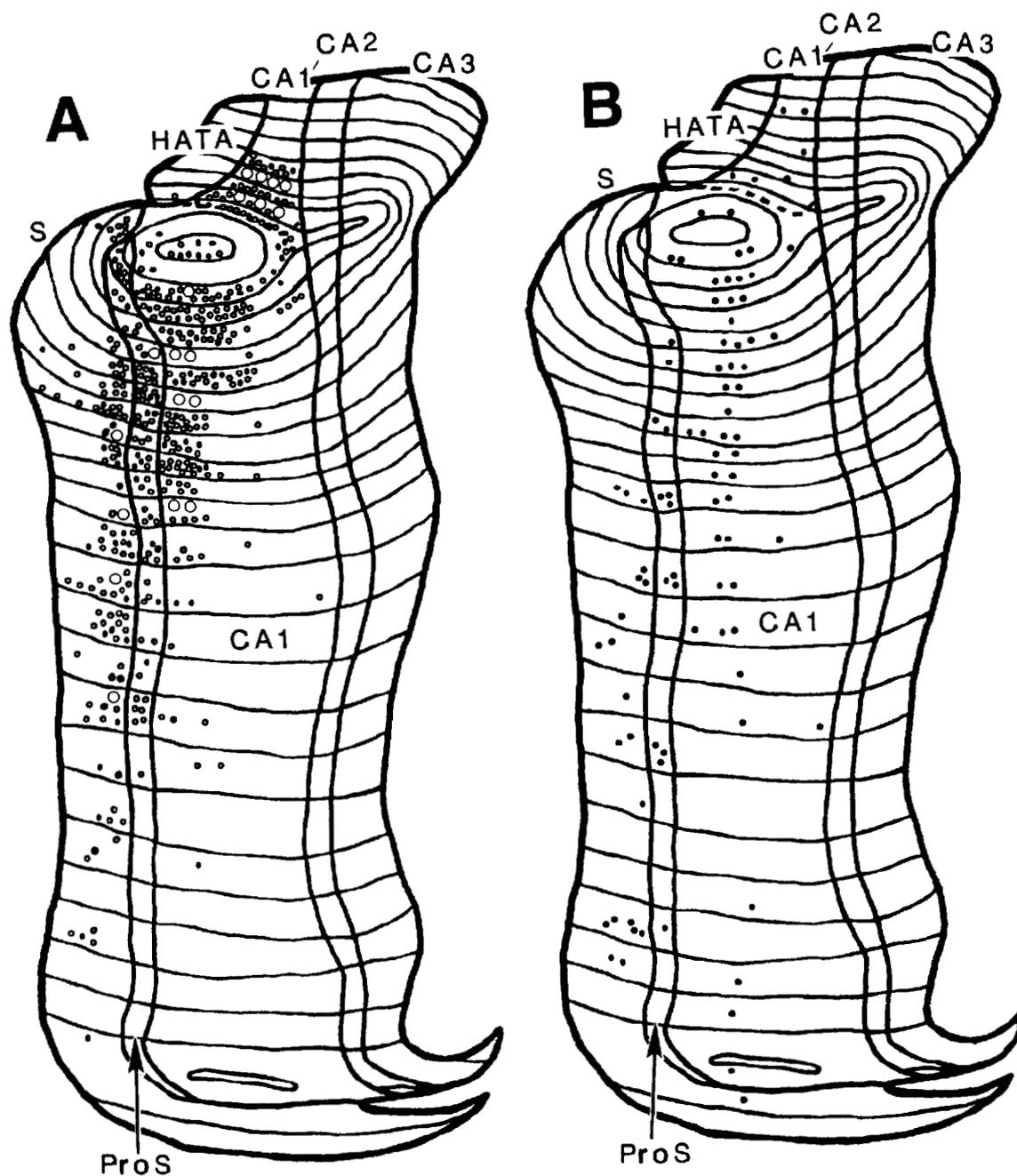
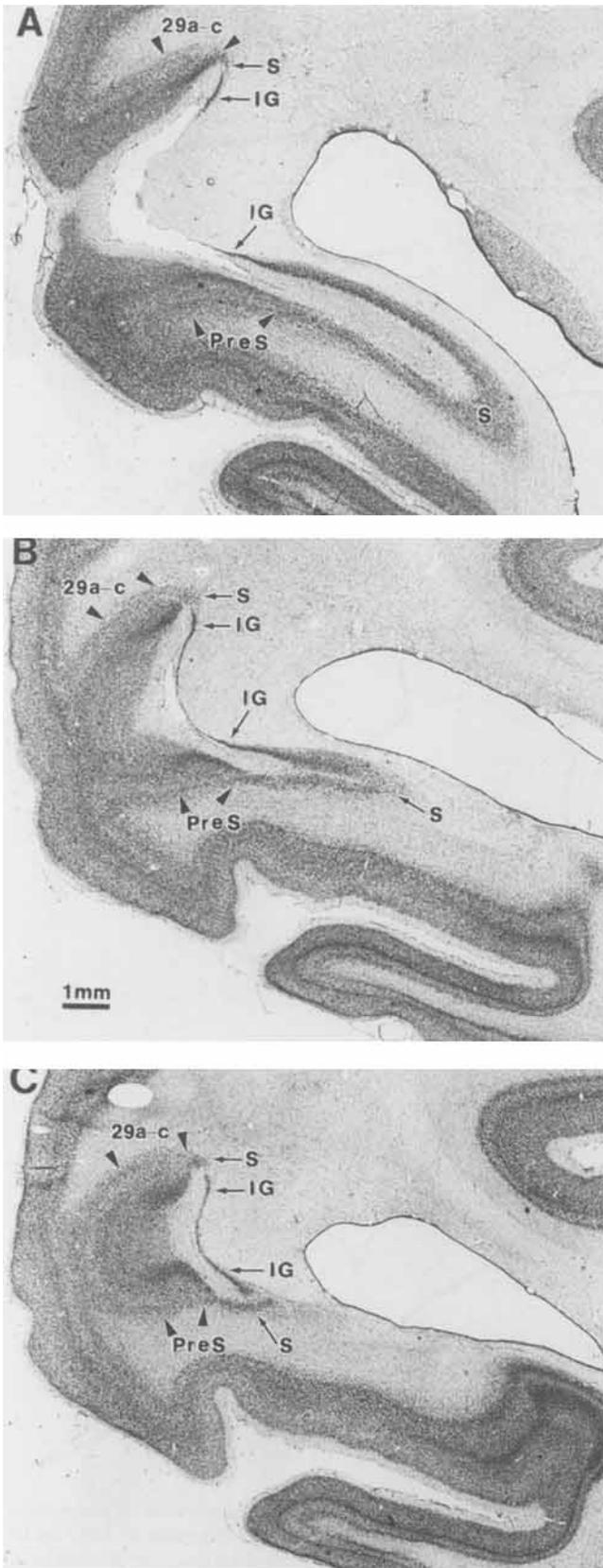


FIGURE 11. Summary diagram showing the relative density of labeled neurons in the hippocampal formation projecting to medial (A) and to orbital (B) prefrontal cortices. Each small symbol represents two neurons. Each large symbol represents 40 neurons.

verse was observed in case AM (Fig. 3, bottom). In two cases we found labeled neurons in the hippocampal formation but not in the presubiculum or parasubiculum (Fig. 3, top and bottom: cases AKb and AJb), and in nine cases we observed the reverse (Fig. 3, center: cases W, X, Y, Z, AD, AC, AB, PZ, MBH). At one extreme, this was exemplified in case AKb with an injection in the lower part of area 14, where the number of labeled neurons was high in the hippocampal formation (Figs. 3A, 5), but none were noted in the presubiculum or parasubiculum. The reverse rela-

tionship was seen in case AB with an injection in the caudal part of dorsal area 46, where many labeled neurons were noted in the presubiculum and area 29a-c, but none were found in the hippocampal formation (Fig. 3, center).

Only a few labeled neurons were found in the parasubiculum; most projected to medial prefrontal areas ( $n = 92$ ), and very few projected to lateral ( $n = 13$ ), or orbital cortices ( $n = 7$ ). By comparison with the parasubiculum, there were considerably more labeled neurons in the presubiculum and area 29a-c (Figs. 13, 14).



**FIGURE 12.** Brightfield photomicrographs of coronal sections stained with cresyl violet to show the cytoarchitecture of the caudalmost extent of the hippocampal formation. Section A was taken from a level at the posterior tip of the corpus callosum, and B and C are posterior to the splenium of the corpus callosum. A dorsal area (29a-c) situated near the supracallosal aspect of the subiculum (S) is architecturally similar to a comparably situated area below the corpus callosum, the presubiculum (PreS). The topographic continuity and structural similarity of area 29a-c and the presubiculum suggest that they may be parts of the same architectonic area.

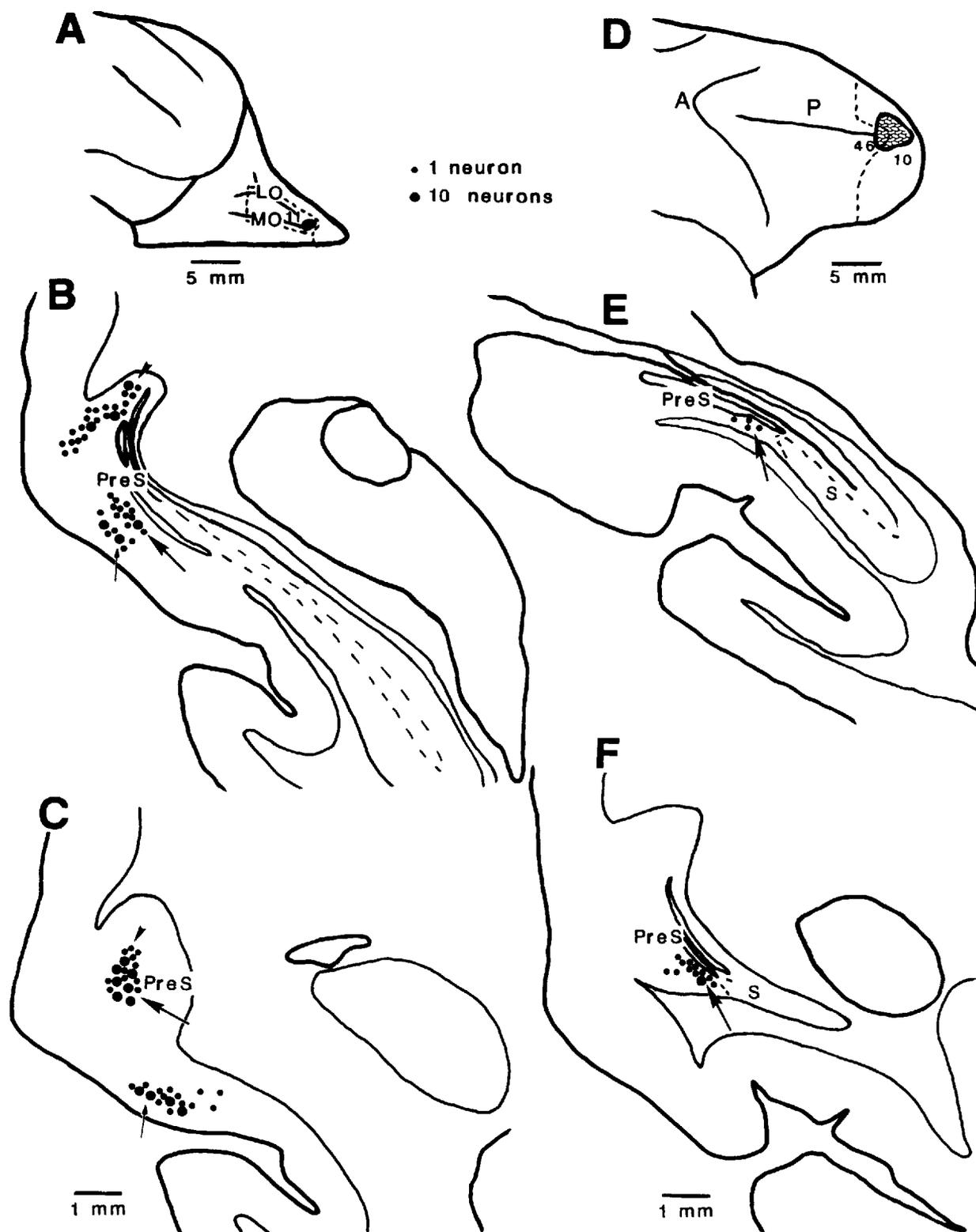
On a regional basis, most labeled neurons in the presubiculum and area 29a-c were noted in cases with injections in lateral areas ( $n = 683$ ). In six of 11 lateral cases seen in Figure 3B, the number of labeled neurons was within the moderate or high range (cases SF, W, X, AB, PZ, MBH), whereas in five cases it was in the low range (cases Y, Z, AD, AC, and MAV). The next highest concentration of labeled neurons was noted in orbital cases ( $n = 359$ ). In four orbital cases the number of labeled neurons was within the moderate or high range (cases AM, MBJ, DLr, ALb), and in five others it was within the sparse range (cases MBY, AF, AG, Aiy, AIr). Cases with medial injections accounted for the smallest number of labeled neurons ( $n = 224$ ) and fell within the moderate range in cases AE, DLb, and Alb or the sparse range in cases AO, AKy, and AH. With respect to density of labeled neurons, the pattern of labeling was the reverse of that observed for projections from the hippocampal formation.

Viewed on a case-by-case basis, most labeled neurons in the presubiculum and area 29a-c were noted after tracer injection in the rostral part of area 11 (case AM,  $n = 231$ ; Fig. 13A-C) and the dorsal part of caudal area 46 (case AB,  $n = 198$ ). Moderate numbers of labeled neurons in the presubiculum and area 29a-c were noted in medial cases with tracer injections in rostral area 32 (case AE,  $n = 130$ ), the upper part of medial area 14 (case DLb,  $n = 24$ ), and the area 32/24 border (case Alb,  $n = 52$ ); in lateral cases with injections in area 8 (case X,  $n = 28$ ), ventral area 46 (case PZ,  $n = 146$ ), dorsal area 46 (case W,  $n = 112$ ), and rostral area 46/10 (case SF,  $n = 75$ ; Figs. 13D-F; 14); and in orbital cases with injections in the central part of area 11 (MBJ,  $n = 29$ ) and rostral area 13 (cases DLr,  $n = 24$ ; and ALb,  $n = 54$ ). In the remaining cases only scattered labeled neurons were noted in the presubiculum and area 29a-c, and these included most area 8 sites investigated on the lateral surface, all caudal orbital sites, and medial area 9 (Fig. 3).

In medial cases more labeled neurons were found in the ventrally situated presubiculum ( $n = 142$ ) than in area 29a-c ( $n = 82$ ). The pattern differed in orbital cases where more labeled neurons were found in area 29a-c ( $n = 247$ ) than in the ventrally situated presubiculum ( $n = 112$ ). In lateral cases the majority of afferent neurons were found in area 29a-c ( $n = 574$ ), and fewer were seen in the presubiculum ( $n = 109$ ).

### Laminar Distribution of Labeled Neurons

Most labeled neurons in the presubiculum and area 29a-c were found in the deep cellular layer. However, there was a difference in the laminar origin of labeled neurons which depended upon



**FIGURE 13.** A-C: An injection of HRP in orbital area 11 in case AM (A, black area) labeled neurons in the presubiculum (B,C, large arrows), and in area 29a-c (arrowheads). In C, area 29a-c (arrowhead) and the presubiculum (large arrow) merge. The small ar-

rows in B and C show clusters of labeled neurons in neocortical areas, outside the presubiculum. D-F: An injection of HRP in lateral area 10/rostral 46 in case SF (D, cuboidal pattern) labeled neurons in the presubiculum (E,F, large arrows).

## DISCUSSION

**Specific Hippocampal Projections Target Functionally Distinct Prefrontal Areas**

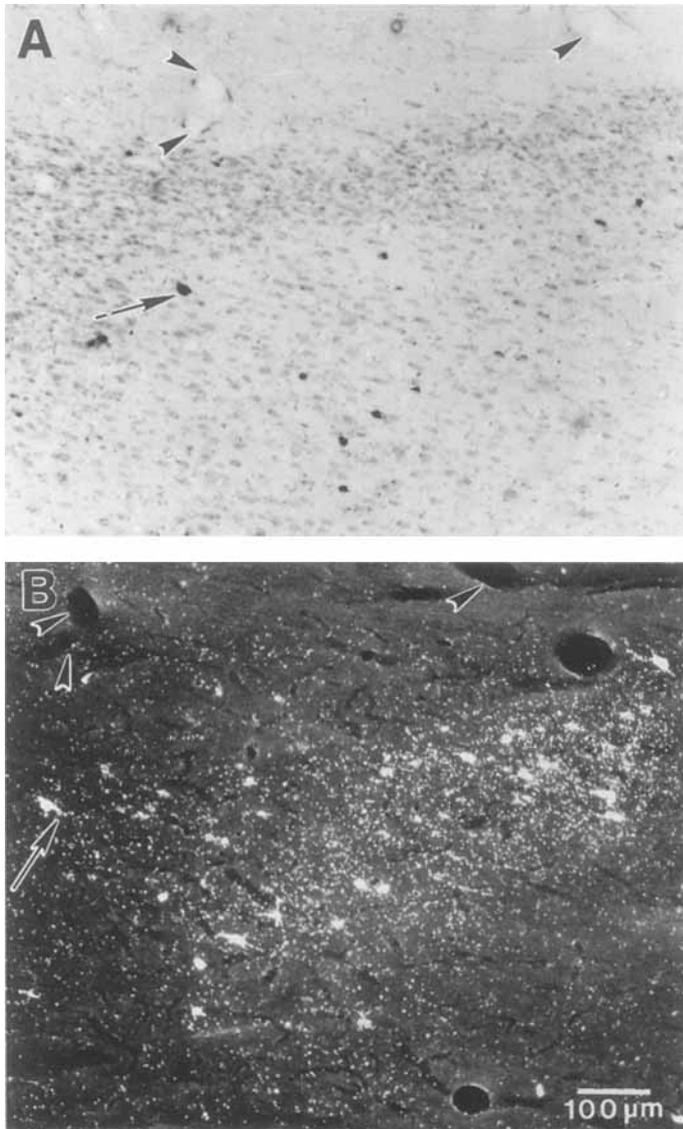
Afferent neurons from the hippocampal formation, the presubiculum, and the parasubiculum projected to specific prefrontal cortices. Our findings extend previous studies (Rosene and Van Hoesen, 1977; Goldman-Rakic et al., 1984; Cavada and Reinoso-Suarez, 1988; Morecraft et al., 1992) in several ways: The hippocampal formation innervates preferentially ventromedial prefrontal cortices, to a lesser extent orbital areas, and only to a minor extent lateral prefrontal cortices. In contrast, projections from the presubiculum and area 29a–c targeted prefrontal areas in the reverse order: the presubiculum and area 29a–c innervate preferentially specific lateral prefrontal cortices, then orbital cortices, and to a lesser extent medial areas.

Moreover, the present findings revealed that hippocampal projections to prefrontal areas were longitudinally extensive. Most projection neurons were found rostrally within the hippocampal formation. However, longitudinal strips of afferent neurons extended to the most caudal part of the hippocampal formation as well. Medial areas received the richest and most diverse afferent projections from the hippocampal formation. In medial cases the rostrocaudal extent of hippocampal projection neurons was topographically fragmented: Afferent neurons originated preferentially in CA1 in the rostral half of the hippocampal formation, and from the subicular fields in its caudal half. In contrast, hippocampal neurons projecting to orbitofrontal cortices were more sparse, more focal, and longitudinally continuous in the rostrocaudal dimension. Thus, for any given case projection neurons originated from a specific field and had a remarkably similar position within the field along its rostrocaudal extent. This evidence suggests that the topographic origins of hippocampal projection neurons differ along the rostrocaudal dimension depending on their destination.

Previous studies have emphasized the importance of the subiculum in cortical projections in both cats and monkeys (Rosene and Van Hoesen, 1977; Schwerdtfeger, 1979; Irle and Markowitsch, 1982; Cavada et al., 1983). Here we report that in the rhesus monkey the CA1 field included a significant proportion of the afferent neurons that projected to medial and orbital prefrontal cortices as well. There are also extensive projections from CA1 to specific areas in the posterior parahippocampal gyrus (Blatt and Rosene, 1988). Taken together, the above data demonstrate that the subiculum is not the only output of the hippocampal formation to the cortex.

**Parallel Limbic Pathways to Prefrontal Cortices**

The preponderance of afferent neurons from the hippocampal formation innervating medial and orbital areas followed the opposite trend from what we had observed previously for projections from the amygdala (Barbas and De Olmos, 1990). Thus, the orbitofrontal cortex, and particularly its caudal extent, appears



**FIGURE 14.** A: Brightfield photomicrograph of coronal section in case SF with an injection of HRP in area 10/46, showing labeled neurons in the presubiculum, characterized by a dense upper cellular layer. The labeled neurons are found in the lower layer. The labeled neurons are also seen in B in a darkfield photomicrograph of the section depicted in A. Scale bar = 100  $\mu\text{m}$ . Arrowheads in A and B point to the same blood vessel for reference.

their destination. Thus, when they projected to lateral prefrontal areas, afferent neurons in the presubiculum and area 29a–c were found overwhelmingly in the deep layer (99%). However, those projecting to orbital areas were distributed both in the superficial and deep layers in a ratio of 1:2, and those projecting to medial areas were more evenly distributed in a ratio close to 1:1. Most labeled neurons in the parasubiculum were found below the upper cellular layer. Because they were few in number, it was not possible to ascertain whether there were differences in their distribution with regard to destination.

to be the principal target of amygdaloid projections (Barbas and De Olmos, 1990). In contrast, the hippocampal formation projected preferentially to medial prefrontal cortices. The differential pattern of hippocampal and amygdaloid projections suggests the existence of parallel and functionally distinct limbic pathways to medial and orbital prefrontal cortices, comparable to the parallel sensory pathways to the two regions (Barbas, 1988; for reviews see Barbas, 1992, 1995a). In addition to robust projections from the amygdala, orbitofrontal cortices receive input from sensory cortices where the emphasis in processing appears to be in the significance of features of stimuli and their memory (for reviews, see Barbas, 1992, 1995a). The coupling of sensory and amygdaloid signals in orbitofrontal cortices may be related to remembering emotionally significant events where the amygdala appears to have a dominant role (for reviews, see Davis, 1992; Damasio, 1994; LeDoux, 1994). The functional inter-relationship of the orbitofrontal cortex and the amygdala has been demonstrated in behavioral studies. Lesions of the orbitofrontal cortex or the amygdala deprive monkeys of appropriate emotional responses necessary for maintaining normal social interactions (for review, see Kling and Steklis, 1976).

The robust hippocampal projections to medial prefrontal areas raises the possibility that medial prefrontal cortices may have some mnemonic properties comparable to those attributed to the hippocampal formation (Mahut, 1971, 1972; Mahut et al., 1982; Parkinson et al., 1988; Zola-Morgan et al., 1989a,b; for reviews, see Squire, 1992; Zola-Morgan and Squire, 1993). Damage to the hippocampus in humans has long been associated with severe anterograde amnesia, which is characterized by selective inability to remember information acquired after the lesion (Scoville and Milner, 1957; DeJong et al., 1968; Muramoto and Kuru, 1979; Cummings et al., 1984). In their classic study, Scoville and Milner (1957) suggested that the amnesic syndrome could be attributed to the hippocampal lesion and was not likely to be due to damage to the amygdala. This hypothesis has been substantiated in recent findings of anterograde mnemonic deficits after restricted lesions in the hippocampus in both humans and monkeys (Zola-Morgan et al., 1986; Beason-Held, 1994; Rempel-Clower, 1994; Alvarez et al., 1995). Moreover, recent evidence indicates that selective bilateral damage to CA1 in humans is sufficient to produce marked anterograde amnesia (Zola-Morgan et al., 1986; Rempel-Clower, 1994). Our findings indicate that CA1 included more afferent neurons innervating prefrontal areas than any other hippocampal field.

The idea that medial prefrontal cortices may share mnemonic functions with the hippocampus is supported by behavioral studies as well. For example, lesions of the ventromedial prefrontal cortices in monkeys produce visual recognition deficits (Bachevalier and Mishkin, 1986; Voytko, 1985). In humans, rupture of the anterior communicating artery, which supplies the ventromedial prefrontal areas (Crowell and Morawetz, 1977) results in severe anterograde amnesia comparable to the classic amnesic syndrome seen after hippocampal lesions (Talland et al., 1967; Alexander and Freedman, 1984). The areas supplied by the anterior communicating artery in humans (Crowell and Morawetz, 1977) include Brodmann's area 25 and the parts of areas 32 and

24 situated adjacent to the genu and the rostrum of the corpus callosum. Our findings indicate that these ventromedial prefrontal areas in the rhesus monkey receive the most robust projections from the hippocampal formation. The mnemonic deficits observed in both monkeys and humans (Bachevalier and Mishkin, 1986; Voytko, 1985; Talland et al., 1967; Alexander and Freedman, 1984) may be due to interruption of a mnemonic pathway linking the hippocampus with ventromedial prefrontal cortices.

Prefrontal limbic cortices, in general, receive robust projections from thalamic midline, anterior, the magnocellular, and the caudal part of the parvocellular mediodorsal nuclei (Barbas et al., 1991; Morecraft et al., 1992; Ray and Price, 1993; Dermon and Barbas, 1994), all of which have been implicated in mnemonic processes (Isseroff et al., 1982; Aggleton and Mishkin, 1983; for review, see Markowitsch, 1982). Our findings provide support for the idea that the medial prefrontal cortices, which are robustly connected with the hippocampus as well as with the limbic thalamus, may be part of a classic mnemonic loop. Further ablation studies in monkeys are necessary, however, since neither the experimental lesions in monkeys nor the damage in humans were sufficiently restricted to allow specific assessment of the role of medial prefrontal cortices in mnemonic processes.

The differential projections from the hippocampus and the amygdala may contribute significantly to the specific functional attributes of orbital and medial prefrontal cortices. The segregation of inputs from the hippocampus and the amygdala to medial and orbitofrontal limbic cortices, however, was not complete, as is the case with all other parallel pathways (Merigan and Maunsell, 1993). Thus, there was partial intermixing of input from functionally distinct limbic pathways in medial and orbitofrontal limbic cortices as well. This finding suggests that damage to each area will increase the probability of incurring a deficit biased in one direction, rather than producing completely dissociative effects.

### **The Presubiculum and Area 29a–c Target Predominantly Lateral Prefrontal Areas**

In marked contrast to medial and orbitofrontal cortices, we noted that projections to the lateral prefrontal areas originated preferentially from the presubiculum and area 29a–c, and only to a minor extent from the subiculum. These findings are consistent with observations made by other investigators for dorsolateral prefrontal areas around the principal sulcus (Goldman-Rakic et al., 1984).

The role of input from the presubiculum and parasubiculum to lateral prefrontal cortices is not known. Lesions of lateral prefrontal cortices do not render nonhuman or human primates amnesic (Milner and Petrides, 1984; Janowsky et al., 1989; Petrides, 1989), although the lateral prefrontal cortex has been implicated in a specific type of mnemonic processing. Thus, damage to lateral prefrontal cortices around the principal sulcus impairs the performance of nonhuman primates when they must remember a stimulus after a short delay. The lateral prefrontal cortices thus have a role in working memory during cognitive tasks (Jacobsen,

1936; Fuster, 1973; Bauer and Fuster, 1976; Kubota et al., 1980; Kojima and Goldman-Rakic, 1982; Sawaguchi et al., 1988; Funahashi et al., 1989; Kubota and Niki, 1990; Sawaguchi and Goldman-Rakic, 1991; for reviews, see Goldman-Rakic, 1988; Fuster, 1989). It is possible that this type of mnemonic processing may be supported by a pathway linking lateral prefrontal areas with the presubiculum and area 29a-c. This hypothesis may be explored further in physiologic and behavioral experiments.

### Feedback Projections From the Hippocampal Formation and Neighboring Areas

The present findings indicated differential projections from the hippocampal formation and the adjacent presubiculum/parasubiculum to prefrontal cortices. Thus, the hippocampal formation was preferentially linked with medial and orbital cortices, which have a lower laminar differentiation than lateral prefrontal cortices (Barbas and Pandya, 1989). In this regard it is interesting that hippocampal fields, which are allocortical, projected preferentially to limbic prefrontal cortices or to their immediate neighbors. In contrast, the presubiculum and parasubiculum, which are periallocortical, projected to lateral prefrontal cortices, which have a better laminar definition than either medial or orbital prefrontal cortices.

The specific depth of projection neurons within the different hippocampal fields varied in a consistent manner among cases. Thus, whereas most projection neurons in CA1 occupied the deepest position within the pyramidal cell layer, in the presubiculum most neurons were found in an intermediate position, a pattern that was further exaggerated in the subiculum and CA1'. In this context CA1' was comparable to the subiculum, to which it appears to be closely related, as was suggested by Amaral and Insausti (1990).

The relative depth of projection neurons may indicate the type of information transmitted from the hippocampus and adjoining areas to the cortex. In the sensory cortices, neurons from deep layers project to subcortical structures or to cortices that are closer to the sensory periphery than the neurons which issue the projections. For this reason, neurons in deep cortical layers have been ascribed a feedback role (Rockland and Pandya, 1979). We previously showed that projections from limbic cortices to culminate areas also originate in deep layers and may be considered feedback on account of their deep laminar origin (Barbas, 1986). By analogy with the cortex, hippocampal neurons from CA1 may provide feedback projections to the prefrontal cortex. It is not clear whether projections from the subicular fields and CA1', which originate from an intermediate level within the pyramidal cell layer, can be considered feedback as well.

Projection neurons from the presubiculum and area 29a-c were found predominantly in deep layers as well, a pattern accentuated in the projection to the lateral neocortices. Thus, afferent neurons innervating lateral prefrontal cortices were situated almost exclusively in a deep position within the presubiculum or area 29a-c. In contrast, although most afferent neurons still occupied a deep position, a significant number in the upper layer of the presubiculum and area 29a-c innervated medial and or-

bitar cortices as well. These findings suggest that the laminar origin of afferent neurons depends on their specific cortical destination, as well as on the type of cortex giving rise to them (Barbas, 1986).

## CONCLUSIONS

The hippocampal formation and the adjacent presubiculum target functionally distinct prefrontal sectors. Afferent neurons from CA1 and the subicular fields of the hippocampal formation innervate predominantly ventromedial followed by orbital prefrontal cortices, whereas the presubiculum/area 29a-c preferentially innervate lateral prefrontal areas. The specific projections from topographically distinct hippocampal areas may contribute to the different roles in mnemonic processing of medial/orbital in comparison with lateral prefrontal areas. Thus, a projection from the hippocampal formation to medial/orbital prefrontal cortices may be part of a network involved in long-term mnemonic processing, and its disruption may elicit a classic amnesic syndrome. In contrast, a link between the presubiculum/area 29a-c with lateral prefrontal cortices may be an important network for working memory. Moreover, based on the present findings as well as a previous study (Barbas and De Olmos, 1990), the connective attributes of medial and orbital prefrontal cortices can be differentiated further. Thus, orbital prefrontal cortices have a stronger association with the amygdala than with the hippocampus, whereas the opposite is true for medial prefrontal areas. On the basis of these findings we suggest that orbital prefrontal cortices have a role both in emotional behavior as well as in emotional memory. In contrast, medial prefrontal cortices may have mnemonic functions comparable to those associated with the hippocampal formation.

## Acknowledgments

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## REFERENCES

- Aggleton JP, Mishkin M (1983) Visual recognition impairment following medial thalamic lesions in monkeys. *Neuropsychologia* 21:189-197.
- Alexander MP, Freedman M (1984) Amnesia after anterior communicating artery aneurysm rupture. *Neurology* 34:752-757.
- Alheid GF, Heimer L, Switzer RC III (1990) Basal Ganglia. In: *The human nervous system* (Paxinos G, ed), pp 483-582. San Diego: Academic Press, Inc.
- Alvarez P, Zola-Morgan S, Squire LR (1995) Damage limited to the hippocampal region produces long-lasting memory impairment in monkeys. *J Neurosci* 15:3796-3807.
- Amaral DG (1987) Memory: anatomical organization of candidate brain regions. In: *Handbook of physiology—the nervous system V* (section

- 1, part 2) (Plum F, ed), pp 211–293. Washington DC: Am Physiol Soc.
- Amaral DG, Insausti R (1990) Hippocampal formation. In: *The human nervous system* (Paxinos G, ed), pp 711–756. San Diego: Academic Press, Inc.
- Arikuni T, Watanabe K, Kubota K (1988) Connections of area 8 with area 6 in the brain of the macaque monkey. *J Comp Neurol* 277:21–40.
- Bachevalier J, Mishkin M (1986) Visual recognition impairment follows ventromedial but not dorsolateral prefrontal lesions in monkeys. *Behav Brain Res* 20:249–261.
- Bakst I, Amaral DG (1984) The distribution of acetylcholinesterase in the hippocampal formation of the monkey. *J Comp Neurol* 225:344–371.
- Barbas H (1986) Pattern in the laminar origin of corticocortical connections. *J Comp Neurol* 252:415–422.
- Barbas H (1988) Anatomic organization of basoventral and mediodorsal visual recipient prefrontal regions in the rhesus monkey. *J Comp Neurol* 276:313–342.
- Barbas H (1992) Architecture and cortical connections of the prefrontal cortex in the rhesus monkey. In: *Advances in neurology*, vol. 57 (Chauvel P, Delgado-Escueta AV, Halgren E, Bancaud J, eds), pp 91–115. New York: Raven Press, Ltd.
- Barbas H (1993) Organization of cortical afferent input to orbitofrontal areas in the rhesus monkey. *Neuroscience* 56:841–864.
- Barbas H (1995a) The anatomic basis of cognitive-emotional interactions in the primate prefrontal cortex. *Neurosci Biobehav Rev* 19:499–510.
- Barbas H (1995b) Pattern in the cortical distribution of prefrontally directed neurons with divergent axons in the rhesus monkey. *Cerebral Cortex* 5:158–165.
- Barbas H, De Olmos J (1990) Projections from the amygdala to basoventral and mediodorsal prefrontal regions in the rhesus monkey. *J Comp Neurol* 301:1–23.
- Barbas H, Mesulam M-M (1985) Cortical afferent input to the principalis region of the rhesus monkey. *Neuroscience* 15:619–637.
- Barbas H, Pandya DN (1984) Topography of commissural fibers of the prefrontal cortex in the rhesus monkey. *Exp Brain Res* 55:187–191.
- Barbas H, Pandya DN (1987) Architecture and frontal cortical connections of the premotor cortex (area 6) in the rhesus monkey. *J Comp Neurol* 256:211–218.
- Barbas H, Pandya DN (1989) Architecture and intrinsic connections of the prefrontal cortex in the rhesus monkey. *J Comp Neurol* 286:353–375.
- Barbas H, Henion TH, Dermon CR (1991) Diverse thalamic projections to the prefrontal cortex in the rhesus monkey. *J Comp Neurol* 313:65–94.
- Bauer RH, Fuster JM (1976) Delayed-matching and delayed-response deficit from cooling dorsolateral prefrontal cortex in monkeys. *J Comp Physiol Psychol* 90:299–302.
- Beason-Held LL (1994) Contributions of the hippocampus and related ventromedial temporal cortices to memory in the rhesus monkey, Ph.D. Thesis, Boston University School of Medicine, Boston.
- Blatt GJ, Rosene DL (1988) Organization of hippocampal efferent projections to the cerebral cortex in the rhesus monkey. *Neurosci Abstr* 14:859.
- Brodmann K (1909) Vergleichende Lokalisationslehre der Grosshirnrinde in ihren Prinzipien dargestellt auf Grund der Zellenbaues. Leipzig: Barth.
- Cavada C, Reinoso-Suarez F (1988) Connections of the prefrontal cortex with the hippocampal formation in the cat and macaque monkey. *Neurosci Abstr* 14:858.
- Cavada C, Llamas A, Reinoso-Suarez F (1983) Allocortical afferent connections of the prefrontal cortex in the cat. *Brain Res* 260:117–120.
- Crowell RM, Morawetz RB (1977) The anterior communicating artery has significant branches. *Stroke* 8:272–273.
- Cummings JL, Tomiyasu U, Read S, Benson DF (1984) Amnesia with hippocampal lesions after cardiopulmonary arrest. *Neurology* 34:679–681.
- Damasio AR (1994) *Descartes's error: emotion, reason, and the human brain*. New York: G.P. Putnam's Sons.
- Davis M (1992) The role of the amygdala in fear and anxiety. *Annu Rev Neurosci* 15:353–375.
- DeJong RN, Itabashi HH, Olson JR (1968) "Pure" memory loss with hippocampal lesions: a case report. *Trans Am Neurol Assoc* 93:31–34.
- Dermon CR, Barbas H (1994) Contralateral thalamic projections predominantly reach transitional cortices in the rhesus monkey. *J Comp Neurol* 344:508–531.
- Funahashi S, Bruce CJ, Goldman-Rakic PS (1989) Mnemonic coding of visual space in the monkey's dorsolateral prefrontal cortex. *J Neurophysiol* 61:331–349.
- Fuster JM (1973) Unit activity in prefrontal cortex during delayed-response performance: neuronal correlates of transient memory. *J Neurophysiol* 36:61–78.
- Fuster JM (1989) *The prefrontal cortex*. New York: Raven Press, 2nd edition.
- Fuster JM (1990) Prefrontal cortex in motor control. In: *Handbook of physiology—the nervous system II*. (Field J, Magoon HW, Hall VE, eds), pp 1149–1178. Washington, DC: American Physiology Society.
- Fuster JM (1993) Frontal lobes. *Curr Opin Neurobiol* 3:160–165.
- Fuster JM, Bauer RH, Jervey JP (1985) Functional interactions between inferotemporal and prefrontal cortex in a cognitive task. *Brain Res* 330:299–307.
- Gallyas F (1979) Silver staining of myelin by means of physical development. *Neurol Res* 1:203–209.
- Geneser-Jensen FA, Blackstad TW (1971) Distribution of acetylcholinesterase in the hippocampal region of the guinea pig. *Z Zellforsch Mikrosk Anat* 114:460–481.
- Goldman-Rakic PS (1988) Topography of cognition: parallel distributed networks in primate association cortex. *Annu Rev Neurosci* 11:137–156.
- Goldman-Rakic PS, Selemon LD, Schwartz ML (1984) Dual pathways connecting the dorsolateral prefrontal cortex with the hippocampal formation and parahippocampal cortex in the rhesus monkey. *Neuroscience* 12:719–743.
- Irle E, Markowitsch HJ (1982) Widespread cortical projections of the hippocampal formation in the cat. *Neuroscience* 7:2637–2647.
- Isseroff A, Rosvold HE, Galkin TW, Goldman-Rakic PS (1982) Spatial memory impairments following damage to the mediodorsal nucleus of the thalamus in rhesus monkeys. *Brain Res* 232:97–113.
- Jacobsen CF (1936) Studies of cerebral function in primates: I. The functions of the frontal association area in monkeys. *Comp Psychol Monogr* 13:3–60.
- Janowsky JS, Shimamura AP, Kritchevsky M, Squire LR (1989) Cognitive impairment following frontal lobe damage and its relevance to human amnesia. *Behav Neurosci* 103:548–560.
- Kling A, Steklis HD (1976) A neural substrate for affiliative behavior in nonhuman primates. *Brain Behav Evol* 13:216–238.
- Kojima S, Goldman-Rakic PS (1982) Delay-related activity of prefrontal neurons in rhesus monkeys performing delayed response. *Brain Res* 248:43–49.
- Kubota K, Niki H (1990) Prefrontal cortical unit activity and delayed alternation performance in monkeys. *J Neurophysiol* 34:337–347.
- Kubota K, Tonoike M, Mikami A (1980) Neuronal activity in the monkey dorsolateral prefrontal cortex during a discrimination task with delay. *Brain Res* 183:29–42.
- LaVail JH (1975) Retrograde cell degeneration and retrograde transport techniques. In: *The use of axonal transport for studies of neuronal connectivity* (Cowan WM, Cuenod M, eds), pp 217–248. New York: Elsevier.
- LeDoux JE (1994) Emotion, memory and the brain. *Sci Am* 270:50–57.
- Lorente de N6 R (1934) Studies on the structure of the cerebral cortex. II. Continuation study of the ammonic system. *J Psychol Neurol* 46:113–177.
- Mahut H (1971) Spatial and object reversal learning in monkeys with partial temporal lobe ablations. *Neuropsychologia* 9:409–424.

- Mahut H (1972) A selective spatial deficit in monkeys after transection of the fornix. *Neuropsychologia* 10:65–74.
- Mahut H, Zola-Morgan S, Moss M (1982) Hippocampal resections impair associative learning and recognition memory in the monkey. *J Neurosci* 2:1214–1229.
- Markovitsch HJ (1982) Thalamic mediodorsal nucleus and memory: a critical evaluation of studies in animals and man. *Neurosci Biobehav Rev* 6:351–380.
- Matelli M, Camarda R, Glickstein M, Rizzolatti G (1986) Afferent and efferent projections of the inferior area 6 in the Macaque monkey. *J Comp Neurol* 251:281–298.
- Merigan WH, Maunsell JHR (1993) How parallel are the primate visual pathways? *Annu Rev Neurosci* 16:369–402.
- Mesulam M-M (1982) Principles of horseradish peroxidase neurohistochemistry and their applications for tracing neural pathways—axonal transport, enzyme histochemistry and light microscopic analysis. In: *Tracing neuronal connections with horseradish peroxidase* (Mesulam M-M, ed), pp 1–151. Chichester: John Wiley.
- Mesulam M-M., Hegarty E, Barbas H, Carson KA, Gower EC, Knapp AG, Moss MB, and Mufson EJ (1980) Additional factors influencing sensitivity in the tetramethyl benzidine method for horseradish peroxidase neurohistochemistry. *J Histochem Cytochem* 28:1255–1259.
- Milner B, Petrides M (1984) Behavioural effects of frontal-lobe lesions in man. *Trends Neurosci* 7:403–407.
- Morecraft RJ, Geula C, Mesulam M-M (1992) Cytoarchitecture and neural afferents of orbitofrontal cortex in the brain of the monkey. *J Comp Neurol* 323:341–358.
- Muramoto O, Kuru Y (1979) Pure memory loss with hippocampal lesions. *Arch Neurol* 36:54–56.
- Nauta WJH (1971) The problem of the frontal lobe: a reinterpretation. *J Psychiatr Res* 8:167–187.
- Nauta WJH (1972) Neural associations of the frontal cortex. *Acta Neurobiol Exp* 32:125–140.
- Nauta WJH (1979) Expanding borders of the limbic system concept. In: *Functional neurosurgery* (Rasmussen T, Marino R, eds), pp 7–23. New York: Raven Press.
- Nishijo H, Ono T, Nishino H (1988) Single neuron responses in amygdala of alert monkey during complex sensory stimulation with affective significance. *J Neurosci* 8:3570–3583.
- Parkinson JK, Murray EA, Mishkin M (1988) A selective mnemonic role for the hippocampus in monkeys: memory for the location of objects. *J Neurosci* 8:4159–4167.
- Petrides M (1989) Frontal lobes and memory. In: *Handbook of neuropsychology*, vol. 3 (Boller F, Grafman J, eds), pp 75–90. New York: Elsevier Science Publishers B.V. (Biomedical Division).
- Ray JP, Price JL (1993) The organization of projections from the mediodorsal nucleus of the thalamus to orbital and medial prefrontal cortex in macaque monkeys. *J Comp Neurol* 337:1–31.
- Rempel-Clower N (1994) Human amnesia and the hippocampal region: neuropsychological and neuropathological findings from two new patients. Ph.D. thesis, University of California at San Diego.
- Rockland KS, Pandya DN (1979) Laminar origins and terminations of cortical connections of the occipital lobe in the rhesus monkey. *Brain Res* 179:3–20.
- Rosene DL, Van Hoesen GW (1977) Hippocampal efferents reach widespread areas of cerebral cortex and amygdala in the rhesus monkey. *Science* 198:315–317.
- Rosene DL, Van Hoesen GW (1987) The hippocampal formation of the primate brain. A review of some comparative aspects of cytoarchitecture and connections. In: *Cerebral cortex*, Vol. 6 (Jones EG, Peters A, eds), pp 345–455. New York: Plenum Publishing Corporation.
- Rosene DL, Roy NJ, Davis BJ (1986) A cryoprotection method that facilitates cutting frozen sections of whole monkey brains from histochemical and histochemical processing without freezing artifact. *J Histochem Cytochem* 34:1301–1315.
- Saunders RC, Rosene DL (1988) A comparison of the efferents of the amygdala and the hippocampal formation in the rhesus monkey: I. Convergence in the entorhinal, prorrhinal, and perirrhinal cortices. *J Comp Neurol* 271:153–184.
- Sawaguchi T, Goldman-Rakic PS (1991) D1 dopamine receptors in prefrontal cortex: involvement in working memory. *Science* 251:947–950.
- Sawaguchi T, Matsumura M, Kubota K (1988) Dopamine enhances the neuronal activity of spatial short-term memory task in the primate prefrontal cortex. *Neurosci Res* 5:465–473.
- Schwerdtfeger WK (1979) Direct efferent and afferent connections of the hippocampus with the neocortex in the marmoset monkey. *Am J Anat* 156:77–82.
- Scoville WB, Milner B (1957) Loss of recent memory after bilateral hippocampal lesions. *J Neurol Neurosurg Psychiatry* 20:11–21.
- Squire LR (1992) Memory and the hippocampus: a synthesis from findings with rats, monkeys, and humans. *Psychol Rev* 99:195–231.
- Talland GA, Sweet WH, Ballantine T (1967) Amnesic syndrome with anterior communicating artery aneurysm. *J Nerv Ment Dis* 145:179–192.
- Van Essen DC, Maunsell JHR (1980) Two-dimensional maps of the cerebral cortex. *J Comp Neurol* 191:255–281.
- Vogt BA, Pandya DN, Rosene DL (1987) Cingulate cortex of the rhesus monkey: I. Cytoarchitecture and thalamic afferents. *J Comp Neurol* 262:256–270.
- Von Bonin G, Bailey P (1947) *The neocortex of Macaca mulatta*. Urbana: The University of Illinois Press.
- Voytko ML (1985) Cooling orbital frontal cortex disrupts matching-to-sample and visual discrimination learning in monkeys. *Physiol Psychol* 13:219–229.
- Wilson FA, Scalaidhe SP, Goldman-Rakic PS (1993) Dissociation of object and spatial processing domains in primate prefrontal cortex. *Science* 260:1955–1958.
- Zola-Morgan S, Squire LR (1993) Neuroanatomy of memory. *Annu Rev Neurosci* 16:547–563.
- Zola-Morgan S, Squire LR, Amaral DG (1986) Human amnesia and the medial temporal region: enduring memory impairment following a bilateral lesion limited to field CA1 of the hippocampus. *J Neurosci* 6:2950–2967.
- Zola-Morgan S, Squire LR, Amaral DG (1989a) Lesions of the hippocampal formation but not lesions of the fornix or the mammillary nuclei produce long-lasting memory impairment in monkeys. *J Neurosci* 9:898–913.
- Zola-Morgan S, Squire LR, Amaral DG (1989b) Lesions of the amygdala that spare adjacent cortical regions do not impair memory or exacerbate the impairment following lesions of the hippocampal formation. *J Neurosci* 9:1922–1936.
- Zola-Morgan S, Squire LR, Alvarez-Royo P, Clower RP (1991) Independence of memory functions and emotional behavior: separate contributions of the hippocampal formation and the amygdala. *Hippocampus* 1:207–220.