

Physiological Patterns in the Hippocampo-Entorhinal Cortex System

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ABSTRACT: The anatomical connectivity and intrinsic properties of entorhinal cortical neurons give rise to ordered patterns of ensemble activity. How entorhinal ensembles form, interact, and accomplish emergent processes such as memory formation is not well-understood. We lack sufficient understanding of how neuronal ensembles in general can function transiently and distinctively from other neuronal ensembles. Ensemble interactions are bound, foremost, by anatomical connectivity and temporal constraints on neuronal discharge. We present an overview of the structure of neuronal interactions within the entorhinal cortex and the rest of the hippocampal formation. We wish to highlight two principle features of entorhinal-hippocampal interactions. First, large numbers of entorhinal neurons are organized into at least two distinct high-frequency population patterns: gamma (40–100 Hz) frequency volleys and ripple (140–200 Hz) frequency volleys. These patterns occur coincident with other well-defined electrophysiological patterns. Gamma frequency volleys are modulated by the theta cycle. Ripple frequency volleys occur on each sharp wave event. Second, these patterns occur dominantly in specific layers of the entorhinal cortex. Theta/gamma frequency volleys are the principle pattern observed in layers I–III, in the neurons that receive cortical inputs and project to the hippocampus. Ripple frequency volleys are the principle population pattern observed in layers V–VI, in the neurons that receive hippocampal output and project primarily to the neocortex. Further, we will highlight how these ensemble patterns organize interactions within distributed forebrain structures and support memory formation. *Hippocampus* 2000;10:457–465.

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PHYSIOLOGICAL PATTERNS IN THE HIPPOCAMPAL FORMATION

Distinct ensemble patterns define the activity of entorhinal neurons in relation to the behavioral, or sleep, state of the rat. Whenever the rat explores

its environment, the collection of entorhinal neurons that convey neocortical input into the circuitry of the hippocampus becomes temporally structured (see Fig. 1 for anatomical overview). When a rat runs down the arm of a maze, sniffs the scent of fresh cheese, or listens to the sound of a conditioned stimulus, layer II and layer III entorhinal neurons discharge gamma frequency volleys (40–100 Hz) every 80–200 ms (see Fig. 2). Layer II and layer III entorhinal neurons convey these collective signals to their targets in the dentate gyrus, CA1, CA3, and subicular cortices. Neurons in the dentate gyrus and CA1 are also entrained into theta-modulated gamma volleys in conjunction within their entorhinal inputs (Chrapak et al., 1995; Bragin et al., 1995; Csicsvari et al., 1999). Neurons in layer V and layer VI of the entorhinal cortex appear only weakly modulated by either a gamma or theta rhythmicity in their discharge, but the data on this issue are not particularly strong (Chrobak and Buzsáki, 1998a; Frank and Wilson, 1999).

In the absence of theta, the collective of neurons that convey hippocampal formation output to the neocortex discharges aperiodic bursts at about 200 Hz. Thus, when the rat stops to eat or drink, simply sits quietly, or is in slow wave sleep, layer V and layer VI entorhinal neurons discharge volleys at 140–200 Hz (ripple frequency) in conjunction with hippocampal formation sharp waves (Buzsáki et al., 1992; Chrobak and Buzsáki, 1994, 1996). This output is correlated to a concurrent discharge of neurons in at least two neocortical targets of the entorhinal cortex: the perirhinal cortex (Collins et al., 1999) and medial prefrontal cortex (Siapas and Wilson, 1998). Surprisingly, neurons in layer II and layer III of the entorhinal cortex do not robustly increase their discharge in relation with these events (Chrobak and Buzsáki, 1994).

We previously suggested that theta/gamma dynamics allow large ensembles of entorhinal neurons to “write” upon, and alter the synaptic connectivity of intrinsic hip-

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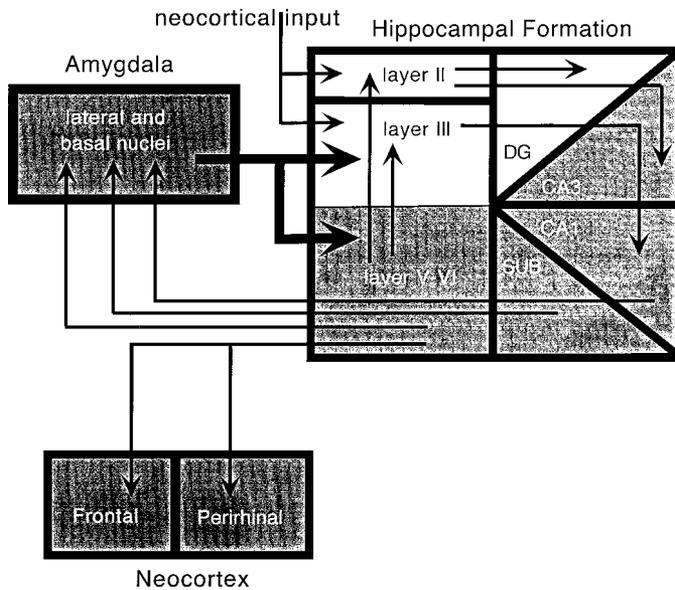


FIGURE 1. Schematic diagram of hippocampal formation (hippocampus and entorhinal cortex). Afferents from primary and associative cortices innervate the superficial (I–III) layers of the entorhinal cortex. Layer II neurons innervate the dentate gyrus and the CA3 subfield. Layer III neurons innervate the CA1 and subiculum (not shown). Intra-hippocampal pathways include the dentate projection to CA3, and the CA3 projection to CA1. CA1 neurons innervate the subiculum, and CA1 and subicular neurons project to the deep layers (V–VI) of the entorhinal cortex. Deep-layer entorhinal neurons provide the neocortically directed output from the hippocampal formation. Connections between the amygdala and entorhinal cortex are shown schematically (for more details, see Amaral and Witter, 1995).

pocampal circuits, during exploratory activity. In contrast, sharp wave/ripple dynamics allow large ensembles of hippocampal neurons to “readdress” and alter the synaptic connectivity of neocortical circuits (Chrobak and Buzsáki, 1998b). Thus, the sharp wave/ripple pattern may support an off-line memory consolidation process. Several findings seem to support this hypothesis. First, neuronal pairs that fire together during exploration discharge together during subsequent sharp waves (Wilson and McNaughton, 1994; Skaggs and McNaughton, 1996). Second, specific spatio-temporal spike sequences among small groups of CA1 neurons during sensory experience reoccur in a compressed time frame during subsequent sharp waves/ripple events (Nadasdy et al., 1999). Lastly, the sharp wave-related discharge of hippocampal outputs is correlated to the discharge of neurons in the prefrontal and perirhinal cortex (Siapas and Wilson, 1998; Collins et al., 1999). Thus, each 200-Hz ensemble pattern is influenced by recent sensory experience and appears to engage neocortical targets.

The temporal discontinuity between the organized discharge of the superficial (layers II and III) and deep (layers V and VI) layer entorhinal neurons suggests that when organized neural activity patterns are conveyed into the hippocampus, few if any organized patterns seem to exit the hippocampus. In turn, when organized neural activity patterns occur within the output neurons of the hippocampus, few if any organized patterns appear to enter the hippocampus. However, experiments to date have only tested the

global states of neuronal networks in the entorhinal cortex, and have characterized them by their average activity. One can take the view, therefore, that the lack of robust excitatory layer V to layer II/III effects reflect effective gating of feed-forward circuits. Against this relatively silent background, the few cells in the superficial layers that discharge in response to deep-layer input may carry important information. A similar argument can be made for CA1 output during theta activity. As a result, a few cells in the deep layers of the entorhinal cortex may be activated by inputs from CA1 pyramidal cells (Frank and Wilson, 1999). In the face of very low background “noise,” however, their activity may be absolutely critical.

THE INPUT STREAM

Layer II and layer III neurons provide entorhinal input to the hippocampus (Steward and Scoville, 1976; Witter and Groenewegen, 1984; Amaral and Witter, 1995; Dolorfo and Amaral, 1998a,b). Via these neurons, the product of neocortical associative processes is fed into the circuitry of the hippocampus. Several studies have demonstrated that superficial layer (I–III) neurons are theta-modulated (Mitchell and Ranck, 1980; Alonso and Garcia-Austt, 1987a,b; Stewart et al., 1992). Current source density analysis demonstrates that theta field potentials reverse across layer II (Alonso and Garcia-Austt, 1987a; Chrobak and Buzsáki, 1998a), indicating that synchronous synaptic input likely impinges on the dendritic fields of layer II and layer III neurons. Alonso and Garcia-Austt (1987b) and Dickson et al. (1995) observed that the majority of theta-modulated cells were located in layer II or layer III, with less than 10% of deep layer neurons being theta-modulated under anesthesia. We did not observe a significant theta modulation of neurons in the deep layers in freely behaving rats (Chrobak and Buzsáki, 1994). A recent report suggests that some deep-layer neurons are theta-modulated (Frank and Wilson, 1999). The location and differences between theta- and nontheta-modulated neurons in the deep lamina need further elucidation.

In conjunction with entorhinal theta potentials, a fast-frequency gamma field oscillation can be observed in the entorhinal cortex (Chrobak and Buzsáki, 1998a; de Curtis et al., 1999). The gamma field oscillations increase in amplitude and regularity in association with entorhinal theta waves, and show an amplitude minimum and phase reversal near the perisomatic region of layer II. Thus, the gamma oscillations likely represent synchronized synaptic potentials in the dendritic field of layer II and layer III entorhinal neurons. We observed that the vast majority of layer II and layer III entorhinal neurons discharged in phase relation to the negative peak of the layer III gamma field oscillation. Thus, layer II and layer III neurons discharge gamma frequency volleys on each theta wave (Fig. 2).

This population dynamic emerges quite independently within the dentate gyrus and CA1 region of the hippocampus (Bragin et al., 1995b; Charpak et al., 1995). Importantly, interconnected domains of neurons exhibit similar local structure in their collec-

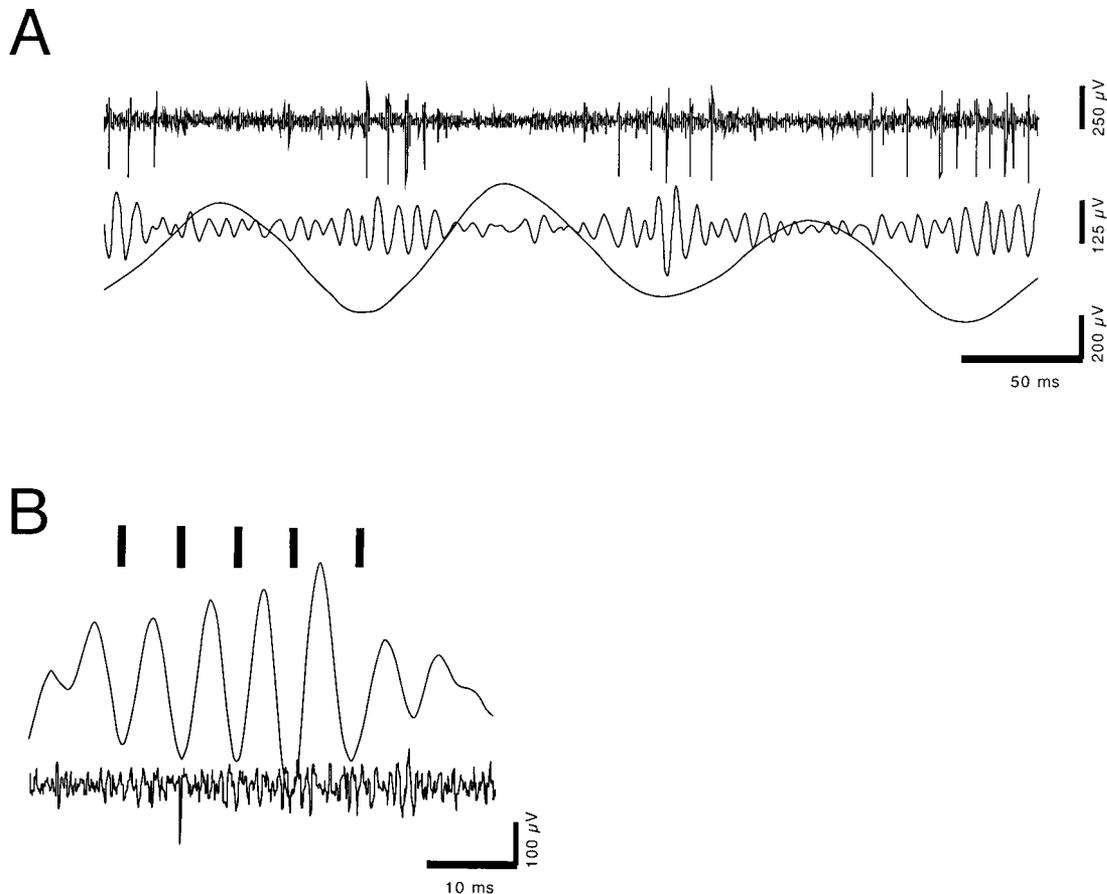


FIGURE 2. Neuronal activity in layers II and III of the entorhinal cortex during theta-modulated gamma waves. **A:** Top trace illustrates the activity of putative layer II interneurons discharging multiple spikes on each theta cycle (filter, 0.5–5.0 kHz). Middle trace illustrates the amplitude-modulated gamma oscillation (filter, 50–150 Hz). Bottom trace illustrates rhythmic theta waves (filter, 1–20 Hz). Note increase in gamma amplitude on each theta cycle. Note also the

relationship between spike discharges and the negative peak of the local gamma field oscillation. **B:** Bottom traces illustrate relationship of a single layer III neuron to the local field gamma oscillation. Note that while putative interneurons discharge multiple spikes on some theta cycle, layer III neurons typically discharge a single spike on each theta cycles, in phase relation to the negative peak of the layer III gamma field (modified after Chrobak and Buzsáki, 1998).

tive discharge properties. We have argued that this temporal structure is a necessary component of ensemble interaction within the circuits that convey neuronal activity into hippocampal formation (Buzsáki and Chrobak, 1995; Chrobak and Buzsáki, 1998).

Layer II is composed primarily of stellate and pyramidal projection neurons. Stellate cells are found to be mainly superficial in layer II and to make up about 65% of the neuropil, while pyramidal cells are found mainly deep in layer II (Klink and Alonso, 1997). These neurons give rise to the perforant path projection to the dentate gyrus and CA3 (Hjorth-Simonsen and Jeune, 1972; Steward and Scoville, 1976; Tamamaki and Nojyo, 1993). In some, but not all, areas of the entorhinal cortex, layer II stellate and pyramidal cells form clusters or cell islands. These entorhinal cell islands are present in many mammals, including rat, monkey, and human. While the cell islands may in some manner represent “functional” modules (Hevner and Wong-Riley, 1992), no known anatomical connections, including the intrinsic entorhinal connections, differentiate adjacent islands. Thus intrinsic entorhinal connections innervate adjacent cell islands, and no patchy organization like that observed in the neocortex (Lund et al., 1993) is

evident. Layer III is a very broad layer made up primarily of pyramidal neurons that project to CA1 and the subiculum.

Stellate neurons have distinct electrophysiological characteristics that could endow them with distinct functional properties (Alonso and Llinás, 1989; Klink and Alonso, 1993a,b). Chiefly, stellate neurons exhibit subthreshold membrane potential oscillations at theta frequency upon depolarization due to a relatively unique voltage-dependent persistent sodium current (Alonso and Llinás, 1989; Magistretti et al., 1999). The membrane oscillations provide for an intrinsic rhythmic depolarization, allowing the neurons to periodically oscillate near discharge threshold. On each depolarizing wave, the neurons can then discharge nonaccommodating spike clusters. These properties have been observed in both layer II stellate and layer II pyramidal neurons, but not in layer III neurons (Alonso and Llinás, 1989). These properties would allow distinct subsets (perhaps morphologically distinct) to convey unique patterns of neuronal activity to hippocampal targets. The unique character of layer II neurons should allow them to discharge more gamma frequency spikes on each theta train compared to layer III neurons, while layer III neurons should exhibit more

irregular discharge patterns. The physiology of layer III neurons is distinct from layer II stellate neurons and is more like that of regular spiking neocortical neurons (Dickson et al., 1997; Gloveli et al., 1997). Thus, it is expected that layer III neurons should exhibit less spikes per cluster (wave) as compared to layer II neurons, and should transmit neuronal patterns with a "higher fidelity" to the pattern of cortical input (Dickson and Alonso, 1997; Gloveli et al., 1997; Klink and Alonso, 1997).

In vivo, the majority of layer II and layer III neurons discharged one or two spikes per theta/gamma cycle. We could not clearly differentiate neurons in the deep and superficial aspects of layer III, and thus we could not confirm the differential firing properties of layer II vs. layer III neurons. However, we did observe two units in layer II that exhibited unique discharge properties. These neurons exhibited trains of action potentials on almost every theta cycle (see Fig. 2). These neurons had higher overall firing rates, exhibited short duration (<0.5 ms) action potentials, and exhibited trains of action potentials on almost every theta cycle (see Fig. 2). We suggest that the two fast-spiking neurons could be layer II GABAergic basket cells, similar to those observed in vitro (Jones and Buhl, 1993).

Jones and Buhl (1993) observed sustained firing of up to 200 Hz in a small subset of layer II interneurons observed in an in vitro slice preparation. These cells have extensive axonal arbors that form basket-like complexes around stellate and pyramidal neurons in layer II. Wouterlood et al. (1995) described the morphology of layer II parvalbumin (PV) GABA neurons. It is likely that the very fast-spiking interneurons observed by Jones and Buhl (1993) were PV+ interneurons. These neurons have extensive axonal arbors within layer II and surround the soma of stellate, pyramidal, and other PV+ GABA neurons. Thus, a rich network of highly interconnected, fast-spiking GABA neurons is present in layer II of the entorhinal cortex. A similar network of basket/chandelier cells is present in CA1, and these neurons contribute to the generation of theta/gamma and ripple oscillations in area CA1 (Sik et al., 1995; Freund and Buzsáki, 1996; Csicsvari et al., 1999). A technical difficulty in the entorhinal cortex is the lack of objective and reliable criteria for cell classification and unit separation methods, as well as the more complex cortical connectivity compared to the "simple" hippocampal regions. These methodological difficulties need to be worked out before any meaningful hypothesis can be advanced about the function of single-cell interactions in the entorhinal cortex.

In summary, layer II and layer III neurons have unique electrophysiological characteristics that may allow them to respond differentially to unique input patterns and more effectively engage unique sets of hippocampal targets. At present, such functional differences are purely hypothetical. We do know, however, that they possess unique characteristics, and that each participates in a temporally structured population volley (a gamma frequency volley) on each theta cycle. Further, the axon collaterals of stellate and pyramidal neurons exhibited recurrent collaterals to layers II and III, particularly around the dendritic arbor of the originating neuron (Klink and Alonso, 1997). The axonal extent of these projections within the entorhinal cortex is an interesting question for inquiry. Anterograde tracing studies demonstrate that focal label-

ing of layer II and layer III neurons results in widespread projections along the entire rostrocaudal axis of the entorhinal cortex (Dolorfo and Amaral, 1998a). Thus, a rich autoassociative network, similar to CA3 but with different electrophysiological properties, may exist in layer II, layer III, or both superficial entorhinal layers. The presence of a large population of fast-spiking stellate neurons with intrinsic oscillatory properties, and a potentially rich autoassociative network, would make the generation of fast-frequency dynamics more complex in layer II than in either the dentate gyrus or CA1, where there are less direct interactions among the principal neurons.

THE OUTPUT STREAM

A lamina dissecans or cell-sparse layer divides layer III from layer V neurons. The first three layers, as described above, contain neurons that contribute to the perforant path projection to the hippocampus. Few, if any, layer V or layer VI neurons contribute projections to the hippocampus proper. While a few bulk labeling studies have indicated a minor projection to the hippocampus (Kohler, 1985), single-cell studies will be needed to confirm the existence of this input.

Layer V and layer VI are the principle output neurons of the hippocampal formation and contribute a substantial projection to the adjacent perirhinal and postrhinal cortices and other cortical targets (Suzuki and Amaral, 1994; Burwell and Amaral, 1998). Further, these neurons originate projections to subcortical targets including the amygdala, lateral septum, and nucleus accumbens (Alonso and Kohler, 1984). Layer V and layer VI neurons also provide associational projections throughout the entorhinal cortex. These neurons project along the rostrocaudal extent of the deep layers of the entorhinal cortex, and provide an intralaminar projection to layers II and III (Kohler, 1986, 1988; Dolorfo and Amaral, 1998a). A few studies have explicitly examined the morphology and physiology of layer V and VI neurons. Lingenhohl and Finch (1991) demonstrated the broad dendritic fields and intraentorhinal axonal branching of both deep- and superficial-layer neurons. These authors labeled a few layer V and VI axons, some of which projected into the superficial layers. Additional anatomical studies of deep-layer neurons are needed to complement our understanding of the anatomy within the superficial layers.

Both layer V and VI neurons participate in ripple-frequency volleys in association with hippocampal and entorhinal sharp waves (Chrobak and Buzsáki, 1994, 1996). The generation of ripple oscillations and the participation of CA 3 and CA1 neurons in hippocampal sharp waves have been well-described (Buzsáki et al., 1992; Ylinen et al., 1995; Csicsvari et al., 1999). A concurrent discharge of subicular and deep-layer presubicular, parasubicular, and entorhinal neurons occurs in conjunction with the CA1 discharge. We also reported on a slow (50–150 ms), large-amplitude field potential in the entorhinal cortex (Chrobak and Buzsáki, 1994, 1996). This entorhinal sharp wave likely reflects the sequen-

tial activation of layer V and layer VI neurons by its CA1 and subicular input. The discharge of deep-layer neurons had minimal impact on layer III and II neurons. Thus, we observed only a small fraction of layer III neurons that responded with a modest (<25%) increase in discharge rate. Further, no layer II neurons increased their discharge rate in conjunction with hippocampal sharp waves (Chrobak and Buzsáki, 1994). This contrasts with the >100% increase in the discharge rate of deep-layer neurons. Despite the prominent deep-to-superficial projection, this input does not effectively engage a large number of superficial-layer neurons during sharp waves.

Several studies have demonstrated the distinctive inhibition of superficial-layer neurons in response to experimental stimulation of the deep layers (Finch et al., 1986, 1988; Jones and Heinemann, 1988; Bartesaghi et al., 1989; Jones, 1993; Paré et al., 1996). It may be that this dominant inhibition allows for a focal activation of superficial-layer neurons. Note that we reported a small percentage of layer III neurons that did increase during sharp waves. Based upon recent anatomic findings (Dolorfo and Amaral, 1998a,b) (see Fig. 3) that demonstrate distinct subdomains within the entorhinal cortex, a more comprehensive analysis of the response of layer III neurons during sharp waves is warranted. Alternatively, failure of inhibition within the superficial layers is known to play a critical role in intensifying epileptiform bursts within the entorhinal cortex into reverberating epileptiform activity (Paré et al., 1992; Jones, 1993). Spontaneous epileptiform bursts occur in the deep layers of the entorhinal cortex in human patients (Spencer and Spencer, 1994; Bragin et al., 1999) and in several models of epilepsy (Jones and Heinemann, 1988; Dickson and Alonso, 1997; Scharfman, 1996).

Epileptiform burst in these models will propagate to layer II, particularly after excitotoxic lesion of layer III with aminooxyacetic acid (Scharfman et al., 1998) or pharmacological manipulation such as the application of carbachol *in vitro* (Dickson and Alonso, 1997). Following excitotoxic lesions of layer III, Scharfman et al. (1998) observed exaggerated, repetitive responses to white-matter stimulation in layer II neurons *in vitro* in contrast to brief EPSPs and single discharges in control slices. It appears that neurons in the deep layers and layer II have a special propensity to generate synchronous population activity (Dickson and Alonso, 1997). For example, application of carbachol to layers V–VI or II can generate rhythmic epileptiform events, while application of carbachol to layer III cannot. Layer III may therefore act as a powerful gate, limiting the responsiveness of layer II to afferent inputs from neocortical and deep-layer entorhinal neurons. In the absence of this gate, layer II neurons are highly responsive to inputs from deep-layer neurons. While it is clear that these processes play a role in the generation of epileptiform activity, it is unclear what role they play in the normal physiology of the entorhinal cortex. Further studies examining the physiological response of layer III neurons during sharp waves should clarify some of these issues. Altogether, these studies indicate that at the population level, propagation of excitatory activity from deep to superficial layers is quite limited in the intact brain. Once this “gate” is open, the facilitated excitatory transmission can give rise to epileptic activity. However, the exact

physiological role of this “filtering” mechanism for the normal operation of the EC has yet to be resolved.

Interestingly, layer III and layer V of the entorhinal cortex receive prominent input from the lateral and basal as well as other amygdala nuclei (Krettek and Price, 1977; Petrovich et al., 1996; Pikkariainen et al., 1999). Paré et al. (1996) demonstrated, in the anesthetized cat, that amygdala neurons discharge as early as 40 ms prior to the peak of a sharp field event in the entorhinal cortex, which was abolished by an amygdala lesion. Thus, the amygdala may play an important role in the generation of synchronous entorhinal discharges. Direct CA1 inputs, particularly from the temporal two thirds of the hippocampus, target several amygdala nuclei, including the lateral and basolateral nuclei (Ottersen, 1982; Van Groen and Wyss, 1990; Canteras and Swanson, 1992; Christensen and Frederickson, 1998). Thus, the amygdala discharge itself may be dependent on the intrahippocampal discharge. However, it is not clear at the moment how population discharge in the amygdala is affected by hippocampal outflow or, vice versa, how the amygdala affects population activity in the hippocampus.

Bragin et al. (1995a) described a distinct population event in the dentate gyrus, termed the field dentate spike. Field dentate spikes are large-amplitude (2–4 mV), short-duration (<30 ms) field potentials in the dentate hilus, that occur in the same behavioral states as sharp waves/ripples. Based on current source density analysis, these events appear to be driven directly by layer II/III neurons within the entorhinal cortex. While dentate spikes are hardly ever followed by sharp waves, dentate spikes occasionally follow sharp waves. Furthermore, entorhinal lesions abolished dentate spikes. We hypothesize that the entorhinal sharp field events, described by Paré et al. (1996), and the discharge of layer II/III neurons that accompany them, are responsible for triggering dentate spikes (Fig. 4). In response to the sharp wave burst initiated in CA3 and relayed via CA1 to amygdala projections, amygdala neurons could send a signal to the entorhinal cortex. This signal may then gate the efficacy of layer V to layer II/III connections and, indirectly, the excitability of the intrahippocampal pathways by way of the dentate spikes. We suggest that in response to a “query” determined by a specified pattern of input from the hippocampus during sharp waves, an amygdala representation of the “affective” value of a stimulus or event may be conveyed to the entorhinal cortex. This input can then gate both the strength or pattern of the entorhinal input to the dentate gyrus by means of the dentate spike, as well as entorhinal outputs via layer V to the neocortex (Fig. 3).

FORMATION OF MEMORIES BY THE HIPPOCAMPAL-ENTORHINAL CORTEX NETWORK: A VIEWPOINT

We suggested earlier that during theta/gamma-associated behaviors, information about the external world reaches the hippocampus via the entorhinal cortex, whereas during behavioral states associated with sharp wave bursts, the direction of information flow is reversed: population bursts initiated in the CA3 recur-

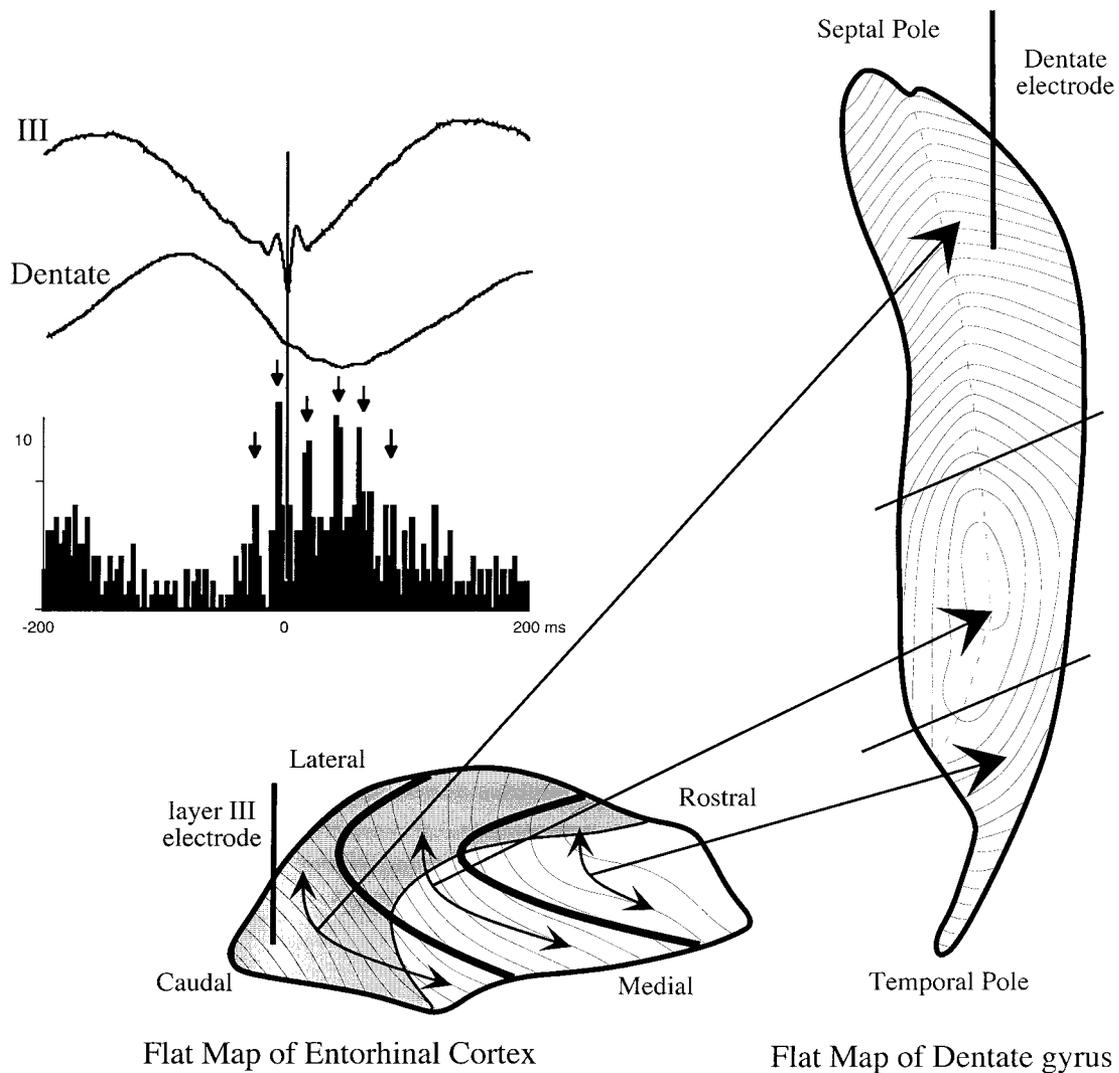


FIGURE 3. Top left: synchrony between gamma field oscillation in layers II–III of the caudolateral entorhinal cortex and the ipsilateral dorsal dentate gyrus. Approximate locations of electrodes are illustrated in maps at bottom and right. Top traces illustrate average gamma field potential ($N = 262$) from layer II of the entorhinal cortex and within the dentate gyrus. Cross-correlogram illustrates moment-by-moment synchrony (arrows), using negative peak of gamma oscillation as zero reference (modified after Chrobak and Buzsáki, 1998). Illustrations at left and bottom use reconstructed flat maps (Swanson et al., 1978) to illustrate approximate locations of electrodes and anatomical connectivity of entorhinal projections to the dentate gyrus. The entorhinal cortex has been traditionally divided into a medial (MEA; white area on entorhinal map) and a lateral (LEA; grey area on entorhinal map) subdivision (Blackstad, 1956). Based on the pattern

of both intrinsic entorhinal projections and entorhinal projections to the dentate gyrus, Dolorfo and Amaral (1998) distinguished three relatively segregated domains within the entorhinal cortex, orthogonal to the MEA/LEA boundaries. Each domain contains rich intrinsic connections (double-headed arrows) within the domain and relatively little overlap of target neurons within the dentate gyrus. The functional implication of this anatomical organization is that segregated cortical processing streams enter the hippocampal formation in relatively distinct, albeit broad, channels. It remains to be investigated whether synchrony across sites in the entorhinal cortex and dentate varies as a function of these three connectivity domains. The data shown here illustrate that a high degree of synchrony can occur within anatomically interconnected regions of the entorhinal cortex and dentate.

rent collateral matrix invade the neocortex (Buzsáki, 1989, 1998; Chrobak and Buzsáki, 1994). The advantage of this two-stage operation has been emphasized by recent computer simulations (Hinton et al., 1995; Lörincz and Buzsáki, 2000). The framework of the hypothesized hippocampus-EC model is characterized as follows. First, the EC-hippocampal formation can be conceived as a “novelty”-detecting “reconstruction network” (Grastyan et al., 1959; Sokolov, 1963; Vinogradova, 1975). In computational jar-

gon, the term “novelty” can be defined as “error,” i.e., the difference between the expected (top-down) and experienced (bottom-up) neuronal representations. A second assumption is that neocortical representations are funneled to the hippocampus primarily by way of layer III of the entorhinal cortex (primary input). The third assumption is that the main function of layer II EC neurons is to compare neuronal representations between the neocortical (bottom-up) inputs and feedback (top-down) inputs from

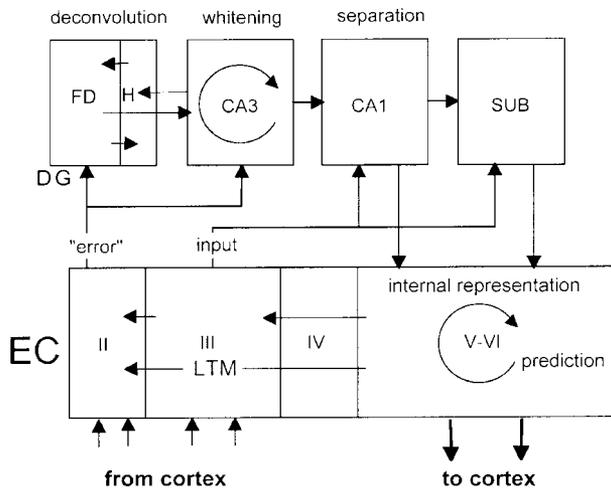


FIGURE 4. Schematic of major fields and connections in the entorhinal cortex (EC)-hippocampal formation and their hypothesized functions in the model. The hippocampus communicates with the perirhinal, parahippocampal, and neocortical regions by way of the EC (from the cortex and to the cortex). The main information flow in the EC is from the deep (V–VI) to superficial (II and III) layers. Long-term memory (LTM) is stored in the excitatory synapses between the deep and the superficial layers of the EC. The EC directly innervates all major hippocampal fields. Layer III of the EC is remapped onto the CA1 field. The difference between neocortical information and processed signal in the hippocampus-layer V-layer II loop (the “reconstructed input”) is the “reconstruction error.” This error signal drives the dentate gyrus and the CA3 field. Granule cells of the fascia dentata (FD) and mossy cells of the hilus (H) form delay lines. These reciprocal excitatory loops of the dentate gyrus (FD and H) function primarily as a deconvolution network. The EC provides the input to the CA3 field (perforant path and mossy fiber synaptic matrices), and this input is decorrelated (whitened) by the CA3 field. The whitened representation undergoes blind source separation in the CA1 field. The resulting independent components are used to train LTM in the EC. All fields receive subcortical innervation, and the activities of these pathways determine the operational modes of the EC-hippocampal region (theta vs. sharp wave phases). The anatomical connectivity and certain physiological properties of the subiculum are similar to those of the CA1 region. However, the present model does not specifically deal with this important region (reprinted from Lörincz and Buzsáki, 2000).

the hippocampus, i.e., to compute and input the “error.” The top-down information is conveyed by the hippocampus-EC layer V-layer II projections. The fourth assumption is that a main operation of the hippocampus is to reconstruct (or re-represent) the template of the neocortical input in order to optimize neocortical circuits to represent temporal sequences, i.e., events and series of events (Lörincz and Buzsáki, 2000).

With the above ingredients, the operation of the model hippocampus-EC can be described as follows. The neocortical representations (primary input) and EC layer V-transformed hippocampal output (i.e., the reconstructed input) are compared by layer II of the EC. The difference between the primary input and the reconstructed input (termed the reconstruction error) is the novel information that activates the dentate gyrus and CA3 circuits, resulting in alterations of the internal connectivity of the CA3 recurrent matrix (plasticity). If the neocortical input to the

EC is not novel (e.g., a rat is placed into a highly familiar environment), then the output of the hippocampus simply iterates previously stored hippocampal representations to the EC. Novel inputs, on the other hand, will induce synaptic changes in the circuits of the hippocampus. Because of the change in the hippocampal output, the EC will be trained until the difference (error) between the input from the neocortex and the reconstructed input conveyed by the hippocampal output is eliminated. Error elimination is also constrained by novelty-driven learning in the hippocampus. We conjecture that hippocampal synaptic modification gives rise to hippocampal outputs, represented in mathematical terms as independent components. Development of the independent component requires a two-phase operation: a nonlinear operation phase that tunes the circuits within the hippocampus (theta-associated behaviors), and a linear operation phase that tunes the entorhinal and neocortical circuits in a supervised manner. The spatiotemporal organization of the sharp-wave event (Wilson and McNaughton, 1996; Nadasdy et al., 1999) is assumed to serve the function of the supervisor.

A prediction of this two-stage entorhinal cortex-hippocampus model involves the differential roles attributed to the entorhinal outputs from layers II and III. Our assumption is that increased activity in layer II represents the reconstruction “error” between the neocortical input and its reconstructed representation by the hippocampus. In turn, the “error” signal is the main driving force to the granule cells and CA3 pyramidal cells, an input necessary for the induction of synaptic plasticity in the CA3 recurrent collateral system. The prediction then is that elimination of entorhinal cortex layer II neurons will prevent modification of the intrahippocampal circuits by the neocortical inputs and results in the preservation of previously trained patterns. Conversely, selective elimination of the layer III input would deprive the hippocampus of its ability to predict (temporally advance information). This should result in an instability and a large variability of place fields of CA1 pyramidal cells.

REFERENCES

- Alonso A, Garcia-Austt E. 1987a. Neuronal sources of theta rhythm in the entorhinal cortex of the rat. I. Laminar distribution of theta field potentials. *Exp Brain Res* 67:493–501.
- Alonso A, Garcia-Austt E. 1987b. Neuronal sources of theta rhythm in the entorhinal cortex of the rat. II. Phase relations between unit discharges and theta field potentials. *Exp Brain Res* 67:502–509.
- Alonso A, Kohler C. 1984. A study of the reciprocal connections between the septum and the entorhinal area using anterograde and retrograde axonal transport methods in the rat brain. *J Comp Neurol* 225:327–343.
- Alonso A, Llinás RR. 1989. Subthreshold Na⁺-dependent theta-like rhythmicity in stellate cells of entorhinal cortex layer II. *Nature* 342:175–177.
- Amaral DG, Witter MP. 1995. Hippocampal formation. In: Paxinos G, editor. *The rat nervous system*, 2nd edition. New York: Academic Press. p 443–494.
- Amaral DG, Price JL, Pitkanen A, Carmichael ST. 1992. Anatomical organization of the primate amygdaloid complex. In: Aggleton JP,

- editor. The amygdala: neurobiological aspects of emotion, memory and mental dysfunction. New York: Wiley-Liss. p 1–66.
- Bartasaghi R, Gessi T, Sperti L. 1989. Electrophysiological analysis of the hippocampal projections to the entorhinal area. *Neuroscience* 30:51–62.
- Blackstad TW. 1956. Commissural connections of the hippocampal region in the rat, with special reference to their mode of termination. *J Comp Neurol* 105:417–537.
- Bragin A, Jando G, Nadasdy Z, van Landeghem M, Buzsáki G. 1995a. Dentate EEG spikes and associated interneuronal population bursts in the hippocampal hilar region of the rat. *J Neurophysiol* 73:1691–1705.
- Bragin A, Jando G, Nadasdy Z, Hetke J, Wise K, Buzsáki G. 1995b. Gamma (40–100 Hz) oscillation in the hippocampus of the behaving rat. *J Neurosci* 15:47–60.
- Bragin A, Engel J Jr, Wilson CL, Fried I, Mathern GW. 1999. Hippocampal and entorhinal cortex high-frequency oscillations (100–500 Hz) in human epileptic brain and in kainic acid-treated rats with chronic seizures. *Epilepsia* 40:127–137.
- Burwell RD, Amaral DG. 1998. Perirhinal and postrhinal cortices of the rat: interconnectivity and connections with the entorhinal cortex. *J Comp Neurol* 391:292–321.
- Buzsáki G. 1989. Two-stage model of memory trace formation: a role for “noisy” brain states. *Neuroscience* 31:551–570.
- Buzsáki G. 1998. The hippocampo-neocortical dialogue. *Cereb Cortex* 6:81–92.
- Buzsáki G, Chrobak J. 1995. Temporal structure in spatially organized neuronal ensembles: a role for interneuron networks. *Curr Opin Neurobiol* 5:504–510.
- Buzsáki G, Leung L, Vanderwolf CH. 1983. Cellular bases of hippocampal EEG in the behaving rat. *Brain Res Rev* 6:139–171.
- Buzsáki G, Horvath Z, Urioste R, Hetke J, Wise K. 1992. High-frequency network oscillation in the hippocampus. *Science* 256:1025–1027.
- Canteras NS, Swanson LW. 1992. Projections of the ventral subiculum to the amygdala, septum and hypothalamus: a PHAL anterograde tract-tracing study in the rat. *J Comp Neurol* 324:180–194.
- Chapak S, Paré D, Llinás RR. 1995. The entorhinal cortex entrains fast CA1 hippocampal oscillations in the anesthetized guinea pig: role of the monosynaptic component of the perforant path. *Eur J Neurosci* 7:1548–1557.
- Christensen M-K, Frederickson CJ. 1998. Zinc-containing afferent projections to the rat corticomедial amygdaloid complex: a retrograde tracing study. *J Comp Neurol* 400:375–390.
- Chrobak JJ, Buzsáki G. 1994. Selective activation of deep layer retrohippocampal neurons during hippocampal sharp waves. *J Neurosci* 14:6160–6170.
- Chrobak JJ, Buzsáki G. 1996. High-frequency oscillations in the output networks of the hippocampal-entorhinal axis of the freely behaving rat. *J Neurosci* 16:3056–3066.
- Chrobak JJ, Buzsáki G. 1998a. Gamma oscillations in the input network of the entorhinal-hippocampal axis of the freely-behaving rat. *J Neurosci* 18:388–398.
- Chrobak JJ, Buzsáki G. 1998b. Operational dynamics in the hippocampal-entorhinal axis. *Neurosci Biobehav Rev* 22:303–310.
- Collins DR, Lang EJ, Paré D. 1999. Spontaneous activity of the perirhinal cortex in behaving cats. *Neuroscience* 89:1025–1039.
- Csicsvari J, Hirase H, Czurko A, Mamiya A, Buzsáki G. 1999. Oscillatory coupling of hippocampal pyramidal cells and interneurons in the behaving rat. *J Neurosci* 19:274–287.
- de Curtis M, Dickson C, Panzica F, Pizzi R, Spreafico R. 1999. Pharmacology and organization of carbachol-induced gamma activity in the medial entorhinal cortex. *Soc Neurosci Abstr* 363:16.
- Dickson CT, Alonso A. 1997. Muscarinic induction of synchronous population activity in the entorhinal cortex. *J Neurosci* 17:6729–6744.
- Dickson CT, Kirk IJ, Oddie SD, Bland BH. 1995. Classification of theta-related cells in the entorhinal cortex: cell discharges are controlled by the ascending brainstem synchronizing pathway in parallel with hippocampal theta-related cells. *Hippocampus* 5:306–319.
- Dickson CT, Mena AR, Alonso A. 1997. Electroresponsiveness of medial entorhinal cortex layer III neurons *in vitro*. *Neuroscience* 81:937–950.
- Dolorfo CL, Amaral DG. 1998a. Entorhinal cortex of the rat: organization of intrinsic connections. *J Comp Neurol* 398:49–82.
- Dolorfo CL, Amaral DG. 1998b. The entorhinal cortex of the rat: topographic organization of the cells of origin of the perforant path projection to the dentate gyrus. *J Comp Neurol* 398:25–48.
- Finch DM, Wong EE, Derian EL, Babb TL. 1986. Neurophysiology of limbic system pathways in the rat: projections from the subicular complex and hippocampus to the entorhinal cortex. *Brain Res* 397:205–213.
- Finch DM, Tan AM, Isokawa-Akesson M. 1988. Feedforward inhibition of the rat entorhinal cortex and subicular complex. *J Neurosci* 8:2213–2226.
- Frank L, Wilson M. 1999. *Soc Neurosci Abstr*.
- Freund TF, Buzsáki G. 1996. Interneurons of the hippocampus. *Hippocampus* 6:347–470.
- Gloveli T, Schmitz RM, Empson T, Dugladze T, Heinemann UHF. 1997. Morphological and electrophysiological characterization of layer III cells of the medial entorhinal cortex of the rat. *Neuroscience* 77:629–648.
- Grastyan E, Lissak K, Madarasz I, Donhoffer H. 1959. The hippocampal electrical activity during the development of conditioned reflexes. *Electroencephalogr Clin Neurophysiol* 11:409–430.
- Hevner RF, Wong-Riley MTT. 1992. Entorhinal cortex of the human, monkey, and rat: metabolic map as revealed by cytochrome oxidase. *J Comp Neurol* 326:451–469.
- Hinton GE, Dayan P, Frey BJ, Neal RM. 1995. The “wake-sleep” algorithm for unsupervised neural networks. *Science* 268:1158–1161.
- Hjorth-Simonsen A, Jeune B. 1972. Origin and termination of the hippocampal perforant path in the rat studied by silver impregnation. *J Comp Neurol* 144:215–232.
- Jones RSG. 1993. Entorhinal-hippocampal connections: a speculative view of their function. *Trends Neurosci* 16:58–64.
- Jones RSG, Buhl EH. 1993. Basket-like interneurons in layer II of the entorhinal cortex exhibit a powerful NMDA-mediated synaptic excitation. *Neuroscience* 59:1476–1496.
- Jones RSG, Heinemann UHF. 1988. Synaptic and intrinsic responses of medial entorhinal cortical cells in normal and magnesium-free medium *in vitro*. *J Neurophysiol* 59:1476–1496.
- Klink R, Alonso A. 1993. Ionic mechanisms for the subthreshold oscillations and differential responsiveness of medial entorhinal cortex layer II neurons. *J Neurophysiol* 70:144–157.
- Klink R, Alonso A. 1997a. Morphological characteristics of layer II projection neurons in the rat medial entorhinal cortex. *Hippocampus* 7:571–582.
- Klink R, Alonso A. 1997b. Muscarinic modulation of the oscillatory and repetitive firing properties of entorhinal cortex layer II neurons. *J Neurophysiol* 77:1813–1819.
- Kohler C. 1985. A projection from the deep layers in the entorhinal area to the hippocampal formation in the rat brain. *Neurosci Lett* 56:13–19.
- Kohler C. 1986. Intrinsic connection of the retrohippocampal region in the rat brain. II. The medial entorhinal area. *J Comp Neurol* 271:149–169.
- Kohler C. 1988. Intrinsic connection of the retrohippocampal region in the rat brain. III. The lateral entorhinal area. *J Comp Neurol* 271:208–228.
- Krettek JE, Price JL. 1977. Projections from the amygdaloid complex and adjacent olfactory structures to the entorhinal cortex and to the subiculum in the rat and cat. *J Comp Neurol* 172:723–752.
- Lingenhohl K, Finch DM. 1991. Morphological characterization of rat entorhinal neurons *in vivo*: soma-dendritic structure and axonal domains. *Exp Brain Res* 84:57–74.

- Lörincz A, Buzsáki G. 2000. Two-phase computational model training long-term memories in the entorhinal-hippocampal region. NY Acad Sci In press.
- Lund JS, Yoshioka T, Levitt JB. 1993. Comparison of intrinsic connectivity in different areas of macaque monkey cerebral cortex. *Cereb Cortex* 3:148–162.
- Magistretti J, Ragsdale DS, Alonso A. 1999. Direct demonstration of persistent Na⁺ channel activity in dendritic processes of mammalian cortical neurons. *J Physiol [Lond]* 15:629–636.
- Mitchell SJ, Ranck JB Jr. 1980. Generation of theta rhythm in medial entorhinal cortex of freely moving rats. *Brain Res* 189:49–66.
- Nadasdy Z, Hirase H, Csicsvari J, Czurkó A, Buzsáki G. 1999. Replay and time compression of recurring spike sequences in the hippocampus. *J Neurosci* 19:9497–9507.
- Ottersen OP. 1982. Connections of the amygdala of the rat. IV: corticoamygdaloid and intraamygdaloid connections as studied with axonal transport of horseradish peroxidase. *J Comp Neurol* 205:30–48.
- Paré D, deCurtis M, Llinás R. 1992. Role of hippocampal-entorhinal loop in temporal lobe epilepsy: extra- and intracellular study in the isolated guinea pig brain in vitro. *J Neurosci* 12:1867–1881.
- Paré D, Ong J, Gaudreau H. 1996. Projection cells and interneurons of the lateral and basolateral amygdala: distinct firing patterns and differential relation to theta and delta rhythms in conscious cats. *J Neurosci* 16:3334–3353.
- Petrovich GD, Risold PY, Swanson LW. 1996. Organization of projections from the basomedial nucleus of the amygdala: a PHAL study in the rat. *J Comp Neurol* 374:387–420.
- Pikkarainen M, Ronkko S, Savander V, Insausti R, Pitkanen A. 1999. Projections from the lateral, basal, and accessory basal nuclei of the amygdala to the hippocampal formation in the rat. *J Comp Neurol* 403:229–260.
- Scharfman HE. 1996. Hyperexcitability of entorhinal cortex and hippocampus after application of aminooxyacetic acid (AOAA) to layer III of the entorhinal cortex in vitro. *J Neurophysiol* 76:2986–3001.
- Scharfman HE, Goodman JH, Du F, Schwarcz R. 1998. Chronic changes in synaptic responses of entorhinal and hippocampal neurons after amino-oxacetic acid (AOAA)-induced entorhinal cortical neuron loss. *J Neurophysiol* 80:3031–3046.
- Siapas AG, Wilson MA. 1998. Coordinated interactions between hippocampal ripples and cortical spindles during slow-wave sleep. *Neurons* 21:1123–1128.
- Sik A, Penttonen M, Ylinen A, Buzsáki G. 1995. Hippocampal CA1 interneurons: an in vivo intracellular labeling study. *J Neurosci* 15:6651–6665.
- Skaggs WE, McNaughton BL. 1996. Replay of neuronal firing sequences in rat hippocampus during sleep following spatial experience. *Science* 271:1870–1873.
- Sokolov EN. 1963. Perception and the conditioned reflex. London: Pergamon Press.
- Spencer SS, Spencer DD. 1994. Entorhinal-hippocampal interactions in medial temporal lobe epilepsy. *Epilepsia* 35:721–727.
- Steward O, Scoville SA. 1976. Cells of origin of entorhinal cortical afferents to the hippocampus and fascia dentata of the rat. *J Comp Neurol* 169:347–370.
- Stewart M, Quirk GJ, Barry M, Fox SE. 1992. Firing relation of medial entorhinal neurons to the hippocampal theta rhythm in urethane anesthetized and walking rats. *Exp Brain Res* 90:21–28.
- Suzuki WA, Amaral DG. 1994. Topographic organization of the reciprocal connections between the monkey entorhinal cortex and the perirhinal and parahippocampal cortices. *J Neurosci* 14:1856–1877.
- Swanson LW, Wyss JM, Cowan WM. 1978. An autoradiographic study of the organization of intrahippocampal association pathways in the rat. *J Comp Neurol* 181:681–715.
- Tamamaki N, Nojyo Y. 1993. Projection of the entorhinal layer II neurons in the rat as revealed by intracellular pressure-injection of neurobiotin. *Hippocampus* 3:471–480.
- Van Groen T, Wyss JM. 1990. Extrinsic projections from area CA1 of the rat hippocampus: olfactory, cortical, subcortical, and bilateral hippocampal formation projections. *J Comp Neurol* 302:515–528.
- Vinogradova OS. 1975. Registration of information and the limbic system. In: Horn G, Hinde RA, editors. Short-term changes in the neural activity and behavior. Cambridge, UK: Cambridge University Press. p 95–148.
- Wilson M, McNaughton BL. 1994. Reactivation of hippocampal ensemble memories during sleep. *Science* 265:676–679.
- Witter MP, Groenewegen HJ. 1984. Laminar origin and septotemporal distribution of entorhinal and perirhinal projections to the hippocampus in the cat. *J Comp Neurol* 224:371–385.
- Wouterlood FG, Hartig W, Bruckner G, Witter MP. 1995. Parvalbumin-immunoreactive neurons in the entorhinal cortex of the rat: localization, morphology, connectivity and ultrastructure. *J Neurocytol* 24:135–153.
- Ylinen A, Sik A, Bragin A, Nadasdy Z, Jandó G, Szabó I, Buzsáki G. 1995. Sharp wave-associated high-frequency oscillation (200 Hz) in the intact hippocampus: network and intracellular mechanisms. *J Neurosci* 15:30–46.