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The evolution of patch-clamp electrophysiology: robotic, multiplex, and dynamic

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Running title: Perspectives on automated electrophysiology

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Abbreviations: BK, big potassium channel; DRG, dorsal root ganglion; FACS, fluorescence-activated cell sorting; HCN, hyperpolarization-activated cyclic nucleotide–gated; hERG, the human ether-à-go-go-related gene; iPSC, induced pluripotent stem cell; Kv, voltage-gated potassium channel; Nav, voltage-gated sodium channel; TRP, transient receptor potential channel

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ABSTRACT

The patch-clamp technique has been the gold standard for analysis of excitable cells. Since its development in the 1980s it has contributed immensely to our understanding of neurons, muscle cells, and cardiomyocytes, and the ion channels and receptors that reside within them. This technique, predicated on Ohm's law, enables precise measurements of macroscopic excitability patterns, and ionic and gating conductances that can be assessed even down to the single channel level. Over the years, patch-clamp electrophysiology has undergone extensive modifications, with the introduction of new applications that have enhanced its power and reach. The most recent evolution of this technique occurred with the introduction of robotic high throughput automated platforms that enable high quality simultaneous recordings, in both voltage- and current-clamp modes, from 10s to 100s of cells, including cells freshly isolated from their native tissues. Combined with new dynamic-clamp applications, these new methods provide increasingly powerful tools for studying the contributions of ion channels and receptors to electrogenesis. In this brief review, we provide an overview of these enhanced patch-clamp techniques, followed by some of the applications presently being pursued, and a perspective into the potential future of the patch-clamp method.

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SIGNIFICANCE STATEMENT

The patch-clamp technique, introduced in the 1980s, has revolutionized understanding of electrogenesis. Predicated on Ohm's law, this approach facilitates exploration of ionic conductances, gating mechanisms of ion channels and receptors, and their roles in neuronal, muscular, and cardiac excitability. Robotic platforms for high-throughput patch-clamp, and dynamic-clamp, have recently expanded its reach. Here, we outline new advances in patch-clamp including high throughput analysis of freshly-isolated neurons, and discuss the increasingly powerful trajectory of new patch-clamp techniques.

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INTRODUCTION

The fundamental processes underlying electrical signalling within biological systems have long tantalized scientists. In 1902, Julius Bernstein first hypothesized that nerve impulses represent a transient "breakdown" of the membrane resistance, where the peak of an action potential represents the point of minimum resistance (Bernstein, n.d.). Although this hypothesis was not entirely accurate, it inspired subsequent generations of investigators to take further steps to unravel the mysteries of electrical signaling. In a leap beyond Bernstein's membrane theory's significant impact to understand impulse transmission, the seminal work of Alan Hodgkin and Andrew Huxley in the early 1950s significantly advanced our understanding of excitability (A. L. Hodgkin and Huxley, 1952a, c; b; AL L Hodgkin and Huxley, 1952; Hodgkin *et al.*, 1952). Their exquisite experiments and subsequent mathematical model continue to be the foundational building blocks for much of modern molecular neuroscience. Hodgkin and Huxley (along with John Eccles) were awarded the Nobel Prize in 1963 for their contributions.

The next significant milestone occurred in the late 1970s, when Bert Sakmann and Erwin Neher first reported single channel patch-clamp recordings (Neher and Sakmann, 1976; Neher *et al.*, 1978; Hamill *et al.*, 1981). The development of the patch-clamp technique led to a much more rapid progress in understanding of the biophysical, physiological, pathophysiological, and pharmacological properties of ion channels and receptors. Today, the patch-clamp technique is regarded as the gold-standard technique for investigating the function of excitable cells (Sigworth and Neher, 1980; Hamill *et al.*, 1981). This technique has allowed investigations of ionic and gating conductances from a wide range of ion channels and receptors. For their monumental contributions, Sakmann and Neher were awarded the Nobel Prize in 1991.

Since the development of the patch-clamp technique (and in conjunction with other allied methodologies, i.e., cloning, computational, and structural biology) the story of ion channels and their importance in the physiology of neurons, muscle cells, and cardiac myocytes has continued. This technique has continued to be a powerful ally in the discovery and characterization of important biological channels and receptors, including various sodium, potassium, calcium, chloride, TRP, and Piezo channels, among others. Most recently, Ardem Patapoutian and David Julius were awarded the Nobel Prize in 2021 for their seminal discoveries, based in part on patch-clamp studies, of receptors for temperature and touch (Peier *et al.*, 2002; Chuang *et al.*, 2004; Catterall *et al.*, 2005; Coste *et al.*, 2010; Catterall and Swanson, 2015; Peters *et al.*, 2015).

One of the most notable figures in shaping the field of ion channels for more than four decades was William Catterall. His immense contributions helped define our modern understanding of voltage-gated sodium and calcium channels (Catterall, 2023). As this issue is

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dedicated to his enormous legacy and memory, it is fitting that we acknowledge that he will be greatly missed, and his contributions will never be forgotten.

CONCEPTUALIZATION & CONFIGURATIONS

The patch-clamp technique is predicated on Ohm's law, which describes the relationship between voltage (V), current (I), and resistance (R), or V=IR (Hille, 2001). In a general electrophysiological context, the volage (V) indicates the local membrane potential that modulates the gating of voltage-gated ion channels, the current (I) indicates the ionic current that flows through the ion channels, and the resistance (R, which has an inverse relationship with conductance (R=1/conductance (G)) indicates the status of the ion channels. The patch-clamp technique enables the clamping of each of the three variables. All patch-clamp amplifiers make use of an electric circuit that is based on current-to-voltage converter with a high feedback resistance, with some set-ups using a capacitor instead of a resistance which could help in the reduction of the noise in electronics, as a capacitor represents infinite resistance (Sigworth, 1995; Sigworth *et al.*, 1995). The general concept behind the set-up is to have current flow through an electrode inside a glass pipette without changing the voltage at which the electrode is clamped (i.e., in voltage-clamp). Once this pipette is lowered onto the membrane of the cell, the input signal goes into an amplifier, and then using other electronics, the signal gets filtered and/or compensated. Depending on the goals of a particular experiment, recordings can be obtained in various modes, including cell-attached, whole-cell, inside-out, and outside-out (Fig. 1). Each of these modes has advantages and disadvantages; however, the most commonly used technique in an ion channel electrophysiologist's arsenal is the whole-cell conformation, in which the membrane at the point of contact between the tip of the glass pipette and the cell is ruptured, thereby providing access to the inside of the cell. Once the cytosolic side of the cell becomes continuous with the inside of the glass pipette, via the introduction of another external electrode, the membrane can be electrically clamped.

In the voltage-clamp configuration, the experimenter sets and applies a voltage, which elicits changes in the conformation of the voltage-gated channels that conduct current. This type of experiment is typically utilized in standard experiments that seek to assess the thermodynamics (voltage-dependence) and/or kinetics of ion channels. These studies have unravelled the mysteries behind how a particular genetic mutation may underlie disorders of excitability in various tissues, such as epilepsy (Lehmann-Horn and Jurkat-Rott, 1999), neuropathic pain (Rush *et al.*, 2007), myotonia (Cannon, 2015), arrhythmia (Schwartz *et al.*, 2020), among many others (Ghovanloo *et al.*, 2016; Fouda *et al.*, 2022). Another key utility of the voltage-clamp is to investigate how various pharmacological agents modulate ion channels. For instance, the mechanism through which the anticonvulsive drug, zonisamide, inhibits sodium channels was determined using this methodology (Schauf, 1987). Voltage-clamp experiments are

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typically utilized when the goal is to investigate the function of a single or a limited group of ion channel types (or subtypes), as various ion channels have their own signature voltage-dependent and/or kinetic properties (Ghovanloo, Tyagi, *et al.*, 2023). As the number of the types of ion channels increases, the resulting signals become mixed and more challenging to interpret. To overcome this confound, experiments have often been performed in heterologous expression systems in which a particular ion channel of interest is overexpressed in a relatively quiescent cell background (Ahuja *et al.*, 2015; Ghovanloo *et al.*, 2018; Ghovanloo, Estacion, *et al.*, 2022; Goodchild *et al.*, 2024). This mode of study, however, introduces another challenge, since the biophysical attributes of a channel expressed in a heterologous system may not fully mimic its properties in native neurons (Cummins *et al.*, 2001). However, voltage-clamp experiments in native environments and primary cells are also feasible, when adequate solutions and pharmacological agents are used to isolate a given ion channel of interest (Sleeper *et al.*, 2000; Cummins *et al.*, 2009; Tyagi *et al.*, 2024).

In the current-clamp configuration, patterns of cell excitability are investigated as the change in voltage across the membrane, which occur as a result of the opening and closing of the membrane-embedded ion channels. In contrast to the voltage-clamp configuration, current-clamp experiments are most meaningful when applied to real excitable cells that fire action potentials, including neurons and cardiomyocytes (Sakakibara *et al.*, 1993; Cummins *et al.*, 2009; Tai *et al.*, 2014; Seibertz *et al.*, 2022). Current-clamp experiments have also contributed immensely to our understanding of how for instance a mutation that substitutes a single amino acid within the channel protein can induce neuronal hyperexcitability leading to a pain disorder, such as inherited erythromelalgia (Dib-Hajj *et al.*, 2005) and paroxysmal extreme pain disorder (Dib-Hajj *et al.*, 2008).

A more recent enhancement of patch-clamp has been provided by the introduction of the conductance-clamp (often called dynamic-clamp) configuration, in which mathematical differential equations that are based on empirical models of various ion channels are used to artificially alter conductances within patch-clamped neurons (Sharp *et al.*, 1993; Prinz *et al.*, 2003, 2004; Desai *et al.*, 2017). This technique is especially powerful in that it enables the subtraction or addition of precisely titrated amounts of current attributable to a selected channel (or several channels) to each recording, in a way that permits before-and-after analysis of the role of that current (channel) within the same intact neuron. This is a big advantage compared to transfection, which can add a wild-type channel (or a mutant channel) to a particular cell-type, but where the level of expression cannot be controlled. Examples of the power of this methodology are provided by studies which assessed the contribution of Nav1.7 (a sodium channel isoform that is predominantly expressed in the peripheral neurons) to excitability of dorsal root ganglion (DRG) neurons, and then compared this to the contribution of a gain-of-function mutant Nav1.7 channel known to produce pain (Vasylyev *et al.*, 2014). Dynamic-clamp

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analysis has also been used to quantitatively study the physiological interaction of different channels, e.g., hyperpolarization-activated cyclic nucleotide–gated (HCN) channels and Nav1.7, within living neurons (Vasylyev *et al.*, 2023). Finally, dynamic-clamp is being used to address questions about the degree of receptor occupancy needed for efficacy during drug development. For example, dynamic-clamp was recently used to address, in single neurons, the question "how many Nav1.7 channels must be blocked to normalize excitability in a cellular model of pain?". This study used graded, fractionated subtraction of precisely titrated portions of the Nav1.7 current as a proxy for channel block, in a study on iPSC (induced Pluripotent Stem Cell)-derived sensory neurons from patients with a genetic model of neuropathic pain, to show that even partial block could attenuate hyperexcitability associated with pain (Alsaloum *et al.*, 2023).

The applications of a patch-clamp study are broad. As another example, in a series of elegant studies, Bruce Bean and colleagues revealed key insights into the roles of A-type potassium currents, sodium conductances, and BK and Kv2 channels in regulating pace-making and firing patterns of dopaminergic neurons in the ventral tegmental area and substantia nigra (Khaliq and Bean, 2008, 2010; Kimm *et al.*, 2015). The applications of patch-clamp electrophysiology have been broadly utilized to address questions on a range of physiological and pathophysiological topics from pain to pace-making, among many others.

Patch-clamp configurations provide a complementary set of tools, each informative in its own way, to the experimenter: the readout of current-clamp experiments is analogous to a recording from an orchestra (a cell), where multiple different musical instruments (channels) work in harmony to produce a symphony (the current-clamp recording of the cell's output). The voltage-clamp readout is analogous to focusing on a single type or one particular instrument (i.e., recordings of current from a specific type of channel). The dynamic-clamp permits an artificial enhancement or subtraction of a single note produced by a single type of instrument to determine the instrument's impact on the overall music (i.e., to determine the contribution of a particular channel to cell function), or the substitution of a mis-tuned instrument for a properly tuned one (to determine the effect of a mutant channel on cell function). These three configurations of patch-clamp, when used to study a single cell type, provide an unparalleled view of electrogenesis and its molecular substrates.

APPLICATIONS IN STRUCTURE-FUNCTION STUDIES

Electrophysiological tools, and in particular the patch-clamp technique, have been crucial to understanding not only of the roles and functional properties of various ion channels and receptors, but also have helped to delineate the roles of various structural parts, within a given channel or receptor, to the biophysical attributes of that channel. In this regard, the patch-clamp technique has been a powerful ally to structural and computation biology within the last three

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decades. Prior to Roderick MacKinnon's seminal work in the 1990s describing the first crystal structure of an ion channel (Doyle *et al.*, 1998) for which he was awarded the Nobel Prize in 2003, investigators primarily relied on empirical models, functional analysis, and hypothesis-driven trial-and-error to decipher the intricate structural underpinnings of ion channel physiology and pharmacology (Hille, 1977, 2001; Bezanilla, 1985; Catterall, 1988; Armstrong, 1992; Armstrong and Hille, 1998; Hille *et al.*, 1999). These approaches have been and continue to be immensely powerful in elucidating structure-function mechanisms. For instance, Bertil Hille's 1977 modulated receptor hypothesis, based on observations describing the pathways of local anesthetic binding inside the channel pore, provided a novel and accurate model of channel pharmacology that continues to be foundational in modern molecular drug discovery in ion channels (Hille, 1977). Building upon this, with the emergence of modern crystal and cryo-EM structures (Montini *et al.*, 2018; Li *et al.*, 2024; Yao *et al.*, 2024), investigators can now visually interrogate ion channel three-dimensional structures, using biochemical means to manipulate these structures; again, patch-clamp analysis has been crucial in assessing these structural substrates of channel function. William Catterall and Ning Zheng successfully utilized this approach to unravel many aspects of sodium and calcium channel physiology and pharmacology (Payandeh *et al.*, 2012; Chakrabarti *et al.*, 2013; Jiang *et al.*, 2018, 2020; Wisedchaisri *et al.*, 2019; Catterall, 2023). An example of this approach was published in 2018, in which the pathway through what is now known as sodium channel fenestrations described by Hille in 1977, was not only structurally visualized, but also cleverly manipulated to describe the relationship between local anesthetic sizes and fenestration diameters underlying resting-state channel block (Gamal El-Din *et al.*, 2018).

More recently, computational approaches have been added as an additional tool to patch-clamp and structural techniques to form more empirically-driven hypotheses as well as validating/providing explanations for functional/structural data (Elbahnsi and Delemotte, 2021). These approaches include molecular dynamics and Rosetta modelling. An example of this is a study on the sliding-helix model of channel gating that was published in 2012 (Yarov-Yarovoy *et al.*, 2012). This study showed that the channel activation involves conformational changes in the voltage-sensing domain, which is tightly coupled to movements in a critical linker peptide chain (known as S4-S5 linker), which pulls on the pore domain of the channel, leading to the opening of the pore and ionic conduction.

In summary, despite the emergence of other methodologies to investigate the properties of ion channels and receptors, the patch-clamp technique continues to be as invaluable as ever for functional analyses, and indeed its utility continues to expand as other methodologies are developed and its applications continue to become more precise.

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TRADITIONAL PRACTICE & LIMITATIONS

Despite the power of patch-clamp electrophysiology, like other methodologies, it comes with limitations and challenges. The traditional practice of the patch-clamp technique is done in a manual configuration, which is labour intensive. It can take an experienced experimenter about 10-15 minutes for a single successful recording. This practice includes identifying a cell using a microscope that meets visual criteria of "looking right" (e.g., transfected/fluorescent), pulling of glass pipettes, filling the glass pipette with an internal solution with a syringe, making sure that there are no air bubbles inside the pipette, screwing the recording electrode into the pipette, lowering it into the cell of interest, breaking into the cell by applying a gentle air pressure, and waiting a few minutes post-breakage to ensure the system is stable before recordings can begin. Errors at any of these steps can delay or prolong the time needed to acquire a recording.

In addition to technical issues, the traditional manual patch-clamp has other notable limitations. The first limitation is the experimenter's implicit bias in cell selection (Fig. 2A-B). Patch-clampers typically select cells that in their experience provide the best results; these include cell shape, how cell membrane morphologies visually appear, and size of the cells, among other considerations. There is considerable opportunity for experimenter-to-experimenter variability or for subtle bias in choosing cells for study.

As noted above, the patch-clamp technique could be used to investigate ion channels in both heterologous and native cell types. Because of its low throughput, both types of studies necessitate culturing of cells on glass coverslips. This practice causes two additional drawbacks. First, in the case of freshly dissociated cells from intact tissues, the cells will have been placed on glass coverslips overnight (or many hours), exposing them to an environment that is different than normal, which may conceivably alter cell biology. Second, because incubation times prior to recordings may vary, the recording conditions for each cell may not be uniform. This inconsistency also carries over to the conditions associated with saline/drug perfusion.

A final limitation is the low success rates when attempting to perform voltage- and current-clamp within the same cell (Ghovanloo, Tyagi, *et al.*, 2023). Each of these configurations can provide important information and, when combined on the same cell, the results can become increasingly informative. However, the transition between voltage- to current-clamp induces stress on the cells, and the low throughput nature of the manual patch-clamp makes it challenging to investigate singular macroscopic conductances and excitability patterns within the same cell.

AUTOMATION & EVOLUTION

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Given the immense power of the patch-clamp technique and its notable limitations, this technique has continued to evolve to both expand its applications, but also to reduce its limitations. The earlier attempts to upgrade this methodology primarily involved using automation to increase the speed of obtaining a recording and to reduce the relatively steep learning curve for becoming a proficient patch-clamper. In the 2000s, the development of the Port-a-Patch system demonstrated how automation could be used to simplify some aspects of the laborious nature of the technique (Fertig *et al.*, 2009). In subsequent years, several research groups across academia and industry made improvements to the technique, while also increasing throughput (Kodandaramaiah *et al.*, 2012; Kolb *et al.*, 2016, 2019; Annecchino *et al.*, 2017; Annecchino and Schultz, 2018; Bell and Dallas, 2021; Perszyk *et al.*, 2021; Dallas and Bell, 2024). These efforts produced multiple robotic approaches and machines with diverse applications.

One of the most promising approaches was the introduction of robotic high throughput methods that can achieve high-resistance seals, and perform patch-clamp recordings of hundreds of cells at a time, such as the Sophion Qube and the Nanion SyncroPatch (Fig. 2C-D). Importantly, these machines (e.g., the Qube) use 384-well chips with a separate amplifier per well, to ensure robust recording quality. These newer methods utilize suction on a cell-on-a-hole basis (in contrast to lowering electrode onto the cell in manual patch-clamp), mitigating several of the previously noted limitations of the traditional methodology (Chambers *et al.*, 2016). Increased throughput alone represents several important advantages, including greater statistical power, increased speed, and higher probability of obtaining successful recordings when performing challenging experiments, such as the voltage- to current-clamp transition (Ghovanloo, Tyagi, *et al.*, 2023). The cell-on-a-hole concept further removes experimenter's bias as cells are applied to the recording chip using a robotic liquid-handler, ensuring the machine is completely blinded to cell selection. Thus, each of the recorded cells gets sucked into the hole in each well completely randomly. Finally, this methodology reduces one contributor to variability between recordings: because the cells get recorded simultaneously and under the exact same conditions, differences between recordings are minimized and experimental robustness further improves.

The steep costs of acquiring and operating these high throughput platforms made them primarily targeted to well-funded industrial laboratories, including the pharmaceutical sector. Therefore, the initial applications of these platforms focused on drug screening using heterologous cell-lines. This is because with drug discovery pipelines, pharmaceutical companies typically develop large numbers of interrelated compounds and isomers with the goal of hitting a desired molecular target (e.g., a particular ion channel). By employing robotic high throughput platforms, research and development teams can efficiently work through their vast chemical libraries in relatively little time. More recently, however, as these platforms became more readily

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available, they have begun to be used in academic, as well as biopharma, laboratories to assess more complex electrophysiological and pharmacological properties of both cell-lines and iPSC-derived neurons and cardiac myocytes (Franz *et al.*, 2017; Ghovanloo *et al.*, 2018; Potet *et al.*, 2020; Ghovanloo, Estacion, *et al.*, 2022; Ghovanloo, Goodchild, *et al.*, 2022; Rosholm *et al.*, 2022; Ghovanloo, Effraim, *et al.*, 2023, 2024). For instance, this methodology has been used to describe the biophysical properties of mutations in Nav1.7 that act as risk factors for development of trigeminal neuron hyperexcitability that produces pain following axonal injury (Ghovanloo, Effraim, *et al.*, 2023), the functional and pharmacological heterogeneity of *KCNQ2* variants associated with epilepsy (Vanoye *et al.*, 2022), assess the effects of drugs on the sodium currents in iPSC-derived cardiomyocytes (Potet *et al.*, 2020), and to determine the kinetics of hERG blockers (Windley *et al.*, 2022). As another example, the pharmacological effects of several non-psychoactive cannabinoid compounds were recently deciphered using these types of platforms; studies using this new methodology have, for example, demonstrated state-dependent sodium channel inhibition of several therapeutically promising compounds including cannabidiol, cannabigerol, and cannabinol that may contribute to their clinical efficacy (Ghovanloo *et al.*, 2018; Ghovanloo and Ruben, 2021; Ghovanloo, Dib-Hajj, *et al.*, 2022; Ghovanloo, Estacion, *et al.*, 2022; Ghovanloo, Effraim, *et al.*, 2024).

Recently, the utility of these robotic technologies has been further extended by development of methods permitting study of freshly isolated primary neurons. Ghovanloo et al. (2023) have developed a new methodology using robotic technology that for the first time enables functional analysis of populations of neurons that have been freshly isolated from intact animals. This permits simultaneous recording and head-to-head comparison of neurons freshly removed from their native environment, all assessed at the same time and under the same conditions. These neurons can be assessed both by voltage-clamp and current-clamp, sequentially carried out in the same cell (An example/application of this is shown in Fig. 3) (Ghovanloo, Tyagi, *et al.*, 2023). An advantage of this methodology is that it can assess freshly isolated cells, thus avoiding the confounds that accompany culturing. Studies to date have demonstrated, for example, that this methodology can be used to functionally define sub-populations of a biologically diverse neuronal cell type (e.g., from the dorsal root ganglion) on the basis of ionic conductances (Ghovanloo, Tyagi, *et al.*, 2023, 2024), and to simultaneously assess the differential effects of pharmacological agents on various neuronal populations under identical experimental conditions (Ghovanloo, Effraim, *et al.*, 2024), with a throughput much higher than for manual patch-clamp. This new approach will be enhanced by improvements in cell isolation and purification methods that will further maximize the yield of successful recordings. It is conceivable that this most recent evolution of patch-clamp electrophysiology may adapt it for use, in tandem, with omics studies of excitable cells, permitting a new level of integration of molecular and functional analyses.

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FUTURE PROSPECTS & HORIZONS

The evolution of the patch-clamp technique over decades has significantly enhanced its capabilities. However, despite recent advances in robotic automated platforms for functional omics, the current iteration of patch-clamp still faces limitations in certain areas. For example, while whole-cell and perforated patch-clamp techniques are feasible, other configurations remain challenging. It is also not currently possible to use these robotic approaches to patch the axons or dendrites of neurons, or brain slices. Although performing these types of studies is also challenging in the manual configuration, they have contributed substantially to our understanding of neurophysiology. For instance, by patch-clamp recording, Vasylyev and Waxman (2012) documented the sequential activation of Nav1.7 and then Nav1.8 within the axons of sensory neurons in culture (Vasylyev and Waxman, 2012), and by patching soma, axons, and dendrites, Hu and Bean elucidated the importance of the balance of ion channel ensembles in setting resting potential in layer 5 pyramidal neurons (Hu and Bean, 2018). This type of investigation is not yet achievable using the new robotic high throughput patch-clamp, but hopefully this will be possible in the future.

Dynamic-clamp has also not yet been implemented using robotic platforms, but this should be achievable in future iterations of this methodology. Moreover, new modes of optogenetics and chemogenetics will likely be integrated into the robotic platforms described above, introducing new tools into automated high throughput patch-clamp methodology that will further enhance its utility for modern electrophysiologists. Notably, with the advancements in fluorescence-activated cell sorting (FACS), which is an advanced variant of flow cytometry, it is becoming increasingly feasible to isolate transfected cells (if necessary), which could be assessed using these new robotic platforms. As these approaches become further developed and optimised, they further add yet another power dimension to the capabilities of modern patch-clamp electrophysiology. We envision that within the next 5-10 years, it will be feasible not only to functionally characterize ionic conductances and excitability patterns of neuronal populations with defined phenotypic roles isolated from intact animals, but also to manipulate specific ionic conductances using an array of pharmacological, optical, and dynamic-clamp techniques. This capability may enable a high-resolution mapping of physiological and pathophysiological processes at a cellular and systemic level.

As these robotic platforms and the experimental conditions (e.g., solutions, etc.) get further optimized, we envision that primary cells will be maintainable while being patched for longer, and with greater stability. Given that both extracellular and intracellular perfusion are currently implementable in some of the robotic platforms described above, it is not unreasonable to expect that further optimization will enable isolation and study of multiple conductances assessed by voltage-clamp on an individual basis, together with evaluation of excitability of the

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cell under study as assessed by current-clamp, all within the same cells shortly after isolation, and all studied simultaneously.

In our analogy to music, we envision the future ability to provide and analyze a functional atlas of voltage, current, and conductance for numerous neuronal populations simultaneously (an atlas from each neuron within each sub-population), akin to orchestrating multiple ensembles in a model organism. By subjecting model organisms to various experimental manipulations, this approach could elucidate the link between specific biophysical pathways at the protein and/or cellular levels and macroscopic phenotypic expression, thus connecting precise functional pathways to observable traits, which would have undergone behavioral studies prior to experimentation.

The patch-clamp technique has enhanced our understanding of virtually every ion channel and receptor. It has undergone a series of modifications and upgrades, each extending its utility and reach, and continues to evolve. The prospect of further evolution of this technique should be exciting to every scientist who studies channels, receptors, or excitable cells. The questions it will continue to help address are enormously exciting.

AUTHOR CONTRIBUTIONS

M.-R.G., S.G.W., and S.D.D-H. wrote or contributed to the writing of the manuscript.

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DATA AVAILABILITY STATEMENT

This review article contains no datasets generated or analyzed during the current study.

COMPETING INTERESTS

None. The authors declare that this research was conducted in the absence of competing interests.

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FIGURE LEGENDS

Figure 1 – Diagrammatic representation of various configurations of the patch-clamp technique.
A) Shows the glass pipette filled with intracellular solution attached to the cell membrane. This configuration is called cell-attached. Once this configuration is established, a rapid pull of the pipette can break a piece of the membrane with ion channel of interest in it. This configuration is called B) inside-out. C) Alternatively, from the cell-attached configuration, the application of suction pressure can rupture the contact point between the tip of the pipette and the cell, thereby providing access to the intracellular side of the cell. This configuration is called whole-cell. D) From the whole-cell configuration, with the pulling of the pipette away from the cell, the outside-out configuration can be obtained.

Figure 2 – Cartoon comparison of traditional manual methodology to automated high throughput patch-clamp.
A) Shows a cartoon representation of how the manual methodology is performed. This method begins with a laborious set of steps in preparation for recording (see text), and necessitates a subjective cell selection process in which the experimenter has to visually select a particular cell for recording. In this case the green cell is the one that gets patched. B) Once the patch is formed, it is possible to perform both voltage- and current-clamp (also dynamic-) recordings, on a single cell basis. However, due to stress on the cells, the transition between the two modes is challenging. This methodology could be used for investigating cell lines and native neurons. A limitation is that different cells get recorded at different times after isolation or culture. C) Describes the general pathway for the new methodology. This method utilizes 384-well chips and cell suspension which D) increases throughput, removes cell selection biases (as well as other sources of confound, as described in the text), and enables a more seamless transition between voltage- to current-clamp in the cells that are successfully patched. This methodology permits study of acutely isolated cells, recorded under the exact same conditions (e.g., same time since isolation). The dynamic-clamp configuration is not yet implemented in the new multiplex patch-clamp methodology. APC indicated automated patch-clamp.

Figure 3 – Voltage-clamp (VC) and current-clamp (CC) recordings in the same dorsal root ganglion (DRG) neurons.

This figure is a reformatted figure from: (Ghovanloo, Tyagi, *et al.*, 2023), illustrating the ability of the new robotic high-throughput methodology to achieve voltage- and current-clamp recordings within the same freshly isolated neurons. A) Inactivating current-voltage (IV) relationship of Nav channels in potassium fluoride (KF) internal solution. Protocols are on the right. B) As DRG neurons are known to express different ensembles of ion channels, with different voltage-dependent properties, across varying diameters (as measured in cell capacitance

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(pF)), single ($V_{1/2}$ from single Boltzmann = V_S) vs. double (first $V_{1/2}$ from double Boltzmann = V_{D1}, second $V_{1/2}$ from double Boltzmann = V_{D2}) Boltzmann equations were used to describe to characterize the conductances within each neuron. Comparison of double vs. single Boltzmann approach. * Indicates statistical significance. C) Number of action potential spikes that were elicited using a standardized ramp current-clamp protocol. D-F) Sample voltage-clamp and current-clamp traces, for the same cell, for high and low spiking neurons are shown.

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Figure 1

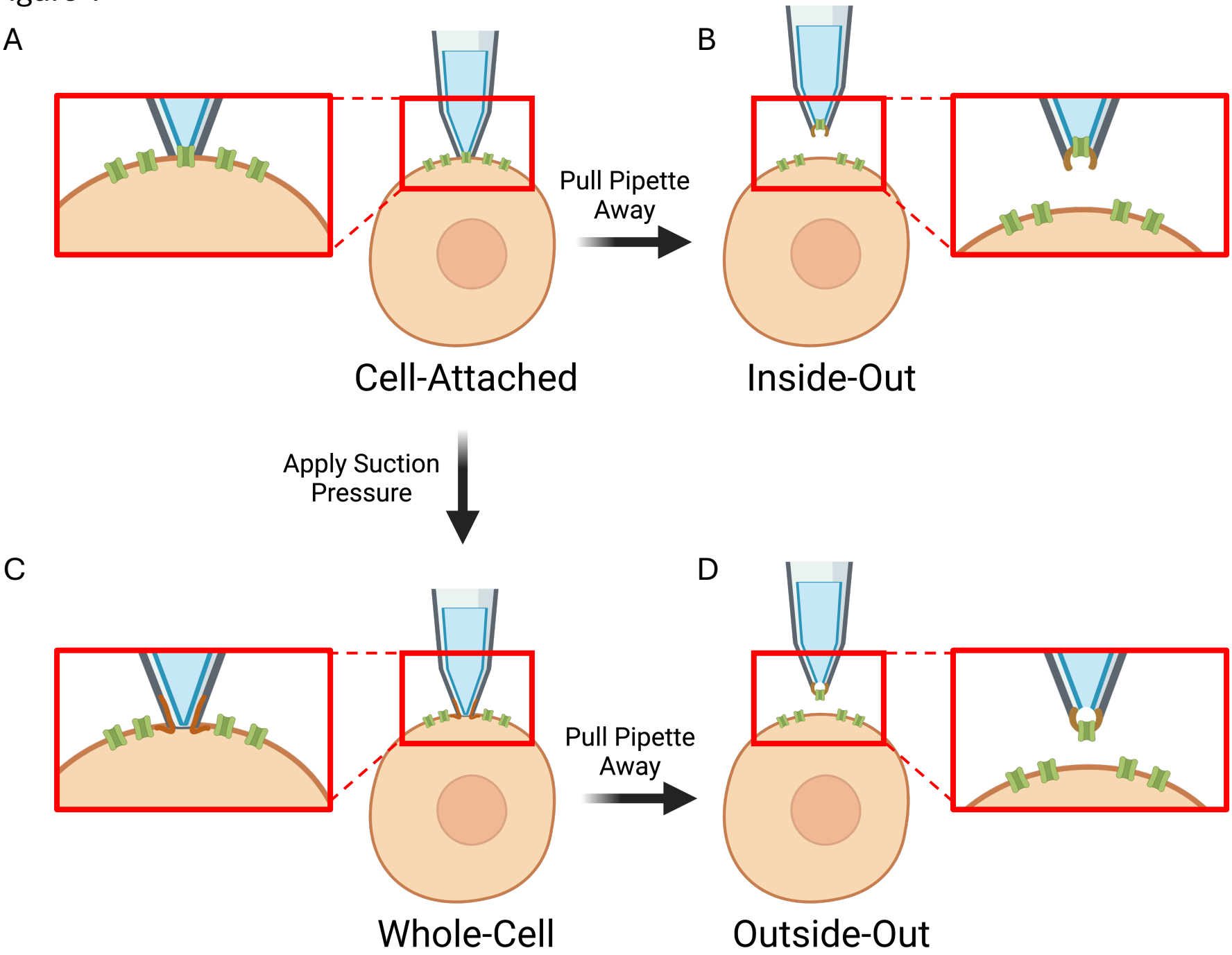

A
B
Pull Pipette Away
Cell-Attached
Inside-Out
Apply Suction Pressure
C
D
Pull Pipette Away
Whole-Cell
Outside-Out

Figure 2

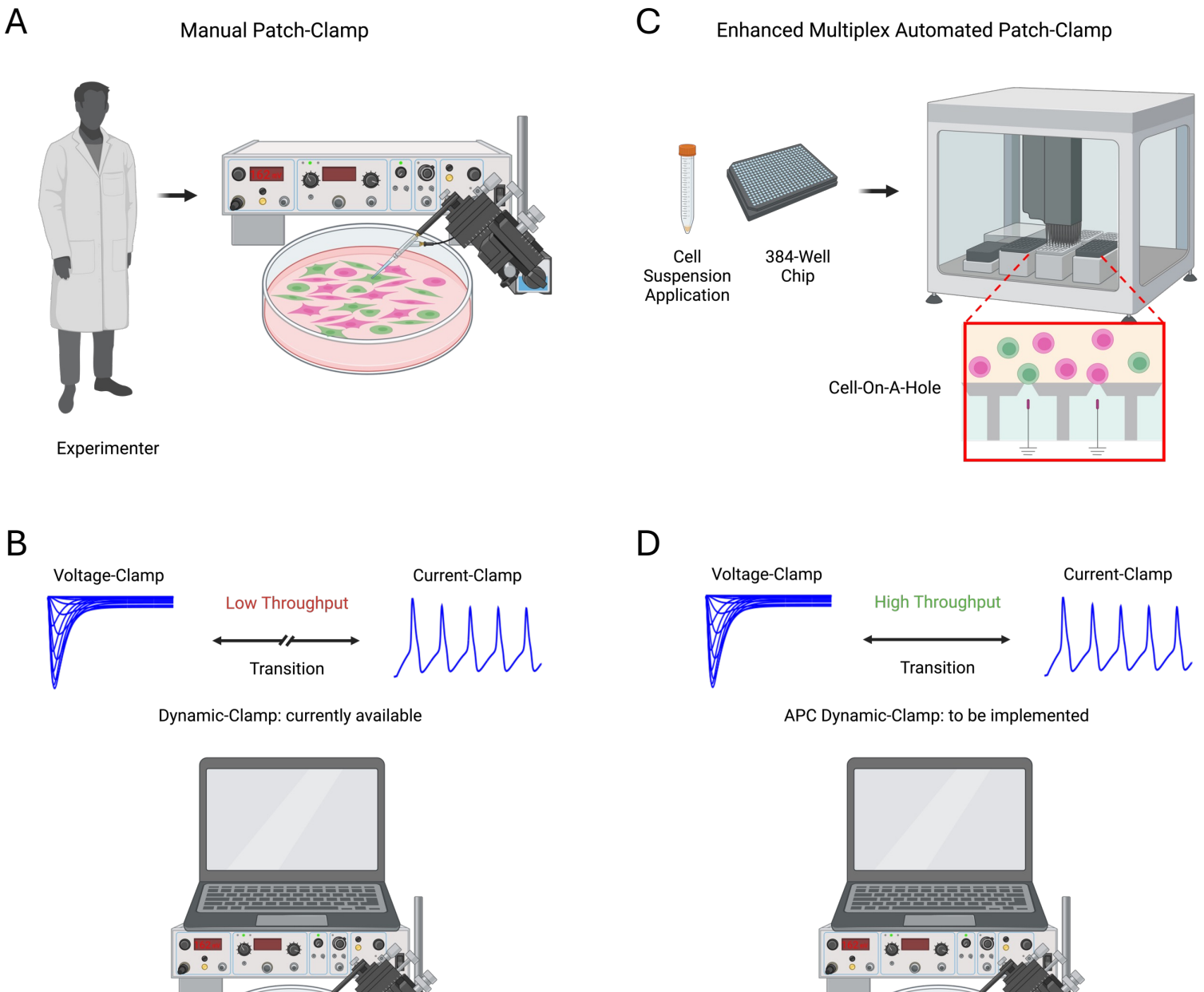
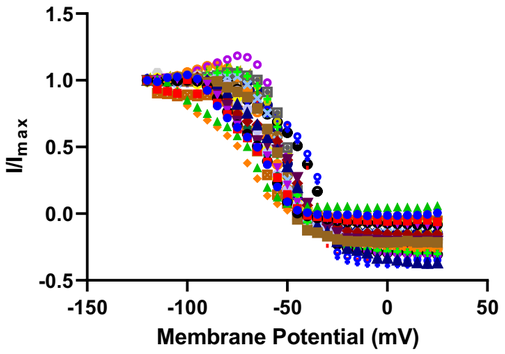

A
Manual Patch-Clamp
Experimenter
C
Enhanced Multiplex Automated Patch-Clamp
Cell Suspension Application
384-Well Chip
Cell-On-A-Hole
B
Voltage-Clamp
Low Throughput
Transition
Current-Clamp
Dynamic-Clamp: currently available
D
Voltage-Clamp
High Throughput
Transition
Current-Clamp
APC Dynamic-Clamp: to be implemented

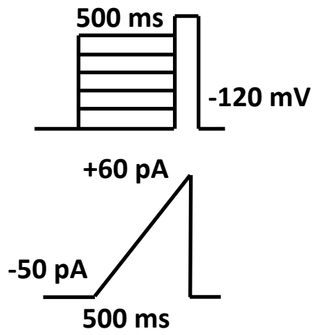
Figure 3

A

VC – KF ICS Inactivation

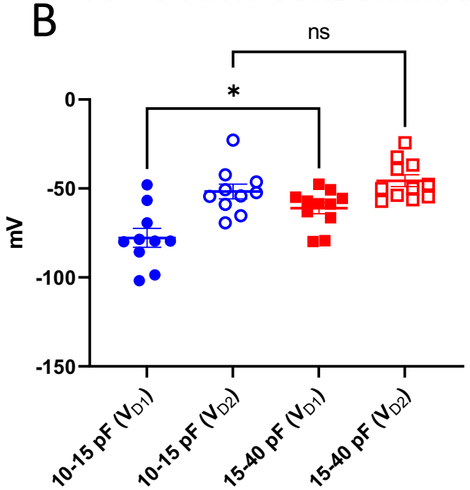


VC & CC Protocols

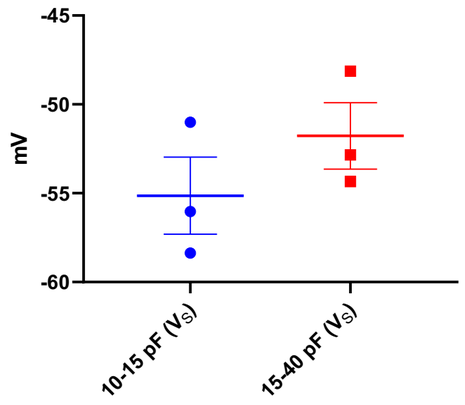


B

VC – Double Boltz Distribution

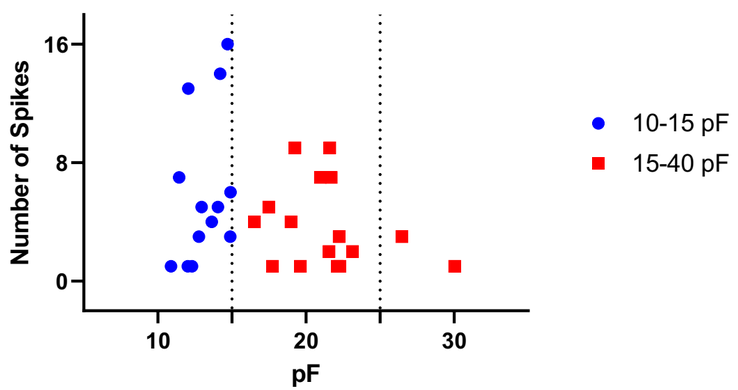


VC – Single Boltz Distribution



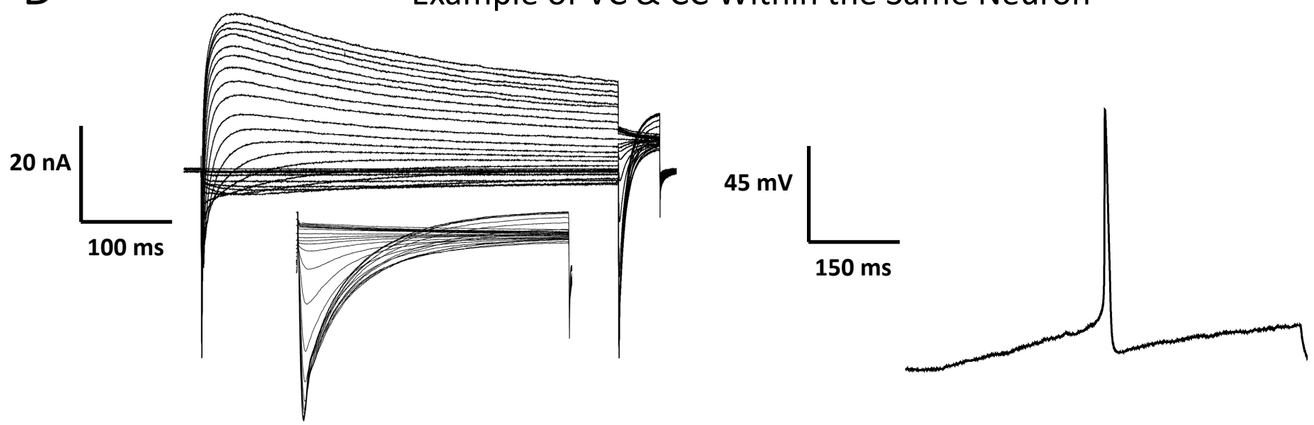
C

CC – # of Spikes vs. Capacitance

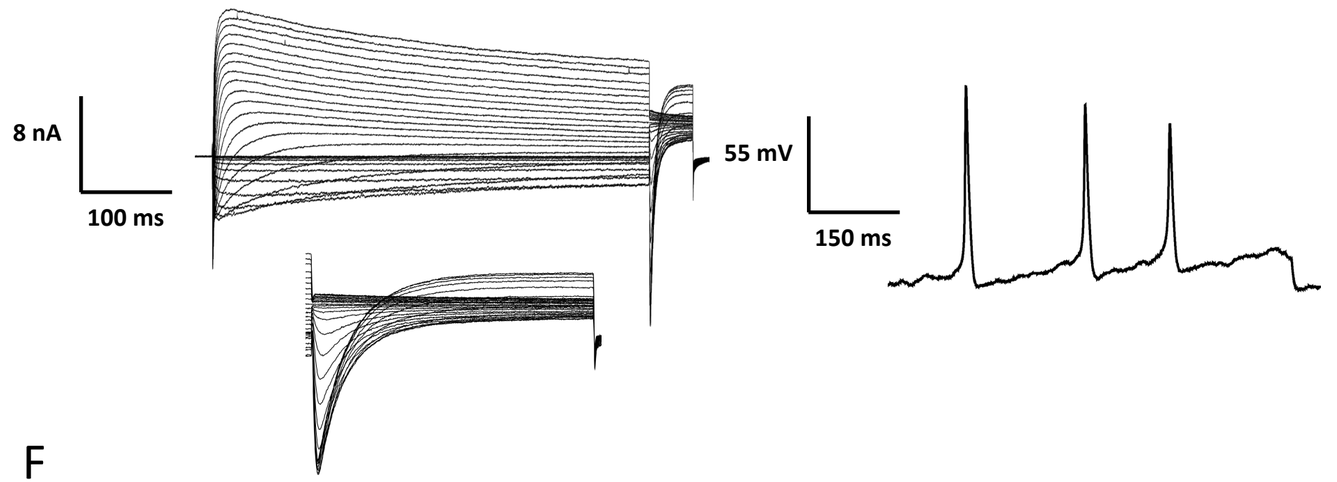
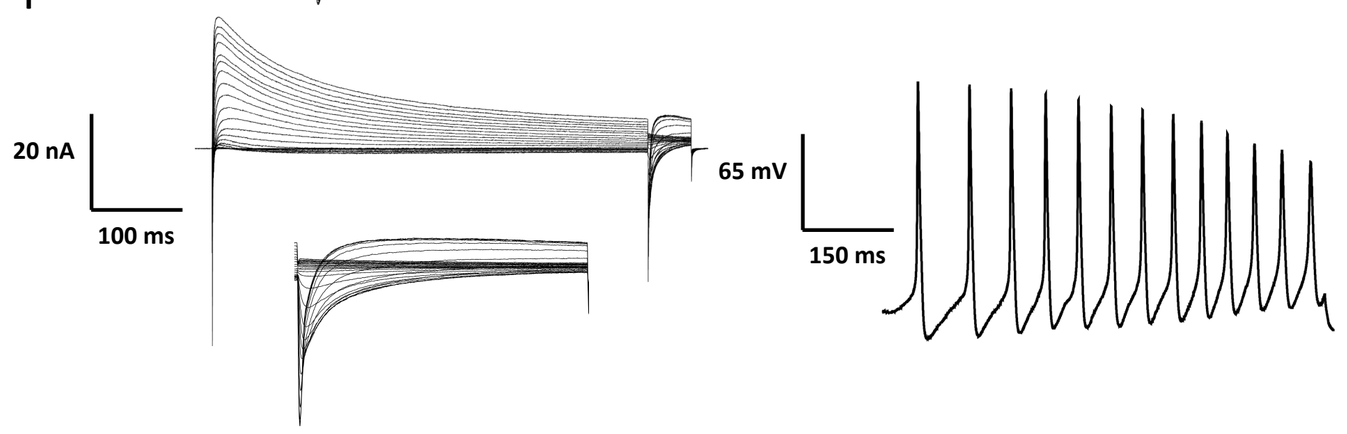


D

Example of VC & CC Within the Same Neuron



E



F