Voltage-sensitive Dyes will replace Electrophysiology in Neuroscience Research

For the Motion

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1 Introduction

The nervous system, responsible for the coordination of actions and sensory information, is made up of neurons. Neurons perform this function by communicating with each other using electrical and chemical signals. Electrophysiology is the study of electrical activity in living neurons and, thus, the molecular and cellular processes that control this communication. Analysing the electrical activity, measuring the signals and identifying the neurons involved helps reestablish neural functions, study the movement of action potential and the physiological characteristics of neurons [4].

1.1 Traditional Techniques

The traditional 'gold standard' electrophysiology techniques [13] involve the use of microelectrodes (both individual and multi-electrodes) to record voltage spikes. These recordings can be extracellular or intracellular, depending on the placement of the electrode.

Small electrodes may be inserted into a single cell, allowing direct observation of intracellular electrical activity. However, this reduces the life of the cell and may cause a leak of substances across the cell membrane. Using patch-clamp techniques ionic currents and membrane potentials may be studied. However, this is susceptible to issues such as vesicle formation (inside out configuration), dialyzing of cell contents (in case of whole-cell configuration), rupture and contamination (in case of perforated patch configuration) etc. Extracellular multi-electrode recordings can give us measures of voltage spikes for a relatively large number of neurons, as well as the repolarization timings. However, these methods are often demanding and slow [6].

1.2 Calcium Imaging

Calcium ion flow between cells is essential for action potential transmission between neurons and plays a critical role in the release of neurotransmitters. Studies have shown that calcium ion changes are indirectly correlated with membrane voltage changes. Fluorescent calcium indicators have been engineered as dyes, and optical measurement techniques such as ratiometric methods are used to study these concentration changes. Calcium imaging has advantages such as better spatial resolution but suffers from low temporal resolution. [5]

1.3 Limitations

Despite their widespread use today and a lot of research put into them, electrophysiology techniques have certain limitations:

1. Recordings through electrodes don't give us cellular level changes that take place (physiological and morphological) during and at the end of Action potentials and repolarization. [6]
2. The electrode shafts are basically blind. By the definition of these methods, we only see the spikes formation. We also don't get the information about which type of neurons are responsible for the visible spikes, and which ones are not active at all.

3. Long shafts enable recordings at a deep level, but that also makes it more invasive, thus posing problems to perform the experiment in vivo.

4. The temporal resolution ranges from milliseconds to seconds, which is lower than what imaging can give (microseconds) [5].

5. Multielectrodes do record many neurons at once, but the recordings are not dense, i.e. within a specified volume, they don't sample enough neurons. [11]

Some of the issues mentioned above were addressed using Calcium Imaging, but trying to correlate calcium concentration changes with membrane and action potential has it's own issues:

1. We try to correlate the change in calcium concentration with changes in voltage, release of neurotransmitters, etc. But this correlation is non-linear, and most of the measurements were recorded based on the assumption that calcium changes occur in all neurons during action potential, which is not true. [12]

2. Calcium changes are slow, thus a very low temporal resolution. [5] This means that if certain spikes and voltage changes are very rapid, then calcium imaging can't effectively record them.

3. Information about changes in the membrane potential that are small or below a threshold are often not recorded as voltage gated calcium ion channels open only after a threshold. [12]

An ideal method would combine benefits of the methods mentioned above, and have other benefits as well. What we really want to know is where do these spikes occur, in which neurons, and if possible, information about slight membrane potential changes directly. This is where Voltage Sensitive Dyes come in.

2 Voltage Imaging

Voltage-sensitive dyes (VSD) are dyes which change their spectral properties in response to voltage changes, which can thus measure changes in cell membrane potential. These can be used to indicate the site of action potential origin, action potential velocity and also direction. VSDs can be used to monitor activity inside cell organelles which cannot be probed by electrodes. They also enable measurement of spatial and temporal variations in membrane potential along the surface of single cells.

The types of VSDs are:

1. Absorption Dyes: The sample is illuminated with a light which the dye maximally absorbs, and the absorbance changes with change in the membrane potential of the neuron.

2. Fluorescence Dyes: Changes in the emission spectrum and intensity with respect to different potential differences across the membrane are observed

Based on kinetics, VSD are also characterized as:

1. Slow Response: Provide a greater contrast, i.e., better signals.

2. Fast Response: Enable measurement of transient potential changes i.e. high temporal resolution.

What dye to use for which samples depends on empirical and chemical investigations. We now consider several arguments which suggest that VSDs will indeed replace electrophysiology in neuroscience research.

2.1 Spatial Resolution

Voltage-sensitive dyes provide an outstanding spatial resolution, allowing signals in even the smallest neuronal structures to be resolved.[13] Additionally, VSDs offer the possibility for recordings in a wider variety of locations less invasively as compared to electrodes. Thus, we get both the 'when (firing patterns)' information and 'where (spacial location and type)' information with high resolution. Using VSDs, we have been able to probe the finest dendrites and axon terminals and detected activities in the synapses too, and discovered facts like the degree of chemical and electrical compartmentalization of dendritic spines[10] and the spatial extent and plasticity of dendritic spikes[8] which could not be probed by electrophysiological methods.

2.2 Recording from a population of neurons

One area where voltage-sensitive dyes have already begun to outperform electrode-based electrophysiology techniques is simultaneous recordings from a population of neurons[1]. The information from the sensory system in response to a stimulus, as well as the representations of the responses going to the motor system are expressed in ensembles of neurons instead of one or two cells. Each particular neuron/synapse encodes one or more aspects of the stimulus or the response.

To be able to talk about the neurobiological backing of stimulus-response trials, we should be to be able to record every activity from each neuron in the ensemble/area being studied as well as other relevant ensembles, from the time of the stimulus presentation to the end of the successive behavioral response.

Electrodes fail to achieve this because of finite spacing (making adjacent regions inaccessible) and size constraints and being unable to identify the origin of recorded signals precisely.

Optical probes and voltage-sensitive dyes allow simultaneous access to the temporally resolved activity (local potential changes) and the precise spatial location of a large number of active neurons. This can not be done with electrodes as the size of the arrays become impractical, and it becomes too invasive. Voltage-sensitive dyes (VSDs) have allowed us to unravel many fundamental aspects of functional and structural organization in the brain[13]. Additionally, VSDs can also detect sub-threshold fluctuations in the membrane potential[3]. This is especially helpful in determining the connections between neurons because a spiking neuron typically causes only a sub-threshold potential in the next neuron, and the ability to map these changes, together with the supra-threshold potentials (actual spikes), can help us infer the wiring of neurons at scales unattainable with traditional microelectrodes.

2.3 Specificity

Neurons in the brain have various gene expression profiles/patterns and types, characterized by the location of the soma, their developmental histories, the morphology of the dendrites and axon projections, and functions. Traditional methods using microelectrodes are unspecific concerning the cell types and cell structure that they probe. The type and other features of the neuron and its relationship to the underlying neural circuit are poorly known in case of the use of electrodes. VSDs, combined with imaging techniques, are targeted probes which allow us to access specific cell types and sub-cellular domains of neurons.[13]

Further, if we genetically encode the voltage-sensitive indicator protein, we can differentiate based on the genetic expression profile of the neuron rather than its surface/membrane properties. Genetically encoded voltage indicators are fusions of fluorescent proteins and voltage-dependent phosphatases and parts of proteins that make up voltage-sensitive ion channels.[9] Genetically encoded indicators of neuronal activity provide a way to understand genetically defined, specific neuronal populations. This directs us closer to the goal of deciphering the brain's exact wiring diagram.

3 Overcoming current issues

Some of the issues which have hindered the use of VSDs are:

1. Incomplete control over which neurons take up dye: At the same position, a dye can get absorbed by different neurons within the same tissue.

2. Low Signal to Noise ratio: The change in fluorescence due to voltage change can be scant. Thus, the signal ratio form the neuron's body is comparable to the noise from the surrounding tissue.

These issues are being actively resolved. The problem of signal to noise ratio was initially partially solved by looking at larger neurons, and applying wide-field voltage imaging of cortex with electrode recordings of a single neuron within that field [14], to get respectable results.
As recently as the past few weeks, breakthrough works on Voltage Imaging by Adam et al. [1] and Piatkevich et al. [11] have engineered techniques and dyes that give a very high signal to noise ratio and thus enable the measurement of not just the spikes, but even the slightest changes in membrane potential, that too in live animals. The problem of surrounding tissue blurring the images has also been addressed by Piatkevich et al. [11], where they are able to produce images with distinct spheres for each cell and light from one cell does not blur it's neighbours.

Some other advancements include:

1. Increase in the time before the dye bleaches out, i.e., the stability time.

2. Fibre optic recordings: deep brain imaging, to get images from deep within a live tissue was initially a difficult task for VSDs, but works such as Barreto et al. [2] provide techniques to overcome this.

3. Dr. Cohen's group at Yale University is working on finding improved fluorescent voltage-sensitive proteins, which can be specifically expressed in individual neuron types in the brain. The fluorescent properties of these dyes keep improving [7].

4 Conclusion

Traditional techniques of neural imaging, such as the use of electrodes and calcium imaging are very powerful and widely used techniques. However, both these techniques have drawbacks. Electrode shafts allow the recording of voltage spikes from both individual and a small population of neurons, but are both invasive and blind, and give us no information about which neurons are responsible for the spikes. Calcium imaging has excellent spatial resolution, but has low temporal resolution, and has a threshold on the changes in membrane potential that can be recorded. To move forward in neuroscience research, there is a need for a technique that combines the strengths of both these methods.

Voltage Sensitive Dyes allow us to study the action potentials generated by each neuron, as we learn from electrodes, and also allow us to understand where the neuron is, as we learn from calcium imaging, all in one neat technique. Recent advances have also enabled the imaging from large populations of neurons simultaneously, image from genetically tagged neurons, image deep-tissue neurons and image for a longer time. This has led to various scientific breakthroughs, such as the probing of fine dendrites, the functional and structural organization of the brain, and has provided a way to infer the connections in the brain. The remaining problems of Voltage Sensitive Dyes are being actively researched and resolved every day.

Voltage Sensitive Dyes thus open up the possibility of recording the voltage and membrane potentials of thousands of neurons, of known location, type, and number, of a live animal, simultaneously. This data can be used to easily reconstruct very accurate and detailed connections in precise locations, at the time of recording. In simpler animals, we can even construct a complete wiring map. In the words of Dr. Mark Humphries, this seems worth a shot. And so, voltage sensitive dyes are the door to the future of neuroscience research.

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