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COMMENTARY

Functional Neuroanatomy of the Parahippocampal Region: The Lateral and Medial Entorhinal Areas

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ABSTRACT: The entorhinal cortex (EC) serves a pivotal role in corticohippocampal interactions, but a complete description of its extrinsic connections has not been presented. Here, we have summarized the cortical, subcortical, and hippocampal connections of the lateral entorhinal area (LEA) and the medial entorhinal area (MEA) in the rat. We found that the targets and relative strengths of the entorhinal connections are strikingly different for the LEA and MEA. For example, the LEA receives considerably heavier input from the piriform and insular cortices, whereas the MEA is more heavily targeted by the visual, posterior parietal, and retrosplenial cortices. Regarding subcortical connections, the LEA receives heavy input from the amygdala and olfactory structures, whereas the MEA is targeted by the dorsal thalamus, primarily the midline nuclei and also the dorsolateral and dorsoanterior thalamic nuclei. Differences in the LEA and MEA connections with hippocampal and parahippocampal structures are also described. In addition, because the EC is characterized by bands of intrinsic connectivity that span the LEA and MEA and project to different septotemporal levels of the dentate gyrus, special attention was paid to the efferents and afferents of those bands. Finally, we summarized the connections of the dorsocaudal MEA, the region in which the entorhinal "grid cells" were discovered. The subregional differences in entorhinal connectivity described here provide further evidence for functional diversity within the EC. It is hoped that these findings will inform future studies of the role of the EC in learning and memory. © 2007 Wiley-Liss, Inc.

KEY WORDS: memory; attention; hippocampal; spatial; medial temporal lobe; anterograde; retrograde

INTRODUCTION

It is well established that the medial temporal lobe is essential for memory formation in the mammalian brain. The rodent brain does not have a medial temporal lobe, but it does contain the structures that are thought to contribute to episodic memory. These structures, sometimes collectively designated as the hippocampal system or region, include the hippocampal formation (dentate gyrus, fields CA3, CA2, and CA1, and the subiculum) and the parahippocampal region (the entorhinal cortex (EC), perirhinal (PER) areas 35 and 36, the postrhinal (POR) cortex, the presubiculum, and the parasubiculum). The EC is uniquely positioned to serve as an interface between the neocortex and hippocampal formation (Fig. 1A). As such, it is a gateway to the hippocampal formation through which bidirectional information passes.

In the rat, the EC is located at the most caudal, ventral, and lateral aspect of the brain (Fig. 1A). The EC was originally defined as area 28 by Brodmann (1909). It was later subdivided into the lateral entorhinal area (LEA) and the medial entorhinal area (MEA) on morphological grounds (Krieg, 1946a,b; Blackstad, 1956). More recently, the EC was further subdivided into six subregions (Insausti et al., 1997). For the purposes of this commentary, however, we will use the LEA and MEA subdivisions. The LEA occupies the rostrolateral portion of the EC, and the MEA occupies the caudomedial portion of the EC (Insausti et al., 1997; Dolorfo and Amaral, 1998). The rostral border of the LEA is occupied by the piriform and periamygdaloid cortices. Its dorsal border is occupied by the PER and POR cortices (Burwell, 2001). The MEA is located at the caudomedial border of the LEA. Lateral and medial portions of the MEA are bordered dorsally by the POR cortex and parasubiculum, respectively.

The primary cortical input to the hippocampal formation arrives via the perforant pathway. The perforant pathway comprises the entorhinal layer II projection to the dentate gyrus and field CA3 as well as the layer III projection to CA1 (Witter et al., 2000). The temporoammonic pathway provides an additional route for entorhinal layer III input to field CA1 and the subiculum (Steward, 1976). Return projections, originating in the subiculum and in field CA1, target the deep layers of the EC. These deep layer cells are the source of the projections back out to cortical regions, as well as the associational projections to the superficial layers of the EC (Kohler, 1985; Dolorfo and Amaral, 1998; van Haeften et al., 2000).

Dolorfo and Amaral (1998) reported that the EC contains three discrete regions or bands of interconnected neurons. The intrinsic connections were

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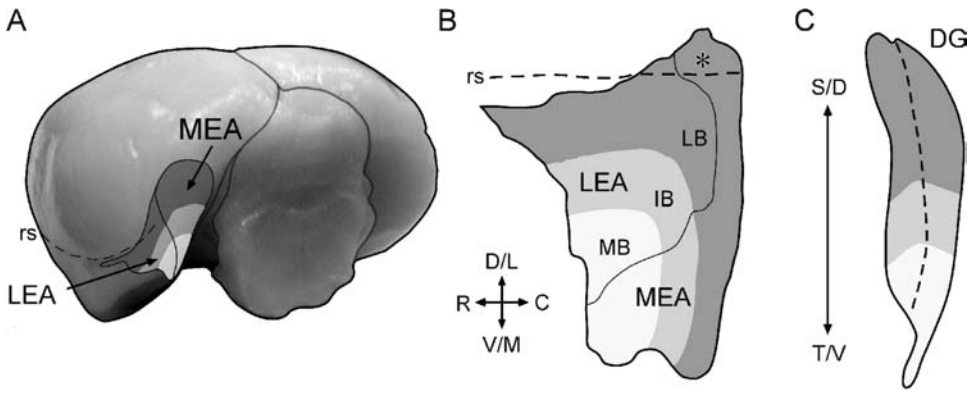

FIGURE 1. The entorhinal cortex in the rat. A: Ventral posterior view of the rat brain with the cerebellum removed. A schematic overlay shows the position of the lateral (LEA) and the medial (MEA) areas of the entorhinal cortex (adapted from Fyhn et al., 2004). In each panel, the lateral band (LB) is shown in dark gray, the intermediate band (IB) in medium gray, and the medial band (MB) in light gray. B: An unfolded map of the pial surface of the entorhinal cortex. The asterisk is located in the dorsocaudal MEA, the location in which grid cells have been described. C: An unfolded map of the dentate gyrus (DG) showing the topography of the entorhinal projections. The crest of the DG is indicated by the dashed line. The DG-projecting bands are shown in levels of gray matching their terminal areas in the DG. The lateral band targets the septal-half of the DG, the intermediate band targets the third-quarter of the DG, and the medial band targets the temporal-quarter of the DG. Other abbreviations: C, caudal; D, dorsal; L, lateral; M, medial; R, rostral; rs, rhinal sulcus; S, septal; T, temporal, V, ventral.

reported to be confined to the band of origin, although it should be noted that discrete cytoarchitectonic borders for the three bands have not been described. As shown in Figure 1B, each of the bands of intrinsic connectivity spans the LEA and MEA. The striking finding reported by Dolorfo and Amaral (1998) was that each band projects to a different septotemporal level of the dentate gyrus (Fig. 1C). The most lateral band projects to the dorsal half of the dentate gyrus; the intermediate band projects to the third quarter of the dentate gyrus; and the medial band projects to the ventral quarter of the dentate gyrus. The intrinsic and extrinsic connectivity of these bands suggests that they are functionally distinct. Indeed, research has shown that lesions in the dorsal or septal area of the hippocampus drastically impair spatial learning, whereas lesions of comparable size in the ventral or temporal area of the hippocampus result in no spatial impairments (Moser et al., 1995).

Available evidence suggests that the MEA contributes predominately spatial information to the hippocampus. Neurons located in the dorsal and caudal part of the MEA lateral band, which project to the septal hippocampus, fire in multiple discrete areas of an environment (Fyhn et al., 2004). These firing fields are stable, smaller, and more coherent when compared with firing fields of neurons located in the MEA intermediate or medial bands that project to temporal portions of the hippocampus (Fyhn et al., 2004). In addition, when the hippocampus was lesioned, the MEA firing fields persisted. Alternatively, when the dorsolateral portion of the EC was lesioned, animals exhibited spatial memory deficits in the Morris water maze. Spatial memory is not impaired following lesions to ventromedial EC (Steffenach et al., 2005). Together, these data suggest that neurons of the dorsocaudal MEA accurately express spatial information regarding an animal's location in an environment, and that the precise spatial firing of these cells is not merely a reflection of hippocampal activity (Fyhn et al., 2004).

In a groundbreaking discovery, Hafting et al. (2005) found that neurons in the superficial layers of dorsocaudal MEA exhibited a novel form of spatial tuning. The population's firing fields formed a topographic spatial map of the environment represented as a grid of tessellating equilateral triangles spanning the entire spatial environment. These neurons, termed "grid cells," are thought to provide a spatial representation of an animal's location (Hafting et al., 2005). Unlike place cells in the hippocampus, which seem to create multiple representations of an environment based on context, grid cells appear to compute context-independent information about the environment (Hafting et al., 2005; Smith and Mizumori, 2006). These findings highlight the importance of understanding the structure and connectivity of the EC.

We have completed a series of anatomical studies documenting the extrinsic connections of the rat EC, PER, and POR cortices in an attempt to better understand the structural and functional differences among these structures (Burwell and Amaral, 1998a,b; Agster, 2007). Here, we have reanalyzed and summarized the cortical, subcortical, and hippocampal system inputs and outputs, specifically for the EC. In addition, we present new data illustrating the topography of the cortical afferents of the EC. Finally, we provide a detailed analysis of the afferents and efferents of the dorsocaudal MEA, the grid cell area.

OVERVIEW OF CONNECTIONS

A total of 80 cortical, subcortical, and hippocampal structures were evaluated for connectivity with the EC. The 26 cort-

ical structures were collected into functional areas including the piriform, frontal, insular, temporal cingulate, parietal, and occipital cortices. The 36 subcortical regions included the olfactory regions, the claustrum, amygdala nuclei, septal nuclei, basal ganglia, dorsal and ventral thalamic nuclei, and the hypothalamus. We also assessed connections with the substantia nigra and ventral tegmental area. The hippocampal system connections were assessed in two groups, the hippocampal formation (dentate gyrus, CA fields, and the subiculum) and the parahippocampal region (the POR, PER, EC, presubicular, and parasubicular cortices). For a detailed description of the subregional nomenclature, please see the commentary in this issue (Furtak et al., in press).

The inputs to the EC were documented by retrograde tract tracing experiments in which tract tracer injections were placed in the EC, and total numbers and densities of cells were estimated in cortical, subcortical, and hippocampal structures. Individual cases and methods for quantifying retrogradely-labeled cells for the cortical structures were previously described (Burwell and Amaral, 1998a). Methods for the subcortical and hippocampal structures were similar with the exception that counting of labeled cells was automated (Neurolucida, MBF Bioscience, Williston, VT; Agster, 2007). For the purposes of the present report, we are most interested in the impact of afferent regions on the EC. Thus, we have tabulated the retrograde data in terms of the percentages of total labeled cells estimated in each of the three sets of afferent regions. We also show new unfolded maps representating densities of labeled cells in the cortical regions.

The outputs of the region were documented by injections of anterograde tracers placed in the EC. The amount of fiber labeling arising from each injection was quantified for the same structures used for the retrograde tract tracer studies. Briefly, the structure was outlined in coronal sections and divided into voxels of a specified area. The density of fiber labeling was rated for each voxel for a series of sections spaced at 0.3 mm intervals along the rostrocaudal axis. The ratings for each voxel for a particular region were then summed to obtain an index of fiber labeling that was weighted for the volume of the structure (Agster, 2007). We elected to use this index, because it best describes the relative impact of the EC on the efferent structures that it targets. Unfolded maps showing the topography of densities of labeled fibers for the cortical regions are also presented.

For the six projections among the PER, POR, and EC, we have both the fiber indexes and estimates of total numbers of labeled cells. For a summary of the connectivity of the POR and PER, see commentary in this issue (Furtak et al., 2007). We were able to validate our fiber index by examining the correlation between the two sets of numbers for each of the projections (PER → POR, POR → PER, EC → POR, POR → EC, PER → EC, and EC → PER). For example, for the PER → POR projection, the pair of entries in the correlation table would be (a) the mean fiber labeling index of POR labeling following anterograde tracer injections in the PER, and (b) the mean estimated total cells labeled in the PER cortex following retrograde tracer injections in the POR. For these six pathways, the two measures were strongly correlated ($r = 0.77$). Thus, for determining the strength of a projection, our method for quantifying anterograde fiber labeling appears to be as effective as quantifying retrogradely labeled cells.

It should be noted that our quantitative studies of retrogradely labeled cells and fiber labeling do have a limitation; neither measure quantifies the number or efficacy of synaptic connections. Nevertheless, the systematic nature of the studies of the entorhinal efferents and afferents allows us to draw a number of sound conclusions. Because we used the same animals, data collection procedures, and analysis methods across all studies, it is possible to make comparisons about connectivity across entorhinal subregions. For example, the complement of inputs to the LEA is evenly divided across cortical, subcortical, and hippocampal regions of origin. In contrast, the MEA receives a substantially larger component from hippocampal structures compared with cortical or subcortical regions (Fig. 2).

CORTICAL CONNECTIONS OF THE LEA

Roughly, 30% of the total afferent input to the LEA arrives from cortical areas (Fig. 2). Of the cortical inputs, the strongest is from the piriform cortex, followed by insular regions, and then the frontal regions (Table 1; Burwell and Amaral, 1998a). The insular connections of the LEA are strongest with the agranular insular cortex. The frontal connections are distributed across all frontal subdivisions. These large percentages of inputs to the LEA are distributed uniformly to the lateral, intermediate, and medial bands of the LEA with the following exceptions: the medial band of the LEA receives considerably less input from the piriform region and more input from the insular region compared with the lateral and intermediate bands of the LEA (Table 1). The LEA also receives somewhat weaker input from the temporal, cingulate, parietal, and occipital areas. The origins of LEA afferents are visualized in unfolded maps of densities of labeled cells arising from retrograde tract tracers in the LEA bands (Figs. 3A,B).

The LEA is reciprocally connected with the piriform, insular, and frontal regions (Agster, 2007). In general, the strongest outputs to cortical structures originate in the lateral band of the LEA and the weakest originate in the intermediate band of the LEA (Table 2). The projection to the piriform cortex is strongest, followed by projections to the frontal and insular regions. The lateral and medial bands of the LEA both contribute strong input to the piriform cortex and the insular region, while the lateral band alone projects more strongly to the frontal regions (Table 2). The LEA lateral band provides a moderately strong input to the cingulate, temporal, and parietal regions, but the projections to visual

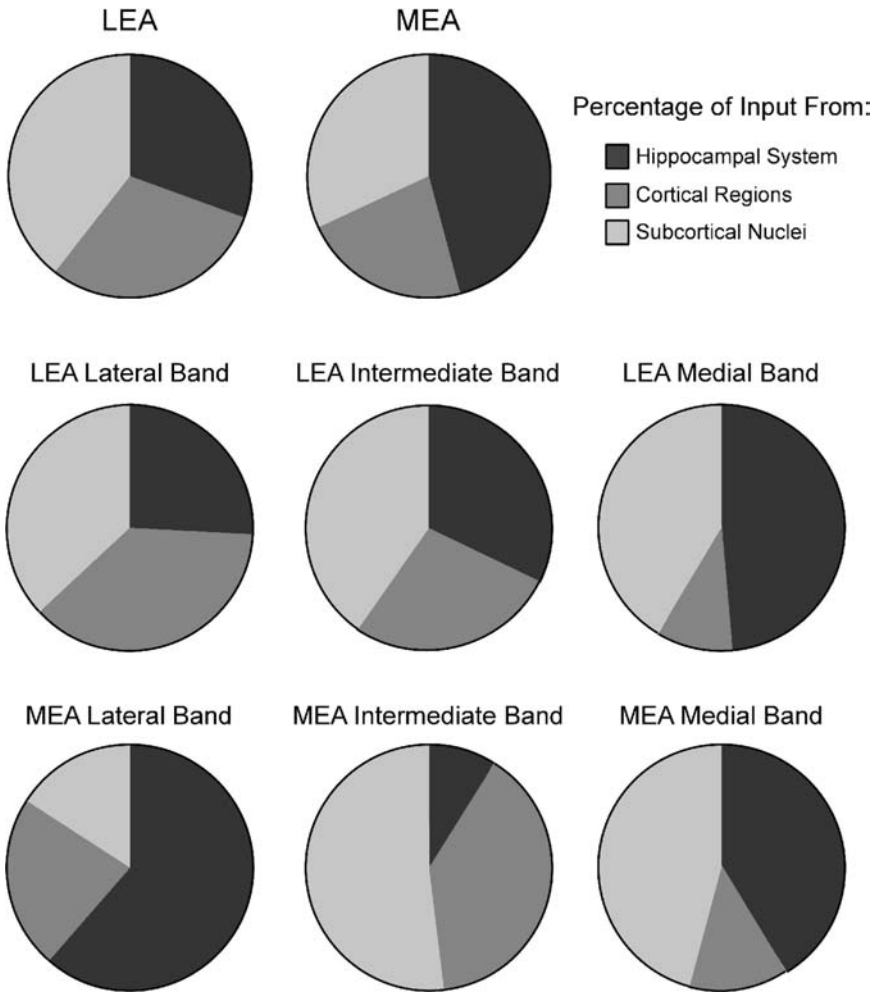

FIGURE 2. Proportions of input from the hippocampal system, cortical regions, and subcortical nuclei. The upper panel shows average proportions for the lateral (LEA) and the medial (MEA) areas of the entorhinal cortex. Total input is then calculated for each of the three dentate-projecting bands (Fig. 1) of the LEA (middle panel) and the MEA (lower panel).

TABLE 1.

Cortical Afferents of the Entorhinal Cortex: Percent Retrogradely-Labeled Cells

	LEA	LEA bands			MEA	MEA bands			MEA
Origins	Average	Lateral	Inter	Medial	Average	Lateral	Inter	Medial	Grid area
Piriform	43.7	48.0	49.8	25.9	34.0	2.5	83.9	53.3	2.9
Frontal	13.4	13.4	11.7	16.9	11.7	17.0	0.9	6.7	12.9
Insular	24.0	25.6	24.1	46.9	6.8	7.6	6.3	8.6	9.1
Temporal	7.3	5.3	5.7	5.7	8.4	11.1	1.2	10.4	14.1
Cingulate	4.1	1.7	3.4	2.0	13.3	15.3	0.4	15.5	12.3
Parietal	4.2	5.3	2.6	1.4	10.4	17.6	5.5	1.6	16.1
Occipital	3.3	0.7	2.7	1.2	15.3	28.9	1.8	3.9	32.6
Total (%)	*100.0*	*100.0*	*100.0*	*100.0*	*100.0*	*100.0*	*100.0*	*100.0*	*100.0*

Numbers represent the mean percent of the total number of retrogradely labeled cells in the originating subcortical regions arising from injections in the lateral entorhinal and medial entorhinal areas (LEA and MEA). See text for details.

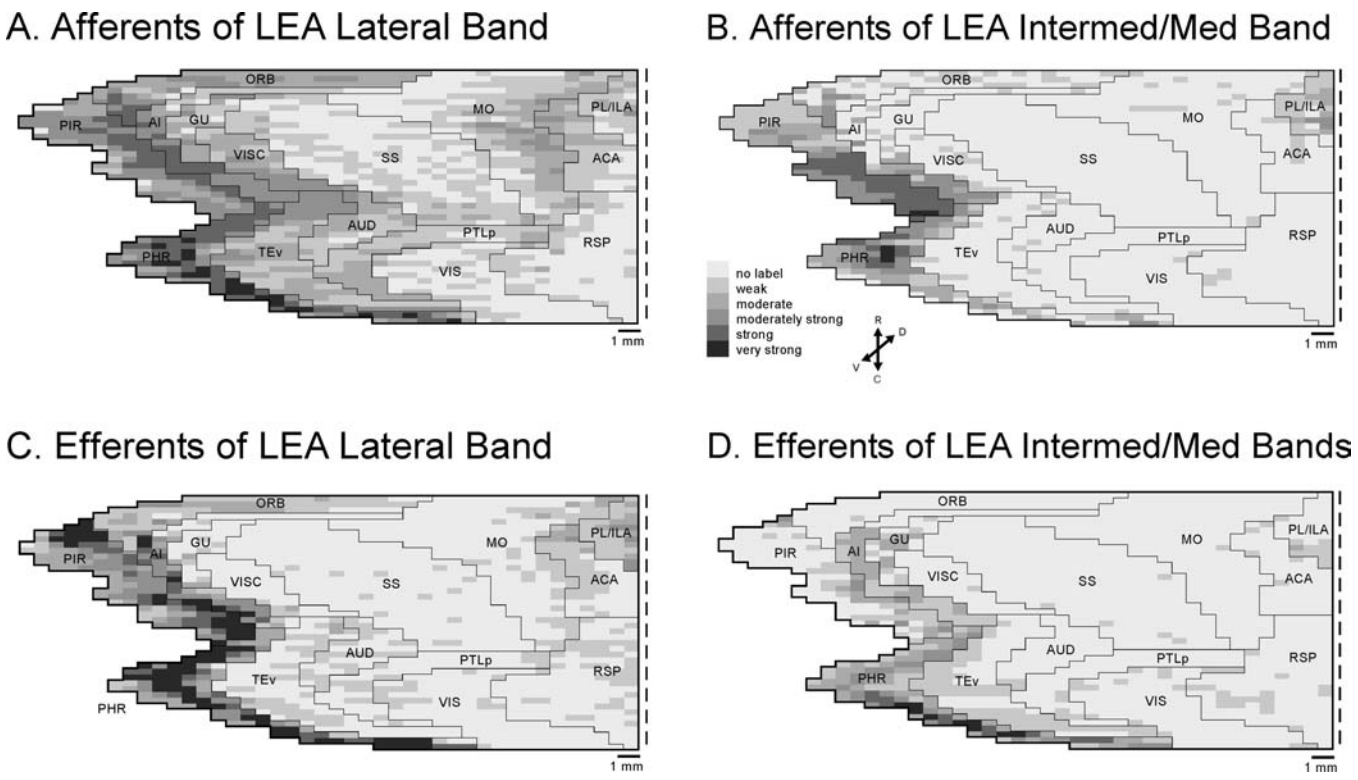

FIGURE 3. Unfolded template maps showing the topography of the cortical connections of the lateral entorhinal area (LEA). Levels of gray represent densities of labeled cells or fibers resulting from tracer injections. A. This map is adapted from a representative retrograde tract tracer injection into the lateral band of the LEA. B. This map represents a retrograde tract tracer case in which the injection site spanned the intermediate and medial bands of the LEA. C. This unfolded map is a composite of two representative anterograde tract tracer cases showing the density of fiber labeling in cortical regions resulting from injections in two locations in the LEA lateral band. D. This unfolded map is a composite of two representative anterograde tract tracer cases showing the density of fiber labeling in cortical regions resulting from injections in the intermediate and medial bands of the LEA. Abbreviations for this and the next figure: ACA, anterior cingulate; AI, agranular insular; AUD, auditory cortex; GU, gustatory; MO, motor cortex; ORB, orbital frontal regions; PIR, piriform cortex; PHR, parahippocampal region; PL/ILA, prelimbic/infralimbic areas; PTLp, posterior parietal; RSP, retrosplenial; SS, comatosensory cortex; TEv, ventral temporal association cortex; VISC, visceral cortex; VIS, visual cortex.

regions are weak. The terminations of the LEA efferents are represented in Figures 3C,D in unfolded maps of the densities of fiber labeling resulting from anterograde tract tracers injected into the LEA. Although the patterns of terminations are similar across bands, the labeling arising from injections in the lateral band is stronger.

TABLE 2.

Cortical Efferents of the Entorhinal Cortex: Index of Fiber Labeling

	LEA	LEA bands			MEA	MEA bands			MEA
Terminations	Average	Lateral	Inter	Medial	Average	Lateral	Inter	Medial	Grid area
Piriform	33.6	42.0	4.8	37.3	18.8	8.3	18.9	50.2	0.0
Frontal	19.8	31.2	0.9	4.7	6.5	5.0	7.1	10.5	0.0
Insular	17.9	22.5	3.1	18.6	9.5	5.4	5.9	25.2	0.3
Temporal	5.8	8.5	3.7	0.0	16.0	21.5	8.7	6.6	24.2
Cingulate	9.4	12.2	2.5	8.0	11.6	10.1	22.3	5.1	2.0
Parietal	11.8	18.6	1.0	2.0	15.7	23.8	6.5	0.3	17.6
Occipital	1.9	2.1	2.6	0.7	7.3	11.2	1.9	1.2	12.1

Numbers represent indexes of fiber labeling in terminal cortical regions arising from anterograde tracer injections in the lateral entorhinal and medial entorhinal areas (LEA and MEA). Density of fiber labeling was rated and then normalized by the volume of the terminal structure. See text for details.

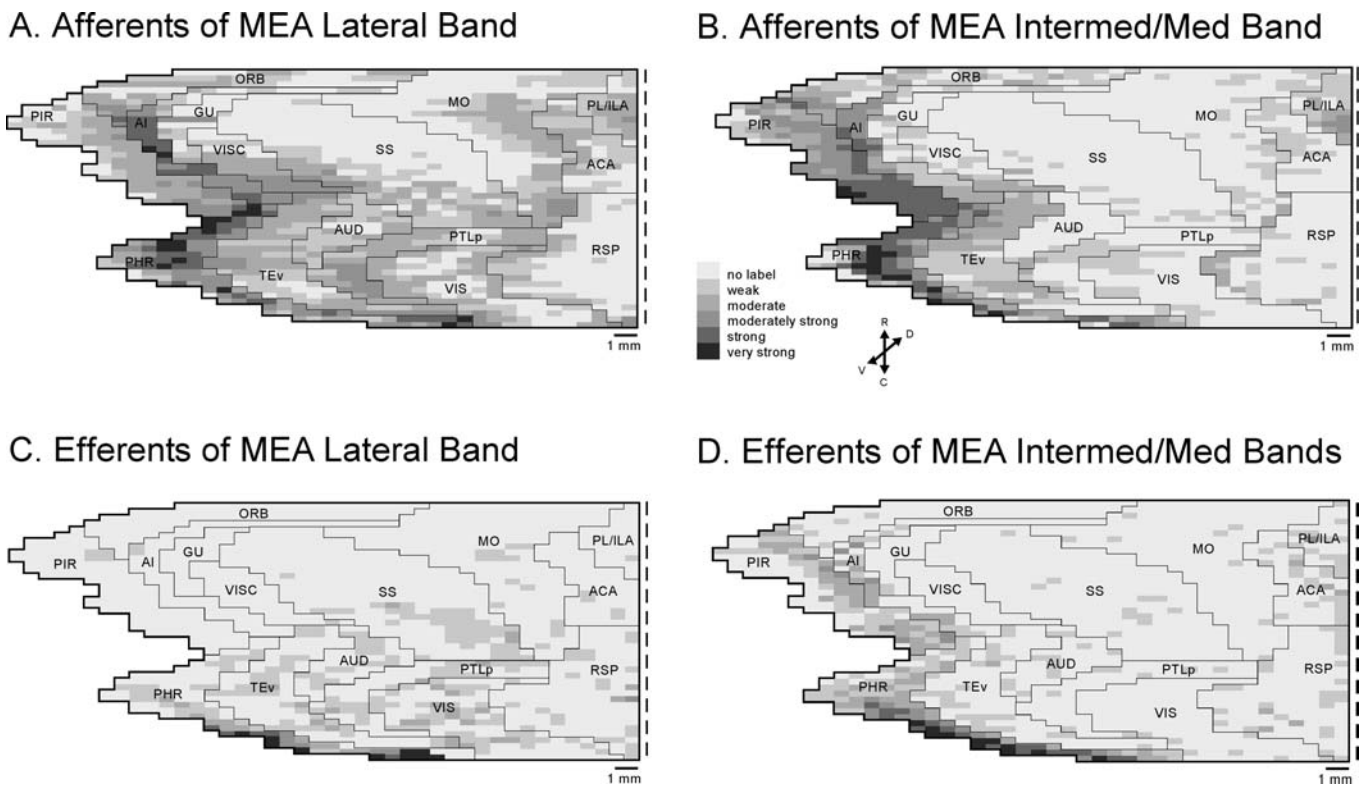

FIGURE 4. Unfolded template maps showing the topography of the cortical connections of the MEA. Levels of gray represent densities of labeled cells or fibers resulting from tracer injections. A. This unfolded map is a composite of two representative retrograde tract tracer cases showing the density of labeled in cortical regions resulting from injections in two locations in the MEA lateral band. B. This map represents a retrograde tract tracer case in which the injection site spanned the intermediate and medial bands. C. This unfolded map is a composite of two representative anterograde tract tracer cases showing the density of fiber labeling in cortical regions resulting from injections in two locations in the LEA lateral band. D. This unfolded map is a composite of two representative anterograde tract tracer cases showing the density of fiber labeling in cortical regions resulting from injections in the intermediate and medial bands. See Figure 3 for abbreviations.

CORTICAL CONNECTIONS OF THE MEA

Cortical input to the MEA accounts for a little more than one-fifth of the total afferent input (Fig. 2). The heaviest input arises in the piriform cortex and preferentially targets the intermediate and the medial bands (Table 1; Burwell and Amaral, 1998a). There is also a moderate projection from frontal regions, arising largely from the secondary motor area and terminating in the lateral band of the MEA. In contrast to the LEA, the MEA receives modest input from insular regions and much stronger inputs from the occipital, parietal, and cingulate regions. Projections from the occipital and parietal regions to the MEA preferentially target the lateral band, while input from the cingulate region preferentially targets the medial and lateral bands. The patterns of afferentation are evident in unfolded maps of densities of labeled cells following injections of retrograde tracers in MEA (Figs. 4A,B).

Most of the cortical connections are reciprocal, although the efferent projections seem weaker than the afferent projections. (Tables 2 and 3, Figs. 4C,D). The MEA projects strongly to the piriform cortex, ventral temporal cortex, and parietal regions (Table 2). The piriform and insular projections originate in the medial band, whereas the temporal and parietal projections originate in the lateral band. There is also a moderately strong projection to cingulate regions that originates in the lateral and intermediate bands of the MEA. Additionally, while frontal and occipital regions contribute a high percentage of cortical input to the MEA, return projections are relatively weak.

SUBCORTICAL CONNECTIONS OF THE LEA

Subcortical structures provide substantial input to the LEA (Agster, 2007). About one-third of all retrogradely labeled cells were located in subcortical structures (Fig. 2). As a general rule, the strength of subcortical projections to the LEA tended to be similar across bands. The strongest afferents are from the olfactory areas (Tables 3 and 4). The olfactory input arises predominately from the endopiriform nucleus and the piriform transition area and is distributed to the lateral, intermediate, and medial bands of the LEA. The amygdala input is also substantial. It arises in the olfactory amygdala as well as in the lateral

TABLE 3.

Subcortical Afferents of the Entorhinal Cortex: Percent Retrogradely-Labeled Cells

	LEA	LEA bands			MEA	MEA bands			MEA
Origins	Average	Lateral	Inter	Medial	Average	Lateral	Inter	Medial	Grid area
Olfactory	36.9	45.1	37.6	29.4	18.4	6.4	40.7	27.8	7.1
Claustrum	20.2	23.2	17.1	19.1	19.6	30.9	5.4	9.7	37.3
Amygdala	23.0	18.6	23.8	36.9	17.2	5.7	10.1	41.0	7.3
Septal nuclei	3.5	3.2	3.3	3.1	5.5	6.6	3.9	4.3	6.2
Basal ganglia	1.2	1.6	1.2	0.7	2.5	3.8	1.3	0.8	3.3
Dorsal thalamus	11.4	6.0	13.1	6.7	25.9	29.6	36.8	12.3	28.6
Ventral thalamus	1.3	0.7	1.5	0.7	3.8	6.2	0.7	0.9	4.9
Hypothalamus	2.5	1.5	2.5	3.5	7.1	10.8	1.2	3.1	5.3
Total (%)	*100.0*	*100.0*	*100.0*	*100.0*	*100.0*	*100.0*	*100.0*	*100.0*	*100.0*

Numbers represent the mean percent of the total number of retrogradely labeled cells in the originating subcortical regions arising from injections in the lateral entorhinal and medial entorhinal areas (LEA and MEA). See text for details.

and basomedial nuclei and is distributed to all three bands. The claustrum provides strong input that is distributed to all three bands.

Thalamic input to the LEA arises primarily in the midline dorsal thalamic group and terminates in all three bands. Input from the septal nuclei is relatively weak, arises in the medial septum, and terminates in all bands. Inputs to the LEA from the basal ganglia, ventral thalamus, and hypothalamus are weak (Table 3).

The olfactory input to the LEA is strongly reciprocated (Table 4). Projections to the olfactory regions originate mainly from the lateral and intermediate bands of the LEA and terminate primarily in the endopiriform nucleus (Table 4). The LEA projections to the amygdala target all nuclei, but the projection to the central nucleus is weakest. Also, noteworthy are the projections to basal ganglia structures. Although the input is relatively weak, the output to the basal ganglia structures is very strong. The projection originates largely in the lateral and intermediate bands of the LEA, and terminates in the accumbens, the caudate putamen, and the substantia innominata.

The dorsal thalamic efferents are weak and terminate primarily in the lateral group of the dorsal thalamus. There are essentially no LEA projections to ventral thalamic structures. Very weak hypothalamic projections terminate in the lateral, medial, and periventricular zones.

SUBCORTICAL CONNECTIONS OF THE MEA

About one-third of the labeled cells resulting from MEA retrograde tracer injections were found in subcortical regions (Fig. 2), suggesting that the MEA is heavily influenced by these structures (Agster, 2007). In the MEA, subcortical inputs differ substantially across bands. For example, input from the claus-

TABLE 4.

Subcortical Efferents of the Entorhinal Cortex: Index of Fiber Labeling

	LEA	LEA bands			MEA	MEA bands			MEA
Terminations	Average	Lateral	Inter	Medial	Average	Lateral	Inter	Medial	Grid area
Olfactory	54.5	58.9	68.1	5.4	9.4	4.1	4.0	36.1	7.5
Claustrum	12.4	13.9	14.2	1.5	2.1	1.7	0.6	5.2	3.1
Amygdala	42.2	50.1	41.7	4.0	5.7	2.2	0.9	24.1	6.1
Septal nuclei	15.7	16.3	21.5	1.4	5.8	3.8	6.7	12.7	1.5
Basal ganglia	121.0	138.4	134.3	7.5	38.1	33.3	11.5	83.7	91.8
Dorsal thalamus	1.8	1.8	1.1	2.7	1.2	0.9	0.0	3.5	0.9
Ventral thalamus	0.4	0.5	0.5	0.0	0.2	0.1	0.0	0.4	0.0
Hypothalamus	1.3	0.5	3.6	0.8	2.2	0.2	4.9	7.1	0.0

Numbers represent indexes of fiber labeling in terminal subcortical regions arising from anterograde tracer injections in the lateral entorhinal and medial entorhinal areas (LEA and MEA). Density of fiber labeling was rated and then normalized by the volume of the terminal structure. See text for details.

TABLE 5.

Hippocampal Afferents of the Entorhinal Cortex: Percent Retrogradely-Labeled cells[a]

	LEA	LEA bands			MEA	MEA bands			MEA
Origins	Average	Lateral	Inter	Medial	Average	Lateral	Inter	Medial	Grid area
Dorsal HC	15.2	16.7	18.4	4.6	19.5	34.6	2.4	2.3	54.0
DG/CA3	0.5	0.3	0.2	1.2	0.5	0.3	0.7	0.9	0.1
CA2/CA1	10.5	8.2	13.9	1.7	16.0	29.2	0.7	1.0	46.4
SUBd	4.3	8.2	4.3	1.8	3.0	5.0	1.0	0.4	7.5
Ventral HC	51.2	41.0	51.0	71.2	32.8	4.3	25.2	76.8	6.5
DG/CA3	1.1	0.3	0.6	5.0	5.7	0.0	2.4	15.5	0.0
CA2/CA1	37.5	29.9	34.2	49.9	14.9	2.0	11.7	35.9	3.4
SUBv	12.7	10.9	16.2	16.3	12.2	2.3	11.0	25.4	3.1
ParaHC region	33.6	42.3	30.6	24.2	47.7	61.2	72.4	20.9	39.4
Presubiculum	3.8	7.8	4.4	4.9	19.5	22.6	33.1	9.3	18.8
Parasubiculum	5.9	5.8	6.4	9.7	17.8	30.2	14.0	6.4	8.6
Postrhinal	4.9	3.8	5.1	2.3	5.6	4.5	17.1	1.8	5.6
Perirhinal	19.0	24.9	14.8	7.3	4.8	3.9	8.3	3.5	6.5
Total (%)	*100.0*	*100.0*	*100.0*	*100.0*	*100.0*	*100.0*	*100.0*	*100.0*	*100.0*

Numbers represent the mean percent of the total number of retrogradely labeled cells in the originating hippocampal regions arising from injections in the lateral entorhinal and medial entorhinal areas (LEA and MEA).

[a]Note: A few cells in field CA3 were observed arising from LEA injection sites. The cells may belong to field CA2. Two cases with retrograde tracer injections in the MEA medial band resulted in substantial numbers of labeled CA3 cells. This may be the result of contamination with the dentate gyrus. See text for details.

trum and the dorsal thalamus preferentially targets the lateral band, but input from the olfactory structures and the amygdala preferentially targets the medial band (Table 3).

Olfactory input arises mainly from the endopiriform nucleus and targets the intermediate and medial bands of the MEA (Table 3). The amygdala input arises largely from the olfactory amygdala. The olfactory amygdala input is very strong to the medial band and moderately strong to the intermediate band. The lateral and basomedial amygdala also provide moderately strong input to the medial band of the MEA.

As is true for the LEA, the midline dorsal thalamic nuclei provide the heaviest thalamic input to the MEA. There is also substantial input from the lateral and anterior groups of the dorsal thalamus. The ventral thalamic contribution is minimal (Table 3). Input from the hypothalamic afferents arises in the mammillary bodies and the lateral zone, and terminates preferentially in the lateral and intermediate bands of the MEA.

With the exception of the grid cell area, which is in the lateral band, the strongest efferent projection from the MEA arises in the medial band and targets the basal ganglia (Table 4). The projection terminates preferentially in the caudate putamen and, to a lesser extent, the nucleus accumbens. The medial band also provides substantial input to olfactory, amygdala, and septal structures. The strongest olfactory projections are to the taenia tecta and the endopiriform nucleus, followed by the accessory olfactory nucleus and the olfactory tubercle. The projection to the piriform transition area is weaker, but still substantial. The amygdala projection preferentially targets the olfactory amygdala structures. The septal projection terminates in the lateral and medial septal nuclei. A smaller projection terminates in the bed nucleus of the stria terminalis. Finally, a modest projection to the claustrum also arises in the medial band of the MEA.

Projections to the thalamus are negligible (Table 4). The MEA does provide weak input to the hypothalamus. The medial band of the MEA provides modest input to the lateral, medial, and periventricular zones. The intermediate band provides a slightly heavier input to the mamillary bodies.

HIPPOCAMPAL CONNECTIONS OF THE LEA

About one-third of the total afferent input to the LEA originates in hippocampal areas (Fig. 2; Agster, 2007). Of the hippocampal inputs, the dorsal and ventral hippocampus both project to the LEA, but the ventral hippocampus provides the heavier projection (Table 5). The input arises largely in field CA1 and the subiculum, and terminates in all three LEA bands with a bias toward the medial band. The parahippocampal region contributes a strong input, largely originating in the PER and targeting the lateral to medial bands of the LEA in a graded fashion with the heaviest input to the lateral band and the weakest to the medial band (Burwell and Amaral, 1998b; Agster, 2007). The POR, presubiculum, and parasubiculum provide modest input terminating in all three bands. The dorsal hippocampus provides a modest input to the LEA originating in the CA1 and subiculum, and terminating in the lateral and intermediate bands of the LEA.

The LEA projections to the hippocampal formation structures show the patterns expected based on studies of the topography of the dentate gyrus projecting bands (Table 6; Dolorfo

TABLE 6.

Hippocampal Efferents of the Entorhinal Cortex: Index of Fiber Labeling

	LEA	LEA bands			MEA	MEA bands			MEA
Terminations	Average	Lateral	Inter	Medial	Average	Lateral	Inter	Medial	Grid area
Doral HC									
DG/CA3	21.5	26.7	17.4	3.4	26.5	20.2	76.7	1.8	2.5
CA2/CA1	22.8	30.2	15.1	0.8	26.0	33.3	22.5	0.3	25.3
SUBd	3.0	4.0	2.0	0.0	2.0	2.5	2.0	0.0	3.5
Ventral HC									
DG/CA3	7.5	2.8	13.2	19.6	9.2	4.4	14.5	23.0	0.0
CA2/CA1	8.4	7.6	13.2	2.8	8.1	9.7	1.8	8.2	0.0
SUBv	6.4	4.9	11.8	2.8	5.1	3.7	8.2	7.7	0.2
ParaHC region									
Presubiculum	4.3	5.0	4.6	0.0	3.2	2.8	6.3	1.6	2.1
Parasubiculum	1.1	0.9	2.2	0.1	2.4	2.7	1.5	1.8	0.4
Postrhinal	44.1	40.2	33.0	43.8	56.6	25.4	58.2	136.6	5.9
Perirhinal	167.3	166.7	116.4	167.0	212.2	76.4	237.0	526.9	25.8

Numbers represent indexes of fiber labeling in terminal hippocampal regions arising from anterograde tracer injections in the lateral entorhinal and medial entorhinal areas (LEA and MEA). Density of fiber labeling was rated and then normalized by the volume of the terminal structure. See text for details.

and Amaral, 1998). For dorsal hippocampal structures the lateral band of the LEA provides the heaviest input to the dentate gyrus and CA3, and the medial band provides the weakest input. The dentate gyrus/CA3 and CA1 projections are heavier than the subiculum projection. Efferents directed to the ventral hippocampus are moderately strong from specific regions of the LEA. The majority of these projections originate in the medial band followed by the intermediate and lateral bands of the LEA. These fibers largely terminate in the ventral subiculum, field CA1, and dentate gyrus.

Of the hippocampal system efferents, the strongest LEA outputs target the parahippocampal region (Table 6). The heaviest projection terminates in the PER, but the POR projection is also strong.

HIPPOCAMPAL CONNECTIONS OF THE MEA

Overall, nearly one-half of the afferent input to the MEA arises in hippocampal system structures (Fig. 2; Agster, 2007). The heaviest hippocampal system input to the MEA originates in the parahippocampal region and terminates preferentially in the lateral and intermediate bands (Table 5). Caudal parasubiculum provides the strongest afferent input to the MEA followed by the dorsal part of the dorsal presubiculum and the POR. The majority of this input is directed to the lateral band of the MEA. In contrast to the LEA, which receives a heavier PER input, the MEA receives input equally from both the PER and the POR.

The ventral hippocampus preferentially projects to the medial band of the MEA (Table 5). Field CA1 provides the majority of this afferent input to the medial band of the MEA. The ventral subicular afferent inputs to the MEA are also quite strong. The dorsal hippocampus also contributes a substantial proportion of the input to the MEA. These projections largely arise from field CA1 and preferentially project to the lateral band of the MEA.

As is well known from many prior studies, the MEA originates a heavy projection to the hippocampus (Table 6). Most notably, the lateral and intermediate bands provide the heaviest input to the dorsal dentate gyrus and dorsal field CA1. The subiculum, field CA3, and field CA2 also receive moderate projections from the MEA. The MEA input to the ventral hippocampus originates primarily in the intermediate and medial bands and targets the dentate gyrus/CA3, field CA1, and subiculum.

Similar to the LEA, the MEA efferent projections to the parahippocampal region are the strongest (Table 6). Projections arising in the MEA and terminating in the parahippocampal region are denser than projections arising in the LEA. Moreover, the parahippocampal projections originate largely in the medial and intermediate bands of the MEA. These regions preferentially project to the PER, but the POR projection is also strong. It should be noted, however, that the fiber labeling index is weighted by the volume of the target structure and the PER is larger in volume than the POR. The projections to the presubiculum and parasubiculum are modest. There is, however, a substantial presubicular projection that terminates in the most dorsal part of the presubiculum, sometimes called the postsubiculum.

CONNECTIONS OF THE DORSOCAUDAL MEA

Twenty seven percent of the total input to the dorsocaudal MEA, the grid cell area, arrives from cortical structures

(Burwell and Amaral, 1998a). Not surprisingly, the occipital regions provide the heaviest input as measured by percent of labeled cells (Table 1). Strong input also arises in parietal, temporal, cingulate, and frontal regions (Burwell and Amaral, 1998a). Frontal input arises primarily in the secondary motor region. Strong reciprocal projections target temporal, parietal, and occipital regions (Table 2; Agster, 2007).

Overall, the dorsocaudal MEA receives relatively little input from the subcortical regions. The largest proportion arises from the claustrum (Table 3; Agster, 2007). The dorsal thalamic input, which is the next strongest, originates in the dorsal midline group as well as the anterior and lateral thalamic groups. Smaller projections arise in the lateral and basolateral nuclei of the amygdala and the medial septum. Ventral thalamic inputs largely arise from the intralaminar nuclei. The mammilary bodies and lateral zone provide the majority of the weak hypothalamic input. The endopiriform nucleus contributes the olfactory input to the dorsocaudal MEA. Basal ganglia input is weak but arises from all subregions.

With the exception of the basal ganglia, the dorsocaudal MEA return projections to subcortical regions are relatively weak (Table 4; Agster, 2007). By far, the strongest efferent projections to subcortical regions target the basal ganglia. The projection terminates preferentially in the caudate putamen and to a lesser extent in the nucleus accumbens. Modest projections target the central and the basolateral nuclei of the amygdala and the claustrum. Output to olfactory structures terminates in the taenia tecta and olfactory tubercle. The septal projection is relatively weak and targets the lateral septum and to a lesser extent, the medial septum.

The hippocampal system provides the most input to the dorsocaudal MEA accounting for more than half of the grid cell area's total afferent input. The dorsal hippocampus provides the largest hippocampal system input (Table 5). These afferents arise primarily from field CA1. The parahippocampal region also contributes a considerable amount of input to the grid cell area. These inputs largely originate in the dorsal presubiculum, the component sometimes termed the postsubiculum. The ventral hippocampus contributes minimal afferent input to the region.

The hippocampal system is reciprocally connected to the dorsocaudal MEA (Tables 5 and 6). Moderately strong projections terminate in the PER and dorsal CA1. In contrast, the ventral hippocampus receives few return projections from the grid cell area.

CONCLUSIONS

The EC is well positioned to act as a gatekeeper for the hippocampal formation. By this, we mean that the region appears to be actively involved in the preprocessing and selection of information ultimately directed to the hippocampus, rather than merely funneling information to the hippocampus. Available evidence suggests that the entorhinal subdivisions, the LEA and MEA, have distinct information processing functions (Hargreaves et al., 2005). Based on functional studies, the MEA contributes spatially specific information, whereas firing fields of the LEA neurons show considerably less spatial specificity (Fyhn et al., 2004; Hafting et al., 2005; Steffenach et al., 2005). Such findings are consistent with the neuroanatomy. The LEA receives considerably more input from the piriform cortex and insular regions and less from the visual, posterior parietal, and retrosplenial cortices (Fig. 5). Additionally, the LEA receives a considerable input from the amygdala and olfactory structures (Fig. 5). These inputs, as well as inputs from the insular cortex, may play a role in providing nonspatial information about context, including the emotional significance. Consistent with this idea, the LEA is strongly and reciprocally connected with the PER, a region thought to be involved in polymodal sensory processing.

In contrast to the LEA, the MEA receives much heavier input from the visual association, posterior parietal, and cingulate cortices. These afferents preferentially target the lateral band of the MEA. These data are consistent with studies showing that neurons located in the MEA encode spatial information (Fyhn et al., 2004; Hafting et al., 2005). Additionally, the MEA receives considerable input from the dorsal thalamus, primarily the midline nucleus and also the dorsal lateral and dorsal anterior thalamic nuclei. Dorsal thalamic afferents preferentially target the lateral and intermediate band of the MEA, which in turn targets the dorsal hippocampus. All of these structures are implicated in spatial learning and memory. Neurons within the dorsal thalamic regions exhibit head direction specificity (Mizumori and Williams, 1993; Taube, 1995). Regarding the grid cell area, our assessment of the available neuroanatomical data suggests that the dorsocaudal MEA is heavily influenced by posterior parietal and visual association areas, as well as the dorsal thalamic nuclei (Fig. 5). These connections may account for the unique spatial firing patterns of cells in the dorsocaudal MEA.

The MEA lateral and intermediate bands receive the heaviest input from parahippocampal structures, including the presubiculum, parasubiculum, and POR cortices. These bands are also heavily targeted by visuospatial input from cortical and subcortical regions. When these dentate-projecting bands were first described, it was noted that the intrinsic connections were confined to the band of origin and were largely nonoverlapping (Dolorfo and Amaral, 1998). Thus, the lateral and intermediate bands of the EC may provide a locus of convergence for nonspatial information projecting to the LEA and spatial information projecting to the MEA before arriving in the hippocampus.

The connections of the medial band of the MEA are distinct and deserve special consideration. The medial band of the MEA receives ~75% of its hippocampal system input from the ventral hippocampus. These afferent projections largely arise from field CA1 and the subiculum. The medial MEA projects strongly to the ventral dentate gyrus and provides noticeable

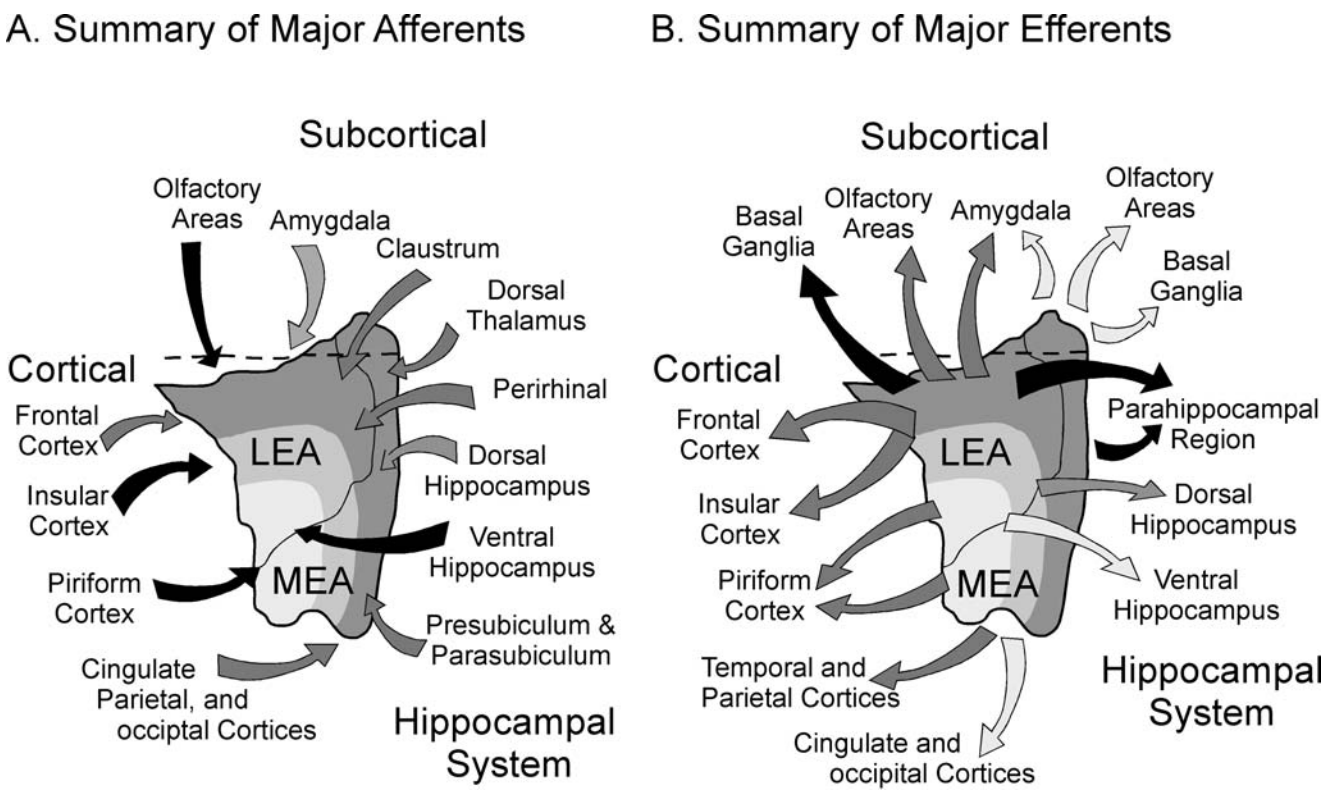

FIGURE 5. Summary of the major connections of the lateral (LEA) and the medial (MEA) entorhinal areas. A. A summary diagram of the afferent connections of the entorhinal cortex. B. A summary diagram of the efferent connections of the entorhinal cortex. Note, strong connections are represented by black arrows, moderate connections are denoted by dark gray arrows, and weak connections are indicated by light gray arrows.

inputs to Field CA1 and the ventral subiculum. Because CA1 provides a principal route for hippocampal output, it has been suggested that the entorhinal-CA1 pathway modulates the plasticity and output of the dorsal hippocampus (Remondes and Schuman, 2002). It is possible that the medial band of the MEA provides a similar function for the ventral hippocampal outputs. Dense efferent projections from the medial band of the MEA also preferentially target the POR and PER. These parahippocampal projections represent the strongest efferents arising from the EC.

Substantial differences in the cortical, subcortical, and hippocampal connections of the LEA and MEA indicate that the information processing functions differ for the two subregions. The nature of the connectional differences suggests that the processing of spatial and nonspatial information may be somewhat segregated with LEA having a greater role in processing nonspatial information and the MEA in processing spatial information. At the same time, the patterns of intrinsic connections suggest that spatial and nonspatial information are combined in entorhinal bands before transmittal to the hippocampal formation. Although it is clear that the EC is involved in processing both spatial and nonspatial information, there are still many unanswered questions about its contribution to memory and learning. Our hope is that this summary of the extrinsic connections of the EC will provide insight into its role in memory and other cognitive functions.

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