Synapse-specific representation of the identity of overlapping memory engrams. Abdou K, Shehata M, Choko K, Nishizono H, Matsuo M, Muramatsu SI, Inokuchi K (2018) **Science. 360(6394):1227-1231.** Article

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Re-interpretation in terms of the IPL mechanism

Semblance hypothesis has provided a mechanistic explanation for both memory (inner sensation of features of the item/event whose memory is being retrieved) and motor action reminiscent of arrival of that item/event. Work by Abdou et al., has two implicit assumptions. One is that engram cells interconnect between memories and secondly, synapse-specific plasticity ensures the identity and storage of individual memories. The findings in this work need mechanistic explanations for both behavioral motor action (withdrawal of foot) and, if possible, for inner sensation of memory of foot shock. Following is an explanation of how the findings in this paper can be explained in terms of semblance hypothesis. Alternatively speaking, findings in this paper allow us to look at semblance hypothesis from a new angle.

In fear conditioning experiments, two stimuli are associated. Out of this one (foot shock) generates a motor response (foot withdrawal). The other one has no motor response. The one with motor response is called unconditioned stimulus (US). The one that does not trigger any motor response on its own is called conditioned stimulus (CS). Authors associated between US and CS. After learning when CS arrives, motor action in response to the US (that occurred prior to learning) takes place.

At this juncture, we would like to find an explicit answer to the questions a) where associative learning is getting stored and how does memories get retrieved, and b) how behavioral motor activity reminiscent of arrival of a stimulus whose memory is being retrieved.

We should understand them with such a clarity that we can explain them to an engineer who wants to replicate the mechanism in an engineered system. For this, the issue can be simplified as given in **figure 1**.

CS arrives through the auditory cortex (AC) & medial geniculate body. Medial division of the medial geniculate body (MGm or MGN) receives both auditory and somatosensory inputs (LeDoux et al., 1987; Bordi and LeDoux, 1994) and project to LA (LeDoux et al., 1990). Furthermore, it was verified that the US (foot shock) activates lateral amygdala neurons (Lanuza et al., 2008). Medical geniculae body is only a relay station for both auditory and foot shock stimuli without any interactions between them at this level. So, we need to imagine that AC-LA pathway is the auditory stimulus pathway and MGm-LA (MGN-LA) pathway is the footshock pathway. At the same time, we have to keep in mind that MGm (MGN) is the path through which AC connects to the LA. LA neurons receive inputs from both AC (sound) and MGm (foot shock). All the diagrams here refer to MGm path as MGN.

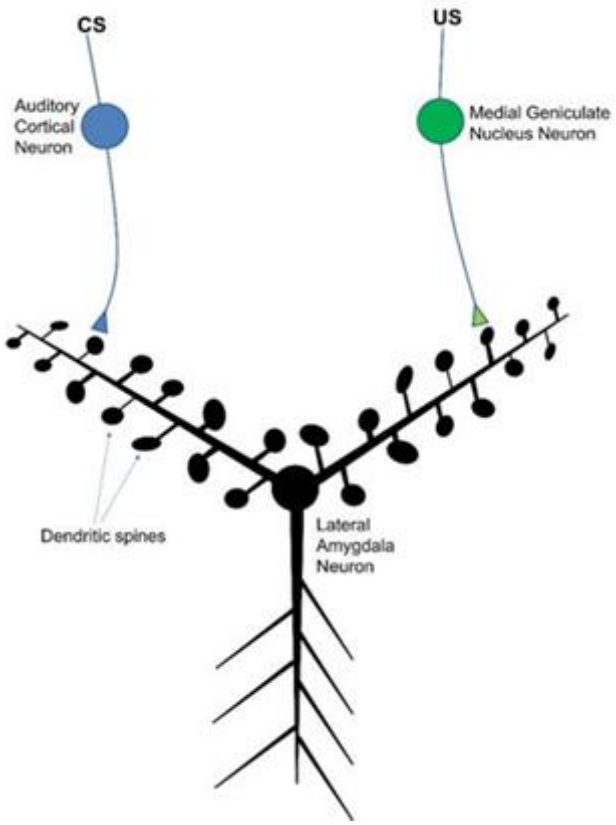

Figure 1. Conditioned stimulus (CS) arrives through neurons of the auditory cortex (ACN). Unconditioned stimulus (US) arrives through

neurons of the medical geniculate nucleus (MGN). They synapse with different spines on a lateral amygdala (LA) neuron. All three of the above neurons are referred to as engram neurons. The question is "After associative learning, when the CS alone arrives, how does it trigger motor response (foot withdrawal) as if it is receiving a foot shock?" To answer this, it is necessary to show at least some evidence for an interaction between the spines of LA that synapse with stimuli arriving through both ACN and MGN in the figure.

The first question is, “What is the mechanistic explanation for the firing of lateral amygdala (LA) neuron when US comes after the associative learning between US and CS?” Authors provide and implicit explanation that plasticity changes occur at the spines of LA neuron on which inputs from CS and US synapse. A mechanistic explanation needs to meet 2 requirements. 1) How does arrival of CS alone cause firing of LA neuron reminiscent of arrival of US? 2) How does arrival of CS generate an internal sensation of arrival of US? If a single explanation can provide answers to both these two questions, then there is a good chance that it can be found correct after verification. At this time, readers can have more questions. First, why can't the inputs from two associatively learned item be shown synapsing to two neighboring spines on one LA neuron as follows (**Figure 2**)?

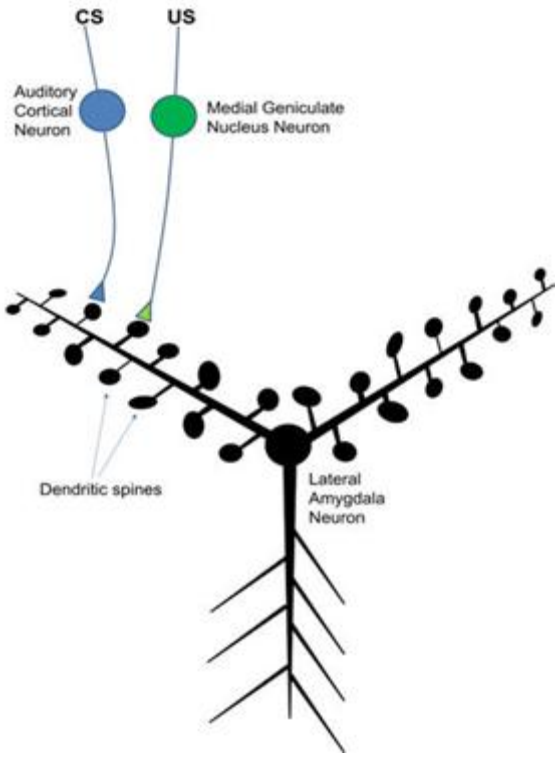

Figure 2. Inputs from two associatively learned stimuli arrive and synapse on to two neighboring spines on one LA neuron. Can this explain associative learning mechanism?

Looking at **figure 2**, we can ask the question, "Can it provide an explanation?" An interpretation of clustered plasticity along with synaptic tagging was explained previously by Govindarajan et al., 2006. The problems with this model are 1) there is no evidence for the formation of an electric cable between two neighboring spines, 2) there are no evidence for the formation of such a cable through the extracellular matrix space, 3) no evidence for the formation of specific tag molecules that can form in physiological timescales of milliseconds to explain function.

So, let us examine the underlying issue more closely by asking some questions.

1) How does arrival of CS cause firing of LA neuron reminiscent of arrival of US? An engineer will want to see at least the formation of a cable between the spines to which CS (ACN neuron) and US (MGN neuron) synapses. But there is no evidence for an electrical cable between them. Synaptic tagging was put forward as a mechanism (Frey

and Morris, 1998). But sufficient number of specific tag molecules that can form and act at physiological timescales are not found. The solution must provide a mechanistic explanation.

2) Since there is no evidence for a long-lasting direct interaction between two spines that receive CS and US on an LA neuron, the configuration given **Figure 2** is not compatible with the actual mechanism of learning. So, is there an alternative?

3) We can also ask, "How does arrival of CS generate an internal sensation of memory of arrival of US?" An ideal solution for the first question is expected to answer this question as well. Currently, we are not searching for a mechanism that generates inner sensations of memory due to several reasons that we can only assume. But an engineer who wants to replicate the mechanism will need to see a blueprint that explains a mechanism for this.

So, the question is, "How to move forward to provide an explanation for both behavioral motor action reminiscent of memory retrieval and the very process of memory retrieval itself?" Both these are intricately connected parts of a single mechanism. Hence, we need to find such a mechanism that will also allow us to explain how the brain can store very large number of associated memories that are associated with firing of neuronal ensembles. Results from the present paper demands that the mechanism should be able to explain how memories are stored apparently using the same neuron in such way that they can be distinguished from each other.

This needs a renewed approach. First, let us see how the dendritic tree is organized. Are the tree branches of a neuron similar to the tree branches of a tree in a forest? Tree branches of a tree in a forest usually don't overlap with each other so that the leaves can get maximum sunlight. But the dendritic arbor of neighboring neurons overlap intensely so that we cannot separate the arbor of one neuron from another one (**Figure 3**). It is important to note that the sister branches on a neuron's dendritic tree often avoid overlapping (Grueber and Sagasti, 2010).

Figure 3. Left side: Trees in a forest. The arbors of even closely located trees do not overlap with each other. Basic reason is that more trees beyond a limit cannot grow in forests due to limited sunlight that they can get. Right side: Drawing by Ramon y Cajal taken from "Comparative study of the sensory areas of the human cortex" page 363 showing Golgi-stained cortex of a 1.5-month-old infant. Cajal once referred to them as "impenetrable jungle". Here, we can see overlapping and intermingling of dendritic arbors of neighboring neurons. If we take a picture of the top view of the cortex to get a comparable view (like that of the forest), we won't be able to recognize any single neuronal arbor even if we paint each neuron differently. Note that Golgi staining stains only a small fraction of neurons! So, in reality, it is an "impenetrable jungle" (Figure on the left side is taken from Wikipedia). It is important to note that the sister branches on a neuron's dendritic tree often avoid overlapping (Grueber and Sagasti, 2010). To get a clear picture of the functional importance of overlapping dendritic arbors, we must ask, "A spine of one neuron abut with spines of how many other neurons?"

What does this indicate? This means that there can be some functional significance for this. Also, dendritic branches of neurons from different cortical layers overlap with each other since their apical tuft regions stay attached to the inner surface of the pia mater, which is the covering of the cortex. This happens due to the descent of neurons from the sub-pial region towards the ventricle during development. I have explained this in figure 1 in a paper (Vadakkan, 2016). The advantage for such an organization is that a spine from one neuron can be present in between spines of a second neuron. Now we can ask, "Is there a possibility for interactions between neuronal processes of different neurons?" Specifically, since spines are the projecting parts on the dendrites of a neuron, we can ask, "Is there a possibility for the spines of a neuron to interact with neuronal process of other neurons?" For the occurrence of some physical interaction between the spine of one neuron and one of the neuronal processes of another neuron, it is reasonable to expect some space between the neighboring spines on the dendrite, even though in a 3D space this is not a necessity. So, we can ask, "Is the inter-spine distance greater than spine diameter?". Yes, it was found that mean inter-spine distance is more than mean spine diameter (Konur et al., 2003).

Remember, the US is a stimulus that will evoke foot withdrawal by itself. CS is a stimulus that does not evoke foot withdrawal. So naturally, CS won't synapse to an LA neuron that is connected to lower motor neurons to cause foot withdrawal. Hence, a configuration where inputs from both CS and US synapsing to the same LA neuron may appear to beat the very purpose of conditioned learning. So, for now we must change the configuration/components of all the above figures (**Figures 1, 2 & 3**) such that the LA neuron that receives input from CS is a different neuron than the LA neuron that receives input from US for demonstration purpose. Once everything becomes explainable, we will come back and ask this question again. So, for now inputs arriving towards LA neurons should be as in **Figure 4**. We can also assume that in the background state, LAN1 neuron is being held at a sub-threshold activated state that will allow sufficient potentials from MGN neuron (by the foot shock) to fire it. Let us see if this works.

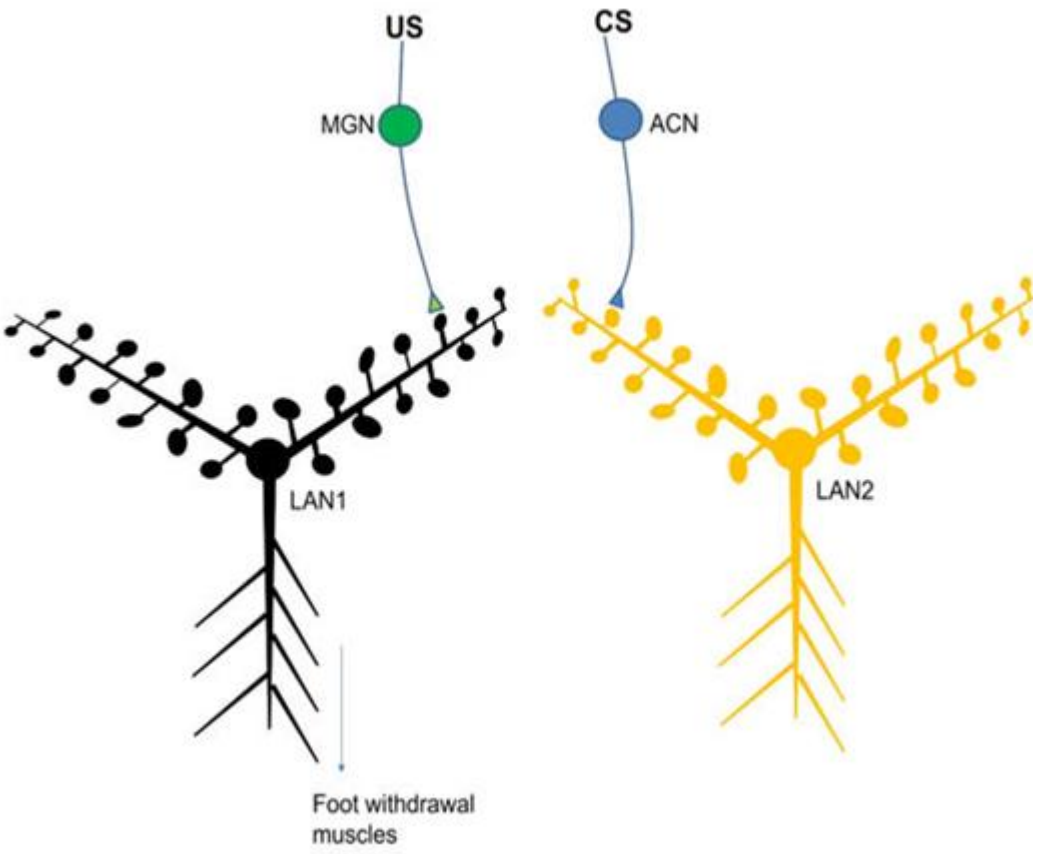

Figure 4. In this configuration, inputs from associatively learned stimuli arrive to two different LA neurons through AC and MG neurons, so that only US stimulus can result in foot withdrawal before learning. According to this configuration, LA neurons that receive inputs from AC alone will not fire those LA neurons so that there won't be any foot withdrawal.

Now, if the spines of one LA neuron are located in the inter-spine spaces of the other LA neurons, then an inter-neuronal inter-spine interaction called inter-postsynaptic functional LINK (IPL) is formed between spines that belong to two different lateral amygdala (LA) neurons during learning. After learning, when the CS arrives, it can depolarize the spine on the second LA neuron that propagated stimuli from the US before learning and cause foot withdrawal (**Figure 5**). This can explain how motor action takes place reminiscent of the arrival of US even though US does not arrive at that time. Furthermore, reactivation of IPL by CS will depolarize the inter-LINKed spine of LAN1 in the absence of arrival of any stimulus from US. This will generate

units of inner sensations (semblance) on the inter-LINKed spine of LAN1. For details, see FAQ section of this site.

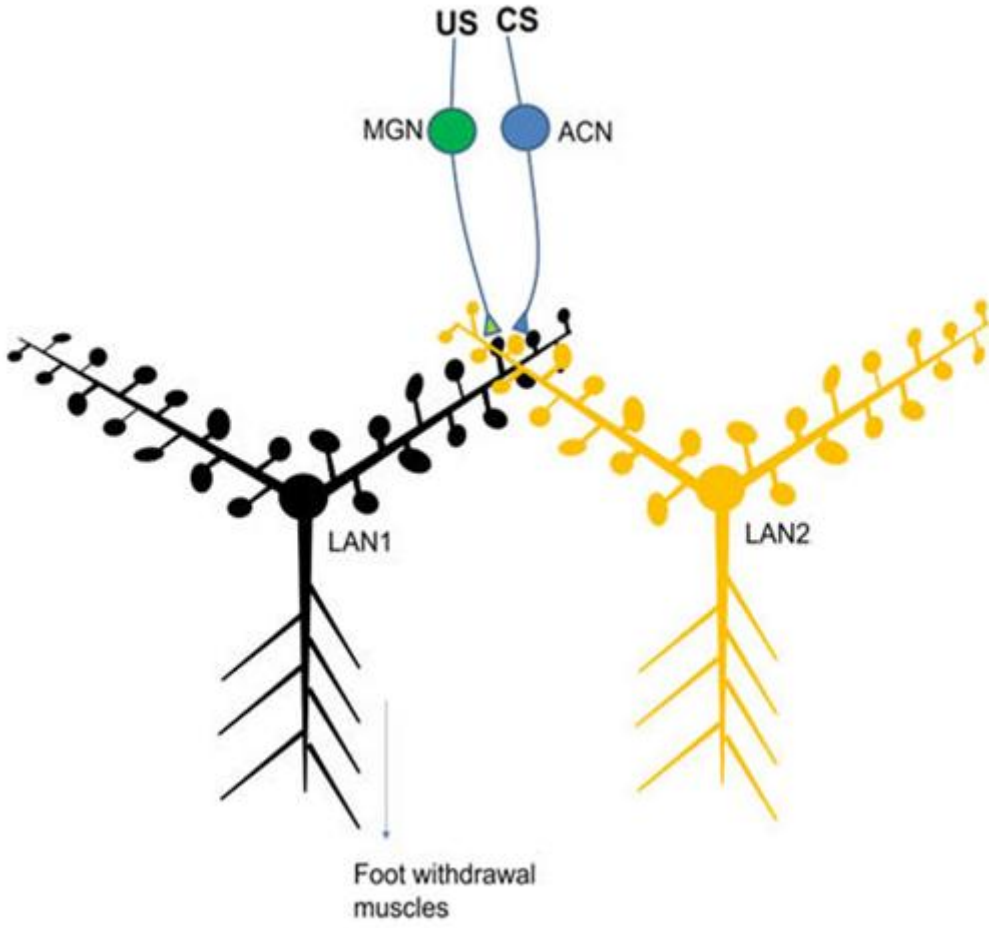

Figure 5. Formation of inter-neuronal inter-spine IPL. Here, output from only LAN1 causes foot withdrawal. After learning, arrival of CS to LAN2 results in propagation of depolarization of the spine of LAN2 through the IPL to the spine of LAN1 to which US arrived at the time of learning. This will cause firing of sub-threshold activated neuron LAN1 and cause foot withdrawal reminiscent of arrival of US. Also, the reactivation of spine of LAN1 through the IPL will result in inner sensation of arrival of US (even though US does not arrive). For details, see FAQ section. The fact that a) dendritic arbors of neighboring neurons overlap with each other, and b) mean inter-spine distance is more than mean spine diameter (Konur et al., 2003), provide an opportunity for the presence of abutted spines that belong to different LA neurons. If associative learning can remove hydration layer between the outer layer of neuronal cell membranes over their spines, then it can lead to an electrical connection between them. When CS arrives,

potentials will propagate through this connection and reach the LA (which is being maintained at a sub-threshold level) that can fire and result in a motor action (foot withdrawal) reminiscent of the arrival of US. For the duration of presence of inter-neuronal inter-spine connection, conditioned reflex can be elicited. The same electrical connection is referred to as inter-postsynaptic functional LINK (IPL) by semblance hypothesis. Reactivation of IPL by CS can explain generation of first-person inner sensation of memory of US (please see FAQ section of this website for details). ACN = Auditory cortical neuron. MGN = Medial geniculate neuron. LAN = Lateral amygdala neuron.

Now, one may ask a question, "Are there LA neurons with inputs only from CS (ACNs)?". The answer is "Not necessarily". LA neurons can have inputs from both CS and US. So contrary to what was said in the paragraph before **figure 4**, how does this work? Two conditions need to be satisfied. Before learning when CS arrives, LA neurons should not fire to generate foot withdrawal. Before learning when US arrives, LA neurons should fire to generate foot withdrawal. So, we can think of a configuration where an LA neuron receives inputs from both CS and US. The inputs from US provide more inputs (potentials) to allow the LA neuron to cross the threshold for firing; whereas inputs from CS provide very minimal inputs that will not allow LA neurons to fire. During learning, IPLs are formed between spines (that receive inputs from CS and US) that belong to different LA neurons. This will lead to the following change after learning - i. e. when CS arrives after learning, the stimuli will propagate through the IPLs formed during learning to generate foot withdrawal. It is reasonable to assume that after learning enough inputs will arrive from CS through several IPLs to one LA neuron that is held at sub-threshold activation state to cause its firing and lead to foot withdrawal. So **figure 5** can be modified as **figure 6**.

Effect of inducing LTP & LTD

A mechanistic explanation for LTP occlusion experimental results in the paper can be explained in terms of the explanation of LTP based on semblance hypothesis (Vadakkan, 2019). This is as follows. Based on semblance hypothesis, both addition and removal of vesicles at the

lateral borders of spines can increase and decrease membrane length respectively at these regions has an important consequence in the formation and reversal of IPLs. AMPA receptor exocytosis takes place at the lateral borders of spine head close to the synapse, a location where IPL formation is expected to take place.

In normal conditions, GluR1subunits of AMPA receptors are located on the spine membrane up to 25nm away from the synaptic junction (Jacob and Weinberg, 2015). This indicates a high probability that vesicles containing AMPAR subunits get exocytosed at this location and along with providing AMPAR subunits to form functional AMPARs, these vesicles increase spine surface areas at their lateral margins that can favor the formation of different types of IPLs. Studies have shown synaptic addition of GluR1 subunit–containing AMPA receptors during experimental induction of long-term potentiation (LTP) (Hayashi et al., 2000; Passafaro et al., 2001).

Since removal of membranes from lateral borders of spines is expected to occur during formation of vesicles for endocytosis, any endocytosis of AMPA receptors can reverse the IPLs. It is known that modest depolarization used in inducing long-term depression (LTD) causes AMPAR endocytosis (Lüscher and Malenka, 2012). Furthermore, it is known that modest depolarization by LTD stimulation protocols cause AMPA receptor endocytosis following activation of phosphatases that dephosphorylate AMPA receptors (Lüscher and Malenka, 2012). It was demonstrated that surface AMPARs are removed during induction of both NMDAR-dependent LTD (Beattie et al., 2000; Carroll et al., 2001), and mGluR-LTD (Waung et al., 2008; Park et al., 2008).

Fear conditioning generates large number of IPLs. So, between the stimulating and recording electrodes there are a large number of IPLs. Hence, LTD stimulation is expected to reverse some of these IPLs located between the electrodes. Since endocytosis of the AMPAR subunits and reduction in the size of enlarged spines can explain LTP decay (Dong et al., 2015), LTD can be explained in terms of reversal of existing IPLs in the brain tissue formed as a result of fear conditioning.

Effect of inducing autophagy

Effect of tat-beclin in erasing a memory can be explained as follows. Induction of autophagy by peptide tat-beclin leads autophagosome to fuse with endosome-lysosome system and degrades contents of the latter. This will lead to degradation of endosomes including those that contain AMPA receptors (glutamate receptor subtype with fast kinetics). When endosomes are formed and degraded continuously, IPLs will reverse back to form independent spines. This will result in failure to induce semblances when the cue stimulus (CS) arrives. Without further specific associative learning, these specific sets of IPLs will not be formed. This can explain how tat-beclin cause long-lasting memory erasure.

Independent storage of two memories in a shared ensemble of neurons

Authors used two types of CS (CS1 & CS2) to associate with one type of US. They have shown that erasing the association between one type of CS and US does not affect the association between second CS and US. Based on the semblance hypothesis, associations between different types of CS and US result in formation of specific IPLs. Autophagy results in memory destabilization and erasure of auditory fear memory associated with AMPAR endocytosis (Shehata et al., 2018). This can also lead to loss of membrane segments for endocytosis from the lateral borders of spines since GluR1subunits of AMPA receptors are located on the spine membrane up to 25 nm away from the synaptic junction (Jacob and Weinberg, 2015). This can lead to reversal of IPL formed with another spine of a second neuron. Reactivation of specific IPLs by a specific CS makes those IPLs vulnerable to reversal by endocytosis using membrane segments from lateral borders of the spines (how this occur in contrast to a resting IPL need to be studied) along with autophagy of the endocytosed vesicles containing AMPA receptor subunits.

Functional connectivity between engram cells (LA neurons). Where is it taking place?

Authors suggest the occurrence of a "functional connectivity" between engram cells. What contributes to this functional connectivity? How does the brain store enormous number of specific memories, retrieved in response to specific cue stimulus, in shared cell assemblies? Explanations of the findings in the paper by Abdou et al., were made based on the views that memories are formed by changes in synaptic plasticity and are stored in specific neuronal ensembles. Semblance hypothesis used inductive reasoning approaches to derive a mechanism that takes place during learning that has the potential for generating both behavioral motor actions and induce first-person inner sensation of memory. Based on this, inter-neuronal inter-spine functional LINKs are formed during learning and are reactivated during memory retrieval providing the functions. This mechanism operates in synchrony with the normal synaptic functions and neuronal firing.

Based on the semblance hypothesis, specific IPLs are formed between spines that belong to specific neurons during a specific learning. Depending on the specific features of a cue stimulus, a set of IPLs get reactivated. Those IPL reactivations that contribute to potentials allowing subthreshold neurons to cross the threshold will fire. These firing cells are called "engram cells". Along with this, IPL reactivations generate inner sensations (semblance) of memory of the associatively learned item. This takes place as a system property of systems where synaptic transmission and propagation of potentials across the IPL contribute vector components to the oscillating extracellular potentials. Note that the brain functions only during a narrow range of frequency of oscillating extracellular potentials. Thus, IPLs provide what the authors refer to as "functional connectivity" between assemblies of firing cells. Since large number of different IPL reactivations can independently contribute potentials to neurons that are being held at subthreshold states, it will provide an apparent view that large number of memories are stored using shared cell assemblies. Explanations provided here constitute a retrodictive (postdictive) evidence in support of the semblance hypothesis.

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