Entorhinal cortex directs learning-related changes in CA1 representations Grienberger and Magee, November 2nd 2022. Nature. doi: 10.1038/s41586-022-05378-6 PubMed

Rapid synaptic plasticity contributes to a learned conjuctive code of position and choice-related information in the hippocampus (Zhao X, Hsu CL, Spruston N, January 5, 2022) Neuron. 110(1):96-108.e4. PubMed

Re-interpretation based on the semblance hypothesis

It is known that Entorhinal cortex (EC) input to hippocampus (HP) is via two different projections. 1) A trisynaptic path from EC layer II (ECII) to CA1 neurons via CA3 neurons and a monosynaptic pathway directly connecting EC layer III (ECIII) to CA1 neurons (**Fig.1**).

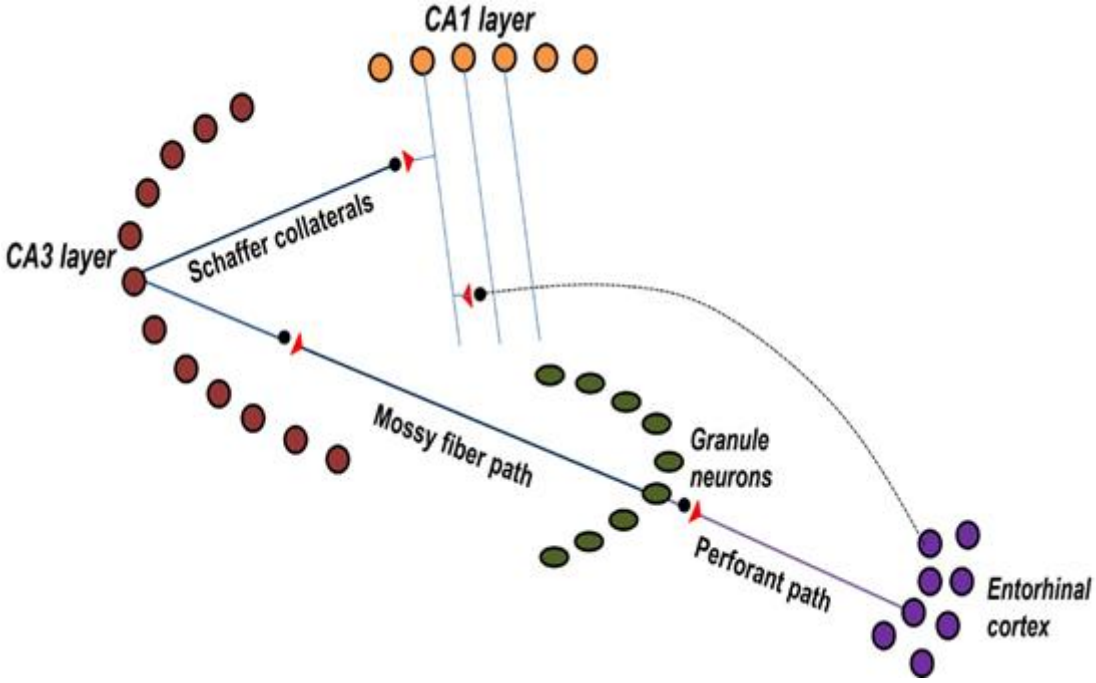

Figure 1. Figure showing both trisynaptic and monosynaptic pathways between one entorhinal cortical (EC) neurons and one CA1 pyramidal neuron. Trisynaptic path from EC2 neurons connects through granule neurons and neurons of the CA3 layer. Monosynaptic path from EC3 neurons synapse to a CA1 neuron located at stratum lacunosum-moleculare layer at apical tuft region of CA1 neuron. At the very least, it is necessary to explain how inner sensation of memory of location is

generated and how it is related to firing of a set of CA1 neurons in the presence of a cue stimulus. Note: Colored circles are neurons.

CA1 pyramidal neurons that fire somatic action potentials when the animal reaches a specific location are called place cells (Moser et al., 2015). Results from one study has led to the inference that EC3 to CA1 connections are involved in temporal association memory (Suh et al, 2010). Later, it was noticed that ability to associatively learn and memorize a location in response to a cue stimulus is correlated with increased firing of CA1 neurons (Zhao et al., 2020) as if there is an "over-representation" of these neurons to place memory. It was also noticed that firing of CA1 neurons is associated with long-term dendritic voltage signals initiated by inputs from EC3 sub-domain of EC (Magee and Grienberger, 2020). Authors attribute this to occurrence of behavioral timescale synaptic plasticity (BTSP) in the EC3-CA1 synapses. Recent experiments that showed increased elevation of both EC3 activity and CA1 place field density in response to a prominent reward-predictive cue stimulus in a new environment led to the interpretation that EC directs learning-related changes in CA1 representations (Grienberger and Magee, 2022). Similar results reported earlier by Zhao et al., 2022 inferred that plateau potentials in CA1 pyramidal neurons rapidly strengthen synaptic inputs carrying conjunctive information about position and choice. Investigators also inferred that learning is due to the formation of a conjunctive population code upstream of CA1. However, a mechanistic explanation with a rigor for replicating the mechanism in an engineered system is not available.

To provide a mechanistic explanation for the above findings, it is necessary to arrive at a mechanism that can provide explanations for the following questions (**Table 1**).

1. How can internal sensation of a particular memory be explained?
2. How can internal sensation of a particular memory in response to a cue stimulus explained?
3. Since memory is associated with internal sensation of a conscious state how can they both be explained in an interconnected manner?

4. Explain how the mechanism provides signals for a motor response at the same time?
5. Explain what learning-mechanism can lead to firing of set of CA1 neurons (place cells) that prompt one to call it as "overrepresentation" of these neurons in place memory?
6. Explain features of the mechanism that qualify it as an evolved mechanism?
7. How to explain formation of long-duration dendritic voltage signals and Ca^{2+} plateau potentials associated with learning changes in a single trial (Takahashi and Magee, 2009; Grienberger et al., 2014; Bittner et al., 2015)?
8. Is it possible to explain various features exhibited by the system at different levels of its operation?

Table 1. Questions that a solution for the nervous system is expected to provide answers for in an interconnected manner.

Challenges and features of a possible solution

Work by Grienberger and Magee (Grienberger and Magee, 2022) infers that there are synaptic plasticity changes at the EC3-CA1 synapses that lead to dendritic voltage signals in the dendrites of CA1 neurons, which is associated with/in turn leads to firing of CA1 neurons. Increased firing of CA1 neurons is being interpreted as “overrepresentation”. A mechanistic explanation is necessary to explain both “plasticity” and “over-representation” with the type of clarity that will allow its replication in engineered systems. The above requirements given in Table 1 can be summarized to two questions. What synaptic changes inferred from dendritic voltage signals, which are being referred to as synaptic plasticity changes, can generate first-person inner sensation of memory? How is the same mechanism linked to sudden firing of previously silent CA1 neurons that made us to infer that they acquire place field property?

The ability to retrieve memory is currently being studied using surrogate markers such as behavioral motor actions and speech. Instead of examining surrogate markers, semblance hypothesis searched for a mechanism for first-person inner sensations directly by asking the question, “At what location and by what mechanism do the first-person

inner sensations get sparked?" Even though third person experimenter cannot sense or identify the formation for first-person properties at this location, reaching a solution point provides a mechanistic explanation for the most important function of the nervous system. It can lead to finding methods to treat its disorders and replicating the mechanism in engineered systems. This motivated derivation of semblance hypothesis (Vadakkan, 2007, 2013, 2019b). It was based on the argument that if it becomes possible to formulate a mechanism for generating inner sensations that can also explain all features of the system exhibited in different levels by an interconnectable mechanism, then the formulated mechanism can be correct. It will then be possible to make testable predictions that can be verified.

Explanation

Towards achieving this, first a conditional definition for memory was made (Vadakkan, 2007). This was followed by examining works that were carried out with an aim to undertake the gold standard test of replicating the mechanism in engineered systems, which will eventually lead to the development of true artificial intelligence (AI). A pioneering work (Minsky, 1980) that viewed memories as hallucinations (internal sensations of something in its absence) matched with the expectations of search for a mechanism of first-person inner sensations. This laid a foundational framework for a testable mechanism. Motivated by this, a search was carried out in the nervous system to identify a change that can occur during associative learning and can be used by one of the associatively learned stimuli to generate hallucinations of second stimulus at the time of memory retrieval.

In the background state, head region of a dendritic spine (postsynaptic or input terminal) is continuously getting depolarized by quantal release of neurotransmitter molecules, in addition to occasional volleys of release of neurotransmitter molecules when action potentials arrive at its presynaptic terminal. Simultaneous activation of two abutted spines by environmental stimuli is expected to form inter-postsynaptic (inter-spine) functional LINK (IPL) during associative learning (Vadakkan, 2013), which forms the linchpin of derived mechanism. At the time of memory

retrieval, reactivation of this IPL by one of the associatively learned stimuli leads to propagation of potentials to the inter-LINKed spine previously activated by the second stimulus whose memory is expected to get retrieved. In the background state of continuous depolarization of spine head by quantal release of neurotransmitter molecules, any sudden lateral activation of inter-LINKed spine (in the absence of arrival of action potentials at its presynaptic terminal) is expected to generate a hallucination that the inter-LINKed spine is receiving a stimulus from the environment through its presynaptic terminal. Qualia of first-person inner sensations of a retrieved memory can be estimated by retrograde extrapolation from the inter-LINKed spine towards identifying all the sensory receptors (see figures 6 and 7 in FAQ section of this website). A unit of semblance (semblion) is equivalent to minimum sensory stimuli capable of stimulating a minimum subset of sensory receptors that will stimulate the inter-LINKed spine. A natural retrograde extrapolation is expected to occur at the time of memory retrieval as a system property of systems where synaptic transmission and propagation of potentials across the IPLs contribute intracellular potentials, whose corresponding changes in the extracellular matrix (ECM) space form vector components of oscillating extracellular potentials taking place within in a narrow range of frequencies. Answers to questions in **Table1** are given in **Table 2**.

1&2. How can the internal sensation of a particular memory in response to a cue stimulus explained? (see above)
3. Since memory is associated with internal sensation of a conscious state how can they both be explained in an interconnected manner? (see Vadakkan, 2010; 2015)
4. Explain how the mechanism provides signals for a motor response at the same time? (see Fig.7 in FAQ section in this website)
5. Explain what learning-mechanism can lead to firing of set of CA1 neurons (Vadakkan, 2013, 2019a; & Fig.11 of FAQ).
6. How to explain formation of long-duration dendritic voltage signals and Ca2+ plateau potentials associated with learning changes in a single trial (Takahashi and Magee, 2009; Grienberger et al., 2014; Bittner et al., 2015)? (see below)

7. Explain features of the mechanism that qualify it as an evolved mechanism? (Vadakkan, 2020).
8. Is it possible to explain various features exhibited by the system at different levels of its operation? (see Vadakkan, 2019b).

Table 2. Answers to questions that a solution for the nervous system is expected to provide answers for in an interconnected manner. The IPL mechanism has provided interconnected explanations for large number of findings in the system and has generated several testable predictions (Vadakkan, 2019b).

Even though semblions from stratum lacunosum-moleculare and stratum radiatum layers appear to be occurring at two different locations, large extracellular waveforms such as theta that connects both these locations (see Fig.1 in Fernández-Ruiz et al., 2017) informs about a mechanism for integrating (binding) semblions from these two locations. Note: Small colored circles are dendritic spines participating in inter-postsynaptic functional LINKs (IPLs). Note: Colored circles are dendritic spines (postsynaptic terminals).

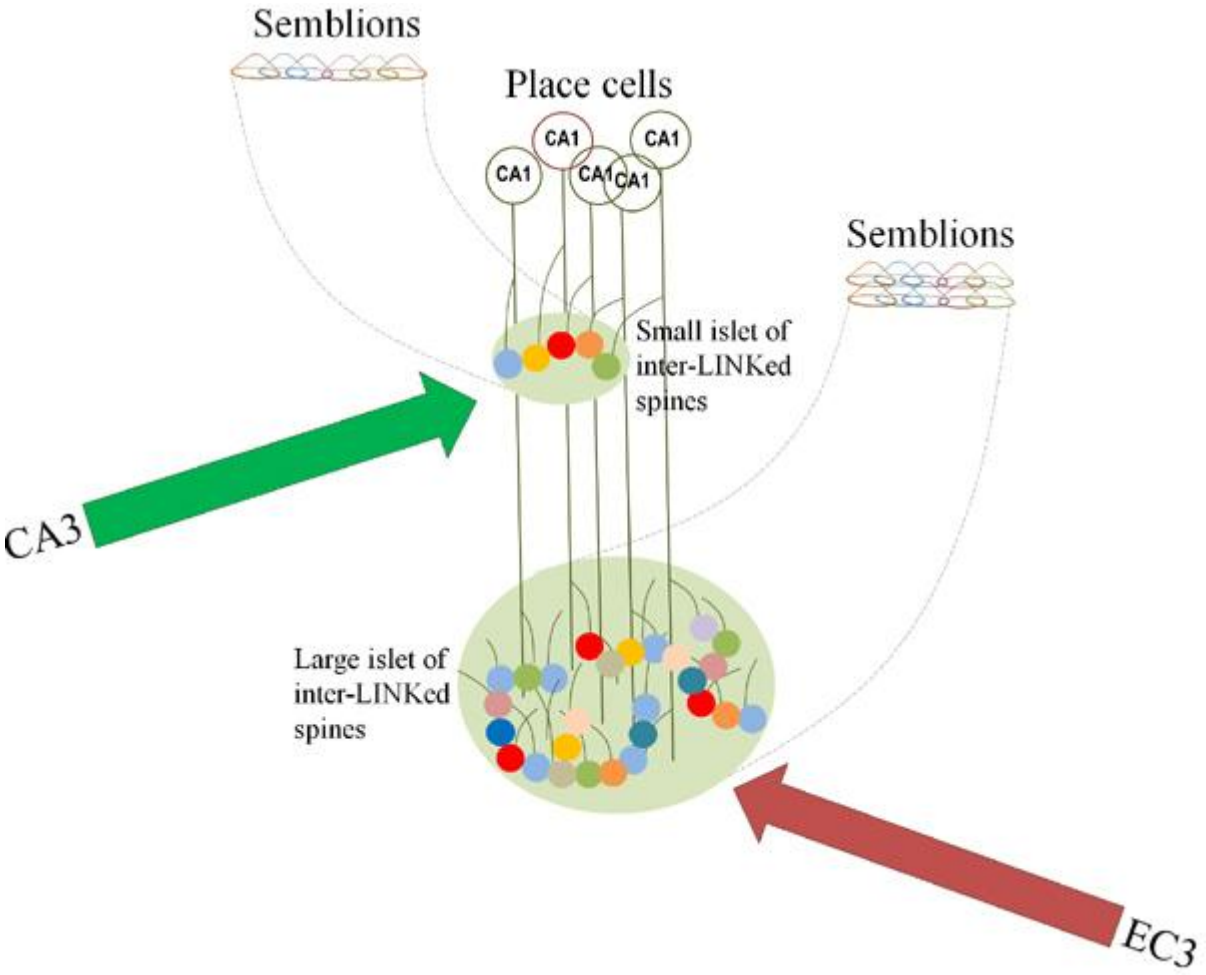

Figure 2. Figure showing how trisynaptic and monosynaptic pathways from entorhinal cortical (EC) neurons and a CA1 pyramidal neuron form islets of inter-LINKed spines in the stratum lacunosum-moleculare and stratum radiatum layers respectively. In this demonstration, only five CA1 neurons with one of their spines participating in the islets of inter-LINKed spines is shown. Also, inter-LINKed spines in one islet are expected to belong to different CA1 pyramidal neurons as indicated by different colors (This can vary. For example, in dendritic excrescences of CA3 neurons). Since dendritic arbor at the apical tuft region where EC3 direct input arrives is relatively bigger, it is reasonable to expect formation of large number of IPLs at this location. This may explain an increased horizontal component contributing to low frequency theta wavefsorms at this location. Even though semblions from stratum lacunosum-moleculare and stratum radiatum layers appear to be occurring at two different locations, large extracellular waveforms such as theta that connects both these locations (see **Fig.1** in Fernández-Ruiz

et al., 2017) informs about a mechanism for integrating (binding) semblions from these two locations. Note: Small colored circles are dendritic spines participating in inter-postsynaptic functional LINKs (IPLs). Note: Colored circles are dendritic spines (postsynaptic terminals).

Oscillating extracellular potentials show characteristic waveforms in these two layers (Fernández-Ruiz et al., 2017) reflecting the nature of spines that are involved in forming IPLs in these locations. Both amplitude and frequency of these wave forms can be explained in terms of vector components contributed by IPLs at these locations. Low frequency theta waveforms can also be explained as the net effect of the vector components contributed by IPLs in a larger area.
(see **Fig.1** in https://www.cell.com/neuron/pdfExtended/S0896-6273(17)30101-0 and extracellular recordings: https://www.nature.com/articles/nn.2894/figures/1). Large number of interneurons are present in the L-M region (Capogna, 2011) indicates presence of IPLs between their spines and spines of CA1 neurons modifying the qualia of internal sensations generated at this location. The net potential generated at the islet of inter-LINKed spines propagates to the axon hillock of the CA1 neurons, allowing some of the sub-threshold activated CA1 neurons to fire somatic action potential.

Firing of EC3 neurons followed by firing of CA1 neurons prompt one to infer involvement of EC3-CA1 synaptic changes. Inhibition of NMDA receptor channels at these synapses causes inhibition of both learning and memory retrieval. The involvement of synapses towards the formation and reactivation of IPLs can be interpreted as synaptic plasticity changes involving EC3-CA1 synapses. Since IPL mechanism can explain generation of inner sensations can occur concurrent with firing of CA1 neurons, it can be viewed as a better explanation. Large amplitude synaptic inputs delivered by EC3 axons in apical dendritic tree of CA1 (Megias et al., 2001; Steward and Scoville, 1976) can lead to the formation of IPLs between spines of different CA1 neurons located in the stratum lacunosum-moleculare layer at apical tuft region of CA1 neuron. Both increased probability and duration of plateau potentials (Takahashi and Magee, 2009; Bittner et al., 2015) can be explained in terms of propagation of potentials through islets of inter-LINKed spines formed

by large number of abutted spines stimulated. IPL reactivation provides additional potentials to sub-threshold activated CA1 neurons allowing them to fire an action potential.

Using CA1 neurons that fire, hippocampal maps were created to study their association for spatial memory performance (Dupppret et al., 2010), which led to the inference that accumulation of place fields is responsible for spatial learning. A pyramidal neuron that has thousands of input terminals can fire a somatic action potential when nearly 140 inputs signals arrive at any combination of input terminals (Eyal et al., 2018). Extreme degeneracy of input signals in firing a CA1 neuron makes firing of a CA1 neuron non-specific with respect to the location from where potentials arrive. Furthermore, large plateau potential anticipated to be generated by islet of inter-LINKed spines leads to more non-specificity of CA1 neuronal firing with respect to the input signals. Inhibition of CA1 firing by AP5 (antagonize NMDA receptors at the synapses of spines of CA1 neuron) or inhibitor of plateau firing CAV2-3 channel blocker SNX-482 occur due to inhibition of neurons. Since synapses are necessary for IPL mechanism to operate, chemicals that block synaptic functions will stop cognitive function and CA1 neuronal firing (place cell firing).

Conclusion

Many times, interpretations of experimental findings are carried out with the presupposition that a single neuron process information. What prompts such interpretations? A neuron having thousands of input terminals and receiving nearly any 140 input signals (less than this during temporal summation) can fire a somatic action potential (Eyal et al., 2018). Hence, the firing property of a neuron cannot be viewed as something that process information. But there is an information processing that must be occurring in the neighborhood of a neuron. Since there is extreme degeneracy of input signals in firing a neuron (Vadakkan, 2018), information processing mechanism is expected to take place in the vicinity of input terminals. It is necessary to ask basic questions to obtain a mechanistic explanation. The solution for the system should be able to explain how first-person property of inner

sensation of a place occur in the nervous system along with remaining features such as 1) optional concurrent behavioral motor actions, and 2) firing of CA1 neurons. Interaction between spines of different neurons separated by narrow extracellular matrix provides a solution for the system. EC3 inputs synapse with spines of CA1 neuron in the stratum lacunosum-moleculare layer at the dendritic apical tuft region. Since spines of adjacent pyramidal neurons overlap with each other, interaction between these spines is expected to occur to generate inner sensations. When these interactions contribute to additional potentials to a subthreshold activated pyramidal neuron, it fires a somatic action potential, which is being viewed as place cells.

The observation that CA1 firing is inhibited by inhibitor of plateau firing $Ca_v2.3$ Ca^{2+}channel blocker indicates that it is capable of blocking somewhere along the route from EC3 to CA1. Explanations for different phenomena indicated presence of IPLs between spines of different CA1 neurons. For example, $Ca_v2.3$ Ca^{2+} channels mediate epileptiform activity such as afterdepolarization, plateau potentials and exacerbation of low-threshold Ca^{2+} spikes resulting in seizure initiation and propagation (Wormuth et al., 2016). It was shown how IPL mechanism can explain seizure generation (Vadakkan, 2016). $Ca_v2.3$ Ca^{2+} channels in the presynaptic terminals are involved in LTP (Breustedt et al., 2003). It was previously explained how synapses are involved in IPL formation and reactivation to influence LTP (Vadakkan, 2019a). Furthermore, $Ca_v2.3$ Ca^{2+} channels have a highly organized spatial distribution with predominant expression in the proximal or distal dendrites (Westenbroek et al., 1995).

The IPL mechanism provided by semblance hypothesis can explain how “engram neurons” that are often seen as “representations” of memory and “synaptic plasticity” change. Conducting experiments to study interaction between abutted spines that belong to different neurons by the arrival of two associatively learned stimuli can be used to verify the presence of IPLs. GFP-reconstitution method across synaptic partners (GRASP) (Feinberg et al., 2008; Gordon and Scott, 2009; Fan et al., 2013; Macpherson et al., 2015; Shearin et al., 2018) was used to study synaptic connections. Trans-Tango (Talay et al., 2017) and TRACT (Huang et al.,

2017) were invented for anterograde trans-synaptic tracing. Retrograde trans-synaptic tracing was carried out using a method called BAcTrace, (Cachero et al., 2020). Recently, retro-Tango method of retrograde synaptic tracing was developed (Sorkaç et al., 2022). Since TRACT, trans-Tango and retro-Tango methods allow neurons to show synaptic partners, similar approaches can be utilized to develop inter-spine tracers to study IPL links between spines that belong to different neurons of one neuronal order. Explanations provided here are expected to motivate verification of interactions between spines belonging to different neurons.

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