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Anatomy and Function of the Primate Entorhinal Cortex

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Abstract

The entorhinal cortex (EC) is a critical element of the hippocampal formation located within the medial temporal lobe (MTL) in primates. The EC has historically received attention for being the primary mediator of cortical information going into and coming from the hippocampus proper. In this review, we highlight the significance of the EC as a major player in memory processing, along with other associated structures in the primate MTL. The complex, convergent topographies of cortical and subcortical input to the EC, combined with short-range intrinsic connectivity and the selective targeting of EC efferents to the hippocampus, provide evidence for subregional specialization and integration of information beyond what would be expected if this structure were a simple conduit of information for the hippocampus. Lesion studies of the EC provide evidence implicating this region as critical for memory and the flexible use of complex relational associations between experienced events. The physiology of this structure's constituent principal cells mirrors the complexity of its anatomy. EC neurons respond preferentially to aspects of memory-dependent paradigms including object, place, and time. EC neurons also show striking spatial representations as primates explore visual space, similar to those identified in rodents navigating physical space. In this review, we highlight the great strides that have been made toward furthering our understanding of the primate EC, and we identify paths forward for future experiments to provide additional insight into the role of this structure in learning and memory.

1. INTRODUCTION

The hippocampal formation has long been implicated in learning and memory (Milner 1972, Mishkin 1982, Squire & Zola-Morgan 1991, Zola-Morgan & Squire 1985). The entorhinal cortex (EC) is a critical element of this network of integrated structures and receives a wide array of converging inputs from polymodal sensory association cortices, as well as subcortical arousal areas (Brodmann & Garey 2006). In turn, the EC itself is the major source of cortical input into the hippocampus proper. The first reported investigations into the EC came from dissections in rodents performed by Santiago Ramón y Cajal at the end of the nineteenth century. His findings highlighted a portion of the rat cortex with substantial reciprocal connections with the hippocampus. Ramón y Cajal (1899) posited that this rich interconnectivity strongly implicated this cortical region as necessary for proper hippocampal function. This hypothesis inspired the current dogma of the EC as a gatekeeper of cortical communication with the hippocampus and has shaped the ways in which this structure has been studied (Amaral et al. 1987). As such, it will come as no surprise that the bulk of contemporary work on EC function has focused on elucidating how the physiology of this structure facilitates hippocampal processing. This field has been driven predominantly by research in rodents performing spatial navigation paradigms (Burgess et al. 2007, Hafting et al. 2005). Through this work, medial (MEC) and lateral (LEC) subdivisions of the rodent EC have been identified that are distinguishable by both their anatomy and their function (Witter et al. 2017). EC input is limited to the distal two-thirds of the molecular layer of the dentate gyrus (DG); the MEC projects to the middle third, whereas LEC projections terminate on the outer third (Witter 2007). Functionally, the MEC's constituent cells, including grid cells, are largely driven by allocentric spatial reference frames (Hafting et al. 2005, Sargolini et al. 2006, Solstad et al. 2008). By contrast, LEC neurons have been reported to elicit temporally modulated firing patterns (Tsao et al. 2018), as well as responses to specific objects (Deshmukh & Knierim 2011, Tsao et al. 2013).

While these findings in the rodent EC have had a significant impact on the trajectory of primate research, several incongruences arise when directly comparing this structure across species. Anatomically, the EC takes a much more rostral position in the primate temporal lobe than in the rodent; in the primate, it sits largely anterior to the hippocampus. Additionally, whereas the EC in rodents is more flat and ellipsoid, this structure adopts a C-like shape in primates (Strange et al. 2014) (**Figure 1*a***). The primate EC contains more pervasive connectivity patterns with visual processing areas compared to that of the rodent (Burwell 2006, Insausti et al. 1987a), a fact that is likely explained by the reliance of primates on vision as their primary exploratory modality, compared to olfaction in rodents (Schroeder et al. 2010). Given the cognitive deficits associated with Alzheimer's-derived atrophy of the EC—one of the initial neuropathologies in humans with this disease (Khan et al. 2014)—it is imperative that we continue to investigate the relationship between this structure's physiology and higher-order cognitive processes, including episodic memory. In this review, we synthesize the growing field of primate EC research aimed at doing just that. We examine how recent research has both elaborated on findings from rodent studies and identified key differences between species. First, we review the anatomy of the primate EC, highlighting its privileged position: The EC serves as a conduit to the hippocampus and as a remarkably efficient site of information convergence. Second, we discuss the diverse network of communications within the hippocampal formation, including interactions between the EC and hippocampus, as well as the robust intrinsic connectivity within the cortical layers of the EC. Finally, we discuss how these anatomical findings have motivated ideas regarding EC function, and we review the lesion and neurophysiological studies that have examined various functional hypotheses. Clearly, the field has made significant headway in uncovering the function of the EC; however, the emergence of

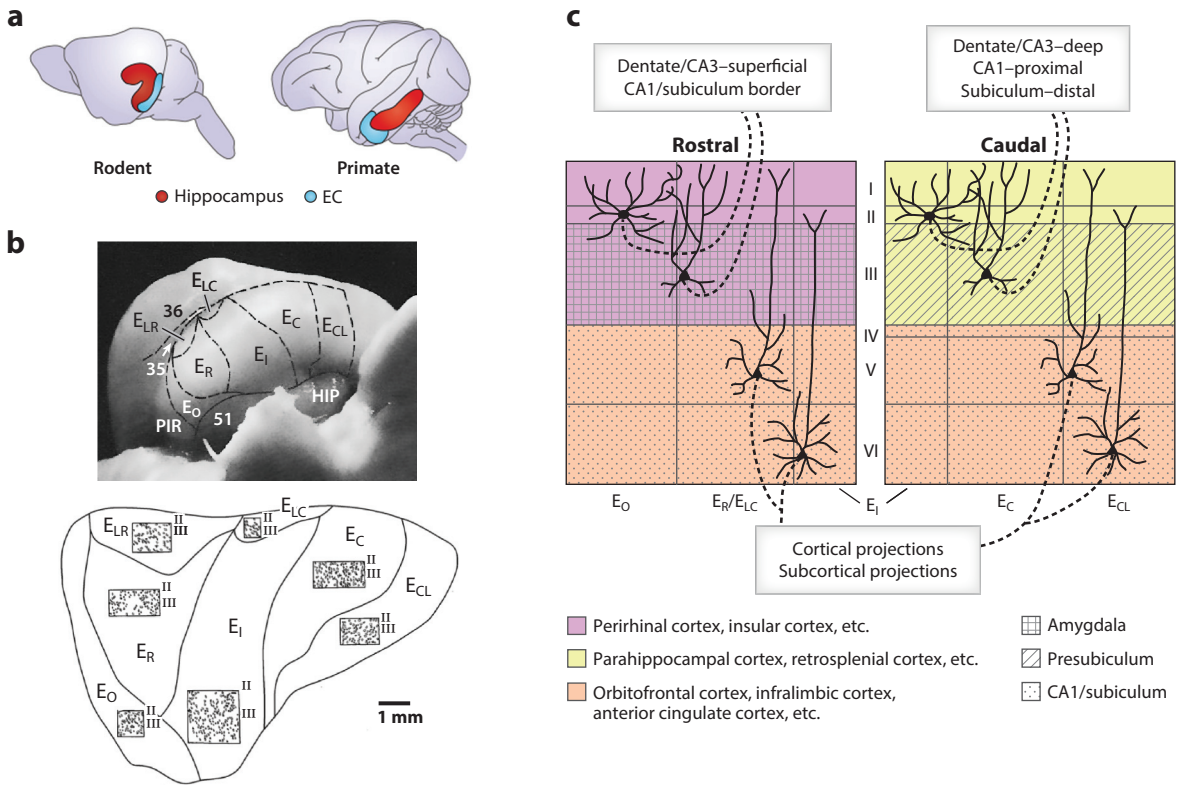

Figure 1

(*a*) Illustration showing the variation of anatomical placement of the EC between rodents and primates. Panel adapted with permission from Strange et al. (2014). (*b*) Photomicrograph of the ventral surface of the temporal lobe and flattened illustration of EC subfields as defined by Amaral et al. (1987). Note the change in layer II and III cell density across the different subregions. Panel adapted with permission from Amaral et al. (1987). (*c*) Schematic of major EC connectivity. Simplified principal neurons in each layer are illustrated, and dashed lines indicate the predominant efferent projections from the EC to the hippocampus (layers I–III) or to other cortical and subcortical sites (layers V and VI). The legend indicates a subset of cortical and subcortical inputs. Panel adapted with permission from Insausti et al. (1987a). Abbreviations: EC, entorhinal cortex; E_C, caudal EC; E_{CL}, caudo-limiting EC; E_I, intermediate EC; E_{LC}, rostro-caudal EC; E_{LR}, rostro-lateral EC; E_O, olfactory EC; E_R, rostral EC.

new tools for neurophysiological recordings and targeted manipulations has the potential to dramatically increase our understanding. We hope that this review will inspire and motivate further studies of this structure in the primate.

2. WHAT IS THE ENTORHINAL CORTEX?

2.1. Etymology and Origin

In the primate, the EC is situated in the anterior medial temporal lobe (MTL). It begins ventral to the amygdala and anterior to the hippocampus. From there, it extends caudally underneath the hippocampus for approximately 5 mm. The earliest usage of the term "entorhinal cortex" was given by Brodmann after his dissections of human tissue, during which he identified a cortical field extending medially from the rhinal sulcus (Brodmann's area 28) with distinct cytoarchitecture from the tissue surrounding the sulcus (perirhinal cortex; Brodmann's area 35) and the tissue extending

laterally from it (ectorhinal or inferior temporal cortex) (Amaral et al. 1987, Brodmann & Garey 2006).

2.2. Cytoarchitecture

Despite the EC being recognized as an independent brain region by virtue of its unique cytoarchitecture, anatomists have historically been in disagreement over this region's exact cytoarchitectonic composition. Ramón y Cajal (1899) proposed a seven-layer architecture in his initial dissections, although follow-up investigations by his student, Lorente di No, reduced that number to six (Amaral et al. 1987). After many subsequent years of disagreement, it is now broadly agreed that the EC is in fact a six-layered structure in rodents (Witter et al. 2017) and primates (Amaral et al. 1987; Van Hoesen & Pandya 1975a,b). However, as we discuss in Sections 3 and 4, the promiscuous connectivity of layers I–V has been most intriguing to researchers studying the functional role of the EC. The proportionate layer sizes and constituent cell densities change across the various subdivisions of the primate EC; however, the cell morphologies (or lack thereof) that constitute each layer remain consistent. Layer I is almost devoid of cell bodies and primarily contains axially directed axons. Layer II is composed of cells that are morphologically modified pyramidal cells, typically referred to as stellate cells. Layer III is the canonical pyramidal cell layer often found in the cortex, and, like layer II, it changes in thickness and density across the various EC subregions. Layer IV is similar to layer I in that it is devoid of cell bodies; accordingly, this area is typically labeled the lamina dissecans. Like layer I, layer IV is a fiber-heavy zone. Layer V, another thick pyramidal cell layer similar to layer III, contains a density gradient of its constituent cells such that they are most densely packed superficially. Because of this cell density gradient, this layer is often subdivided into sublaminae Va, Vb, and Vc. Layer VI—perhaps the least-studied portion of the EC—houses variously sized neurons in bands of differing density. In contrast to the rest of the EC, the sub-bands of this layer are interesting in that they take a much more curved shape compared to those of other layers (Amaral et al. 1987).

2.3. Entorhinal Subregions

The canonical subdivision of the rodent EC into MEC and LEC has its basis in several facets of anatomy and physiology. The cytoarchitecture of the LEC is consistent with the majority of the cortex and has smooth, continuous cell layers, which is a stark contrast from the patchy, interrupted cell layers seen in the MEC (Witter et al. 2017). In addition, differing patterns of connectivity (Witter 2007) and unique neurophysiological responses to behavioral phenomena serve to distinguish these subregions (Deshmukh & Knierim 2011; Fyhn et al. 2004; Hafting et al. 2005; Sargolini et al. 2006; Solstad et al. 2008; Tsao et al. 2013, 2018). Our ability to subdivide the primate EC has also been steeped in a history of contradictions (Amaral et al. 1987). In Brodmann's initial dissections of the EC of various species, he opted not to subdivide the EC of primates despite delineating medial and lateral aspects in the brains of bats, hedgehogs, and other nonprimate species (Brodmann & Garey 2006). Subsequently, others have divided the primate EC into as many as 14 subareas in monkeys and 23 in humans based on cytoarchitectonics alone (Amaral et al. 1987). Just as the disagreements over gross cytoarchitecture were resolved, a subtle agreement has been reached within the field over how to subdivide the primate EC since the era of these initial studies. In rhesus monkeys, pivotal work by Van Hoesen & Pandya (1975a,b) highlighted three predominant subfields—a caudal, an intermediate, and a rostral area. One of the main findings to support this separation was the changing cytoarchitecture between the caudal EC and the more rostral EC elements. Specifically, in the rostral area, layer IV is virtually nonexistent,

and the stellate cells in layer II form small, discontiguous clusters of islands. In the intermediate zone, the layer II islets become more prominent and consistent, moving caudally in conjunction with layer IV becoming increasingly visible. Finally, layer II is a continuous band of cells, and the cell-less layer IV is significantly more prominent in the caudal area. These findings were largely corroborated by Amaral and colleagues (1987) in cynomolgus macaques; however, Amaral and colleagues also observed strong departures from the rhesus brain that led them to identify seven distinct EC subregions (**Figure 1*b***). The most rostral element, the olfactory EC (E_O), is the only subregion delineated by its unique inputs. As its name suggests, this region receives strong, direct terminations from the olfactory bulb. Just behind the E_O lies the region labeled the rostral EC (E_R). The E_R bears many similarities with the rostral area in the rhesus. However, it lacks the cell-less layer IV and contains patchy islets of cells in its superficial layers. The intermediate EC (E_I) sits directly behind the E_R and serves as the transitionary bound between the interrupted cell islands in the more anterior portions of the EC and the continuous layers in more posterior elements. Lateral to both the E_R and E_I, the portion of the EC that runs along the medial edge of the rhinal sulcus is cytoarchitectonically distinct from its more medial subregions. Because of changing cytoarchitecture even along its rostro-caudal extent, this lateral element of the EC has been divided into two distinct areas. The rostral portion (E_{LR}) sits lateral to the E_R, and the caudal element (E_{LC}) lies lateral to the E_I. Layers II and III house thick islands of cells in both regions, with the E_{LC} containing more dense clusters. The cell-less layer I increases in size in the E_{LR} relative to the E_{LC}, although there is no visible layer IV in either area. Interestingly, a rhesus homolog of these regions was identified by Van Hoesen & Pandya (1975a,b), and it was named prorhinal cortex. Yet Amaral et al. (1987) opted not to label this an independent brain area because its projection patterns were similar to other subregions within the EC of cynomolgus macaques. The caudal EC is divided into two fields, E_C and E_{CL}. The E_C sits directly behind the E_I and the caudal-limiting field (E_{CL}) behind the E_C. In both regions, the islands of layer II cells become thicker and more robust as one moves caudally, such that, in the most posterior elements of the E_C and all of the E_{CL}, the layer looks like a uniform band of neurons (Amaral et al. 1987).

3. NONHIPPOCAMPAL ENTORHINAL CONNECTIVITY

3.1. Subcortical Connectivity

The EC receives substantial input from subcortical areas, including the amygdala, claustrum, striatum, basal forebrain, and others (Insausti et al. 1987b). Interestingly enough, with rare exceptions, there is no strong evidence of topographic bias of subcortical projections onto the EC. Additionally, subcortical sites appear to project only ipsilaterally to the EC, as opposed to bilaterally (Insausti et al. 1987b). However, the diversity of these diffuse subcortical inputs, combined with the immense topography of cortical termination patterns onto the EC (see Section 3.2), amplifies the high degree of specialized information that this structure can convey to the downstream hippocampus. The number of subcortical sites that project to the EC is tremendous; in this section, we highlight a few key areas that specifically communicate with the primate EC in the service of learning and memory. In the rodent, the medial septal nucleus (MS) and diagonal band of Broca (DBB), components of the basal forebrain, are considered to be among the most critical cholinergic and GABAergic inputs into both the EC and hippocampus because of their strong influence on the rhythmic mesoscopic properties of neuronal activity in these regions (Carpenter et al. 2017, Dragoi et al. 1999, Mitchell et al. 1982). In the monkey, there exist strong projections between the MS/DBB and the EC. However, in contrast to findings in rodents suggesting a strong topography of connectivity (Kondo & Zaborszky 2016), the primate MS/DBB efferents to the EC appear to

be considerably more diffuse across the structure (Insausti et al. 1987b). In addition, strong cholinergic projections (Liu et al. 2015) from the nucleus basalis of Meynert terminate onto the primate EC (Amaral & Cowan 1980, Insausti et al. 1987b, Mesulam et al. 1983). Projections from this region are virtually nonexistent in the rodent (Kondo & Zaborszky 2016), suggesting that there is an additional excitatory drive into the EC that is primate-specific. This largely unexplored interaction between the nucleus basalis and EC in primates may explain some of the observed differences in the neurophysiology of this region across species (see Section 5.3). Surprisingly, the largest subcortical projections to the EC come not from the basal forebrain nuclei, but rather by way of the amygdala, which has been implicated in numerous behavioral phenomena including fear conditioning, reward processing, and memory-associated processes (Blair et al. 2001, Chudasama et al. 2009, LaLumiere 2014). Most of the projections from the amygdala to the EC originate in the lateral nucleus of the amygdala, with sparser projections arising from the accessory basal nucleus and periamygdaloid complex (Aggleton 1986, Insausti et al. 1987b). In contrast to other subcortical termination patterns, these projections are biased toward rostral elements of the EC (Insausti et al. 1987b), with the subregions most heavily innervated by the amygdala being the E_O, E_R, and E_{LR}. In these regions, anterograde tracing experiments highlighted robust amygdala terminations specifically in layers I, III, and V, whereas amygdalar input is restricted to layer I in more intermediate and caudal EC regions (Insausti et al. 1987b).

With respect to inputs from diencephalic structures, the primate EC receives considerable input from both the thalamus and hypothalamus (Amaral & Cowan 1980, Insausti et al. 1987b). Thalamic input to the EC is dominated mostly by the central lateral nucleus, which has been indicated by rodent studies to be responsive to noxious, visceral stimulation (Ren et al. 2009). Retrograde tracing also suggests noticeable terminations in the EC after injections made in the paraventricular, parataenial, and pulvinar nuclei (Amaral & Cowan 1980, Insausti et al. 1987b). Although efferents from the nucleus reuniens are also present in the primate EC, the density of these projections appears to be sparser than that in rodents (Dolleman-Van Der Weel & Witter 1996, Insausti et al. 1987b). Several nuclei within the hypothalamus also project to the EC. Of these, the most prominent projection comes from the supramammilary area (Amaral & Cowan 1980, Insausti et al. 1987b). This is another region that contributes to rhythmic synchrony in the neurophysiology of the rodent EC, both through its direct influences on the structure and through interactions with the MS/DBB (Vertes & Kocsis 1997). Its promiscuous connectivity in the primate EC suggests an analogous synchronizing function with the hippocampal formation of primates. Several neuromodulatory systems in the brainstem also innervate the EC. Considerable projections from the dorsal raphe nucleus and ventral tegmental area (VTA) have been observed through retrograde tracing (Insausti et al. 1987b). The most anterior EC subregion, the E_O, receives specific input from the substantia nigra and parabrachial nucleus, suggesting a unique interaction of reward-related and visceral brainstem areas with the most rostral element of the EC.

3.2. Sensory Afferents

The EC serves as the main sensory relay into the hippocampus (**Figure 2*a***), and the strongest of these sensory projections is sent directly from the olfactory bulb to the most rostral element of the EC, the E_O (Carmichael et al. 1994, Insausti et al. 1987a). As mentioned above, this is the only entorhinal subregion defined on the basis of its cortical inputs, and no other unimodal sensory area has been shown to project directly to the EC in primates. The piriform cortex, which acts as the first cortical relay for the olfactory bulb, sends modest projections to both the E_R and E_{LR}

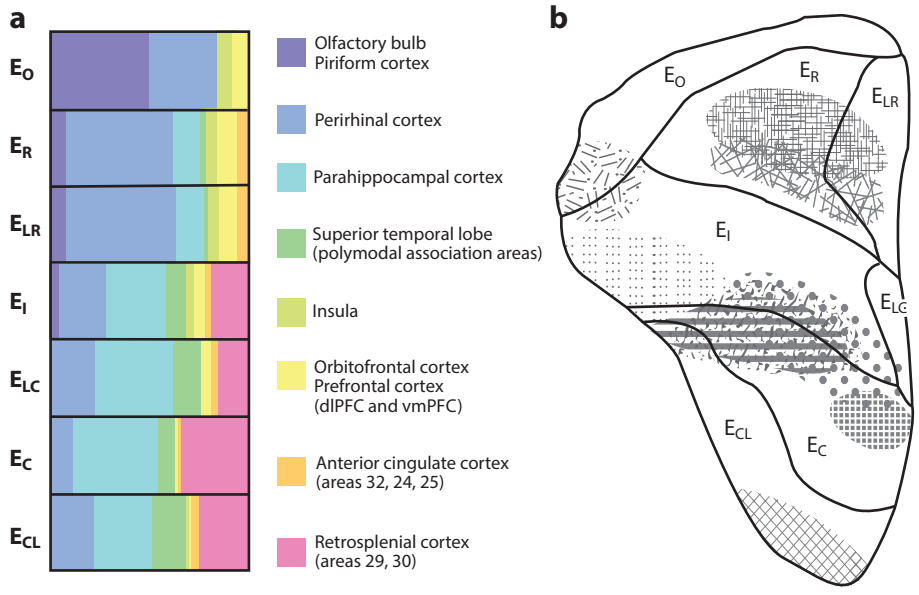

Figure 2

(*a*) Summary of the major sensory and cortical afferents to the primate EC, and the general topography of their respective termination patterns. Data adapted with permission from Insausti et al. (1987a). (*b*) Flattened map of the EC showing the placement of retrograde tracer injections that contribute to panel *a*. Illustrated patterns represent individual injection cases. Image adapted with permission from Insausti et al. (1987a). Abbreviations: dlPFC, dorsolateral prefrontal cortex; EC, entorhinal cortex; E_C, caudal EC; E_{CL}, caudo-limiting EC; E_I, intermediate EC; E_{LC}, rostro-caudal EC; E_{LR}, rostro-lateral EC; E_O, olfactory EC; E_R, rostral EC; vmPFC, ventromedial prefrontal cortex.

and a much sparser projection to the E_I. Retrograde tracing studies have definitively shown that neither auditory cortices nor high-level visual cortices such as area TE in the inferior temporal cortex project to any entorhinal subfield (Insausti et al. 1987a).

As may be expected, the majority of sensory input into the primate EC comes from polymodal association cortices (**Figure 2*a***). Two of the heaviest projections into EC come from perirhinal cortex (area 35/36) and parahippocampal cortex (TF/TH) (Insausti et al. 1987a, Van Hoesen & Pandya 1975b). While these areas have been implicated in the processing of somatosensory and auditory stimuli, they receive predominantly visual input (Baxter et al. 1999, Fritz et al. 2005, Ramos 2014). The perirhinal cortex has been associated with the processing of complex visual objects (Buffalo et al. 1999, 2000; Gaffan & Murray 1992; Murray & Mishkin 1998), and, in the parahippocampal cortex, this culmination of sensory processing is geared toward spatial information processing (Aguirre et al. 1996). It has long been accepted that, in rodents, homologous regions project to distinct parts of the EC and form discrete functional domains by way of parahippocampal–MEC and perirhinal–LEC interactions (Burwell & Amaral 1998). However, this dogma has been recently challenged by Doan and colleagues (2019). Through a combination of tract tracing and in vitro physiology experiments, Doan et al. (2019) identified a heavy bias of both perirhinal and parahippocampal input toward the LEC, with only minimal projections from the parahippocampal cortex to a limited portion of the MEC. These recent findings parallel the diffuse perirhinal and parahippocampal projection patterns to the EC of primates. Anterograde and retrograde tracing experiments have shown that both the perirhinal and parahippocampal

cortices project to all subregions of the EC apart from the most rostral E_O and the lateral elements E_{LR} and E_{LC} (Insausti & Amaral 2008, Insausti et al. 1987a, Suzuki & Amaral 1994) (for retrograde injection placements, see **Figure 2*b***). The perirhinal cortex projects more strongly to rostral elements of the EC, with only sparse terminations on the caudo-lateral EC. In tandem, the parahippocampal cortices are biased toward more caudo-central portions, with only minor projections to the rostro-lateral EC (Insausti & Amaral 2008, Suzuki & Amaral 1994) (**Figure 2*a***). Interestingly, a restricted portion of the posterior parahippocampal cortex originates projections to the most caudo-medial portion of the EC, akin to what has been observed in rodents (Doan et al. 2019, Insausti & Amaral 1987a, Suzuki & Amaral 1997). Thus, the input patterns of the rodent and primate ECs, with their respective perirhinal and parahippocampal cortices, are consistent in that polymodal information converges onto lateral portions of the EC in both species.

The dorsal polymodal areas of the superior temporal gyrus also send projections to the EC, and tracing studies implicate a bias toward more caudal aspects (Insausti & Amaral 2008, Insausti et al. 1987a) (**Figure 2*a***). Similar to the collective functions of the perirhinal and parahippocampal cortices, these inputs from the superior temporal sulcus provide an additional avenue by which the caudal EC receives highly processed visual information for object recognition and biological motion processing (Chaplin et al. 2018, Desimone & Ungerleider 1986). The rostral EC receives unique polysensory input from the insula, and anterior portions of this region (the agranular insula) innervate the anterior EC subregions E_R, E_{LR}, and anterior E_I (Insausti & Amaral 2008, Insausti et al. 1987a). These inputs have been reported to carry audio-visual information, although the insula has been implicated in widespread sensory and cognitive functioning (Evrard 2019). For all sensory areas, the strongest sites of laminar termination were located in layers I–III (Insausti & Amaral 2008) (**Figure 1*c***). Thus, with the exception of the olfactory area E_O, the data suggest that all areas of the EC receive largely visually modulated information about putative objects and relevant motion information from neocortical sites.

3.3. Frontal and Cingulate Afferents

The frontal and cingulate cortices also send extensive projections to the primate EC (**Figure 2*a***). One of the largest inputs from the frontal cortices to the EC in primates originates in the orbitofrontal cortex (OFC; area 13/13a) (Insausti & Amaral 2008, Insausti et al. 1987a, Rempel-Clower 2000, Van Hoesen & Pandya 1975b, Witter et al. 1989). These inputs preferentially innervate the rostro-lateral elements of the EC but synapse on all subregions except the E_{CL}. These projections have been shown in retrograde tracing experiments to most strongly emanate from caudal elements of areas 13/13a. Sparser projections have also been observed from more rostral orbitofrontal areas 11 and 12, ventromedial area 14, and dorsolateral frontal cortices (Insausti & Amaral 2008, Insausti et al. 1987a). Anterior cingulate areas 32, 24, and 25 project strongly to the E_R and E_{LR}, with minor projections to more caudal aspects of the EC. The retrosplenial cortex (areas 29 and 30) constitutes the major projection from the posterior cingulate. This region provides robust input to the more caudal elements of the EC, such as the posterior E_I, E_C, and E_{CL} (Insausti & Amaral 2008, Insausti et al. 1987a). The projections from these frontal and cingulate areas are similar to inputs from sensory areas (Section 3.2) in that they terminate preferentially onto layers I–III, although in the case of the retrosplenial cortex and some of the caudo-medial portions of the OFC, the laminar distribution of projections included deep layers V and VI (Insausti & Amaral 2008, Rempel-Clower 2000). The OFC and anterior cingulate cortices have long been associated with reward-based learning and complex contextual learning, respectively (Setogawa et al. 2019, Walton & Mars 2007). In contrast, the retrosplenial cortex has been shown to be critical in maintaining allocentric heading during navigatory behaviors (Chen

et al. 1994, Clark et al. 2010). The parcellation of these functionally unique inputs onto distinct rostro-caudal sites suggests an additional route of information specialization within the various subregions of the primate EC.

4. ENTORHINAL CONNECTIVITY WITHIN THE HIPPOCAMPAL FORMATION

As mentioned above, the EC is the main source of cortical input to the hippocampus. In all species, the perforant pathway provides the strongest connection between these two regions (Witter et al. 1989). In macaque brains, layers II and III of the EC comprise the majority of perforant path projections, with deeper layers V and VI providing only sparse efferents (Van Hoesen 1975a, Witter & Amaral 1991, Witter et al. 1989). Layers II and VI communicate specifically to the DG and CA3, whereas layer III and V projections are more biased toward CA1 and the subiculum (Witter & Amaral 1991). In turn, feedback from CA1 and the subiculum in the hippocampus terminates predominantly onto the deep layers V and VI, with very sparse projections onto layer III (Witter & Amaral 1991) (**Figure 1*c***).

The various connections between the hippocampus and EC are not homogenously distributed and actually span three distinct topographies. In the first, cells located laterally in layers II and III of the EC project to more caudal aspects of the hippocampus, while more medially situated cells terminate rostrally (Witter & Amaral 1991, Witter et al. 1989). Two distinct topographies have also been identified along the rostral-caudal axis of the EC. Efferent projections to the DG and CA3, which arise largely from layer II, are oriented such that rostral connections from the EC synapse onto more superficial aspects of the dendrites of the principal cells within each area. Specifically, rostral projections terminate onto the dendrites of granule cells in superficial aspects of the molecular layer of the DG, whereas more caudal efferents terminate deeper in the molecular layer. Additionally, rostral projections terminate onto pyramidal cell dendrites more distally in the strata lacunosum moleculare within CA3 in comparison to the more proximal terminations by caudal efferents (Van Hoesen & Pandya 1975a, Witter & Amaral 1991). Projections to CA1 and the subiculum from layer III of the EC also have a rostro-caudal termination pattern, such that rostral efferents target distal CA1 and the proximal subiculum, with increasingly caudal projections synapsing onto more proximal CA1 and the distal subiculum (Suzuki & Amaral 1994).

Alongside its myriad projections to the hippocampus, the diverse intrinsic connectivity of the EC adds an additional level of complexity when interpreting the role of this structure within the hippocampal formation. As in rodents, the EC of primates can be distinguished as having at least three discrete bands of interconnectivity (Chrobak & Amaral 2007). Rostro-medial layer V and VI neurons synapse onto caudo-medial layer II and III cells (and vice versa for caudo-medial layer V and VI cells), and similar recurrent projection patterns are observed for intermediate and lateral aspects of the EC. However, unlike in rodents, the bands do not span the entire length of the primate EC. Instead, they are limited to between one-third and one-half the length of the EC (approximately 3–5 mm), with rostral bands being shorter and less dense than more caudal elements (Chrobak & Amaral 2007). Interestingly, the deep-to-superficial orientation of intrinsic EC connectivity has been identified to be largely excitatory and asymmetric, with 56% of the projections from layers V and VI being glutamatergic and synapsing onto stellate and pyramidal cells, respectively (Chrobak & Amaral 2007). The remaining 44% synapse onto the dendritic shafts of putative interneurons, however, suggesting parallel mechanisms of gain modulation between deep and superficial layers of the EC. Only 5% of total identified projections in superficial layers were identified as recurrent in the monkey EC. Additionally, layer II cells of the lateral EC (E_{LC} and E_{LR}) send projections to layer II of all other subregions of the structure (Chrobak & Amaral 2007).

5. FUNCTION OF THE ENTORHINAL CORTEX

5.1. Functional Implications from Anatomy

Considering the diversity of cortical input to the EC, of EC projections to the hippocampus, and within the intrinsic connectivity of EC, it is reasonable to assume that there are distinct processing domains within the EC that enable it to send unique information to the downstream hippocampus. For example, given the topography of projections from the perirhinal and parahippocampal cortices to the EC (see Section 3.2), we might expect that the rostral and caudal portions of this structure are somewhat biased toward information processing of objects and space, respectively (Insausti & Amaral 2008, Insausti et al. 1987a). The caudo-medial portion of the EC that receives projections from the parahippocampal cortex, but not the perirhinal cortex, seems uniquely specialized for spatial processing, and this is where spatial representations have been observed in electrophysiological recordings from our lab (see Section 5.3). In contrast, the convergence of information on lateral portions of the EC suggests that neurons in this region have the capacity to encode highly conjunctive and complex information. Additionally, in contrast to the rodent EC, the intrinsic circuitry across different rostro-caudal elements of the monkey EC is discontinuous throughout the length of the structure (Chrobak & Amaral 2007). This suggests more distinct functional specialization within the primate EC by virtue of the fact that there is virtually no feedback from portions of the EC that receive different cortical inputs.

Similar functional hypotheses can be posited from frontal connectivity with the primate EC. Both the OFC and retrosplenial cortex project to the EC. The OFC is strongly implicated in value-based decision making (Setogawa et al. 2019), whereas the retrosplenial cortex is more associated with allocentric navigation (Chen et al. 1994, Clark et al. 2010). With respect to EC connectivity, as described in Section 3.3, the OFC synapses primarily onto rostral elements of the EC, and the retrosplenial cortex terminates onto caudal sites. Again, because of limited feedback between different sections along the EC's rostro-caudal extent (Chrobak & Amaral 2007), this anatomical evidence suggests domains in the primate EC that are functionally distinct by virtue of their different frontal influences. Because of the difficulty in accurately targeting putative EC subareas, it has been difficult to test many of these hypothesized functional distinctions. As electrophysiological recordings and elegant manipulation paradigms continue to evolve, it will be important to assess how the diverse connectivity into and within the EC facilitates the specificity of the information that it sends to the hippocampus in the service of learning and memory.

5.2. Lesion Results

Over the past two decades, lesion studies have directly examined the role of the EC in various visually guided memory-dependent behaviors. The most ubiquitous of these paradigms are the delayed match-to-sample (DMS) task and its nonmatch variant, in which subjects are rewarded for choosing a stimulus that either matches or is distinct from an initially viewed sample after a delay period. Monkeys are remarkably skilled at performing these behaviors and achieve scores significantly above chance levels, even when the delays between the sample and choice phases are extended to beyond 10 min (Buckmaster et al. 2004, Leonard et al. 1995, Murray & Mishkin 1998, Zola et al. 2000). Interestingly, unlike the cases of recognition memory deficits observed in animals with lesions of the hippocampus alone (Nemanic et al. 2004, Pascalis & Bachevalier 1999, Zola et al. 2000) or lesions that include the perirhinal and parahippocampal cortices (Buffalo et al. 1999, 2000; Gaffan & Murray 1992; Meunier et al. 1993), animals with EC lesions show only transient impairment, with normal performance on tests of recognition memory observed after approximately 1 year post lesion (Leonard et al. 1995). Leonard and colleagues (1995) showed

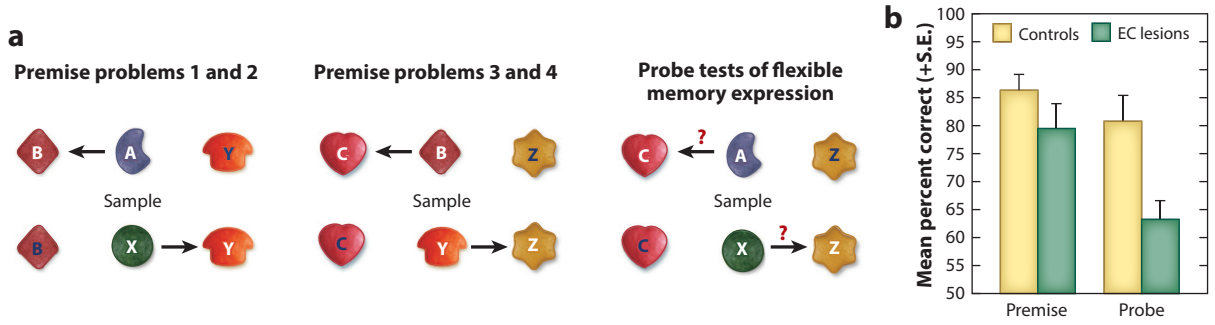

Figure 3

(*a*) Paired-associates (PA) task schematic. Monkeys are trained to select one stimulus in the presence of a centrally located cue. In the following trial, the previously chosen stimulus becomes the sample cue for a novel pair of choice stimuli. The critical inference test utilizes the initially cued stimulus with the latter pair of choice stimuli, and animals are rewarded for using the indirect relationship between objects to infer the rewarded associations. (*b*) Performance scores for the PA task show that monkeys with entorhinal cortex (EC) lesions (*green bars*) perform just as well as unoperated controls (*yellow bars*) at the initial premise pair training. However, monkeys with EC lesions are significantly impaired at discerning the correct paired stimulus during inference probes. Figure adapted with permission from Buckmaster et al. (2004).

through histological analyses that perirhinal cortex projections to CA1 become significantly more robust in animals with EC lesions, providing a potential mechanism by which plasticity can induce the observed behavioral recovery. Perirhinal involvement in object recognition observed through permanent lesions (Buffalo et al. 1999, 2000; Gaffan & Murray 1992; Meunier et al. 1993) has been corroborated by recent work using optogenetic techniques in which neurons in this region were manipulated to bias recognition of a series of objects (Tamura et al. 2017). These transient and highly local manipulations were effective in biasing animals' perception of novel objects as familiar and familiar objects as novel when neurons were activated or inhibited, respectively. Whether acute manipulations of the EC produce a deficit in object recognition remains to be tested.

Intriguingly, EC lesions produce deficits on tasks that require the flexible manipulation of learned associations (Buckmaster et al. 2004), suggesting a role for this structure in supporting the relational organization of memory. Buckmaster and colleagues (2004) examined both monkeys with EC lesions and intact monkeys in a series of memory-related paradigms that probed the animals' ability to form associational relationships between objects and flexibly access those relationships. The first of these was the paired-associates (PA) task, in which subjects are rewarded for choosing the correct object from two choices, based on a centrally located visual cue (**Figure 3**). The correct choice item can then serve as a sample for a proceeding pair of objects, one of which is again rewarded by association with the cue. Monkeys with EC lesions were just as capable of learning these two premise pairs as unoperated controls, although they did take slightly longer to reach the established acquisition criterion. When presented with another pair of problems, the initial training in controls facilitated much more rapid acquisition. In stark contrast, monkeys with EC lesions were just as slow at learning the associations in these new problems as they were during initial training. Conducting the PA task with overlapping stimuli allowed for a critical inference test in which the sample from the first trial was presented with the choice stimuli from the second trial. Because these objects have never been shown together, the monkey must draw upon the overlapping relationships between the experienced objects to solve this inherently novel problem. During these probe trials, monkeys with EC lesions were incapable of linking the sample to the indirectly associated choice object. By contrast, control monkeys were able to use their previously acquired associations flexibly to correctly solve the probe trials. Similar results

were observed in the transitive inference (TI) paradigm. In TI, overlapping pairs of objects form a transitively associated hierarchy (i.e., A > B > C > D > E). If the EC is critical for either creating the overall reward hierarchy or flexibly manipulating the relationships that constitute it, then monkeys with EC lesions should fail at an inference test where discontinuous objects in the hierarchy are presented together (i.e., B versus D). This is exactly what was observed in the study by Buckmaster et al. (2004). Although monkeys with EC lesions were not impaired at learning the initially presented pairs, they were incapable of successfully employing the learned associations between items to discriminate between nonadjacent items. By contrast, when presented with the first and last objects of the hierarchy (the only items consistently rewarded and not rewarded, respectively), monkeys with EC lesions performed normally, suggesting that they were able to leverage the consistent reward history of the objects to solve the problem. Similar findings have been observed in monkeys with combined lesions of the hippocampus and fornix, although the performance impairment observed in these animals might be explained by the unintended damage of subcortical EC projections running through the fornix (Saunders & Weiskrantz 1989). Given this apparent role of the EC in flexibly linking experiences with objects, one might wonder if this structure plays a role in associating objects with other physical features such as spatial location. Buckmaster and colleagues tested this potential function using a serial-delayed recognition span task, which rewarded monkeys for choosing one item from an identical set on a multisite board when that item was in a novel spatial location. Results showed that monkeys with EC lesions were incapable of holding even one spatial location in memory. Taken together, lesion studies suggest that, although the EC is not critical for remembering putative objects or even simple associations between experienced objects, it is necessary for employing existing relational frameworks between objects to navigate novel experiences.

5.3. Physiological Findings

One of the neurophysiological hallmarks of the rodent hippocampal formation is high-amplitude, rhythmic neuronal activity that occurs in its constituent structures as animals actively explore an environment. This activity is typically present as a consistent, theta-band (6–12 Hz) oscillation in recorded local field potentials as animals translocate (Dragoi et al. 1999, Vanderwolf 1969), and it is strongly correlated with olfactory exploratory behaviors such as sniffing and whisking (Komisaruk 1970). By contrast, rhythmic activity in the theta band observed in the monkey hippocampal formation, including the EC, is not observed as a sustained oscillation, but instead occurs in interrupted bouts (Courellis et al. 2019; Hoffman et al. 2013; Jutras & Buffalo 2010, 2014; Jutras et al. 2013; Killian et al. 2012; Talakoub et al. 2019). These discontinuous bouts of theta are also observed in the hippocampus proper of humans (Aghajan et al. 2017, Bohbot et al. 2017, Lega et al. 2012) and in both the hippocampus and EC of bats (Eliav et al. 2015, Ulanovsky & Moss 2007, Yartsev & Ulanovsky 2013, Yartsev et al. 2011). Additionally, whereas non-REM sleep in rodents is characterized by a lack of sustained theta activity in MTL structures (Constantinou et al. 2016, Vanderwolf 1969), evidence suggests that structures within the hippocampal formation in primates, including the EC, paradoxically elicit stronger and longer bouts of theta-band activity during transitions from awake behavior to sleep (Talakoub et al. 2019). Future work will be critical to advance our understanding of the synchronized network states that organize neuronal activity in the primate hippocampus and EC.

In addition to research focused on the mesoscopic physiology of this region, analysis of the responses of EC neurons during memory-dependent paradigms has begun to elucidate the ways in which the distinct neurophysiology of the EC facilitates its function, as proposed by anatomical

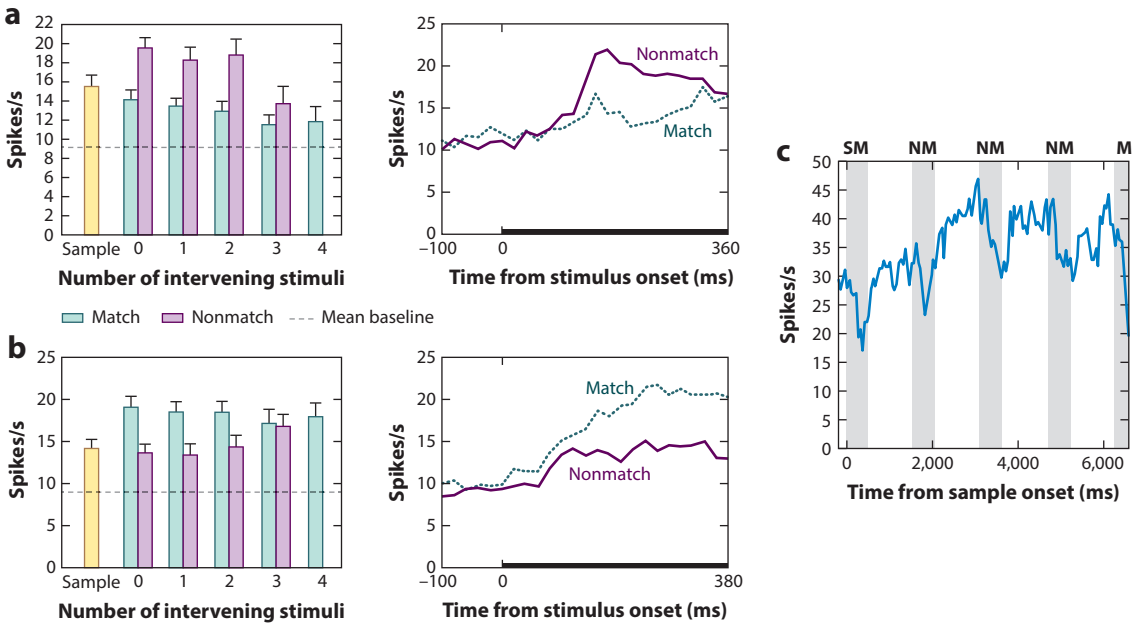

Figure 4

(*a*) Average firing-rate histogram (*left*) and time course (*right*) of stimulus-selective cells in the monkey entorhinal cortex (EC) that show match suppression during delayed match-to-sample (DMS) with various intervening stimuli. (*b*) Average firing-rate histogram (*left*) and time course (*right*) of cells that exhibit match enhancement during DMS under the same conditions as in panel *a*. (*c*) Firing-rate histogram of an EC neuron with significant delay period activity. As memory load increases with intervening irrelevant stimuli, the firing rate of this neuron increases. Figure adapted with permission from Suzuki et al. (1997).

(Sections 3, 4, and 5.1) and lesion investigations (Section 5.2). The EC receives input from neurons in the most anterior portions of the ventral visual stream, which do not themselves respond to simple stimulus features (Miller et al. 1993). It is therefore not surprising that EC neurons do not show selective responses to particular features of object stimuli, such as color or contour (Suzuki et al. 1997). Instead, single-unit recordings in the EC have identified memory-related responses to visual stimuli. For example, EC neurons show stimulus-specific increases in firing rate in response to the presentation of the sample stimulus in a DMS task (Riches et al. 1991, Suzuki et al. 1997). If the EC were simply relaying object identity to the downstream hippocampus, then one would expect the firing rates of sample-selective neurons to be similar between repeated presentations of the same stimulus. However, during match trials, EC neurons demonstrated modulations in firing rates, showing both increases and decreases (Fahy et al. 1993, Riches et al. 1991, Suzuki et al. 1997) (**Figure 4*a–b***), and these match enhancement and suppression effects on firing rate are observed before the animal makes its response during the test phase. Similar patterns of activity have been observed in the hippocampus (Jutras & Buffalo 2010), perirhinal cortex (Miller & Desimone 1994), and prefrontal cortex (Miller et al. 1996); these patterns suggest that the EC is potentially tuning its responses to facilitate memory-based decision making. Alternatively, given the dopaminergic inputs from the VTA mentioned in Section 3.1, stimulus novelty may drive the responses in this structure. In fact, when unique nonmatching stimuli are presented between the sample and matching test item, EC neurons elicit elevated firing (Suzuki et al. 1997). Interestingly, during interstimulus delays, EC neurons show sustained firing across numerous delay lengths, and in the majority of cases, these responses are sample selective, suggesting a

possible influence of working memory on these neurons (Suzuki et al. 1997) (**Figure 4*c***). These delay-sustained responses stand in contrast to responses of the upstream perirhinal cortex, in which any delay activity was eliminated upon presentation of intervening stimuli (Miller et al. 1993). Although lesion studies suggest only a transient role of the EC in simple object recognition (see Section 5.2), these memory-related neural responses may underlie the role of the EC in the flexible manipulation of learned associations (Buckmaster et al. 2004).

The most prominent neural response that has been identified in the rodent hippocampal formation relates to allocentric spatial representations (Fyhn et al. 2004, Hafting et al. 2005, O'Keefe & Dostrovsky 1971, Sargolini et al. 2006); one of the major hypotheses of the function of the hippocampal formation is that of a cognitive map that represents not just physical space but also relational object space (Buffalo 2015, Eichenbaum 2017, O'Keefe & Nadel 1978, Schiller et al. 2015). During a spatial task in which monkeys were rewarded for responding to a stimulus located in the same position as it was during a sample phase (delayed match-to-place), a proportion of EC neurons elicited biased responses for particular locations (Suzuki et al. 1997). Recent studies have also explored the extent to which EC neurons show place-specific firing relative to visual exploration. In rodents, exploration in physical space elicits a range of neuronal responses in the EC, including firing in symmetric, triangular patterns (grid cells) (Fyhn et al. 2004, Hafting et al. 2005, Sargolini et al. 2006); firing in relation to experienced physical boundaries (border cells) (Solstad et al. 2008); and firing relative to an animal's orientation within an environment (head-direction cells) (Sargolini et al. 2006). Work from our own lab in head-fixed animals viewing images on a computer screen shows that some cells in the primate EC elicit spatial responses based on locations of gaze, including grid-like firing fields and responses that are selective to fixations made at the borders of visual stimuli (Killian et al. 2012) (**Figure 5*a–c***). In addition, and potentially analogous to rodent head-direction cells, a population of primate EC neurons were identified that were tuned to fire when the animal made saccades in a particular direction (Killian et al. 2015) (**Figure 5*d***). Grid cells in the monkey EC display a gradient of grid field size and spacing such that both parameters increase in more medial recording sites. These findings are consistent with the gradient of changing grid field structure along the longitudinal extent of the EC that has been observed in both rodents (Hafting et al. 2005) and bats (Yartsev et al. 2011). In more recent work, a large proportion of nongrid but spatially responsive EC neurons have been identified during visual exploration (Meister & Buffalo 2018). The firing fields of these neurons are spatially reliable but more amorphous than those of grid cells. These findings are consistent with a recently described population of rodent EC cells (Diehl et al. 2017). Recordings in the monkey EC identified multiple frames of reference among the population of nongrid spatial cells (Meister & Buffalo 2018). When monkeys viewed an image that shifted to multiple positions on a screen, the firing patterns of one population of EC neurons remained the same relative to the image regardless of its position within the screen. In contrast, a second population of EC neurons elicited egocentric firing preferences. As images shifted on the screen, these neurons stably represented where on the screen the animal was looking, as opposed to boundaries established by the visual image. Finally, spatial responses in the monkey EC were also identified relative to the location of attention, even in the absence of any movement (Wilming et al. 2018). In this study, monkeys were trained to maintain fixation on a small cross presented at the center of the screen and covertly attend to a small dot moving in the periphery. Monkeys were rewarded for releasing a bar in response to a subtle luminance change in the dot. The luminance change was titrated to ensure that selective attention to the cue was required for successful performance. The responses of EC neurons were examined relative to the location of the monkey's attention on successful trials, and a significant proportion of EC neurons demonstrated grid-like responses. These findings, taken together with recent similar findings in humans (Julian et al. 2018, Nau et al. 2018a), suggest that primate EC

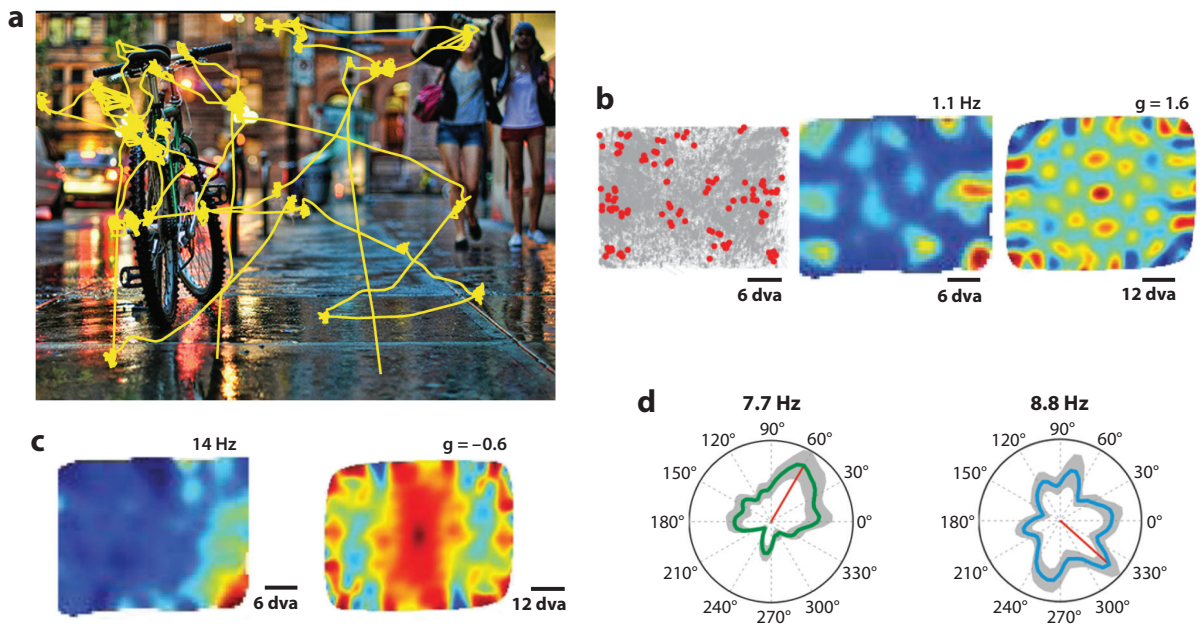

Figure 5

(*a*) Example image presented on a computer screen during head-fixed visual exploration, with an example visual scan path overlaid (*yellow trace*). (*b*) Combined eye trace trajectories (*gray traces*), with spikes from a putative EC grid cell overlaid (*red dots*). Note the regular interval of spike clusters that is also apparent in the adjacent firing-rate map of this cell and best exemplified by the spatial autocorrelation map. (*c*) Firing-rate map (*left*) and autocorrelation map of a border cell isolated during the same visual exploration paradigm. Panel adapted with permission from Killian et al. (2012). (*d*) Example polar histograms of EC neurons that elicited strong firing rate modulations for saccades in a particular direction made immediately before (*green trace*) or after (*blue trace*) spikes were collected. Panel adapted with permission from Killian et al. (2015). Abbreviations: dva, degrees of visual angle; EC, entorhinal cortex.

neurons may have the ability to regularly tile experience, independent of specific sensory or environmental dimensions.

One might expect that, in addition to responding to particular places and things during an experienced event, a structure implicated in episodic memory would also process information about the temporal order of those experiences, and several forms of timing-related responses have been observed in the primate EC. When monkeys are rewarded for remembering the order of a list of individually presented objects, EC neurons demonstrate selective responses for objects in a particular position within the presented list (Naya & Suzuki 2011, Naya et al. 2017) (**Figure 6*a–b***). In contrast to neurons recorded from the ventral prefrontal cortex (Naya et al. 2017), EC neurons also elicit conjunctive object-list position specificity. Recent work has demonstrated timing responses in the monkey EC as monkeys perform a free-viewing task (Bright et al. 2019). Many neurons in the EC responded to image onset, showing large changes relative to baseline shortly after image onset. Interestingly, these neurons showed a wide variety of rates with which they relaxed back to baseline, extending from hundreds of milliseconds to over 5 s (**Figure 6*c–d***). Results from a linear discriminant analysis suggested that elapsed time could be decoded from the population, along with the identity of the presented image. Taken together, these neurophysiological investigations provide evidence that information about what, when, and where is represented within the monkey EC.

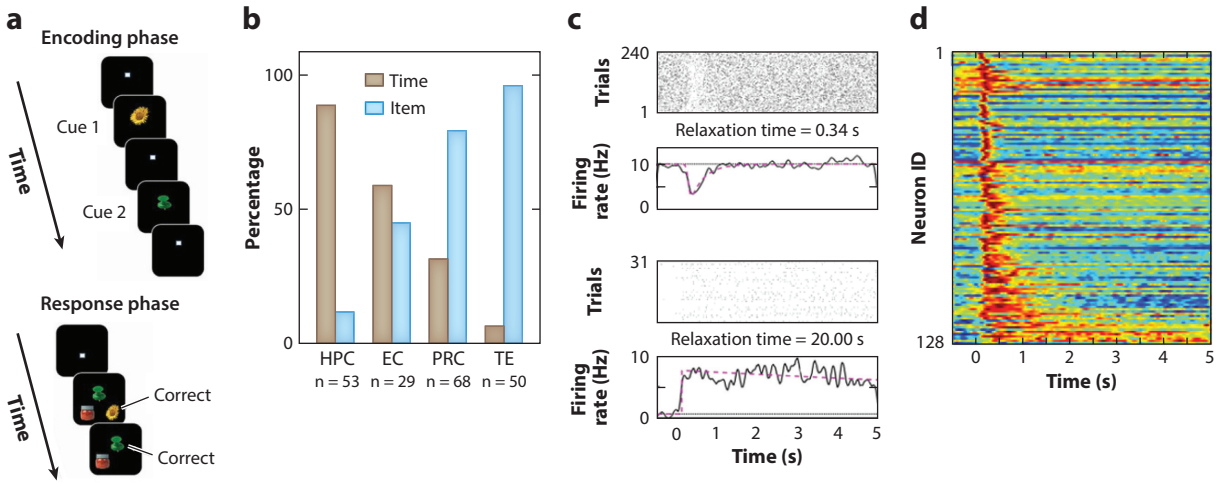

Figure 6

(*a*) Schematic of the temporal order memory task from Naya et al. (2011, 2017). Monkeys were shown two cues separated by brief delays (encoding phase). During the response phase, monkeys were rewarded for selecting the first, then second observed object from a cluster of simultaneously presented items. (*b*) Percentage of order- and item-specific cells isolated from various regions in the medial temporal lobe. Note that, in contrast to the strong temporal selectivity in hippocampal neurons and item selectivity in perirhinal cortex and inferior temporal lobe neurons, cells in the EC are heavily mixed in their temporal order and stimulus selectivities. Panel adapted with permission from Naya & Suzuki (2011). (*c*) Raster plots and firing-rate histograms of putative temporal context cells acquired during a visual exploration paradigm (see **Figure 5*a***) that differentially modulate their firing rate relative to a prestimulus baseline with differing rates of relaxation. (*d*) Normalized firing rate map of 128 EC neurons sorted by their time of return to baseline firing rate. All recorded cells elicited little variance in their response latency to the presented image. However, their variance in relaxation time over the course of presentation suggests the ability of EC neurons to track the passage of time at different timescales. Panel adapted with permission from Bright et al. (2019). Abbreviations: EC, entorhinal cortex; HPC, hippocampus; PRC, perirhinal cortex; TE, inferotemporal cortex.

6. PARALLELS WITH THE HUMAN ENTORHINAL CORTEX

There is a growing body of evidence suggesting that the EC in humans closely mirrors what has been observed in nonhuman primates in both its anatomy and function. Although tracing experiments are not possible in humans, homologous subregions of the human EC were identified in functional connectivity experiments using functional magnetic resonance imaging (fMRI) (Maass et al. 2015, Schröder et al. 2015). Specifically, a strong blood oxygen level–dependent (BOLD) signal was observed in the rostro-lateral EC and caudo-medial EC that was coherent with the BOLD signal measured in the perirhinal and parahippocampal cortices, respectively. As described in Section 3.2, tracing studies in nonhuman primates also strongly imply topographic biases of projections from the perirhinal cortex to the rostro-lateral EC and from the parahippocampal cortex to the caudo-medial EC (Insausti & Amaral 2008, Suzuki & Amaral 1994). Unique functional connectivity of proximal and distal portions of the human subiculum with the rostro-lateral and caudo-medial EC was also observed, which is consistent with the patterns of projections observed through tracing experiments in nonhuman primates (Maass et al. 2015, Schröder et al. 2015, Suzuki & Amaral 1994).

The rostro-lateral and caudo-medial aspects of the human EC also exhibit behavioral selectivity in their respective BOLD activity preferences, with the rostro-lateral elements being more object responsive and the caudo-medial portions eliciting more spatially correlated BOLD responses (Schröder et al. 2015). While additional research is needed to confirm homologous functional

specialization of EC subregions in nonhuman primates, these observed functional specializations in humans parallel the respective object and spatial specificity of the LEC and MEC in rodents (Deshmukh & Knierim 2011; Fyhn et al. 2004; Hafting et al. 2005; Sargolini et al. 2006; Solstad et al. 2008; Tsao et al. 2013, 2018). Deep brain stimulation of the human EC during virtual spatial navigation has shown a critical, albeit still not fully understood, influence of this structure in remembering previously visited locations (Jacobs et al. 2016, Suthana et al. 2012). As mentioned in Section 5.2, EC lesions in nonhuman primates produce profound deficits in spatial memory (Buckmaster et al. 2004). Neurophysiological recordings and imaging studies have also identified neural firing motifs within the human EC homologous to those observed in other species (see Section 5.3). Specifically, fMRI reveals macroscopic activity patterns with hexadirectionally selective, grid-like regularity in healthy subjects navigating a virtual reality open field maze (Doeller et al. 2010). Like rodent grid cells, these grid-like BOLD signals are anchored to allocentric cues, and certain signals were conjunctively modulated by the direction of virtual movement. These hexadirectionally modulated patterns are also present when subjects are tasked with imagining a route to a target stimulus (Horner et al. 2016), during visual scan behaviors (Nau et al. 2018b), and when subjects are required to conceptually organize information along two orthogonal discriminative axes (Constantinescu et al. 2016), suggesting a generalized coding scheme within the human EC for flexibly encoding and retrieving continuous information across behaviors. These imaging findings have been corroborated by single-unit recordings taken from human epileptic patients, in which EC neurons with grid-like firing fields (Jacobs et al. 2013) and those with direction-selective firing patterns (Jacobs et al. 2010) were identified during virtual reality navigation paradigms. Taken together, the growing body of converging evidence suggests that both the anatomical and functional features of the EC are largely conserved across species.

7. SUMMARY

The primate EC provides the majority of the cortical input to the hippocampus; however, data from lesion and neurophysiological studies suggest that the EC functions as much more than a simple information relay. The impairments observed following lesions restricted to the primate EC are distinct from those following hippocampal or perirhinal lesions, and the diverse neural activity observed among EC neurons is similarly distinct from that of its neighboring regions. In particular, the EC is only transiently necessary for simple object recognition but becomes critically important when the relationships between indirectly related objects must be inferred and when previously experienced locations must be remembered. Along with the object-related delay activity and timing responses that have been observed in the EC, these neurons also display robust and reliable spatial responses similar to the exquisite spatial representations identified in the rodent EC. Anatomically, the EC is a site of convergence of multiple cortical information streams, many with their own topographical projection patterns. Combined with the EC's own limited intrinsic connectivity and topographically biased projections to different subregions within the hippocampus, this suggests that the EC is a computationally diverse area with elegant targeting of downstream structures. However, to date, the functions of these distinct processing streams have not been fully characterized. Novel technologies, including large-scale chronic recordings to densely sample neurons across EC subregions simultaneously with other brain regions, >1,000-site laminar probes to more thoroughly investigate network oscillations across laminae, and targeted modulation through cell-type-specific optogenetic manipulations, are becoming accessible in primate research. Such tools will be critical to advancing our understanding of the computations that the EC performs intrinsically and in concert with other memory-associated machinery within the hippocampal formation.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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LITERATURE CITED

Aggleton JP. 1986. A description of the amygdalo-hippocampal interconnections in the macaque monkey. *Exp. Brain Res.* 64(3):515–26

Aghajan ZM, Schuette P, Fields TA, Tran ME, Siddiqui SM, et al. 2017. Theta oscillations in the human medial temporal lobe during real-world ambulatory movement. *Curr. Biol.* 27(24):3743–51.e3

Aguirre GK, Detre JA, Alsop DC, D'Esposito M. 1996. The parahippocampus subserves topographical learning in man. *Cereb. Cortex* 6(6):823–29

Amaral DG, Cowan WM. 1980. Subcortical afferents to the hippocampal formation in the monkey. *J. Comp. Neurol.* 189(4):573–91

Amaral DG, Insausti R, Cowan WM. 1987. The entorhinal cortex of the monkey: I. Cytoarchitectonic organization. *J. Comp. Neurol.* 264(3):326–55

Baxter MG, Hadfield WS, Murray EA. 1999. Rhinal cortex lesions produce mild deficits in visual discrimination learning for an auditory secondary reinforcer in rhesus monkeys. *Behav. Neurosci.* 113(2):243–52

Blair HT, Schafe GE, Bauer EP, Rodrigues SM, LeDoux JE. 2001. Synaptic plasticity in the lateral amygdala: a cellular hypothesis of fear conditioning. *Learn. Mem.* 8(5):229–42

Bohbot VD, Copara MS, Gotman J, Ekstrom AD. 2017. Low-frequency theta oscillations in the human hippocampus during real-world and virtual navigation. *Nat. Commun.* 8:14415

Bright IM, Meister MLR, Cruzado NA, Tiganj Z, Howard MW, Buffalo EA. 2019. A temporal record of the past with a spectrum of time constants in the monkey entorhinal cortex. bioRxiv 688341. **https://doi.org/10.1101/688341**

Brodmann K, Garey LJ. 2006. *Brodmann's: Localisation in the Cerebral Cortex*. Berlin: Springer

Buckmaster CA, Eichenbaum H, Amaral DG, Suzuki WA, Rapp PR. 2004. Entorhinal cortex lesions disrupt the relational organization of memory in monkeys. *J. Neurosci.* 24(44):9811–25

Buffalo EA. 2015. Bridging the gap between spatial and mnemonic views of the hippocampal formation. *Hippocampus* 25(6):713–18

Buffalo EA, Ramus SJ, Clark RE, Teng E, Squire LR, Zola SM. 1999. Dissociation between the effects of damage to perirhinal cortex and area TE. *Learn. Mem.* 6(6):572–99

Buffalo EA, Ramus SJ, Squire LR, Zola SM. 2000. Perception and recognition memory in monkeys following lesions of area TE and perirhinal cortex. *Learn. Mem.* 7(6):375–82

Burgess N, Barry C, O'Keefe J. 2007. An oscillatory interference model of grid cell firing. *Hippocampus* 17(9):801–12

Burwell RD. 2006. The parahippocampal region: corticocortical connectivity. *Ann. N. Y. Acad. Sci.* 911(1):25–42

Burwell RD, Amaral DG. 1998. Perirhinal and postrhinal cortices of the rat: interconnectivity and connections with the entorhinal cortex. *J. Comp. Neurol.* 391(3):293–321

Carmichael ST, Clugnet M-C, Price JL. 1994. Central olfactory connections in the macaque monkey. *J. Comp. Neurol.* 346(3):403–34

Carpenter F, Burgess N, Barry C. 2017. Modulating medial septal cholinergic activity reduces medial entorhinal theta frequency without affecting speed or grid coding. *Sci. Rep.* 7:14573

Chaplin TA, Rosa MGP, Lui LL. 2018. Auditory and visual motion processing and integration in the primate cerebral cortex. *Front. Neural Circuits* 12:93

Chen LL, Lin L-H, Green EJ, Barnes CA, McNaughton BL. 1994. Head-direction cells in the rat posterior cortex. *Exp. Brain Res.* 101(1):8–23

Chrobak JJ, Amaral DG. 2007. Entorhinal cortex of the monkey: VII. Intrinsic connections. *J. Comp. Neurol.* 500(4):612–33

Chudasama Y, Izquierdo A, Murray EA. 2009. Distinct contributions of the amygdala and hippocampus to fear expression. *Eur. J. Neurosci.* 30(12):2327–37

Clark BJ, Bassett JP, Wang SS, Taube JS. 2010. Impaired head direction cell representation in the anterodorsal thalamus after lesions of the retrosplenial cortex. *J. Neurosci.* 30(15):5289–302

Constantinescu AO, O'Reilly JX, Behrens TEJ. 2016. Organizing conceptual knowledge in humans with a gridlike code. *Science* 352(6292):1464–68

Constantinou M, Cogno SG, Elijah DH, Kropff E, Gigg J, et al. 2016. Bursting neurons in the hippocampal formation encode features of LFP rhythms. *Front. Comput. Neurosci.* 10:133

Courellis HS, Nummela SU, Metke M, Diehl GW, Bussell R, et al. 2019. Spatial encoding in primate hippocampus during free navigation. *PLOS Biol.* 17(12):e3000546

Deshmukh SS, Knierim JJ. 2011. Representation of non-spatial and spatial information in the lateral entorhinal cortex. *Front. Behav. Neurosci.* 5:69

Desimone R, Ungerleider LG. 1986. Multiple visual areas in the caudal superior temporal sulcus of the macaque. *J. Comp. Neurol.* 248(2):164–89

Diehl GW, Hon OJ, Leutgeb S, Leutgeb JK. 2017. Grid and nongrid cells in medial entorhinal cortex represent spatial location and environmental features with complementary coding schemes. *Neuron* 94(1):83–92.e6

Doan TP, Lagartos-Donate MJ, Nilssen ES, Ohara S, Witter MP. 2019. Convergent projections from perirhinal and postrhinal cortices suggest a multisensory nature of lateral, but not medial, entorhinal cortex. *Cell Rep.* 29(3):617–27.e7

Doeller CF, Barry C, Burgess N. 2010. Evidence for grid cells in a human memory network. *Nature* 463(7281):657–61

Dolleman-Van Der Weel MJ, Witter MP. 1996. Projections from the nucleus reuniens thalami to the entorhinal cortex, hippocampal field CA1, and the subiculum in the rat arise from different populations of neurons. *J. Comp. Neurol.* 364(4):637–50

Dragoi G, Carpi D, Recce M, Csicsvari J, Buzsáki G. 1999. Interactions between hippocampus and medial septum during sharp waves and theta oscillation in the behaving rat. *J. Neurosci.* 19(14):6191–99

Eichenbaum H. 2017. The role of the hippocampus in navigation is memory. *J. Neurophysiol.* 117(4):1785–96

Eliav T, Geva-Sagiv M, Finkelstein A, Yartsev MM, Rubin A, et al. 2015. *Synchronicity without rhythmicity in the hippocampal formation of behaving bats*. Paper presented at Neuroscience 2015, Oct. 17–21, Chicago, IL

Evrard HC. 2019. The organization of the primate insular cortex. *Front. Neuroanat.* 14:43

Fahy FL, Riches IP, Brown MW. 1993. Neuronal activity related to visual recognition memory: long-term memory and the encoding of recency and familiarity information in the primate anterior and medial inferior temporal and rhinal cortex. *Exp. Brain Res.* 96(3):457–72

Fritz J, Mishkin M, Saunders RC. 2005. In search of an auditory engram. *PNAS* 102(26):9359–64

Fyhn M, Molden S, Witter MP, Moser EI, Moser MB. 2004. Spatial representation in the entorhinal cortex. *Science* 305(5688):1258–64

Gaffan D, Murray EA. 1992. Monkeys (*Macaca fascicularis*) with rhinal cortex ablations succeed in object discrimination learning despite 24-hr intertrial intervals and fail at matching to sample despite double sample presentations. *Behav. Neurosci.* 106(1):30–38

Hafting T, Fyhn M, Molden S, Moser MB, Moser EI. 2005. Microstructure of a spatial map in the entorhinal cortex. *Nature* 436(7052):801–6

Hoffman KL, Dragan MC, Leonard TK, Micheli C, Montefusco-Siegmund R, Valiante TA. 2013. Saccades during visual exploration align hippocampal 3–8 Hz rhythms in human and non-human primates. *Front. Syst. Neurosci.* 7:43

Horner AJ, Bisby JA, Zotow E, Bush D, Burgess N. 2016. Grid-like processing of imagined navigation. *Curr. Biol.* 26(6):842–47

Insausti R, Amaral DG. 2008. Entorhinal cortex of the monkey: IV. Topographical and laminar organization of cortical afferents. *J. Comp. Neurol.* 509(6):608–41

Insausti R, Amaral DG, Cowan WM. 1987a. The entorhinal cortex of the monkey: II. Cortical afferents. *J. Comp. Neurol.* 264(3):356–95

Insausti R, Amaral DG, Cowan WM. 1987b. The entorhinal cortex of the monkey: III. Subcortical afferents. *J. Comp. Neurol.* 264(3):396–408

Jacobs J, Kahana MJ, Ekstrom AD, Mollison MV, Fried I. 2010. A sense of direction in human entorhinal cortex. *PNAS* 107(14):6487–92

Jacobs J, Miller J, Lee SA, Coffey T, Watrous AJ, et al. 2016. Direct electrical stimulation of the human entorhinal region and hippocampus impairs memory. *Neuron* 92(5):983–90

Jacobs J, Weidemann CT, Miller JF, Solway A, Burke JF, et al. 2013. Direct recordings of grid-like neuronal activity in human spatial navigation. *Nat. Neurosci.* 16(9):1188–90

Julian JB, Keinath AT, Frazzetta G, Epstein RA. 2018. Human entorhinal cortex represents visual space using a boundary-anchored grid. *Nat. Neurosci.* 21(2):191–94

Jutras MJ, Buffalo EA. 2010. Recognition memory signals in the macaque hippocampus. *PNAS* 107(1):401–6

Jutras MJ, Buffalo EA. 2014. Oscillatory correlates of memory in non-human primates. *NeuroImage* 85(2):694–701

Jutras MJ, Fries P, Buffalo EA. 2013. Oscillatory activity in the monkey hippocampus during visual exploration and memory formation. *PNAS* 110(32):13144–49

Khan UA, Liu L, Provenzano FA, Berman DE, Profaci CP, et al. 2014. Molecular drivers and cortical spread of lateral entorhinal cortex dysfunction in preclinical Alzheimer's disease. *Nat. Neurosci.* 17(2):304–11

Killian NJ, Jutras MJ, Buffalo EA. 2012. A map of visual space in the primate entorhinal cortex. *Nature* 491(7426):761–64

Killian NJ, Potter SM, Buffalo EA. 2015. Saccade direction encoding in the primate entorhinal cortex during visual exploration. *PNAS* 112(51):15743–48

Komisaruk BR. 1970. Synchrony between limbic system theta activity and rhythmical behavior in rats. *J. Comp. Physiol. Psychol.* 70(3):482–92

Kondo H, Zaborszky L. 2016. Topographic organization of the basal forebrain projections to the perirhinal, postrhinal, and entorhinal cortex in rats. *J. Comp. Neurol.* 524(12):2503–15

LaLumiere RT. 2014. Optogenetic dissection of amygdala functioning. *Front. Behav. Neurosci.* 8:107

Lega BC, Jacobs J, Kahana M. 2012. Human hippocampal theta oscillations and the formation of episodic memories. *Hippocampus* 22(4):748–61

Leonard BW, Amaral DG, Squire LR, Zola-Morgan S. 1995. Transient memory impairment in monkeys with bilateral lesions of the entorhinal cortex. *J. Neurosci.* 15(8):5637–59

Liu AKL, Chang RC-C, Pearce RKB, Gentleman SM. 2015. Nucleus basalis of Meynert revisited: anatomy, history and differential involvement in Alzheimer's and Parkinson's disease. *Acta Neuropathol.* 3:527–40

Maass A, Berron D, Libby LA, Ranganath C, Düzel E. 2015. Functional subregions of the human entorhinal cortex. *eLife* 4:e06426

Meister MLR, Buffalo EA. 2018. Neurons in primate entorhinal cortex represent gaze position in multiple spatial reference frames. *J. Neurosci.* 38(10):2430–41

Mesulam M-M, Mufson EJ, Levey AI, Wainer BH. 1983. Cholinergic innervation of cortex by the basal forebrain: cytochemistry and cortical connections of the septal area, diagonal band nuclei, nucleus basalis (substantia innominata), and hypothalamus in the rhesus monkey. *J. Comp. Neurol.* 214(2):170–97

Meunier M, Bachevalier J, Mishkin M, Murray EA. 1993. Effects on visual recognition of combined and separate ablations of the entorhinal and perirhinal cortex in rhesus monkeys. *J. Neurosci.* 13(12):5418–32

Miller EK, Desimone R. 1994. Parallel neuronal mechanisms for short-term memory. *Science* 263(5146):520–22

Miller EK, Erickson CA, Desimone R. 1996. Neural mechanisms of visual working memory in prefrontal cortex of the macaque. *J. Neurosci.* 16(16):5154–67

Miller EK, Li L, Desimone R. 1993. Activity of neurons in anterior inferior temporal cortex during a short-term memory task. *J. Neurosci.* 13(4):1460–78

Milner B. 1972. Disorders of learning and memory after temporal lobe lesions in man. *Clin. Neurosurg.* 19:421–46

Mishkin M. 1982. A memory system in the monkey. *Philos. Trans. R. Soc. Lond. B* 298(1089):83–95

Mitchell SJ, Rawlins JNP, Steward O, Olton DS. 1982. Medial septal area lesions disrupt θ rhythm and cholinergic staining in medial entorhinal cortex and produce impaired radial arm maze behavior in rats. *J. Neurosci.* 2(3):292–302

Murray EA, Mishkin M. 1998. Object recognition and location memory in monkeys with excitotoxic lesions of the amygdala and hippocampus. *J. Neurosci.* 18(16):6568–82

Nau M, Julian JB, Doeller CF. 2018a. How the brain's navigation system shapes our visual experience. *Trends Cogn. Sci.* 22(9):810–25

Nau M, Navarro Schröder T, Bellmund JLS, Doeller CF. 2018b. Hexadirectional coding of visual space in human entorhinal cortex. *Nat. Neurosci.* 21(2):188–90

Naya Y, Chen H, Yang C, Suzuki WA, Squire LR. 2017. Contributions of primate prefrontal cortex and medial temporal lobe to temporal-order memory. *PNAS* 114(51):13555–60

Naya Y, Suzuki WA. 2011. Integrating what and when across the primate medial temporal lobe. *Science* 333(6043):773–76

Nemanic S, Alvarado MC, Bachevalier J. 2004. The hippocampal/parahippocampal regions and recognition memory: insights from visual paired comparison versus object-delayed nonmatching in monkeys. *J. Neurosci.* 24(8):2013–26

O'Keefe J, Dostrovsky J. 1971. The hippocampus as a spatial map: preliminary evidence from unit activity in the freely-moving rat. *Brain Res.* 34(1):171–75

O'Keefe J, Nadel L. 1978. *The Hippocampus as a Cognitive Map*. Oxford, UK: Clarendon Press

Pascalis O, Bachevalier J. 1999. Neonatal aspiration lesions of the hippocampal formation impair visual recognition memory when assessed by paired-comparison task but not by delayed nonmatching-to-sample task. *Hippocampus* 9(6):609–16

Ramón y Cajal S. 1899. Estudios sobre la corteza cerebral humana. I. Corteza visual. *Riv. Trimest. Microgr.* 4(1):1–63

Ramos JMJ. 2014. Essential role of the perirhinal cortex in complex tactual discrimination tasks in rats. *Cereb. Cortex* 24(8):2068–80

Rempel-Clower NL. 2000. The laminar pattern of connections between prefrontal and anterior temporal cortices in the rhesus monkey is related to cortical structure and function. *Cereb. Cortex* 10(9):851–65

Ren Y, Zhang L, Lu Y, Yang H, Westlund KN. 2009. Central lateral thalamic neurons receive noxious visceral mechanical and chemical input in rats. *J. Neurophysiol.* 102(1):244–58

Riches IP, Wilson FAW, Brown MW. 1991. The effects of visual stimulation and memory on neurons of the hippocampal formation and the neighboring parahippocampal gyrus and inferior temporal cortex of the primate. *J. Neurosci.* 11(6):1763–79

Sargolini F, Fyhn M, Hafting T, McNaughton BL, Witter MP, et al. 2006. Conjunctive representation of position, direction, and velocity in entorhinal cortex. *Science* 312(5774):758–62

Saunders RC, Weiskrantz L. 1989. The effects of fornix transection and combined fornix transection, mammillary body lesions and hippocampal ablations on object-pair association memory in the rhesus monkey. *Behav. Brain Res.* 35(2):85–94

Schiller D, Eichenbaum H, Buffalo EA, Davachi L, Foster DJ, et al. 2015. Memory and space: towards an understanding of the cognitive map. *J. Neurosci.* 35(41):13904–11

Schröder TN, Haak KV, Jimenez NIZ, Beckmann CF, Doeller CF. 2015. Functional topography of the human entorhinal cortex. *eLife* 4:e06738

Schroeder CE, Wilson DA, Radman T, Scharfman H, Lakatos P. 2010. Dynamics of active sensing and perceptual selection. *Curr. Opin. Neurobiol.* 20(2):172–76

Setogawa T, Mizuhiki T, Matsumoto N, Akizawa F, Kuboki R, et al. 2019. Neurons in the monkey orbitofrontal cortex mediate reward value computation and decision-making. *Commun. Biol.* 2:126

Solstad T, Boccara CN, Kropff E, Moser MB, Moser EI. 2008. Representation of geometric borders in the entorhinal cortex. *Science* 322(5909):1865–68

Squire LR, Zola-Morgan S. 1991. The medial temporal lobe memory system. *Science* 253(5026):1380–86

Strange BA, Witter MP, Lein ES, Moser EI. 2014. Functional organization of the hippocampal longitudinal axis. *Nat. Rev. Neurosci.* 15(10):655–69

Suthana N, Haneef Z, Stern J, Mukamel R, Behnke E, et al. 2012. Memory enhancement and deep-brain stimulation of the entorhinal area. *N. Engl. J. Med.* 366(6):502–10

Suzuki WA, Amaral DG. 1994. Topographic organization of the reciprocal connections between the monkey entorhinal cortex and the perirhinal and parahippocampal cortices. *J. Neurosci.* 14(3):1856–77

Suzuki WA, Miller EK, Desimone R. 1997. Object and place memory in the macaque entorhinal cortex. *J. Neurophysiol.* 78(2):1062–81

Talakoub O, Sayegh PF, Womelsdorf T, Zinke W, Fries P, et al. 2019. Hippocampal and neocortical oscillations are tuned to behavioral state in freely-behaving macaques. bioRxiv 552877. **https://doi.org/10.1101/552877**

Tamura K, Takeda M, Setsuie R, Tsubota T, Hirabayashi T, et al. 2017. Conversion of object identity to object-general semantic value in the primate temporal cortex. *Science* 357(6352):687–92

Tsao A, Moser MB, Moser EI. 2013. Traces of experience in the lateral entorhinal cortex. *Curr. Biol.* 23(5):399–405

Tsao A, Sugar J, Lu L, Wang C, Knierim JJ, et al. 2018. Integrating time from experience in the lateral entorhinal cortex. *Nature* 561(7721):57–62

Ulanovsky N, Moss CF. 2007. Hippocampal cellular and network activity in freely moving echolocating bats. *Nat. Neurosci.* 10(2):224–33

Van Hoesen GW, Pandya DN. 1975a. Some connections of the entorhinal (area 28) and perirhinal (area 35) cortices of the rhesus monkey. I. Temporal lobe afferents. *Brain Res.* 95(1):1–24

Van Hoesen GW, Pandya DN. 1975b. Some connections of the entorhinal (area 28) and perirhinal (area 35) cortices of the rhesus monkey. III. Efferent connections. *Brain Res.* 95(1):39–59

Vanderwolf C. 1969. Hippocampal electrical activity and voluntary movement in the rat. *Electroencephalogr. Clin. Neurophysiol.* 26(4):407–18

Vertes RP, Kocsis B. 1997. Brainstem-diencephalo-septohippocampal systems controlling the theta rhythm of the hippocampus. *Neuroscience* 81(4):893–926

Walton ME, Mars RB. 2007. Probing human and monkey anterior cingulate cortex in variable environments. *Cogn. Affect. Behav. Neurosci.* 7(4):413–22

Wilming N, König P, König S, Buffalo EA. 2018. Entorhinal cortex receptive fields are modulated by spatial attention, even without movement. *eLife* 7:e31745

Witter MP. 2007. The perforant path: projections from the entorhinal cortex to the dentate gyrus. *Prog. Brain Res.* 163:43–61

Witter MP, Amaral DG. 1991. Entorhinal cortex of the monkey: V. Projections to the dentate gyrus, hippocampus, and subicular complex. *J. Comp. Neurol.* 307(3):437–59

Witter MP, Doan TP, Jacobsen B, Nilssen ES, Ohara S. 2017. Architecture of the entorhinal cortex a review of entorhinal anatomy in rodents with some comparative notes. *Front. Syst. Neurosci.* 11:46

Witter MP, Van Hoesen GW, Amaral DG. 1989. Topographical organization of the entorhinal projection to the dentate gyrus of the monkey. *J. Neurosci.* 9(1):216–28

Yartsev MM, Ulanovsky N. 2013. Representation of three-dimensional space in the hippocampus of flying bats. *Science* 340(6130):367–72

Yartsev MM, Witter MP, Ulanovsky N. 2011. Grid cells without theta oscillations in the entorhinal cortex of bats. *Nature* 479(7371):103–7

Zola-Morgan S, Squire LR. 1985. Medial temporal lesions in monkeys impair memory on a variety of tasks sensitive to human amnesia. *Behav. Neurosci.* 99(1):22–34

Zola SM, Squire LR, Teng E, Stefanacci L, Buffalo EA, Clark RE. 2000. Impaired recognition memory in monkeys after damage limited to the hippocampal region. *J. Neurosci.* 20(1):451–63