How thick is extracellular matrix (ECM) space that is supposed to insulate between neurons & what does it prompt us to think?

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Any electronic circuit should be insulated from contacts with neighboring circuits. Even though propagation of depolarization is different from electric current, it is necessary for the extracellular matrix (ECM) surrounding neuronal processes to prevent any ephaptic transmission between them. To get an idea of the importance of insulating circuit components, let us have a look at a printed circuit board (PCB) used in electronic instruments. A PCB is a non-conductive plate that electrically connects components through conductive tracks etched on it. In brain, the job of PCB is carried out by the very thin (often negligible) ECM between cellular processes (**Fig.1**). How does ECM faithfully fulfill its functions? Does the thin nature of ECM provide any functional advantages that we can think of?

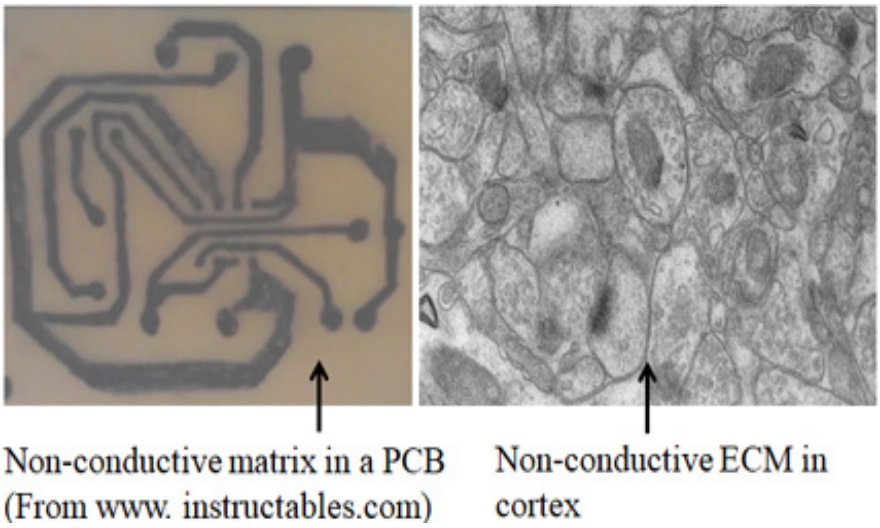

Figure 1*. Difference between a printed circuit board (PCB) and brain. The major difference is that in a PCB, electrical paths that connect electronic components are separated from each other by large area of non-conductive (insulating) material. But in the brain, neuronal processes are separated by very thin (& often negligible) insulating medium of extracellular matrix (ECM).* ***Left side****: A printed circuit board made of a non-conducting plate on which conductive tracks are etched to connect the circuit components. Note that the surface area of non-conducting plate that does not have the conductive tracks is roughly more than 80% of the surface area of this plate.* ***Right side****: An electron microscopic image from the brain cortex. Note that neuronal & glial cell processes occupy most of the surface area with only very negligible insulating ECM space in between them. Note that while acting as an insulting medium, ECM also has two additional functions. 1) Acts as a buffer zone that facilitates ion flux across membranes. 2) Brain functions occur only in a narrow range of frequency of oscillations of potentials within ECM that spans throughout the cortex. It is to be noted that the negligible ECM has to function very faithfully as an insulating medium without causing spread of depolarization to non-targeted neuronal processes. According to the IPL mechanism, the negligible ECM has an added advantage in forming IPLs between abutted spines. Note that even though it may seem easy for*

forming an IPL between abutted spines, very high energy is required to displace the hydration water between two lipid membranes (Cohen and Melikyan, 2004; Martens and McMahon, 2008). Furthermore, ***since the repulsive "hydration force" increases steeply when distance between the two bilayers reduces below 20 Å, fusion between two membranes becomes a very high energy requiring process*** *(Rand and Parsegian, 1984; Harrison, 2015). Negligible ECM also emphasizes the importance of maintaining the expected adaptation that prevents conversion of all IPLs formed during life not to progress towards IPL fusion (Vadakkan, 2020).*

What does the thin ECM prompt us to think?

ECM act a buffer zone of ions that facilitates ion flux across membranes during propagation of depolarization, which also form part of the oscillating extracellular potentials that is being maintained in a narrow range of frequency, which is necessary for all the brain functions. The most striking feature of ECM in cortex is its thin space occupied by it (**Fig.1**). But the fact that it acts as a robust insulating medium by virtue of the need for very high energy for establishing electrical connectivity (Rand and Parsegian, 1984; Cohen and Melikyan, 2004; Martens and McMahon, 2008; Harrison, 2015) between neuronal processes by displacing fluid ECM between them offers functional advantage. If learning generates lipid membrane changes in millisecond time-scales that can overcome the energy barrier, then establishing inter-neuronal electrical continuity can form a robust learning mechanism. According to semblance hypothesis (Vadakkan, 2019), IPL mechanism has this advantage. Furthermore, from studies using artificial membranes (Leikin, 1987), it can be inferred that the area of inter-spine hemifusion is likely to be restricted to approximately $10nm^2$ or even less. Astrocytic pedocytes are present only around 50% of synapses, which too are restricted to only 50% of the perisynaptic space (Ventura and Harris, 1999). Except this restriction, the available surface area of ECM where IPLs can be established is very large. It provides a huge advantage for the operation of the system. The high energy requirement for establishing physical interaction between lipid membranes guarantees that there won't be any non-specific interactions between neuronal processes that lead to electrical continuity between them. **It also informs us that learning must be triggering a biological mechanism to overcome the high energy requirement in millisecond time-scales.**

What molecular mechanisms may be etching information in the thin ECM?

Experimental evidence suggests the role of proteins SNARE and complexin (Vadakkan, 2019) in overcoming this high energy barrier. SNARE proteins are known to provide energy for bringing together membranes against repulsive charges and overcome energy barrier related to curvature deformations during hemifusion between abutted membranes (Oelkers et al., 2016). They also generate force to pull together abutted membranes as tightly as possible (Hernandez et al., 2012). By initiating the fusion process by supplying energy (Jahn and Scheller, 2006), SNARE proteins can lead to the formation of characteristic hemifusion intermediates (Lu et al., 2005; Giraudo et al., 2005; Liu et al., 2008). These properties of SNARE proteins highlight their functional significance in forming hemifusion intermediates between lateral spine head regions of spines. Furthermore, protein complexin present within the postsynaptic terminals (Ahmad et al., 2012) is known to interact with the neuronal SNARE core complex to arrest fusion at the stage of hemifusion (Schaub et al., 2006).

Now, the question is, "Between which two membranes do the hemifusion occur in the postsynaptic terminals (dendritic spines)?" In the presynaptic terminal there are several synaptic vesicles docked to the membrane that faces the synapse. So, there one can assume that they are hemifused with the membrane of the presynaptic terminal. But in the spines, there are no reports of docked vesicles with its membranes. So the question is "Where do the proteins SNARE and complexin act to generate hemifusion intermediates in the spines?" Detailed electron microscopic & nanometer-scale real time studies are needed here to test for inter-spine interactions that range from mere contacts between membranes to partial and complete hemifusion between membranes of spines that belong to different dendrites. Based on the present work, it is expected that inter-spine interactions occur between abutted spines where sensory stimuli converge during learning (or when they are stimulated together) within millisecond time-scales. Since working memory lasts only for a few seconds, most of them are expected to reverse back. However, since animals have already associatively learned many items and events in the environment, is reasonable to expect several stable hemifused areas between spines at locations of convergence of stimuli. Hence, they can be detected relatively easily. Dendritic excrescences present on the dendrites of CA3 neurons provide hints to this and they can be examined to verify presence of hemifused membranes between spines within them.

When an animal moves through the environment, it receives several new associated stimuli. As a result, a large number of IPLs is formed at locations of convergence of signals from those stimuli. What is most interesting is that most IPLs will reverse back within a few seconds. This is expected since majority of our memories of events during day-to-day life are working memories & last only for a few seconds. **Since SNARE-mediated vesicle fusion at the presynaptic terminal takes place within milliseconds, occurrence of a SNARE-mediated IPL formation of similar time-scales is anticipated during learning.** How long these IPLs remain determines whether they will be available for reactivation to generate internal sensation of memory at the time of arrival of a cue stimulus.

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