An indirect proof for the mechanism of memory storage proposed by the semblance hypothesis

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Since third person observers cannot directly access how first-person inner sensations are generated in another brain, it is necessary to hypothesize a mechanism and use indirect methods to test how the brain generates its functions. Associative learning is best studied using conditioned learning paradigms. In fear conditioning experiments, two stimuli are associated. In classical experiments, the one that generates a motor response is called unconditioned stimulus (US). The other one that has no motor response on its own is called conditioned stimulus (CS). When CS arrives after associative learning between US and CS, output response to both the CS and US (that occurred prior to learning) takes place (Fig.1). To understand the learning mechanism, it is necessary to know how the pathways through which CS and US propagate get connected during learning.

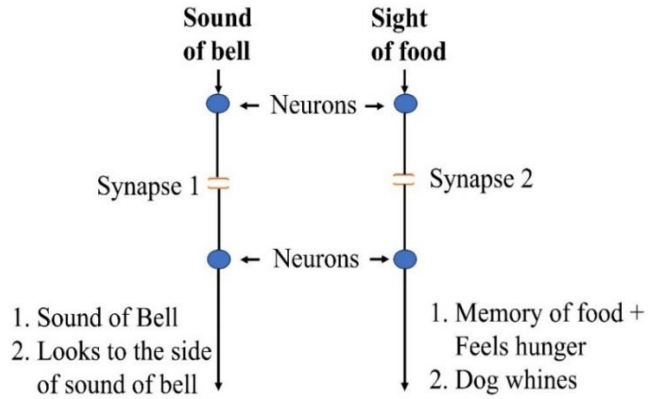

Figure 1. *Conditioned learning paradigm. Association between sound of a bell and site of the food is shown. After learning, the arrival of the sound of the bell alone is expected to generate the output features in response to both sound and food.*

Findings in a modified fear conditioning study

By keeping a) one of the stimuli in two conditioned learning events (foot shock), and b) the output lateral amygdala (LA) neurons that fire the same, a study (Abdou et al., 2018) used two different frequencies of sound (7 and 3 Hz) in two separate learning events. Erasure of associative learning between a specific frequency of sound (7Hz) and foot shock was carried out by injecting tat-beclin (tBC) to stimulate autophagy in LA neurons. This did not affect the association between the second frequency (3Hz) and foot shock. Authors infer that "Sharing of engram cells underlies the linkage between memories, whereas synapse-specific plasticity guarantees the identity and storage of individual memories." However, the solution needs a mechanistic explanation with the level of clarity that it can be replicated in engineered systems.

Constraints from Abdou et al., work

Specific findings in and constraints offered by a specific study (Abdou et al., 2018) are given in **Table 1**.

	Findings	**Author's inference**	**Constraints**
1	Shared set of neurons fire during two separate memory retrievals having shared output function.	Identity of intermingled memories are stored in a shared cell ensemble that fire.	A specific mechanism to store and retrieve different memories is expected to be present among the connections between them.
2	Complete retrograde amnesia (produced by autophagy in the output neuron) of one fear memory did not affect another linked fear memory.	Presence of synapse-specific representation of the identity of overlapping memory engrams.	Autophagy irreversibly abolishes storage mechanism of one memory. Since this action stops soon so that a second learning can be undertaken, it is a reversible action.

3	Optogenetic potentiation (LTP) or depotentiation (LTD) of input pathways as evidenced from motor actions (foot withdrawal) for one specific learning affected recall of only that memory and not the other.	Presence of synapse-specific representation of the identity of overlapping memory engrams.	The mechanism responsible for it should be taking place along or in between the routes through which optogenetic stimulation propagates and leaves a specific mark that can be used for memory retrieval.

Table 1. *Constraints from specific findings from Abdou et al., 's work (Abdou et al., 2018) that can be used to arrive at a testable mechanism of learning changes from which memories can be retrieved. LTP: Long-term potentiation. LTD: Long-term depression.*

Overcoming current challenges in solving the nervous system

First, be explicit about the existing issues in solving the system and then explain a methodology to overcome them. These are listed (**Table 2**).

Where is the missing gap in our current knowledge?	1. We need to discover a learning mechanism from which memories in their true nature as first-person inner sensations are generated. 2. We need to explain how this mechanism is connected with motor actions such movements and speech.# 3. It should be possible to interconnect this mechanism with a large number of findings from different levels of the system.
What were the previous proposals	1. Clustered plasticity model (Govindarajan et al., 2006). Since mean inter-spine distance is more than mean spine diameter (Konur et al., 2003) and since there are no cables/mechanism connecting these spines either

to overcome this gap?	through intracellular or extracellular routes, there is an explanatory gap. 2. Tagging of synapses with certain specific molecules (Fey and Morris, 1997). But the number of specific molecules needed, and a millisecond timescale operated mechanism are lacking.
What is needed for a new approach?	1. A mechanism that can both connect the inputs in **Fig.2**, and which is reactivatible in millisecond timescales is needed. In addition, this mechanism should have a unique property to explain how the foot withdrawal is accomplished when exposed to the CS along after learning. This mechanism should also have the ability to generate a first-person inner sensation of memory of the electric shock. 2. In **Fig.2**, inputs are arriving at the same LA neuron. But for the CS to manifest the output conditions of both CS and US the configuration in **Fig.1** must change. 3. We need to use findings from studies that used third person observations such as behavior and other laboratory findings to deduce a mechanism. 4. Once derived, we should be able to provide testable predictions that can be verified.
What is a possible solution?	A solution should be able to satisfy constraints from findings from different levels of the system. Inter-postsynaptic functional LINKs (IPLs) have succeeded in achieving this (**Fig.3**).
Why should this new approach be correct?	1. It can explain constraints from a very large number of findings from different levels of the system (see **Table 2** on the Home page of this website). 2. Normally, inter-membrane fusion is a very high energy requiring process. Hence, in the baseline state, elements of the system can remain unconnected, which is essential for circuit stability. 3. IPLs can form and get reactivated in milliseconds.

	4. IPLs are reversible (forgetting), stabilizable for different durations (explaining short-term and long-term memories). 5. There is a unique operational mechanism present at the inter-LINKed spines to generate hallucinations expected of a mechanism for memory (Minsky, 1980). 6. Propagation of potentials along the IPLs provides horizontal component for the oscillating extracellular potentials to manifest, whose frequency in a narrow range of frequency is essential for the normal operation of the system. 7. It operates in synchrony with the synaptically-connected neurons in the nervous system.

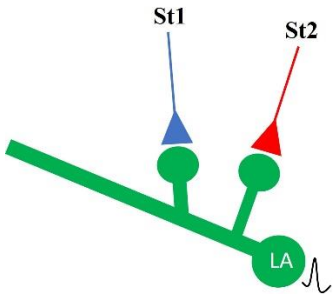

Fig.2. Conventional way used to conceive the mechanism. *Two associated stimuli (St1 and St2) arriving through two input terminals (blue and red) to two adjacent spines on a dendrite of one LA neuron. To associatively learn, a connection must occur between them in millisecond timescales and can be reactivated also in millisecond timescales.*

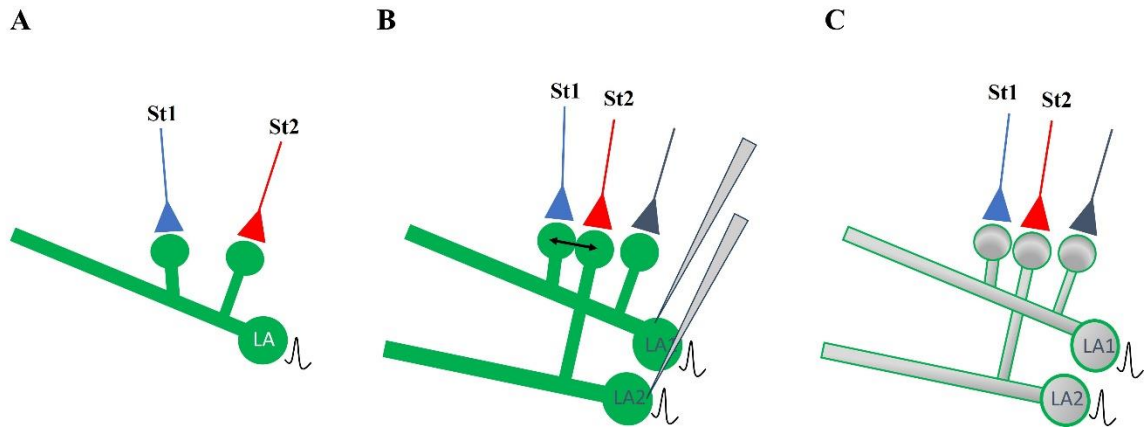

Figure 3. ***Figure showing missing link in the connectome***. *This has taken advantage of the possibility for the output function (foot withdrawal) to take place through more than one lateral amygdala (LA) output neuron.* ***A****) Conventional best possible scenario of two associatively learned input stimuli arriving to adjacent spines on a dendrite of an output LA neuron.* ***B****) During associative learning, inter-postsynaptic functional LINK (IPL) (shown by a double arrowed line) is formed between spines that belong to two neurons (when motor outputs in response to the input stimuli are different). In the case of fear conditioning, output function for different conditioned learning is the same (foot withdrawal). Hence, IPL formation can take place between spines on different dendrites of a single LA neuron (not shown here). Injection of tBC (shown from two pipettes in grey) to the LA neurons to stimulate autophagy is shown. The effect spreads to all its spines.* ***C****) Autophagy removes membrane segments from the lateral spine region, which in turn will reduce the spine diameter and will facilitate reversal of the IPL. When tBC reverses the IPL, it leads to erasure of a specific memory. Since it is possible to make a different associative learning after 5 hours, the effect of tBC is expected to reverse back.*

Can this solution provide first-person inner sensations of foot shock?

Normally, the head regions of dendritic spines are continuously being depolarized by quantally released neurotransmitter molecules from their

presynaptic terminals even during sleep. Occasionally an action potential arrives at the synapse triggering a postsynaptic potential. In this dominant state of continuous depolarization of the postsynaptic terminal (dendritic spine) resulting from the presynaptic terminal, reactivation of IPL by the arrival of the sound of a bell (CS) alone causes an incidental lateral activation of postsynaptic terminal of the synapse through which foot shock passed before. This will spark a cellular hallucination of a sensory stimulus of shock arriving from the environment through its presynaptic terminal, even though no such stimulus is arriving. Details of how qualia are determined are described previously (Vadakkan, 2013).

The above-described mechanism that can generate first-person inner sensation of memory as a hallucination (inner sensation of a stimulus in its absence) matches with the expectation of a mechanism for memory (Minsky, 1980). Furthermore, this configuration of learning-induced change permits all the requirements in **Fig.1.** Synaptic transmission through the synapses and propagation of depolarization along the IPLs contribute vector components of oscillating intracellular potentials among the network of neurons, which is reflected as extracellular oscillating potentials whose frequency needs to be maintained in a narrow range for the normal functioning of the nervous system.

How does autophagy operate to irreversibly erase the memory?

Stimulation of single spines in hippocampal CA1 pyramidal neurons induces the selective enlargement of stimulated spines (Matsuzaki et al., 2004). CA1 cells that receive inputs from CA3 engram cells specifically exhibit increases in both spine volume and density (Choi et al., 2018). Spine enlargement can be viewed as a prior step for facilitating IPL formation as proposed by the semblance hypothesis. The corollary/reverse is also true. Any procedure that leads to a reduction in the size of spines that are inter-LINKed through an IPL can lead to reversal of that IPL.

AMPA receptors (AMPARs) are fast kinetic glutamate receptor subtypes that are formed from different subunits. Translation of AMPAR subunit mRNAs generates corresponding proteins that are inserted to the membranes via vesicles. These inserted AMPAR subunit proteins undergo lateral diffusion along the membrane, reassembled to form functional AMPARs, and move towards the synapses (Opazo and Choquet, 2011; van der Sluijs and Hoogenraad, 2011). AMPARs undergo both constitutive and activity-dependent translocations from the postsynaptic membrane to the cytoplasm via endocytosis and return to the postsynaptic membranes in vesicles (Luscher et al., 1999; Ehlers, 2000; Lee et al., 2004 Henley et al., 2011; Anggono and Huganir, 2012). AMPARs diffuse laterally away from the postsynaptic terminal and are endocytosed at specialized endocytic zones on the plasma membranes adjacent to the post-synaptic density (PSD) (Lu et al., 2007; Opazo and Choquet, 2011). These endocytosed AMPARs either get targeted for degradation in lysosomes or they get recycled back to the plasma membrane.

Induction of autophagy by tBC leads autophagosome to fuse with endosome-lysosome system and degrades contents of the latter including those that contain AMPARs. When degradation of endosomes is stimulated, it can promote formation of vesicles transporting the AMPAR subunits from the spine membranes to the cytoplasm. This augmented endosome formation will need membrane segments from the lateral spine region (**Fig.4**). This will result in a reduction in the lateral spine dimension, which will lead to reversal of IPLs formed during learning. This results in reversal of inter-LINKed spines back to independent ones. With the reversal of IPLs, arrival of one of the associatively learned stimuli (CS) will not be able to generate first-person inner sensation of memory as described before. This explains how tBC irreversibly erases memory.

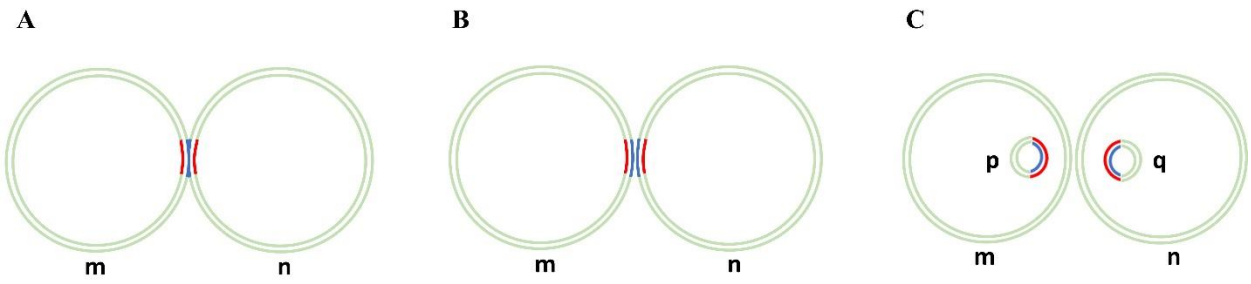

*Figure 4. Figure showing how endocytosis will cause reduction in the size of the dendritic spine and reverse newly formed IPL. **A**) Cross section through two dendritic spines that are inter-LINKed to form a hemifused structure. **B**) Membrane segments invaginate from the spine membranes to form endosomes. In this process, the circumference of the spines reduces pulling the IPLs to separate. Here the hemifused membranes reverse back to the stage of abutted membranes. **C**) When the endosomes are formed by using membrane segments from the spine membranes, IPLs completely reverse back to form independent spines. Note that endosome membranes are made of part of the membrane region that was forming the IPL in figure A. Red: inner membrane segments of the spines become outer membrane segments of the endosomes. Blue: outer membrane segments of the spines become inner membrane segments of the endosomes.*

How do LTP and LTD of specific input pathways related to specific memory?

While on protein synthesis inhibitors, induction of optical long-term potentiation (LTP) by stimulating terminals of specific AC and MGN engram cells (neurons that fire during memory retrieval) responsible for a specific associative learning (that used 7Hz sound) allowed these mice to completely recover from amnesia to the control group's freezing level. Similar results were also obtained in a previous study (Ryan et al., 2015). This shows that a non-protein synthesis dependent mechanism is responsible for learning and that mice can retain this change for a period after learning during which memory remains labile. The IPL mechanism is suitable to describe this.

In the mice treated with both tBC and protein synthesis inhibitor, optical LTP showed only a slight increase in the freezing level that was similar to that which occurred in the unpaired control group. Failure to reinstate the memory by optical LTP in tBC treated mice can be explained as follows. tBC reverses back all the learning generated IPLs. Even optical LTP of input engram cells after tBC treatment can only generate many new non-specific IPLs that can only generate non-specific semblances, which will not result in any specific memory.

When only modest energy is applied at the stimulating electrode, it leads to modest activation of NMDA receptors (glutamate receptors with slow kinetics) that trigger LTD (Malenka, 1994). In contrast to exocytosis of AMPAR subunits during LTP induction using strong depolarization of spines, modest depolarization used in LTD cause AMPAR endocytosis (Lüscher and Malenka, 2012). This shows that high energy used in LTP leads to the generation of large number of IPLs most likely by spine expansion that incorporates membrane segments from AMPAR exocytosis (Vadakkan, 2019). Similarly, endocytosis during LTD reverses IPLs and leads to memory erasure (**Fig.4**).

Conclusion

Many scientific problems have been solved by using indirect methods. For example, we cannot directly visualize DNA in solution. So, we use indirect methods such as the ability of DNA to bind with ethidium bromide, which in turn is visible under UV light. Sometimes we need to use indirectly-indirect methods to obtain evidence. The present work shows a retrodictive evidence for the semblance hypothesis. Several other retrodictive of evidence for the hypothesis is already been presented (**Table 2** of the Home page of semblancehypothesis.org). Testable predictions put forward by the hypothesis will help us to test its veracity. Even with all these, single evidence against this hypothesis will constitute sufficient reason for its rejection.

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