Testable predictions by the semblance theory and how to test them?

- **A short description** (A detailed version will be posted here soon. For more information: www.semblancehypothesis.org)

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Testable predictions are expected from any causal mechanism for a phenomenon [Douglas, 2009]. To understand the operational mechanism of the nervous system, it is necessary to derive a mechanism using constraints from large number of observations from different levels of the system operations. It will then become possible to make testable predictions that can be verified. This is a standard procedure in physics to understand particles and fields that we cannot sense directly through our sensory systems. To understand the operational mechanism of the brain that generates first-person inner sensations of higher brain functions such as perception and memory, it is necessary to make theoretical derivation of an operational mechanism and then test its predictions. Since it was possible to derive an operational mechanism that satisfies all the above requirements, semblance hypothesis can now be considered as a biological theory of brain functions. The major next step is to test its predictions. The following are the testable predictions made by this theory and a brief description of methods that can be used to verify them.

1. **Highly reversible initial stage of inter-postsynaptic functional LINKs (IPLs).** Dendritic spines of different neurons are electrically isolated from each other. IPL occurs when electrical connection between them is established by the removal of a thin layer of water (hydration exclusion) of extracellular matrix space between them. IPL (**Fig.1A, B**) is expected to be of only few squire nanometers in size between the dendritic spines and are expected to reverse back after few seconds.

   How to test them? Total surface area of dendritic spines ranges from 0.61 to 3.14 μm^2 [Wilson et al., 1983]. Associative learning is expected to generate IPLs between spines that belong to different neurons where the associated stimuli converge. Since hydration exclusion reverses back quickly, first electrical connection that is established temporarily between spines that belong to different neurons can be carried out. By simultaneous recording from two neurons whose spines may take part in IPL formation, it will become possible to estimate the electrical connection and its decay over few seconds. Then, it is necessary to find a microscopic method to inspect the physical interaction between the spines by hydration exclusion.

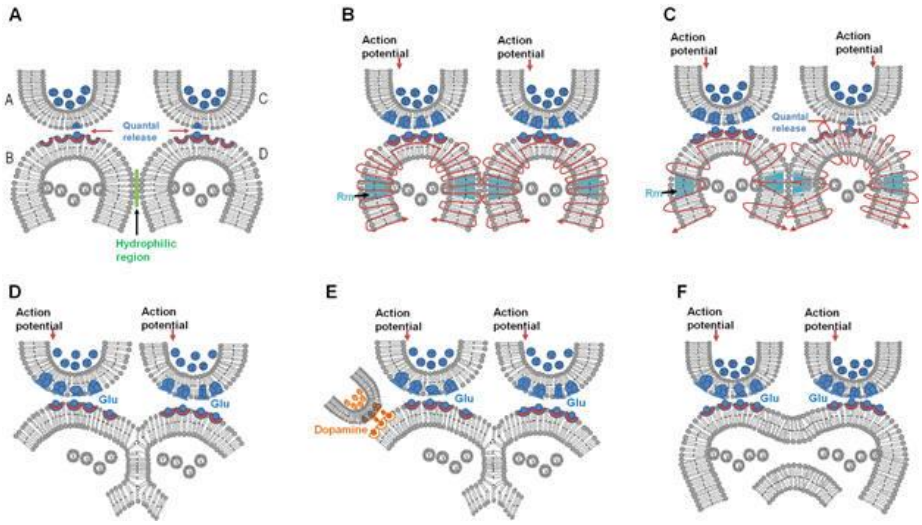

Figure 1. Different types of reversible inter-postsynaptic functional LINKs. **A**) Two abutted synapses A–B and C–D. Presynaptic terminals A and C are shown with synaptic vesicles (in blue color). Postsynaptic terminals (dendritic spines or spines) B and D have membrane-bound vesicles marked V containing subunits of AMPA receptor inside them. Action potential arrives at presynaptic terminal A releasing a volley of neurotransmitters from many synaptic vesicles inducing an excitatory postsynaptic potential (EPSP) at postsynaptic terminal B. From the presynaptic terminal C, one vesicle is shown to release its contents into the synaptic cleft. This quantal release is a continuous process (even during rest) that leads to the generation of very small potentials on postsynaptic membrane D. Note the presence of a hydrophilic region separating postsynaptic terminals B and D. When an action potential arrives at presynaptic terminal A, it activates synapse A–B and generates an EPSP at postsynaptic terminal B. The hydrophilic region prevents any type of interaction between postsynaptic terminals B and D. Very high energy is required for excluding the inter-postsynaptic hydrophilic region (Martens and McMahon 2008). **B**) Membrane expansion occurring at physiological time-scales can provide sufficient energy to exclude the inter-postsynaptic hydrophilic region, allowing close contact between the postsynaptic membranes in this region. This forms a transient inter-postsynaptic LINK that lasts only for a short period of time. During this short period of time, a cue stimulus-generated action potential arriving at synapse A–B reactivates this inter-postsynaptic functional LINK and spreads to postsynaptic terminal D and induces units of internal sensation at the inter-LINKed postsynaptic terminal D. This can explain working memory. **C**) Diagram showing formation of a partial inter-postsynaptic membrane hemifusion. These vesicles contain glutamate receptor subtype 1 (GluA1). Activity arriving at the synapse can lead to exocytosis of vesicles containing AMPA GluA1 receptor-subunits abutted to the cell membranes and expansion of the postsynaptic membrane at physiological time-scales. During exocytosis, the vesicle membrane gets incorporated into the postsynaptic membrane at locations of exocytosis making this region of the membrane highly re-organizable. This matches with the location where AMPA receptor subunits were shown to concentrate at the extra-synaptic locations extending up to 25nm beyond the synaptic specialization (Jacob and Weinberg 2014). Note the interaction between the outer layers of membranes of the postsynaptic terminals. Depending on the lipid membrane composition, the process of close contact between the membranes described in the above section B) can get converted to a partial hemifusion state. **D**) Stage of partial hemifusion can progress to complete hemifusion. The reversible partial and

complete hemifusions are short-lived and can explain the necessary learning-induced changes responsible for short-term memory. Some of the hemifusion changes can get stabilized for different lengths of time. For example, insertion of a transmembrane protein across the hemifused segment can maintain the inter-postsynaptic LINK until this protein gets removed. These changes can be responsible for long-term memory. **E**) Dopamine is known to facilitate motivation-promoted learning. In this diagram dopaminergic input to postsynaptic terminal B results in its expansion, which will augment inter-postsynaptic LINK formation. This can explain the action of dopamine on learning. Furthermore, it can sustain the hemifused LINK for a long period of time, which may facilitate its stabilization. **F**) Hemifusion can advance to a complete fusion state in pathological conditions and it depends on several factors. Fusion of the postsynaptic terminals between two different neurons can lead to cytoplasmic content mixing and cytotoxic cell response. These include dendritic spine loss and eventually triggering of apoptosis leading to neurodegenerative changes. Note that excessive dopamine can lead to excessive expansion of the postsynaptic membrane and can lead to membrane fusion if other factors that resist this get compromised. Rm: membrane segment marked in Turkish blue shows area where membrane reorganization occurs (Figure modified from Vadakkan KI (2015a, b).

2. **IPLs formed by partial and complete hemifusion.** Some of the IPLs formed by hydration exclusion are expected to undergo partial and complete hemifusion (**Fig.1 C, D**).

   How to test them? This stage of IPLs are expected to last for a long duration. Even though many of them are reversible, they can be observed by using high-resolution electron microscopic images. For example, suspected regions of hemifusion are marked in the following diagram (**Fig.2**). Research work along this line can be expanded.

3. **Stabilized hemifusion stage of IPLs.** These are expected to be present by different mechanisms [Vadakkan, 2016b]. One mechanism is formation of islets of inter-LINKed spines where many of the inter-LINKed spines within these islets will be activated regularly. This will maintain stabilization between the spines. In other cases, where isolated inter-LINKed spines are present, there will be some robust mechanisms to stabilize the inter-spine hemifusion. These include presence of special transmembrane proteins.

   How to test it? Presence of islets of inter-LINKed spines can be verified by electron microscopy. Modification of Golgi staining protocols can be undertaken to observe the presence of these islets.

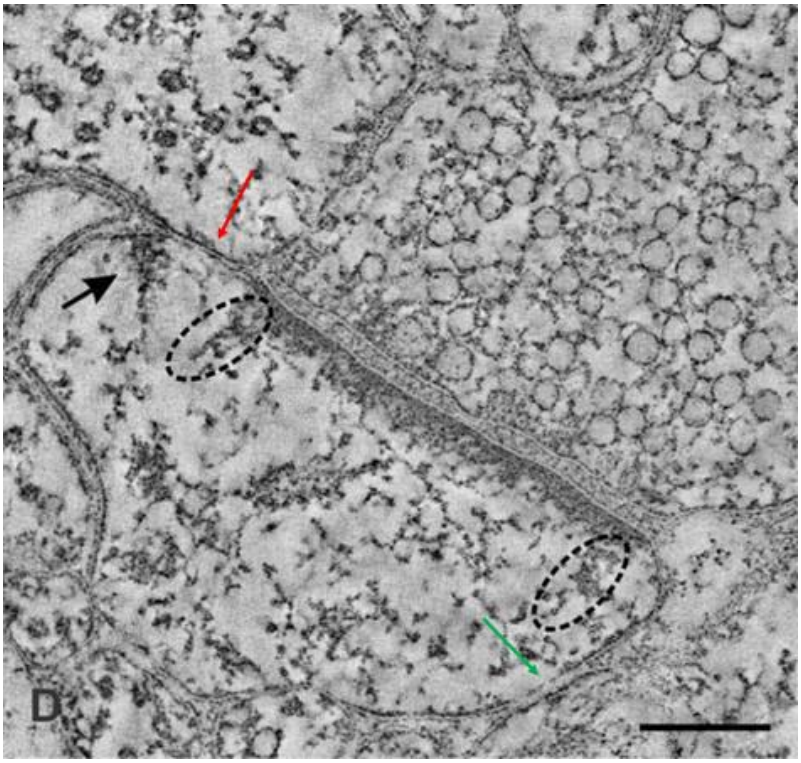

Figure 2. This is Figure 4D from Burette A.C, Lesperance T, Crum J, Martone M, Volkmann N, Ellisman M.H, and Weinberg RJ (2012) Electron Tomographic Analysis of Synaptic Ultrastructure. Journal of Comparative Neurology 520 (12): 2697-2711. This figure is modified by inserting a red arrow. The red arrow points towards a likely inter-postsynaptic area with only 2 layers of membrane instead of the expected 4 layers. Even though tissue distortions during tissue processing and folded membrane are possibilities, such changes that can span for distances of only 100 nm is very unlikely. This observation indicates the possibility that it is an area of inter-postsynaptic membrane hemi-fusion. It needs further dedicated studies for verification. The green arrow points to a likely location where the close contact between the membranes is visible. Since some of the cell processes are likely astrocytic pedocytes, dedicated studies are required to verify these observations. Scale bar = 100nm.

3. **Inter-membrane interactions take place in real time.** Since inter-membrane interaction are expected to take place, it will be possible to demonstrate real-time interaction between membranes of spines that belong to different neurons.

   How to test it? Injecting different neurons, whose spines can undergo IPLs, with different lipophilic fluorophores to stain their membranes [Floyd et al., 2008] followed by associative learning is expected to demonstrate IPL formation [Vadakkan, 2013].

4. **A robust mechanism by specific SNARE proteins**. SNARE proteins such as Q-SNAREs are expected to arrest membrane fusion at the stage of membrane hemifusion by interactions with proteins such as complexin, syntaxin-3, or other postsynaptic proteins [Vadakkan, 2016b].

   How to test them? These can be carried out by both electrophysiological and dye diffusion experiments.

5. **Different types of IPLs are expected to form following LTP stimulation.** A reversal of this process is expected to occur during the reversal phase following LTP induction [Vadakkan, 2019].

   How to test them? This can be demonstrated using the above-mentioned experiments before and after LTP stimulation.

6. **Strength of LTP depends of inter-neuronal inter-spine interaction.** For a specific distance between the stimulating and recording electrodes, strength of LTP induced at different locations will depend on the number of inter-spine LINKs formed during the delay time after stimulation [Vadakkan, 2019].

   How to test them? This can be demonstrated by correlations between spine density at locations between the stimulating and recording electrodes and the strength of LTP at different locations.

7. **Kindling is expected to generate inter-spine fusion.** Kindling is expected to generate inter-spine fusion in the synapse-rich area between the electrodes [Vadakkan, 2016]. (**Fig.1 F**).

   How to test it? This can be tested by injecting different fluorophores into the soma to stain the membranes of different neurons [Floyd et al., 2008] whose spines are expected to interact to form IPL fusion. Kindling is expected to cause dye diffusion as demonstrated previously [Colling et al., 1996]. Imaging techniques can be used to demonstrate the path through with such dye diffusion occur between the fused spines that belong to different neurons.

8. **Generation of internal sensations are dependent on a narrow range of frequency of oscillating extracellular potentials**. Both associative learning and retrieval of memories take place only during a narrow range of oscillating extracellular potentials.

   How to test it? Trying to invent methods to change the frequency of oscillating extracellular potentials in the olfactory glomerulus in the fly *Drosophila*. Based on the IPL mechanism, this will be possible only by disturbing the function of IPLs. Hence, if we succeed in changing the frequency of oscillating extracellular potentials, it is expected to alter smell perception [Vadakkan, 2015].

9. Internal sensations will be generated in artificial systems that operate according to the principles of the semblance theory.

   How to test it? Make circuits and assemble large number of units to test for the occurrence of internal sensations within them [Vadakkan, 2012; 2014].

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