Editorial

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BIOELECTRONICS IN MEDICINE

Single neuron recording: progress towards high-throughput analysis

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"Recently, two key advances have been made that have made fully autonomous patch clamping possible"

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Since its development in the late 70s and early 80s by Nobel laureates Erwin Neher and Bert Sakmann [1], patch clamping has been the gold standard single-cell electrophysiology technique. With the highest temporal and spatial resolution achievable by any recording technology, patch clamping has expanded from its inception of studying frog muscle fibers to studying intracellular synaptic computations within single neurons. Beyond single-cell electrophysiology, intracellular access via whole-cell patch clamping allows the harvesting of cell cytosol for transcriptomic profiling [2] and the infusion of dyes for visualizing morphology of the cells being recorded. Thus, electrophysiological information of the cell can be integrated with genetic and morphological characteristics, providing a comprehensive characterization of the cell. Patch clamping is, however, a delicate process, requiring considerable practice, experience and skill to manipulate a glass micropipette, carefully place it in physical contact with a cell, and modulate the internal pressure to achieve a gigaohm seal. Consequently, despite its many advantages, patch clamping is a relatively low throughput and laborious process as compared with other electrophysiology techniques. Until recently, this has precluded the application of patch clamping for high-throughput analysis.

Automation & parallelization of patch clamping

Automation and parallelization have been two approaches taken to address the low-throughput and laborious nature of patch clamping. Some of the first efforts to automate patch clamping were driven by its utility in studying ion channels. Ion channels are highly attractive targets for a variety of drugs. The pharmaceutical industry has always required high-throughput assays to evaluate the effect of vast libraries of drug candidates on ion channels of clinical importance. To this end, microfluidic planar patch chip-based systems have been developed by several commercial entities for high-throughput patch clamping [3]. These devices typically utilize micromachined multi-well plates with patch-clamp orifices, integrated with robotic cell and fluid handling for multiple parallelized recordings from cells in suspension.

In contrast, progress toward high-throughput patch clamping of single neurons in intact tissue has been much more recent. Neurons within intact tissue have different electrophysiological properties as compared with neurons in culture, and further preserve endogenous connectivity with other neurons. Patch clamping *in vivo* further allows measuring intracellular responses to functional information received by the single neurons within the whole intact brain. In a first demonstration, we showed that the stereotyped process of lowering a pipette into a brain region of interest at high pressure, the slow lowering of a pipette in search of neurons, stopping and establishing a gigaseal and whole-cell patch-clamp could be coded into an algorithm. This algorithm was used to automate a simple robot which uses pipette impedance as the sole feedback signal to control the position and internal pressure of the pipette [4]. These first experiments were conducted in anesthetized animals wherein motion artifacts in the tissue are predominantly due to rhythmic pulsations caused by heartbeat and breathing. It is far more challenging to

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obtain stable whole-cell patch recordings in awake head-fixed animals, where motion artifacts are more pronounced in amplitude, as well as more sporadic and unpredictable. Desai and colleagues overcame these challenges and demonstrated that automated patch clamping could be performed in head-fixed, awake and behaving animals [5].

Computer vision for image-guided targeted patching

The first robotic *in vivo* patch clamping demonstrations were focused on automating 'blind' whole-cell patching, wherein no visual information of the location of the pipette or the cell being patched is available. In many experiments, it is important to be able to target specific types of neurons for patching, for which visual guidance is necessary. For instance, the brain has heterogeneous populations of cells with excitatory neurons far outnumbering inhibitory neuron populations. Inhibitory neurons are important for a number of neuronal computations occurring in the brain, and disruptions to normal functioning of inhibitory neurons is implicated in several brain disorders. Blind whole-cell patching has a bias toward recording predominantly pyramidal cells [6]. To overcome this issue, Wu *et al.* first demonstrated automated computer vision-guided targeted patching of cells in *in vitro* brain slices [7]. Microscopy was used to image the pipette and cells, and computer vision algorithms located coordinates of pipette tips and the centroids of cells of interest. Once the coordinates were determined, trajectories to guide pipettes to specific target cells of interest in brain slices were computed. This strategy worked for both differential interference contrast imaging as well as epi-fluorescence imaging wherein cells were tagged with a fluorescent indicator. Further progress was made a year later when two groups simultaneously demonstrated that two-photon imaging could be used to perform targeted recordings of single neurons *in vivo* in 3D tissue [8,9]. With image-guided targeted patching being automated, more recently, researchers have developed machine learning algorithms to automate and detect healthy cells within brain slices of both rodents and humans for targeted patching [10]. More broadly, the capability of using microscopic imaging, computer vision algorithms, and robotic positioning is being utilized for other applications, such as microinjecting into single neurons and stem cells [11].

Parallel patch clamping of multiple neurons in a circuit

Beyond easing the single-cell patch clamping process, the principles of automation have also been applied to simplify the multi-neuron patch-clamp process. Multi-neuron patch clamping can be used to explore the sub- and supra-threshold electrophysiological characteristics of multiple neurons simultaneously, enabling the study of synaptic plasticity and network connectivity. This comes at the cost, however, of increased complexity associated with manipulating and coordinating multiple pipettes moving in tissue and potentially displacing cells of interest. Thus, unlike extracellular recording techniques, parallel intracellular recordings of multiple neurons simultaneously have been limited to a few highly skilled laboratories. In principle, the algorithms for automating single electrode patch clamping can be parallelized to control several robotic arms. Early work utilized simple robotic routines to semi-automatically guide up to twelve patch pipettes into a single brain slice, close to cells of interest, after which a human operator took over to perform patching manually [12]. Further strides were made in automating the multi-patch process in brain slices by Peng and colleagues [13]. This work adapted algorithms developed for *in vivo* patching [4], developed an open source, microcontroller-based pipette pressure system, and further incorporated an automated cleaning protocol (adapted from [14]) to dramatically increase throughput [13]. This system permitted up to ten simultaneous recordings with the capability for sequential recordings thereafter. Progress has also been made in automating *in vivo* multi-patching via the 'multipatcher'. The 'multipatcher' is a system of four interacting 'blind' patch clamping robots that automate the patch-clamp process from cell detection, sealing and breaking-in [15]. This work demonstrated simultaneous whole-cell recordings from up to four neurons in anesthetized and awake head-fixed animals.

Toward fully autonomous single-cell electrophysiology

With the process of patching being automated, early work still required human practitioners to perform several operations, such as changing pipettes between trials, or operating the data-acquisition software once a patch recording is obtained. Recently, two key advances have been made that have made fully autonomous patch clamping possible. For decades, patch-clamp electrophysiologists have considered the micropipettes used for patch clamping as single-trial use electrodes. Kolb and colleagues demonstrated that readily available cleaning solutions can be used to clean the pipette tips which permitted the pipettes to be reused for subsequent trials without affecting the patch quality. Coupled with simple robotic routines that clean and rinse the pipettes, they demonstrated a robotic platform that can autonomously perform patch-clamp recordings in adherent cells and brain slices [14]. Alternately,

in cases where the contents of the pipette need to be retrieved after recording, like after harvesting the cytosol for transcriptomic analysis, fluid handling robotics can be incorporated into an automated patch clamping platform to exchange pipettes between trials, as demonstrated by Holst and colleagues [16]. Both these works demonstrated fully autonomous patch clamping, recording from hundreds of cells over several hours of operation without any human intervention.

Future outlook

What does the future hold for high-throughput single-cell recordings? The recent progress in automation and parallelization may promote medical advances catalyzed by high-throughput patch clamping. For example, improvements in high-throughput parallel patch clamping could be applied in the study of network synaptic connectivity disruptions in neurological disorders. Likewise, the incorporation of visually guided systems aided by computer vision algorithms permits researchers to target specific cells of interest and could enable identification of neuronal subpopulations implicated in various brain disorders. Future advancements in robotics and automation will potentially decrease the cost of consumables and will re-energize wider support for research in drug discovery. Looking further, the development of cheap microchip amplifiers for patch clamping [17,18] should enable rapid and massive scale-up and parallelization of autonomous patch clamping systems. There are, however, several other challenges that need to be overcome. Patch clamping depends on several factors extraneous to the experimental procedure. These include the quality and health of tissue [6] and the quality of the patch pipettes. These extraneous factors lead to significant intra-lab and inter-lab variability in the yield, throughput, and quality of patch recordings. One approach to mitigate this is to standardize tissue preparation. For instance, cranial microsurgeries used to make craniotomies for *in vivo* patch clamping have been automated [19,20] and similar efforts are ongoing to automate live tissue slicing.

Conclusion

We have a number of enabling technologies developed for truly high-throughput single-cell recordings in a variety of contexts. Thus, the field is truly at an inflection point, and the coming years will have an increasing number of studies incorporating massively high-throughput single-cell electrophysiology.

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