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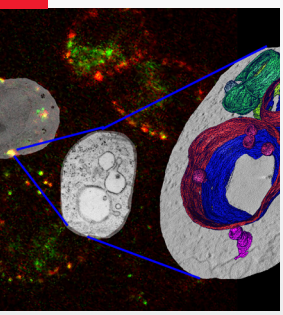

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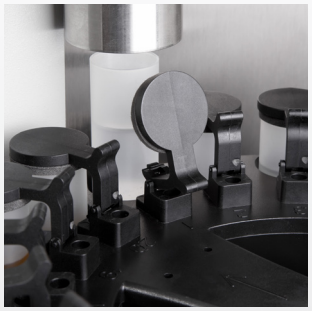

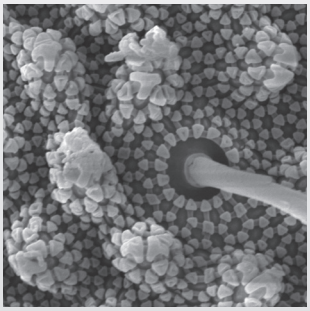

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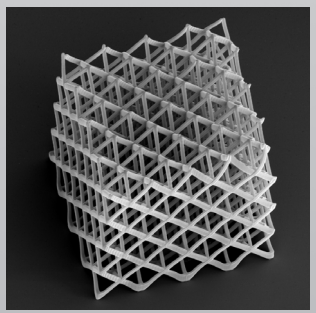

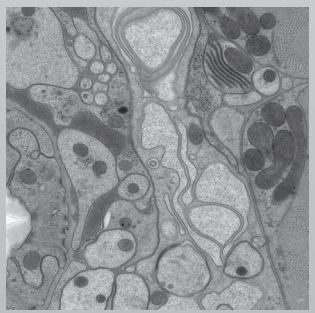

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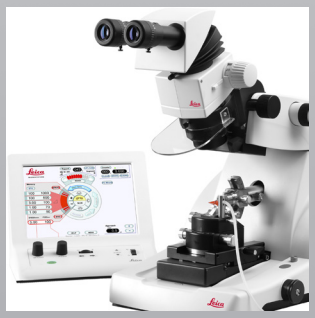

# THE QUEST FOR UNDERSTANDING MEMBRANE DYNAMICS OR WHY ELECTRICAL STIMULATION AND HIGH-PRESSURE FREEZING CAN BE THE WAY TO VISUALIZE SYNAPTIC FUNCTION.

Dr. Shigeki Watanabe, Department of Cell Biology, Johns Hopkins University Baltimore, MD 21205-1832

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It takes one-thousand of a second for a membrane to polarize and initiate an impulse of excitation. The ionic mechanism that governs this process is what makes life possible. It is what maintains all vital process and help us crate memories, it is in any active cells of the heart and the brain, it is a bioelectric potential. But what really makes this process remarkable is that bioelectricity propagates with an incredible speed, faster than the speed of light. Capturing and dissecting such dynamic processes is everything but trivial.

With the dawn of optogenetics light became the way to activate or block cellular processes. The optogenetic tools created plethora of photosensitive proteins that opened up the door for experiments that were not possible before. But to understand complex and dynamic phenomenon it is critical to have a point of reference, a point of synchronization of events at clearly specified temporal resolution.

The only way to accurately capture ultra-fast process is to aligne light stimulation with high pressure freezing. The "flash-and-freeze" method proved to be the method to visualize membrane dynamics with millisecond temporal resolution. It became the tool to dissect the complex picture into easy to comprehend frames. It is by all means a powerful technique, but when it comes to bioelectricity and synapse activity there is one important question to be answered: how close is this process to the physiology of a neuron?

The light sensitive channelrhodopsin is heterologously expressed in neurons and may affect the membrane properties and dynamics. Few important aspects should be considered. The channelrhodopsin is a non-selective cation channel that allows for calcium influx. Calcium is a second messenger that can affect many cellular processes. This calcium influx does not seem to affect synaptic transmission but may have impact on endocytosis and subsequent membrane trafficking. More interestingly, in transfected neurons the time required to generate an action potential will fluctuate from neuron to neuron at given intensity of light stimulation. This inconsistency is directly linked to the expression level of channelrhodopsin as well as the type of channelrhodopsin. The excitation of neurons by light stimulation in such heterogeneous population will trigger multiple, inconsistent action potentials.

For example, a single light pulse of 10 ms would trigger a single action potential is some neurons and two action potentials in others. The result can lead to controversial conclusions. This is a clear drawback that will arise many questions. What would be the correct interpretation of the captured snapshot of an excitation process, as in Figure 1? Are the two fusing vesicles next to each other the results of multiple action potentials? Or do they represent concurrent fusion of two vesicles from a single action potential? Is the absence of fusing vesicles in particular synapses due to the rapid collapse of vesicles, or does it simply reflect low release probability?

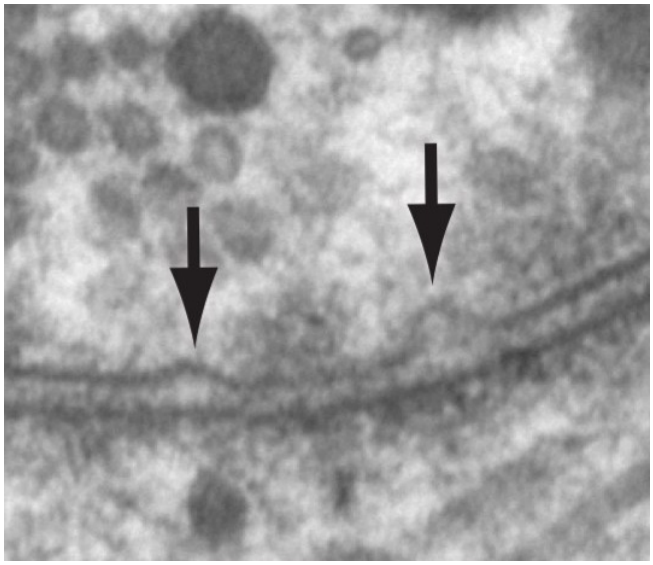


**Figure 1**. Synaptic vesicles fusing with the membrane of hippocampal neurons after 10 ms light stimulation

What would be the alternative, a more physiological way to activate synaptic activity, and alternative that will give us the ultimate zero time point? A method that will avoid plausible interpretation and will become the ultimate reliable approach to study synaptic dynamics.

Nearly two centuries ago Luigi Galvani's experiments on the frog legs (Fig.2) opened up the possibility electrical stimulation to be utilized as a powerful tool not only in research but also in applied physiology. The combination of electrical field stimulation of neurons with high-pressure freezing is the only way towards the quest for the ultimate time resolution of synaptic activity. We called this process "Zap-and-freeze". The process of physiological stimulation resolved at one milliseconf time frames.

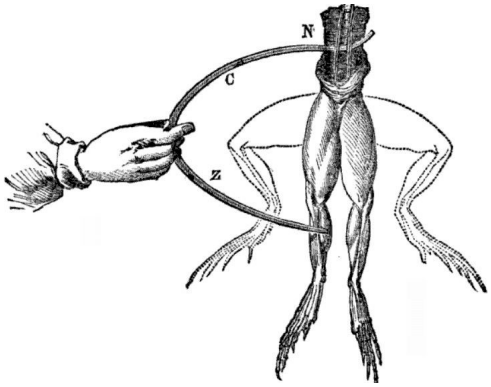



**Figure 2**. Set-up of Luigi Galvani's experiment.
Image from David Ames Wells, The science of common things: a familiar explanation of the first principles of physical science. For schools, families, and young students. Publisher Ivison, Phinney, Blakeman, 1859.

The simulation of applied electrical field of 1 ms across neurons to trigger an action potential is presented in Figure 3. It shows electrical stimulation offers much faster and accurate initiation of neuronal activity. It gives the possibility to visualize and analyse processes faster than the speed of light. The zap-and-freeze can capture the flip-books of neurons in motion under more physiological conditions.

Light stimulation and high pressure freezing, without any doubt, hold the potential for manipulating cellular processes but having the possibility to apply electrical stimulation increases the palette for the cell biology of the neuron.

Zap-and-freeze – reviving an old technique for the better future.

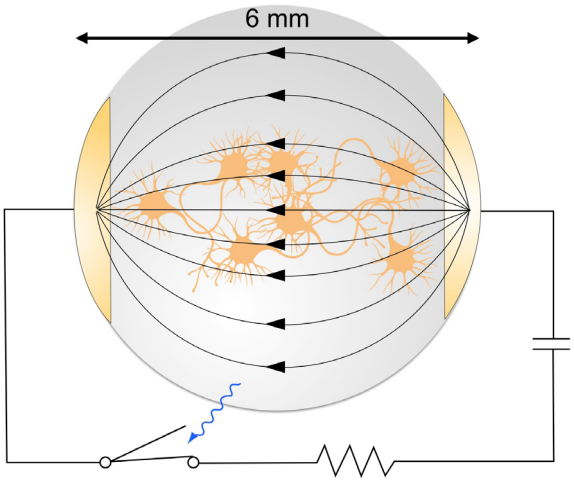
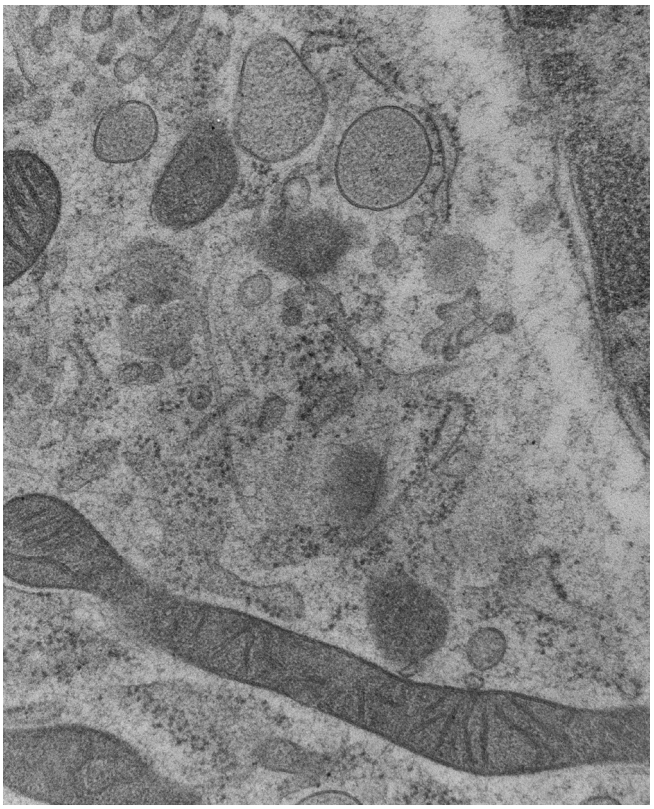



**Figure 3**. Schematic representation of electrical field generated through a hippocampal neurons, cultured on a 6 mm sapphire disk.



Mouse Hippocampal Neurons, Dr. Shigeki Watanabe, Johns Hopkins University

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