Distribution of Calbindin D-28k in the Entorhinal, Perirhinal, and Parahippocampal Cortices of the Macaque Monkey

WENDY A. SUZUKI1 AND ANGEL PORTEROS1,2
1Center for Neural Science, New York University, New York, New York 10012
2Dpto. Biología Celular y Patología, Universidad de Salamanca and Instituto de Neurociencias de Castilla y León, Salamanca E-37007, Spain

ABSTRACT
We examined the distribution of calbindin D-28k–immunoreactive (CB-IR) neurons, fibers, and neuropil in the entorhinal (area 28), perirhinal (areas 35 and 36), and parahippocampal (areas TH and TF) cortices in the macaque monkey. Two main findings are reported. First, except for CB-IR neurogliaform cells that are only observed in the parahippocampal cortex, the morphology of CB-stained pyramidal and nonpyramidal cells were similar across the three cortical areas examined. Second, we find that the topography of CB staining differed between the three areas. The entorhinal cortex exhibits the most striking gradient of CB staining such that the most anterior and medial portions are most strongly labeled, whereas posterior and lateral areas exhibit only weak labeling. The labeling throughout the perirhinal and parahippocampal cortices is more homogeneous. Area 35 contains only lightly stained neuropil and few CB-IR cells. Area 36 and areas TH and TF of the parahippocampal cortex contain a moderate to high density of CB-IR cells and fibers throughout their full rostrocaudal extents, although each area exhibits unique laminar patterns of staining. In all areas examined, the highest density of CB-positive cells and fibers is observed in superficial layers with lower densities of CB-positive cells and fibers present in deep layers. These findings, taken together with our current understanding of the connections of these areas may have implications for understanding the circuit properties of the entorhinal, perirhinal, and parahippocampal cortices areas in both normal and disease states. J. Comp. Neurol. 451:392–412, 2002. © 2002 Wiley-Liss, Inc.

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The entorhinal (area 28), perirhinal (areas 35 and 36), and parahippocampal (areas TH and TF) cortices are situated on the ventromedial aspect of the temporal lobe. Together, these adjacent and strongly interconnected cortical areas provide the major interface for sensory information both into and out of the hippocampus. The perirhinal and parahippocampal cortices have strong and largely reciprocal interconnections with the frontal, temporal, and parietal lobes as well as the cingulate, retrosplenial, and insular cortices (Van Hoesen et al., 1972; Martin-Elkins and Horel, 1992; Suzuki and Amaral, 1994a; Lavenex et al., 2001). The perirhinal and parahippocampal cortices also have strong and reciprocal projections with the entorhinal cortex (Insausti et al., 1987; Suzuki and Amaral, 1994b), the major cortical input of the hippocampus (Dolorfo and Amaral, 1998). Convergent findings from human neuropsychological studies (Scoville and Milner, 1957; Stefanacci et al., 2000) together with findings from lesion (Mishkin, 1978; Zola-Morgan and Squire, 1982) confirm the importance of the entorhinal, perirhinal, and parahippocampal cortices in memory and cognitive functions.
and neurophysiological (Miller et al., 1993; Suzuki et al., 1997) studies have shown that entorhinal, perirhinal, and parahippocampal cortices, together with the hippocampus, are essential for normal declarative memory.

In addition to their role in memory function, there is also substantial evidence that these same medial temporal lobe areas are also highly vulnerable in several human neurologic disease states such as Alzheimer’s disease (AD; Arnold et al., 1991a; Braak and Braak, 1996), schizophrenia (Jakob and Beckmann, 1986; Falkai et al., 1988; Arnold et al., 1991b), and temporal lobe epilepsy (Sloviter, 1989; Sloviter et al., 1991). For example, the region referred to as the “transentorhinal” zone (Braak and Braak, 1985), which appears to correspond to area 35 of the perirhinal cortex (Van Hoesen et al., 2000), is the first brain area to develop neurodegenerative alterations in AD. It has long been appreciated that the entorhinal cortex is devastated in Alzheimer’s disease (Hooper and Vogel, 1976; Van Hoesen et al., 1991, 2000), and both area 36 of the perirhinal cortex as well as the parahippocampal cortex also exhibit striking neuropathology in AD (Van Hoesen et al., 2000).

Despite the important role of the medial temporal lobe in normal memory function and its susceptibility in disease states, surprisingly little is known about the neurochemical organization of these areas. The cytosolic calcium-binding protein calbindin D-28K (CB) is one neurochemical marker that has received considerable attention. CB is localized to distinct distributions of neurons throughout the cortex in monkeys, humans, and rats (DeFelipe et al., 1989; Celio, 1990; Van Brederode et al., 1990; Kobayashi et al., 1990; Hof and Morrison, 1991; DeFelipe and Jones, 1991; Beall and Lewis, 1992; Hof and Nimchinsky, 1992). Although the specific functions of CB remain to be determined, it has been suggested that it may play a protective role for buffering excess calcium influx (Baimbridge et al., 1992; Rintoul et al., 2001; Yenari et al., 2001) as well as a role in plasticity (Hendrickson et al., 1991; Baimbridge et al., 1992).

Previous studies suggest that CB-containing neurons may be differentially vulnerable in AD. For example, in the prefrontal cortex, CB containing pyramidal neurons of layer III and CB-containing interneurons of layer V–VI are both severely affected in AD, although the CB-positive nonpyramidal cells of the superficial layers appear unaffected (Hof and Morrison, 1991). In the medial temporal lobe, one previous study has reported decreases in CB gene expression in the hippocampus in AD (Iacopino and Christakos, 1990). Another study, however, reported that CB-positive neurons in the temporal lobe resist degeneration in AD (Hoffman et al., 1989).

An important prerequisite for interpreting the patterns of CB staining in diseased brain tissue is a clear understanding of the distribution of CB in normal brain tissue. Although several previous studies have described the distribution of CB in the entorhinal cortex in normal human brain tissue (Beall and Lewis, 1992; Tunon et al., 1992; Mikkonen et al., 1997), much less is known about the distribution of CB in animal model systems such as the macaque monkey. In this study, we provide a comprehensive description of the cellular morphology as well as the laminar and regional topography of the distribution of CB-immunoreactive (IR) neurons, fibers, and neuropil throughout the macaque monkey entorhinal, perirhinal, and parahippocampal cortices. The identification of neurochemically distinct populations of neurons can be a useful tool for dissecting the neural circuitry of the medial temporal lobe in both normal and pathologic conditions.

MATERIALS AND METHODS

Subjects and fixation

Brain tissue from three adult male cynomolgus monkeys (Macaca fascicularis) weighing between 3.6 and 4.5 kg, were used for this study (cases M7–98, M14–00, and M12–99). These same animals were also used in nonrelated tract tracing experiments. The monkeys were first tranquilized with ketamine-HCl (10 mg/kg, i.m.) and deeply anesthetized with sodium pentobarbital (50 mg/kg, i.v.). After rinsing the vascular tree with 500 ml of 0.9% saline, a 4% paraformaldehyde solution in 0.1 M sodium acetate buffer (pH 6.5) was perfused at 250 ml/min for 5 minutes and 100 ml/minute for 15 minutes followed by 4% paraformaldehyde in 0.1 M sodium borate buffer, pH 9.5 (100 ml/min for 30 minutes). The brain was dissected out and post-fixed in the 4% sodium borate buffer solution for 6 hours at 4°C.

Histologic processing

After washing in phosphate buffer, the brains were cryoprotected first in a 10% glycerol solution with 2% dimethyl sulfoxide (DMSO) in phosphate buffer for 24 hours followed by 2 days in a 20% glycerol-2% DMSO solution. The brains were frozen with cooled isopentane and stored at −70°C until further processing. A one-in-eight series of section were cut at 30 μm in the coronal plane through the entire extent of the medial temporal lobe with a freezing, sliding microtome. Sections were stored in tissue collecting solution (30% ethylene glycol and 25% glycine in 0.05 M sodium phosphate buffer, pH 7.3) at −20°C until the immunohistochemical processing was started.

Sections were first rinsed in phosphate buffer and processed for CB D-28k immunohistochemistry by using the avidin-biotin-peroxidase method. The sections were sequentially incubated in (1) primary mouse anti-rat-CB antibody (monoclonal Ab 300 antibody, Swant Antibodies, Bellinzona, Switzerland) diluted 1:2,500 in phosphate buffer containing 10% normal horse serum and 0.03% Triton X-100 for 48 hours at 4°C; (2) biotinylated antimouse immunoglobulin G (Vector Labs, Burlingame, CA) diluted 1:250 in phosphate buffer for 2 hours at room temperature; and (3) horseradish peroxidase-coupled avidin complex (1:200 in phosphate buffer, Vector) for 2 hours at room temperature. The peroxidase was visualized with 0.07% 3,3’-diaminobenzidine and 0.003% hydrogen peroxide in 0.1 M Tris-HCl buffer (pH 7.6). The sections were stored in the dark until analyzed. The specificity of the antiserum has been previously described (Celio, 1990) by using radioimmunoassay and bidirectional gel electrophoresis.

Analysis

CB-stained sections were analyzed by using a Zeiss III microscope, an Axioshot microscope, and/or an Olympus SZX12 stereomicroscope with a camera lucida attachment. The cytoarchitectonic boundaries and laminar organization of the different areas were drawn from adjacent
Nissl-stained sections. Pyramidal cells included in the analysis of somatic area were situated in the middle of the thickness of the analyzed section and exhibited a clearly visible nucleus. Nonpyramidal cells were also situated in the middle of the thickness of the section and exhibited at least two primary prolongations. For each cell, the diameter of the maximal visible cross-section was quantified. For each neuronal type and region, 30 neurons were measured. To obtain an estimation of proportions of CB-IR neuronal types, we counted the different cell types observed in three randomly selected 50×500 μm rectangles situated parallel to the pial surface in the entorhinal, perirhinal, and parahippocampal cortices in each of the three animals studied. In Table 1, + = less than 5 cells; ++ = 5 to 20 cells; +++ = 20 to 40 cells; ++++ = more than 40 cells. We used a relative scale to measure the intensity of CB labeling seen in Tables 1–4. Despite the fact that the sections from each of the three cases used were not all stained in the same batch, the relative staining intensities observed for the various cell types across all three animals were consistent. All cell measurements were carried out with a Neurolucida image analysis system. Brightfield digital images were taken with an Olympus Provis AX microscope and a digital camera (1.34 megapixels, Apogee Instruments, Inc., Tucson, AZ). The objectives used were Olympus 1.25× (PlanApo N.A. 0.04) and UPlanFl 4× (N.A. 0.13), 20× (N.A. 0.50), and 40× (N.A. 0.75). The images were captured with Adobe Photoshop 6.0 software (Adobe Systems, Inc., San Jose, CA). The capture software was connected to a trichromatic sequential filter (Cambridge Research & Instrumentation, Inc., Boston, MA). After conversion into black and white, the sharpness, contrast, and brightness were adjusted to reflect the appearance of the labeling seen through the microscope. Unfolded maps were drawn by using a camera lucida attachment to an Olympus SZX 12 stereomicroscope. All unfolded maps were constructed as previously described in Suzuki and Amaral (1994b).

RESULTS

Nomenclature and cytoarchitectonic features

The entorhinal, perirhinal, and parahippocampal cortices surround the rhinal sulcus on the ventromedial surface of the monkey brain. The terminology for the various subdivisions of these regions of cortex as well as the criteria used for establishing the boundaries between the subdivisions have been described previously (Amaral et al., 1987 for the entorhinal cortex; Suzuki and Amaral, unpublished observations for perirhinal and parahippocampal cortices). Briefly, the entorhinal cortex is situated medial to the rhinal sulcus and has six subdivisions (Figs. 3, 4). Rostrally and medially is the olfactory division (EO), the only entorhinal field to receive a direct projection from the olfactory bulb. Area EO is characterized by a thin layer I, a thin layer II made up of lightly stained cells, and a patchy, disorganized layer III made up of large darkly stained cells. Layer IV is more prominent than in any other regions of area 36 (Fig. 9F). The caudal part of the entorhinal cortex is characterized by a clear sublamination in layer VI. The caudal portion of the entorhinal cortex is made up of the larger, more laterally situated caudal division (EC), and a smaller, medially situated caudal limiting division (ECL). Area EC exhibits the most columnar organization of the entorhinal cortex, and there are multiple sublaminae visible in layers V and VI (Fig. 3E). The lamina dissecans is either very thin or not visible in area EC. Layer II of area ECL tends to be thicker than in area EC, the lamina dissecans is also very thin or not visible, and layers V and VI are not as strongly laminated as area EC (Fig. 3F).

The perirhinal cortex is situated lateral to the full rostrocaudal extent of the rhinal sulcus. It is composed of a region situated more posterior to area 35 and a larger, more laterally situated area 36 (Figs. 9, 10). Area 35 is a thin, agranular cortex characterized by irregular patches of large darkly stained pyramidal cells in layer II and a distinctive dense band of large, darkly staining pyramidal and multipolar cells in layer V (Fig. 9A). Area 36 includes five subdivisions. Area 36d is the most anterior and dorsal subdivision of the perirhinal cortex and lies on the medial portion of the temporal pole. Area 36d is characterized by relatively small clumps of darkly stained neurons in the superficial parts of layer II. Deep portions of layer II are composed of neurons that are slightly smaller and less densely packed giving layer II a bilaminate appearance. Although the bilaminate organization of layer II is visible in area 36d, this feature is most prominent in most medial regions of area 36 (i.e., area 36r and 36c). Layers III and IV of area 36d are narrow and layers V and VI are not easily differentiated (Fig. 9D). Area 36r is situated just posterior to area 36d and sits lateral to approximately the anterior half of the entorhinal cortex. Area 36r has been further subdivided into the medial and a lateral subdivision (areas 36rm and 36rl, respectively). Layer II of area 36rm is characterized by a distinctive bilaminate organization and clumps of large darkly stained neurons in the superficial parts of layer II. Layer III is sparsely populated and made up of large, relatively lightly stained rounded cells. Layer IV is thin and layers V and VI are made up of a dense population of large darkly stained cells (Fig. 9B). Area 36rl can be distinguished from area 36rm because the cell aggregates of darkly stained cells in layer II are more irregularly spaced and layer VI is wider than in area 36rm (Fig. 9C). Area 36c lies posterior to area 36r and is also subdivided into medial and lateral subdivisions (areas 36cm and 36cl, respectively). Area 36cm can be distinguished from area 36rm because the cortex is thinner and has a more distinctly radial organization (Fig. 9E). Area 36rl is the most laminated subdivision of the perirhinal cortex and layer IV is more prominent than in any other subdivision of area 36 (Fig. 9F).
The parahippocampal cortex lies caudal to both the perirhinal and entorhinal cortices and is composed of a medially situated area TH and a larger and more laterally situated area TF (Figs. 15, 16). Area TF is subdivided into medial and lateral portions (areas TFm and TFl, respectively). Area TH is a rather primitive looking cortex. Through most of its rostrocaudal extent, area TH is agranular, although a weak layer IV can sometimes be seen in the most caudal sections. Layers II and III of area TH are relatively thin and not well differentiated from each other. The most distinctive characteristic of area TH is its densely populated layer V made up of large, round, and darkly stained cells. Layer VI can be easily distinguished from layer V because cells are clearly smaller (Fig. 15A). Layers II and III of area TFm are more easily differentiable than in area TH. Layer IV in area TFm is thin, and layers V and VI contain darkly staining pyramidal cells that tend to be smaller than the distinctive layer V cells of area TH (Fig. 15B). Area TFl is thicker than area TFm and contains a more prominent layer IV. Layer V tends to have a trilaminar appearance with large pyramidal cells tending to congregate in the middle of the layer, whereas superficial and deep portions are less strongly populated (Fig. 15C).

**General appearance of the CB immunostaining**

The morphologic characteristics and regional and laminar patterns of distribution of the calbindin-
immunoreactive (CB-IR) structures in the monkey medial temporal lobe were consistent across the three animals studied. CB immunoreactivity is found in somata, dendrites, axons, immunoreactive puncta, and neuropil distributed throughout the entorhinal, perirhinal, and parahippocampal cortices. In general, the darkest CB neuropil staining is observed in supragranular layers and is more intense in layers II and III than in layer I. As described in many previous studies, CB immunoreactivity is observed in subpopulations of both pyramidal and nonpyramidal neurons (Stichel et al., 1987; DeFelipe et al., 1989; Sequer et al., 1990; van Brederode et al., 1990; Hof and Morrison, 1991; DeFelipe and Jones, 1991; Hendrickson et al., 1991; Seress et al., 1991; Beall and Lewis, 1992; Hayes and Lewis, 1992; Mikkonen et al., 1997; Kondo et al., 1999). The majority of the stained cells are moderate to weakly stained pyramidal cells identified by the presence of a thick apical dendrite oriented toward the cortical surface and characteristic triangular-shaped cell body (Fig. 1A,B). In contrast, most of the CB-IR nonpyramidal neurons were intensely immunoreactive and displayed a high variability of shapes, sizes, dendritic morphologies, and laminar localization (Figs. 1, 2). We identified five main categories of CB-IR nonpyramidal cells: multipolar, Cajal-Retzius, round, and neurogliaform. Except for neurogliaform cells, which were only observed in the parahippocampal cortex, all other cell types were observed in all three cortices examined with only minor differences in their size or laminar distributions. Tables 1 to 4 summarize the staining intensity, laminar localization, and size for all morphologic cell types observed.

**Pyramidal cells.** CB-IR pyramidal cells in all three cortices of the medial temporal lobe can be subdivided based on their size and staining intensity (Fig. 1A,B; Table 1). Pyramidal neurons range in size from medium (153.5 μ ± 45.5 μ) to large (210.1 μ ± 33.2 μ), display light to medium staining intensity, and are predominantly located in superficial layers II and III, although some lightly stained pyramidal cells are found in layers V–VI (Table 1). Their somata exhibit a characteristic triangular shape, and generally only the initial portions of the basal and apical dendrites are stained (Fig. 1A,B). In areas EO2 and ER of the entorhinal cortex, CB-IR pyramidal cells form dense clusters in layer III. In perirhinal and parahippocampal cortices they are located through layer III. The CB-IR pyramidal cells in infragranular layers are slightly smaller and less intensely stained than pyramidal cells in supragranular layers.

**Nonpyramidal cells**

**Multipolar cells.** A relatively sparse population of large darkly stained, CB-IR multipolar neurons with round or polygonal somata is observed throughout the different cortical layers, although they are most abundant in layers II and III (Fig. 1C,D). These cells had two to four stained dendrites that ramified at short distances from the somata, with no preferential pattern of ramification or orientation of the dendrites. In the entorhinal cortex, and most prominently in the parahippocampal cortices, we observe medium multipolar neurons (Fig. 13A). Like the large multipolar cells, the medium multipolar cells display a heterogeneous pattern of ramification but exhibited a more widespread localization in both supragranular and infragranular layers (II–VI). Small numbers of small, darkly stained multipolar neurons with triangular cell bodies and thin apical, basal, or horizontally oriented dendrites are also seen in layers II to VI of entorhinal and parahippocampal cortices, but they are not present in the perirhinal cortex.

**Bipolar cells.** Intensely stained CB-IR bipolar neurons can be observed in all layers of the entorhinal, perirhinal, and parahippocampal cortices, although the highest density is observed in layers II, III, and V (Fig. 1E,F). CB-IR bipolar cells can be differentiated by the size of their cell bodies. Strongly stained large bipolar cells are observed in all three cortical areas analyzed, and strongly stained medium bipolar cells are observed in the entorhinal cortex. Typically, these bipolar CB-IR cells have elliptical somata with one ascending and one descending primary dendrite (Fig. 1F). Although most of the CR-IR bipolar neurons are vertically oriented (Fig. 1F), some of the medium bipolar cells are oriented horizontally (Fig. 1E).
also observe some lightly stained primarily vertical bipolar cells located in layers II, V, and VI. These cells are most abundant in the perirhinal cortex and are also observed in the parahippocampal cortex.

*Cajal-Retzius cells.* Scattered throughout layer I of entorhinal, perirhinal, and parahippocampal cortices are large and intensely stained fusiform shaped cells with two stained dendrites extending from the soma (Figs. 2A, 5A arrows, 6A arrow, 17A arrows). The morphology and localization of these cells correspond with that of CB-IR Cajal-Retzius cells, which have been described in several cortical areas throughout the monkey brain (Ramon y Cajal, 1911; DeFelipe and Jones, 1988; Ferrer et al., 1992).

*Neurogliaform cells.* One category of CB-IR multipolar neuron that is unique to the parahippocampal cortex is the neurogliaform cell (Fig. 2B). This category of multipolar neuron is distinguished by the presence of numerous dendrites that radiated out from a small round or ovoid cell body and by its small size (41.3 ± 7.2 μm). The stained dendrites are of approximately equal length, thin and lightly stained and resemble the neurogliaform cells that have been described by Conde et al. (1994) in the prefrontal cortex. They are present in layers II–VI, although they are preferentially located in superficial layer II.

*Round cells.* We observe a small number of small, lightly stained cells with very thin and short stained prolongations in layer I (Fig. 2C; Tables 2–4). These cells are seen throughout layer I of the entorhinal, perirhinal, and parahippocampal cortices, and have not previously been described in the monkey cortex.
Distribution of calbindin D-28k in the entorhinal cortex

General distribution of the labeling. The most striking feature of CB staining in the entorhinal cortex is the prominent rostrocaudal gradient (Figs. 4, 19). Although rostral areas EO and ER exhibit high densities of CB-IR cells, fibers, and neuropil, CB staining gradually decreases in area EI and is the lowest in posterior areas EC and ECL. The anterior half of the entorhinal cortex is the most intensely stained subdivision of the entorhinal cortex (EO). Although rostral areas E O and E R exhibit high densities of CB-IR cells, fibers, and neuropil, CB staining gradually decreases in area E I and is the lowest in posterior areas E C and E CL. The anterior half of the entorhinal cortex is the most intensely stained subdivision of the entorhinal cortex (EO). The prominent rostrocaudal topography of labeling is clearly visible in these sections, with rostral sections (A) exhibiting the strongest labeling and caudal sections (D) exhibiting the weakest overall CB staining. Amyg, amygdala; E O, olfactory subdivision of the entorhinal cortex; E R, rostral subdivision of the entorhinal cortex; E L, lateral subdivision of the entorhinal cortex; E C, caudal subdivision of the entorhinal cortex; E CL, caudal limiting division of the entorhinal cortex; Hpc, hippocampus; rs, rhinal sulcus. Scale bar = 1 mm in D (applies to A–D).

Fig. 4. A–D: Low-power brightfield photomicrographs of stained coronal sections demonstrating the distribution of calbindin D-28k (CB) immunoreactivity in different subfields of the entorhinal cortex from rostral (A) to caudal (D). Arrowheads indicate the boundaries between the entorhinal subfields. The prominent rostrocaudal topography of labeling is clearly visible in these sections, with rostral sections (A) exhibiting the strongest labeling and caudal sections (D) exhibiting the weakest overall CB staining. Amyg, amygdala; E O, olfactory subdivision of the entorhinal cortex; E R, rostral subdivision of the entorhinal cortex; E L, lateral subdivision of the entorhinal cortex; E C, caudal subdivision of the entorhinal cortex; E CL, caudal limiting division of the entorhinal cortex; Hpc, hippocampus; rs, rhinal sulcus. Scale bar = 1 mm in D (applies to A–D).

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Olfactory division of the entorhinal cortex (EO). Area E O is the most intensely stained subdivision of the entorhinal cortex (Figs. 4A, 5A, 8). The neuropil and fiber stain in layer I, however, is light. Layer I contains a small number of CB-IR cells, which are typically small and round. In addition to the small round cells in layer I, this layer also contains a small number of large and intensely stained Cajal-Retzius cells (Fig. 5A, arrows). Layer II of area E O is very thin (Fig. 3A), and the neuropil and cell staining in this layer resembles that of layer I. Layer III of area E O contains the darkest neuropil and fiber labeling and the highest density of CB-IR cells of the entorhinal
cortex. This layer contains a high number of lightly stained CB-IR pyramidal cells that are arranged in large irregular patches (Fig. 5A). Layer III also contains a population of densely stained CB-IR nonpyramidal cells. Most of these interneurons have a vertical bipolar morphology, with descending dendrites that extend toward deep layer III, although some multipolar neurons are also observed. The neuropil staining in layers V–VI is less dense than in layer III, and there are far fewer stained pyramidal and nonpyramidal cells present. The stained nonpyramidal cells in the deep layers exhibit both bipolar and multipolar morphology.

**Rostral subdivision of the entorhinal cortex (E_R).** Although rostral and medial portions of area E_R resemble area E_O in its density and pattern of CB staining, the overall density of staining gradually decreases in more lateral portions of this field (Figs. 4A, 5A, 5B, 8, 19). Like area E_O, neuropil staining in layers I and II of area E_R is light. Groups of small, round interneurons with short stained prolongations can also be observed in these superficial layers. Medial portions of layer III of area E_R contain densely stained neuropil and fibers as well as a high density of CB-IR cells. In lateral portions of layer III, the clusters of CB-IR cells and fibers become smaller and more discrete and the zones of light labeling between the clusters become proportionately larger. Clusters of neurons in layer III of area E_R are also characteristic of Nissl-stained sections (Fig. 3B). The CB-IR cells in layer III include both pyramidal as well as nonpyramidal cells that have a vertical bipolar morphology. These bipolar neurons tend to be situated in the superficial portion of layer III (Fig. 5B, arrow). In general, the density of CB-IR nonpyramidal cells is lower than the density of lightly stained CB-IR pyramidal cells in layer III. The neuropil staining in layer V and VI of area E_R is light and is similar in density to the zones of light neuropil staining between the densely stained clusters typical of layer III. The deep layers contain only small numbers of CB-IR cells, and these cells exhibited a mainly vertical bipolar morphology.

**Lateral subdivision of the entorhinal cortex (E_L).** The staining density in area E_L is weaker than in the adjacent area E_R. Area E_L exhibits a clear rostrocaudal gradient with rostral regions exhibiting stronger CB staining than caudal regions (Figs. 4A–C, 6A, 8). Layer I of area E_L contains only lightly stained neuropil and a low density of small and lightly stained cells as well as Cajal-Retzius cells. Layers II and III contain a higher density of CB-IR cells and fibers. In contrast to the patchy organization of CB staining in layer III in medial portions of area E_R, the labeled cells and neuropil in layers II and III of

Fig. 5. Brightfield photomicrographs demonstrating the distribution of calbindin D-28k (CB) in areas E_O (A) and E_R (B) of the entorhinal cortex. In both panels, medial is toward the left and lateral to toward the right. Note the dense cell and neuropil labeling in layer III of area E_O (A). Arrows in A indicate the location of Cajal-Retzius cells in layer I. B illustrates a lateral portion of area E_R in which the overall level of CB staining is decreased relative to E_O, although dense patches of CB labeling are clearly visible in layer III. Arrow in B shows a bipolar cell in superficial portions of layer III. Roman numerals separated by horizontal lines indicate the boundaries of individual cortical layers. E_O, olfactory subdivision of the entorhinal cortex; E_R, rostral subdivision of the entorhinal cortex. Scale bar = 250 μm in B (applies to A,B).
area E_I are more homogeneously distributed. These layers contain pyramidal cells, which are distributed evenly throughout the two layers as well as a relatively large population of nonpyramidal cells. Horizontal bipolar cells are conspicuous in superficial portions of layer II (Fig. 6A, arrow) and other horizontal and vertically oriented bipolar cells are observed throughout layer II and III. The density of staining in layers II and III is strongest in rostral portions of this area and gradually decreases in caudal regions. In rostral portions of layer III, the labeled cells and fibers are distributed homogeneously throughout the full depth of the layer. However, in caudal portions, a stain-free band occupies the deepest portion of layer III and superficial portions of layer V. The neuropil stain in layers V is weaker than that observed in layers II and III, and this layer also contains small numbers of predomi-
antly bipolar cells. Compared with layer V, the density of stained cells in layer VI is similar, although the neuropil label is less pronounced.

Intermediate subdivision of the entorhinal cortex (E_I). The intermediate subdivision of the entorhinal cortex exhibits both a mediolateral and a rostrocaudal gradient of CB staining (Figs. 4C, 6B, 8). Indeed, the CB staining in area E_I is transitional between the dense staining patterns of anterior areas E_R and E_O and the weak staining patterns of posterior areas E_C and E_CL. Throughout area E_I, both layers I and II are only lightly stained and contain small numbers of CB-IR cells. Layer III exhibits the most dramatic variation through its rostrocaudal and mediolateral axes. Rostromedial portions of layer III are relatively homogeneous and contain densely stained neuropil and a moderately high density of stained pyramidal and nonpyramidal cells (Fig. 8). The majority of the intensely stained nonpyramidal cells exhibit bipolar morphology, although some scattered multipolar cells are also present. The lateral portions of rostral layer III contain discrete clusters of densely stained neuropil and labeled cells separated by zones of lightly stained neuropil and lower densities of labeled cells (Fig. 8). Layer III in the posterior half of area E_I is characterized by the presence of a relatively light density of CB-IR neuropil and cells distributed homogeneously throughout the superficial two thirds of the layer. The deepest third of posterior layer III is occupied by a striking stain-free band. This stain-free band is continuous with the cell-free lamina dissecans that is prominent in Nissl-stained sections at this level of area E_I (Fig. 3D). The pattern of CB staining observed in posterior portions of layer III of area E_I is similar to and continuous with the pattern of layer III staining in posteriorly adjacent areas E_C and E_CL (see below). CB staining
deep layers V and VI of area EI is less dense than the staining in layer III. Layer V exhibits a light neuropil stain with just a few labeled cells. The neuropil stain is even lighter in layer VI making the layer V/VI border easily visible (Fig. 6B). Small numbers of CB stained cells are also observed in layer VI. Most of the CB-IR cells in layer V and VI display bipolar morphology, although some multipolar cells are also present. Like other subdivisions of the entorhinal cortex, the deep layers of area EI contain only occasional pyramidal cells.

Caudal division of the entorhinal cortex (EC). Area EC together with area E CL exhibits the lowest density of CB-IR elements of all the subdivisions of the entorhinal cortex (Figs. 4D, 7A, 8). The neuropil staining in layers I and II of area EC is weak, and small numbers of lightly stained and Cajal-Retzius cells are present. Layer III contains light neuropil labeling and moderate numbers of CB-IR pyramidal through its superficial two-thirds. Similar to layer III of area EC, there is a striking staining-free band that occupies the deep portion of layer III. The lamina dissecans is either very thin or not visible in Nissl-stained sections through area EC (Fig. 3E). Layer V exhibits moderately dense neuropil labeling that contrasts sharply with the stain-free band in deep layer III. Small numbers of labeled cells are also observed in layer V. The neuropil staining in layer VI is noticeably weaker than that of layer V, making the layer V–VI boundary clearly visible in CB-stained sections. Like layer V, layer VI also contains small numbers of mainly nonpyramidal CB-IR cells.

Caudal limiting division of the entorhinal cortex (EC L). The laminar pattern CB staining in area EC L is essentially identical to the pattern of labeling in area EC (Figs. 4D, 7B, 8). The only difference of note is that the overall density of labeled cells in superficial layer III is slightly decreased in area EC L compared with area EC.

Distribution of calbindin D-28k in the perirhinal cortex

General distribution of the labeling. The pattern of CB immunostaining in the perirhinal cortex is distinctly different from the staining in the laterally adjacent entorhinal cortex (compare Figs. 4 and 10). In contrast to the striking rostrocaudal gradient of CB staining seen in the entorhinal cortex, the pattern of CB staining in the perirhinal subfields is generally homogeneous through its rostrocaudal axis. Area 35 exhibits the lowest density of CB staining in the perirhinal cortex. Area 36 is strongly stained and the density of staining is similar or slightly less than the density of staining seen in entorhinal areas.
35 exhibiting the highest density of neuropil and more caudal regions exhibiting gradually lighter neuropil and cell staining. Layers V and VI of area 35 exhibit only light neuropil stain and contain a small number of small, mostly lightly stained nonpyramidal cells. The contrast between more densely stained superficial layers and lightly stained deep layers is much less striking than in the adjacent area 36 (see below).

**Area 36**

Dorsal area 36 (area 36d). Layer I of area 36d contains a weakly stained neuropil and occasional Cajal-Retzius cells (Figs. 11B, 14). The density of neuropil staining in layer II is higher than in layer I, and layer II has a bilaminar organization. Superficial portions of layer II contain few if any CB-IR cells. The superficial portions of layer II correspond to a region that sometimes contains the distinctive clusters of cells in Nissl-stained sections (Fig. 9D). In contrast to superficial portions of layer II, deep portions containing a band of densely stained nonpyramidal cells. The neuropil in the deep portion of layer II and layer III is darker than in layer I. Layer III contains a moderately dense population of small and lightly CB-stained pyramidal cells. The CB-IR pyramidal cells of layer III exhibit a density gradient such that the densest population of cells is situated deep in the layer and fewer cells are located superficially. As a consequence, staining in the superficial portion of the layer appears relatively light. Deep layers contain very light neuropil label (even lighter than layer I) and only small numbers of labeled cells. Most of the CB-IR cells in these layers are nonpyramidal cells with bipolar morphology.

Rostromedial subdivision of area 36 (area 36rm). The neuropil staining in layer I of area 36rm is darker than in area 35 (Figs. 10A, 12A, 14). Small numbers of lightly stained, round cells, as well as Cajal-Retzius cells are observed in layer I. Layer II of area 36rm contains a more densely stained neuropil than layer I and, like area 36d, has a bilaminar organization in both Nissl-stained sections (Fig. 9B) as well as CB-stained sections. Although the superficial portion of layer II appears relatively free of CB-stained cells, deep portions of the layer contain a distribution of intensely stained bipolar and multipolar cells. The population of bipolar cells outnumber the multipolar cells in this layer. The neuropil stain in layer III resembles that of layer II. Layer III also contains a dense population of CB-IR pyramidal cells that are distributed relatively homogeneously through the layer. Small numbers of densely stained nonpyramidal cells can also be observed scattered through the layer. In striking contrast to layers II and III, layers IV, V, and VI contain a very lightly stained neuropil, although the neuropil in layer V tends to be slightly darker than in layers IV or VI (Fig. 10). Layer IV is relatively cell free and layer V contains more CB-IR-cells than layer VI. The CB-positive cells tend to be mostly densely stained nonpyramidal cells, including both bipolar and multipolar cells.

Rostrolateral subdivision of area 36 (area 36rl). Layer I of area 36rl resembles that of area 36rm and contains a moderately stained neuropil with small numbers of CB-IR cells (Figs. 10A, 12B, 14). Like area 36rm, the CB staining in layer II of area 36rl is also distinguished by its bilaminar organization. Like layer I, the superficial portion of layer II contains a moderately stained neuropil and only occasionally labeled cells. The superficial portion of layer II corresponds to the distinctive and irregularly spaced

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**Fig. 8.** Schematic diagrams showing the pattern of cell and neuropil staining throughout the different subdivisions of the entorhinal cortex. Note the heaviest staining in rostral and medial portions of the entorhinal cortex with relatively light staining in the most caudal subdivisions. $E_O$ (medial) and $E_L$ (lateral) show the medial and lateral subdivisions of the rostral area $E_I$. The staining pattern in posterior portions of area $E_C$ resembles area $E_O$ and $E_L$, Roman numerals to the left of each cortical area indicate the layer. LD, lamina dissecans; $E_O$, olfactory subdivision of the entorhinal cortex; $E_L$, lateral subdivision of the entorhinal cortex; $E_C$, caudal subdivision of the entorhinal cortex; $E_{1,2}$, caudal limiting division of the entorhinal cortex; CB, calbindin; -IR, immunoreactive.
clumps of large darkly stained cells that are characteristic of area 36rl in Nissl-stained sections (Fig. 9C). The deep portion of layer II contains a population of intensely stained predominantly bipolar cells. This band of nonpyramidal cells in deep layer II corresponds to the population of round, lightly stained cells seen in Nissl-stained material (Fig. 9C). The most superficial portion of layer III also contains a thin band of intensely stained nonpyramidal cells that is continuous with the band of nonpyramidal cells in deep portions of layer II. Just deep to these nonpyramidal cells is a region of relatively sparse CB labeling. The deepest portions of layer III contain a dense population of CB-IR pyramidal cells. Occasional CB-positive nonpyramidal cells are also observed throughout the deep portions of layer III. In general, layer III of area 36rl tends to have a more laminar appearance in CB-stained material than area 36rm. Layers IV, V, and VI of area 36rl resemble the same layers in area 36rm. All three layers contain light neuropil stain and only small numbers of CB-IR cells. The neuropil of layer V in area 36rl tends to be slightly more densely stained than layers IV or VI. This layer also tends to contain the largest proportion of CB-IR cells. The deep layers contain small numbers of CB-IR bipolar and multipolar cells.

Caudomedial subdivision of area 36 (area 36cm). The general staining pattern in area 36cm resembles that of area 36rm, although the density of labeled cells tends to be slightly lower in area 36cm (Figs. 10B, 13A, 14). Layer I of

Fig. 9. Photomicrographs of Nissl-stained sections through areas 35 (A), 36rm (B), 36rl (C), 36d (D), 36cm (E), and 36cl (F) of the perirhinal cortex. These subdivisions are based on the work of Amaral et al. (1987) and Suzuki and Amaral (unpublished observations). Roman numerals separated by horizontal lines indicate the boundaries of individual cortical layers. See text for details of the major cytoarchitectonic features of each area. rm, rostromedial; rl, rostrolateral; d, dorsal, cm, caudomedial; cl, caudolateral. Scale bar = 500 μm.
area 36cm contains a moderately stained neuropil with occasionally small, lightly stained cells. Layer II contains a population of intensely stained bipolar and multipolar cells that are distributed throughout the layer. The neuropil and fiber labeling in layer II is more intense than in layer I. Layer III contains a relatively dense population of stained pyramidal cells that are distributed throughout the layer. The density of pyramidal cells in layer III of area 36cm is slightly lower than that of area 36rm. Small numbers of CB-IR nonpyramidal cells are also observed scattered throughout the layer. Layers IV, V, and VI contain only very lightly stained neuropil. Layer IV is more prominent in area 36cm than in area 36rm and forms a virtually stain-free band (Fig. 13A). Layer V contains a slightly higher density of neuropil and fiber staining as well as a low density of stained nonpyramidal cells. The neuropil stain and CB-IR cell density in layer VI is slightly lower than in layer V.

Caudolateral subdivision of area 36 (area 36cl). The pattern of CB staining in area 36cl is similar to the pattern observed in area 36rl, although the density of CB-IR cells is slightly decreased (Figs. 10B, 13B, 14). Layer I of area 36rl contains a moderate density of CB-IR neuropil. Similar to area 36rl, Layer II of area 36cl has a bilaminar organization. Although the superficial half of the layer contains only moderately stained neuropil and only occasionally labeled cells, the deep half of layer II contains a moderate density of intensely stained nonpyramidal cells. Most of these exhibit bipolar morphology, although some multipolar cells are also observed. Layer III of area 36cl has a striking laminar organization. Superficial portions of the layer contain a population of intensely stained nonpyramidal cells that are continuous with those in deep layer II. The deep portions of layer III contain a dense population of CB-stained pyramidal cells, but the density in area 36cl is lower than area 36rl. Between the stained pyramidal cells deep in the layer and the intensely stained nonpyramidal cells superficially is a zone of relatively low cell density in the middle of layer III. Because the cells in layer III of area 36cl exhibit a more radial organization (Fig. 9F), the different sublamina of area 36cl appear more organized than in area 36rl. Layer IV of area 36cl contains almost no CB-IR neuropil and only occasionally labeled cells. The neuropil label in layer V is slightly higher, although substantially lower than in layers I–III. Layer V also contains a small number of stained nonpyramidal cells. Layer IV contains a slightly lower density of neuropil stain and a smaller number of labeled nonpyramidal cells than layer V.

Distribution of calbindin D-28k in the parahippocampal cortex

General distribution of the labeling. Like the perirhinal cortex, the parahippocampal cortex exhibits a relatively homogeneous pattern of CB immunostaining throughout its rostrocaudal extent (Figs. 16, 19). Area TH exhibits a slightly lower density of CB staining than areas TFl and TFm (Fig. 17A–C). Although the general density of CB staining in the parahippocampal cortex resembles that of the perirhinal cortex, the laminar patterns of staining differ particularly in layer II. Similar to both the entorhinal and perirhinal cortices, superficial layers of areas TH and TF are substantially more heavily stained than deep layers.

Area TH. Layer I of area TH exhibits a moderately stained neuropil and small numbers of Cajal-Retzius and lightly stained, round cells (Figs. 16, 17A arrows, 18). The neuropil is slightly darker in layer II and contains a striking population of large intensely stained nonpyramidal cells. Small lightly stained pyramidal or round cells are also observed in layer II and are most prominent in anterior levels of area TH. Layer III contains a higher density of lightly stained pyramidal cells as well as smaller numbers of strongly stained mostly bipolar cells. In anterior levels of area TH, the neuropil staining in layer V is similar or slightly lower than in layer III. Anterior levels

Fig. 10. Low-power brightfield photomicrographs of stained coronal sections demonstrating the distribution of calbindin D-28k immunoreactivity in different subfields of the perirhinal cortex. A: A more anterior section through areas 36rm and 36rl. B: A more posterior section through areas 36cm and 36cl. Dotted lines indicate the medial and lateral boundaries of area 35. Note the relatively homogeneous pattern of labeling throughout area 36 with superficial layers labeled much stronger than deep layers. rm, rostromedial; rl, rostrolateral; cm, caudomedial; cl, caudolateral. Scale bar = 1 mm in B (applies to A, B).
of layer V contain smaller numbers of both pyramidal and nonpyramidal cells (mostly bipolar) compared with layer III. In contrast, the neuropil label in more posterior portions of layer V is clearly weaker than layer III resulting in an overall more laminar appearance. Layer V of posterior TH also contains small numbers of nonpyramidal cells, including both bipolar and multipolar cells. Layer VI has the lightest CB labeling of area TH. This layer contains only a weakly stained neuropil and smaller numbers of CB-IR nonpyramidal cells. Most the nonpyramidal cells in layer VI exhibit bipolar morphology. Figure 17A shows a section through the more anterior portion of area TH where the labeling across the different lamina is less laminar and more homogeneous. Figure 15A shows a Nissl-stained section at a similar level of area TH for comparison.

Medial subdivision of area TF (TFm). Layer I of area TFm resembles layer I of area TH (Figs. 16, 17B, 18). The neuropil and fiber staining increases slightly in layer II and is distinguished by a population of intensely stained bipolar and multipolar cells. Unlike layer II of area 36, the CB-stained nonpyramidal cells of layer II of area TFm are distributed throughout the width of the layer. These cells tend to be larger than the intensely stained nonpyramidal cells observed in layer II of area 36. Layer III of area TFm contains a homogeneously distributed population of lightly stained pyramidal cells. The density of CB-IR pyramidal cells in layer III is higher than the cell density of the medially adjacent area TH. Layer III of area TFm also contains smaller numbers of nonpyramidal cells scattered throughout the layer. Layer IV is very thin in area TFm (Fig. 15B) and, in CB-stained material, is indistinguishable from layer V. Layers V and VI of area TFm exhibit a light neuropil stain that is even lighter than the staining in layer I. Deep layers also contain a small population of both lightly stained pyramidal cells as well as intensely stained nonpyramidal cells. The more striking laminar staining pattern of CB in area TFm contrasts with the more graded staining pattern observed in anterior portions of area TH. Similarly, in Nissl-stained sections, area TFm exhibits a more laminar organization compared with area TH (Fig. 15B).

Lateral subdivision of area TF (TFl). The staining in both layers I and II of area TFl resembles the staining in area TFm (Figs. 16–18). Like area TFm, layer III of area TFl is characterized by a relatively dense population of lightly stained pyramidal cells. There is a subtle size gradient in layer III such that the deep portions of this layer contain larger cells than superficial portions of the layer. Because of this size gradient, deep portions of layer

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Fig. 11. Brightfield photomicrographs demonstrating the distribution of calbindin D-28k in areas 35 (A) and 36d (B) of the perirhinal cortex. Dotted lines in A indicate the medial and lateral boundaries of area 35. Note the overall very light level of CB staining in area 35. Area 36d exhibits the lightest staining of all subdivisions of area 36. Moderate densities of labeled cells and neuropil are observed in deep layer II and III with very light labeling seen in layers V–VI. In both panels, lateral is toward the left and medial is toward the right. Roman numerals separated by horizontal lines indicate the boundaries of individual cortical layers. D, dorsal. Scale bar = 250 μm in B (applies to A,B).
III tend to look more densely stained than superficial portions. Superficial portions of layer III also contain a small population of intensely stained nonpyramidal cells. Unlike area TFm, layer IV of area TF1 is clearly visible in both CB-stained material and Nissl-stained material (Fig. 15C) and, in CB-stained material, is distinguished by its lack of staining. The neuropil stain in layers V and VI is slightly more dense than layer IV but not as densely stained as layer I. Layers V and VI also contain a relatively sparse population of stained pyramidal and nonpyramidal cells, including both bipolar and multipolar cells.

**General summary**

Figure 19 shows unfolded maps of the entorhinal, perirhinal, and parahippocampal cortices illustrating the topography of CB staining in superficial and deep layers. The striking rostrocaudal topography of CB staining in the superficial layers of the entorhinal cortex is obvious. In contrast, the labeling throughout the perirhinal and parahippocampal cortices is more homogeneous. Most areas show a clear laminar organization of CB staining, with superficial layers exhibiting stronger labeling than deep layers. This pattern is less obvious but still visible in areas E_c, E_CL, and 35. The difference in the laminar organization of CB staining across areas can be best appreciated by comparing Figures 8, 14, and 18.

**DISCUSSION**

In this study, we describe the distribution of CB-IR cells, fibers, and neuropil in the macaque monkey entorhinal, perirhinal, and parahippocampal cortices. We report two main findings. First, we find that the morphology of CB-IR pyramidal and nonpyramidal cells observed in the entorhinal, perirhinal, and parahippocampal cortices is similar. Except for CB-IR neurogliaform cells, which were only observed in the parahippocampal cortex, each of the three cortical areas examined contained similar complements of lightly stained pyramidal cells and intensely stained multipolar, bipolar, and Cajal-Retzius cells. Second, we report that the laminar and regional topography of CB immunoreactivity in each of the three cortical areas examined is unique. The entorhinal cortex is characterized by a striking rostrocaudal gradient of CB staining in its superficial layers. In contrast to the entorhinal cortex, the CB-staining patterns in the perirhinal and parahippocampal cortices are both more homogeneous, although they also exhibit unique laminar staining patterns, par-
particularly in layer II. The distinct patterns of CB staining observed throughout the entorhinal, perirhinal, and parahippocampal cortices taken together with the known connectivity of these areas provide insights into the excitatory and inhibitory circuits that may be influenced by CB in the monkey medial temporal lobe.

Comparison with previous studies

Cell morphology. Consistent with previous reports of CB staining in higher order cortical association areas (DeFelipe et al., 1989; Hof and Morrison, 1991; Beall and Lewis, 1992; Tunon et al., 1992; Hays and Lewis, 1992; Mikkonen et al., 1997; Kondo et al., 1999), we observe a dense population of lightly stained CB-IR pyramidal cells as well as smaller numbers of intensely stained nonpyramidal cells throughout the entorhinal, perirhinal, and parahippocampal cortices. The morphologic characteristics of the nonpyramidal cells in these medial temporal lobe cortical areas (i.e., multipolar, bipolar, and Cajal-Retzius cells) are similar to those described in the human entorhinal cortex (Beall and Lewis, 1992; Mikkonen et al., 1997) as well as in the monkey prefrontal cortex (Conde et al., 1994). The similarities in CB staining in higher order cortical association areas in both monkeys and humans contrast with the CB staining profiles that have been reported in primary sensory areas.

Fig. 13. Brightfield photomicrographs demonstrating the distribution of calbindin D-28k in areas 36cm (A) and 36cl (B) of the perirhinal cortex. Medial is toward the left and lateral is toward the right. The overall density of labeling in these posterior subdivisions of the area 36 are similar or slightly less compared with more anterior regions. Arrow in A indicates the location of a medium multipolar cell in layer V. Roman numerals separated by horizontal lines indicate the boundaries of individual cortical layers. cm, caudomedial; cl, caudolateral. Scale bar = 250 μm in B (applies to A,B).

Fig. 14. Schematic diagrams showing the pattern of cell and neuropil staining throughout the different subdivisions of the perirhinal cortex. All conventions are the same as Figure 8. rm, rostromedial; rl, rostrolateral; d, dorsal, cm, caudomedial; cl, caudolateral.
For example, in contrast to higher order sensory areas that contain large numbers of CB-IR pyramidal cells, primary sensory areas contain very few CB-IR pyramidal cells. Kondo et al. (1999) identified a clear gradient in CB-stained pyramidal cells along the cortical sensory pathways in monkeys with the lowest numbers of stained pyramidal cells observed in the primary sensory areas and the highest observed in and around the medial temporal lobe. Primary sensory areas also appear to contain different subpopulations of CB-IR nonpyramidal cells. For example, primary somatosensory cortex (DeFelipe et al., 1989; DeFelipe et al., 1990; DeFelipe and Jones, 1991) and early visual cortical areas (DeFelipe et al., 1999) contain CB-stained bundles of radially oriented fibers associated with double bouquet cells. CB-stained double bouquet-like profiles are not observed in either the medial temporal lobe (present study; Beall and Lewis, 1992) or in the prefrontal cortex (Conde et al., 1994). Although the description of the various CB-positive cell types throughout cortex remains incomplete, these observations make two points. First, the similarities in the kinds of CB-stained profiles observed across higher order association areas in both humans and monkeys suggest that these higher order areas may share similarities in their basic neurochemical circuitries. Second, the striking differences in CB-staining patterns between higher order and primary sensory areas suggest that CB may be a useful neurochemical marker to differentiate between the unique circuit properties of these functionally distinct areas.

**Topography and laminar organization**

**Entorhinal cortex.** Although very few studies have described the patterns of CB staining in monkey entorhinal cortex (Beall and Lewis, 1992), several previous studies have described the distribution of CB in the human entorhinal cortex (Beall and Lewis, 1992; Tunon et al., 1992; Mikkonen et al., 1997). Given these previous reports, a question of considerable interest is how does the laminar distribution and general topography of CB staining seen in the monkey entorhinal cortex compare with that seen in the human? There are both similarities as well as differences across the two primate species. For example, in both monkeys and humans, the strongest CB staining is observed in the super-
financial layers of the anterior and medial portions of the entorhinal cortex (compare Fig. 8 with Fig. 13 of Mikkonen et al., 1997). Similarly, in both species, the density of cell and neuropil labeling in the superficial layers are substantially stronger than in deep layers. In addition to these similarities, however, two notable differences were observed. First, the layer II cells of the monkey entorhinal cortex appeared to exhibit less prominent CB staining than the layer II cells in the human. This finding is particularly evident in the intermediate, caudal, and caudal limiting subdivisions of the entorhinal cortex (compare Fig. 8 of the present study with Fig. 13 of Mikkonen et al., 1997). Second, the overall staining in areas EC and ECL of the monkey brain is substantially less prominent than in humans. These patterns of similarities and differences, taken together with the well-described topography of entorhinal interconnections with hippocampus in the monkey, have implication for the regulation of information flow between the entorhinal cortex and the hippocampus in these two primate species.

The topography of projections from the monkey entorhinal cortex to the hippocampus in the monkey has been well established (Witter and Amaral, 1991). Layer II and III neurons in the medial portions of the entorhinal cortex project to the anterior dentate gyrus and hippocampus. Progressively more lateral portions of the entorhinal cortex project progressively more posteriorly in the dentate gyrus and hippocampus. Moreover, for a given mediolateral level of the entorhinal cortex, cells located rostrally tend to project superficially in the molecular layer of the dentate gyrus and stratum lacunosum-moleculare of the CA3 and CA2 fields. Cells located progressively more caudally project to deeper portions of molecular layers of the dentate gyrus and stratum lacunosum-moleculare (See Fig. 14 of Witter et al., 1989). These projection patterns suggest that the strong CB staining in the rostral and medial portions of the entorhinal cortex in both monkeys and humans will influence information flow to the superficial portions of the molecular layer of the dentate gyrus.
and CA2 and CA3 of the anterior hippocampus. In contrast, the stronger labeling in superficial layers of the caudal and lateral portions of the human compared with the monkey entorhinal cortex suggests that the more posterior portions of the hippocampus may be more strongly influenced by CB-containing neurons in humans than in monkeys. Because CB-IR pyramidal cells are likely to be excitatory projection neurons, whereas CB-containing nonpyramidal cells are likely to be inhibitory and colocalize with GABA (van Brederode et al., 1990), this finding suggests that these CB-IR neurons may influence both the excitatory transmission as well as the inhibitory control of entorhinal-hippocampal projections. The more prominent staining patterns in the posterior portions of the human entorhinal cortex suggest that CB may play a more prominent role in certain entorhinal-hippocampal projections in humans compared with monkeys.

**Perirhinal and parahippocampal cortices.** We also report high densities of CB-IR pyramidal and nonpyramidal cells in the deep portions of layer II and superficial layer III of the perirhinal cortex and in layers II and III of the parahippocampal cortex. Layer III cells of both perirhinal and parahippocampal cortex provide the major efferent projections to the entorhinal cortex (Suzuki and Amaral, 1990, 1994b). This finding suggests that the CB-positive cells in these areas are in a position to influence or regulate both excitatory and inhibitory transmission to the entorhinal cortex. The entorhinal cortex projects most strongly to deep layer I, layer II, and superficial layer III of both the perirhinal and parahippocampal cortex. Weaker entorhinal projections terminate in layer V and VI in both areas. This finding suggests that the CB-IR neurons in both the perirhinal and parahippocampal cortices may also influence the back projections arriving from the entorhinal cortex. The back projections from the entorhinal cortex to the perirhinal and parahippocampal cortices and then back to the neocortex have been implicated in the process of memory consolidation (Higushi and Miyashita, 1995; Lavenex et al., 2001).

**Comparison with the distribution of other calcium-binding proteins**

Numerous previous studies have shown that the distribution of calbindin together with two other well-studied calcium binding proteins, parvalbumin and calretinin, are localized to largely distinct and nonoverlapping subpopulations of cells (Tunon et al., 1992; Beall and Lewis, 1992; Pitkanen and Amaral, 1993a; Conde et al., 1994; Del Rio and DeFelipe, 1996; Kemppainen and Pitkanen, 2000). Consistent with these previous reports, a comparison of our findings with the distribution of parvalbumin in the monkey entorhinal cortex suggests largely nonoverlapping distributions of immunoreactive cells and fibers. We showed that cells and fibers stained for CB are most prominent in rostral and medial portions of the entorhinal cortex. Cells and fibers immunoreactive for parvalbumin, in contrast, are most prominent in caudal and lateral portions of the entorhinal cortex (Pitkanen and Amaral, 1993b). The distribution of parvalbumin- or calretinin-immunoreactive elements has not been described for the monkey perirhinal or parahippocampal cortex. Because CB, parvalbumin and calretinin all colocalize the inhibitory neurotransmitter GABA (Del Rio and DeFelipe, 1996; Kemppainen and Pitkanen, 2000), the distributions of these three calcium binding proteins has been used as a model for understanding the organization of inhibitory circuits in the brain (Pitkanen and Amaral, 1993a; Conde et al., 1994; Mikkonen et al., 1997; Kemppainen and Pitkanen, 2000). For example, in various neocortical sites, immunohistochemical studies have been combined with detailed EM and tracing studies to identify some of the postsynaptic targets of subpopulations of local circuit neurons containing calcium-binding proteins (Somogyi et al., 1979; DeFelipe et al., 1989, 1990; DeLima and Morrison, 1989; Lewis and Lund, 1990; Williams et al., 1992). In contrast, little information of this kind is available about the monkey entorhinal, perirhinal, or parahippocampal cortices. Studies that define the intrinsic and extrinsic projections of these different classes of neurons containing calcium binding proteins (including CB) will be essential to understanding the organization of inhibitory circuits in the medial temporal lobe and their potential contribution to memory function.

**Role of CB in cortical circuitry**

A growing number of studies have reported that the presence of CB immunoreactivity can differentiate subpopulations of neurons throughout the brain. For example, in the
monkey auditory system, CB identified a subpopulation of medial geniculate neurons that project to layer I of the primary auditory cortex (Hashikawa et al., 1991, 1995; Molinari et al., 1995). CB immunoreactivity has also been associated with extrageniculate pathways in the visual system. In a recent report, Rodman showed that CB content characterizes a subset of geniculoextrastriate projections in the macaque monkey (Rodman et al., 2001). There is also evidence that CB-positive neurons throughout the cortex as well as the hippocampus receive privileged innervation from serotonergic neurons originating in the median and the dorsal raphe nuclei (Hornung and Celio, 1992). Although specific connectional characteristics of the CB-positive neurons in the monkey have not yet been identified, these findings raise the possibility that CB immunoreactivity may also define a specific connectional or functional subpopulation in the medial temporal lobe.

Understanding the circuit characteristics of CB-positive neurons in the medial temporal lobe in normal brain tissue is an important first step toward understanding the complex patterns of degeneration observed in various human neurologic disease states. For example, Hof and Morrison (1991) report that the strongly CB-IR interneurons are resistant to damage in AD but that the CB-IR pyramidal cells were significantly decreased in the prefrontal cortex. Although the cortical medial temporal lobe areas have not been examined explicitly in AD, one previous study reported a specific reduction of CB gene expression in the hippocampus of patients with AD (Iacopino and Christakos, 1990). A detailed description of CB-IR neurons in animal model systems such as the macaque monkey not only have important implications for understanding the functional organization of these medial temporal lobe cortical areas, but will ultimately shed light on the role of these markers in various neurologic disease states.

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LITERATURE CITED


