Chapter 2

Patch Clamp Technology in the Twenty-First Century

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Abstract

In the almost four decades since its inception, the patch clamp technique has transitioned from a specialist skill to a method commonly used among many others in a lab. Development of patch clamp instrumentation has not been steady: A boost of product releases in rapid succession by multiple manufacturers in the 1990s had slowed to a trickle by the mid-2000s. In 2016, Sutter Instrument's entry into the market of turnkey patch clamp amplifier systems, defined as an amplifier with matching data acquisition hardware and software, caused a fresh breeze in a field in danger of going stale. Sutter has meanwhile completed the product line, culminating in the flagship dPatch® Ultra-fast, Low-noise Digital Amplifier. The dPatch System constitutes a contemporary, digital design that features many firsts, including digital signal compensation, an extremely high bandwidth and fully integrated dynamic clamp capability, paired with the increasingly popular SutterPatch® Software.

This chapter compares feature sets of the new Sutter instrumentation with the established platforms by the other two providers of turnkey systems, Axon Instruments by Molecular Devices and HEKA Elektronik by Harvard Bioscience. A variety of products from other manufacturers, who rely on combination with components from other sources rather than offering turnkey systems, are listed, but for their conceptual diversity not compared at a great level of detail. The chapter further covers architectural considerations for patch clamp systems, headstage design, data acquisition strategies and efficient structuring of the recorded data, controlling and monitoring periphery, advanced technologies, such as software lock-in amplifier capability and dynamic clamp features, and application modules for efficient analysis of action potentials and postsynaptic events.

Key words Patch clamp amplifier, Data acquisition, Whole-cell, Single-channel recording, dPatch, IPA, SutterPatch, Dynamic clamp

1 Introduction

In the decades since its invention [1, 2], patch clamp electrophysiology has fared a similar fate to many other scientific techniques: From a bleeding-edge skill, mastered by a relatively small number of well-trained specialists, it has matured and proliferated to a widely used method. Patch clampers used to recognize one another after exchanging a few sentences, a substantial level of electronics skills was part of what was needed to be successful, and an oscilloscope was a mandatory part of each electrophysiology rig. There were

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quite a number of laboratories that defined themselves as patch clamp labs or electrophysiology labs in a broader sense.

Today, patch clamp experiments are usually performed as one out of a wide spectrum of scientific techniques to investigate a problem or a question associated with a particular model system. Researchers do not typically define themselves as patch clampers or electrophysiologists anymore and, consequently, often lack the detail knowledge of the intricacies of the instrumentation they use. Progress in instrumentation design and technology has made this paradigm shift possible for the majority of scientific applications of the patch clamp technique.

The automobile industry today delivers products that do not routinely require roadside repair or maintenance anymore. A car user can easily perform their daily commute and everyday shopping without so much as knowing how to open the hood—even though they will not likely become a successful race driver. Very similarly, the makers of today's patch clamp systems must provide hardware and software that perform the majority of everyday tasks without requiring detailed electronics knowledge or an advanced degree in computer science. Algorithms that automatically set resistance and capacitance compensation to a degree that enables a meaningful recording are expected, as are application modules that facilitate data acquisition and analysis following published and established standards.

Among the suppliers for patch clamp amplifier and data acquisition systems, only three offer turnkey packages, consisting of an amplifier component, a more or less integral data acquisition system, and a software package: Axon Instruments/Molecular Devices, LLC. [3], HEKA Elektronik Dr. Schulze GmbH [4], and Sutter Instrument Company [5]. For the purposes of this chapter, instrumentation and software by these three major suppliers shall be discussed in more detail. Several smaller patch clamp amplifier manufacturers, such as Alembic [6], Dagan [7], npi electronic [8], and Warner Instruments [9], serve niche sectors of the market. A number of laboratories use National Instruments Corporation interfaces for data acquisition [10], often in conjunction with software programmed in-house or in the public domain. Common development platforms for this type of software are MatLab [11] or LabView [12]. The British company Cambridge Electronics Design (CED; [13]) makes computer interfaces and software, including products that can be used for patch clamp. A number of software packages that operate with various mainstream hardware models are available commercially, open-source, or as freeware. A few examples, without any claim of completeness, include AxoGraph [14], Ephus [15], jClamp [16], NeuroMatic [17], QuB [18], or WinWCP [19]. Since most of these products either serve niche applications, have limited feature sets, or require a substantial amount of customization and programming, an

exhaustive discussion is beyond the scope of this chapter. Many of the principles discussed in the context of the hardware and software systems by the three major providers analogously still apply to these products.

2 Headstage Design Considerations

To minimize current noise and maximize signal bandwidth, virtually all current patch clamp amplifiers consist of a separate headstage and a main unit. The headstage contains the feedback amplifier circuitry for voltage clamp operation as well as the current clamp/voltage follower circuit, and acts as the physical link between a micromanipulator and a pipette holder. Connection to the micromanipulator is typically established through a standard dovetail fitting. The dovetail connection was originally agreed on between Sutter Instrument and Axon Instruments in the early 1990s (Dale Flaming and Alan Finkel, personal communication) and has since been adopted by virtually all manufacturers of micromanipulators for electrophysiology and patch clamp amplifiers.

Two common formats for pipette holder fittings to the headstage are Axon Instruments' HL-U standard and HEKA Elektronik's BNC-based connection. Sutter Instrument and several other amplifier manufacturers use connections that are compatible with the HL-U standard. If a threaded connector made of Teflon® is used on the headstage side, as with Axon Instruments headstages and several others, considerable mechanical movement of the joint is possible, which manifests itself as drift of the pipette tip. Since this is often falsely attributed to the micromanipulator, troubleshooting the wrong component can waste precious time. To avoid this effect, Sutter Instrument headstages use a threaded collar that is machined of metal, and the pipette holder is firmly seated against it (Fig. 1). A welcome side effect is substantially better noise shielding of the headstage input. Several third-party suppliers make pipette holders as replacements, or for special applications, which are compatible with one of the two common standards. For the HL-U standard, Sutter Instrument also offers a pipette holder with a barrel made of quartz, which minimizes thermal expansion and may improve noise performance in certain applications [20].

Ground connector receptacles are typically placed at the back of the headstage. With most manufacturers, the headstage case is connected to signal ground and constitutes one of the ground connections to the micromanipulator system. The HEKA EPC 10 headstage case is driven, and therefore the ground connector and the dovetail fitting are insulated. The Sutter Instrument dPatch System comes with headstages that are divided into the analog recording headstage and a preamplifier module that contains the analog-to-digital and digital-to-analog converters, as well as the

Fig. 1 The pipette holder fitting on Sutter Instrument amplifiers is compatible with Axon Instruments' HL-U standard, which has been adopted by several other manufacturers. The threaded collar on Sutter headstages is executed in metal rather than the commonly used Teflon®. This provides both greater mechanical stability and better electrical shielding

compensation circuitry, most of which operates in the digital realm. The preamplifier is designed to be placed inside the Faraday cage (if equipped), and all communication to the main unit is digital, which makes it completely immune to noise interference.

3 Feedback Elements

3.1 Noise Performance in Voltage Clamp

Most experimenters form the gigaseal in voltage clamp mode, irrespectively of whether they are planning a single-channel recording or move on to whole-cell mode and, eventually, may switch to current clamp mode. Currents through individual ion channels are small, typically in the range between 0.1 pA and several tens of pA, depending on the identity of the ion channel, the membrane potential, and the solutions. The demands to noise performance are therefore most stringent in voltage clamp mode. While the experimenter is, obviously, interested in the noise level during an actual recording, having an electrode holder, a pipette, a cell or patch and a bath connected to the headstage is not an appropriate way to determine the noise performance of an amplifier. These components act as antennas, and the noise they pick up describes the environment rather than the amplifier. The established method of measuring the noise specification of a patch clamp amplifier is therefore with the headstage in open-circuit condition, inside a grounded enclosure. The noise specification is given as root mean

square (RMS) noise by convention, since that metric is a good descriptor of broadband noise. However, among amplifier manufacturers, there is no consistent bandwidth at which the noise performance is measured. For single-channel recordings, the RMS noise of the amplifier system should be around 200 fA in a 0.1 Hz–10 kHz band. For whole-cell recordings, a noise level of 1–2 pA_{RMS} in the same frequency band is acceptable.

3.2 Resistive Feedback

The most common headstage design uses the resistor feedback technology, in which a high-MΩ resistor R_f and an operational amplifier A_1 form a sensitive current-to-voltage converter [21]. The feedback circuit is connected to the inverting input of the operational amplifier, and the command voltage to the non-inverting input (Fig. 2a). A second operational amplifier A_2 subtracts the command voltage from the output of A_1, which is proportional to the feedback current. The output of A_2 is, therefore, a voltage that is proportional to the current through the tip of the pipette (V_I). The limited bandwidth of R_f makes it necessary to boost the high-frequency gain at the output of A_2. The principle, its limitations, and further design considerations are discussed in great detail in [21] and shall not be reiterated here.

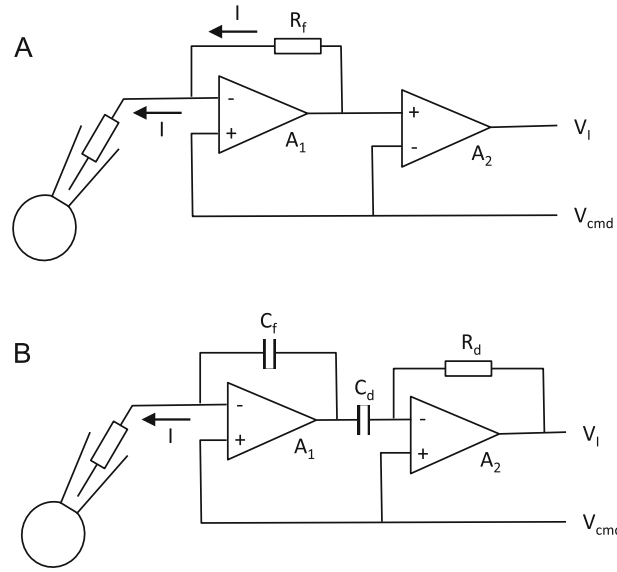

Fig. 2 Resistive and capacitive feedback circuits used in patch clamp headstages. (**a**) Resistive feedback circuit. *I*: The current injected into the pipette is converted into a proportional voltage by operational amplifier A_1 and feedback resistor R_f. Operational amplifier A_2 subtracts the command potential V_{cmd} and puts out voltage V_I, which is proportional to the current *I*. (**b**) Capacitive feedback circuit. The feedback capacitor C_f configures operational amplifier A_1 as an integrator. The following circuit of operational amplifier A_2, capacitor C_d and feedback resistor R_d form a differentiator, which puts out the voltage signal proportional to *I*

3.3 Capacitive Feedback

To minimize noise in the feedback circuit, the Molecular Devices Axopatch 200B amplifier and the Sutter dPatch system feature capacitive feedback stages in addition to resistive ranges. Replacing the feedback resistor R_f with a capacitor C_f creates an integrating headstage circuit, which then must be followed with a differentiator (Fig. 2b). A capacitive feedback circuit provides lower noise when compared to resistive feedback, a greater bandwidth, better linearity and a larger dynamic range. Particularly the better noise performance makes capacitive feedback designs the best choice for single-channel recordings. A capacitive feedback circuit is not suitable for current clamp recordings, however. Therefore, both the Axopatch 200B and the dPatch systems are equipped with two resistive ranges, in addition. The dPatch system also features dedicated voltage follower circuitry, which enable current clamp recordings with minimal distortion. This is not the case for the Axopatch 200B (*see* Sect. 4 below).

3.3.1 Resetting Transients

Large steady-state offsets in the recorded current would drive the integrator circuit in capacitive mode into saturation. Therefore, the capacitor needs to be reset when the circuit gets close to saturation. During this reset, a transient occurs, and the signal is not valid for a short period of time (typically $\ll$1 ms). *See* ref. 21 for an exhaustive discussion of this principle. While the Axopatch 200B uses a sample-and-hold circuit and puts out a "not-valid" signal on a dedicated connector, the digital design of the dPatch system allows for directly flagging the reset to the SutterPatch Software. The user can then decide to mask the resets with a straight line, equivalent to the way the Axopatch 200B treats them, to insert a blank into the recorded data, which may make it easier to exclude the invalid portion from further analysis, or to record the reset transients as they are. The reset transients further comprise a slow component with a complex time course, which can take several ms to fully decay. These are compensated with analog circuitry or by digital subtraction in the Axopatch 200B and dPatch amplifiers, respectively.

4 Voltage Follower Circuitry in Current Clamp

It is important to note that the Axopatch 200 amplifier was designed for ultimate noise performance, but high-fidelity current clamp recordings were not among the design goals. The lack of a dedicated voltage follower circuit in the headstage causes waveform distortions in current clamp mode, which must be taken into consideration when action potential waveform analysis is performed [22]. All other currently available amplifiers by the major providers have dedicated voltage follower circuits and are suitable for recordings in current clamp mode. While there are minute differences in bandwidth and phase correlation between various

amplifier models, which may affect high-resolution analysis of action potential waveforms (e.g., [23]), a detailed discussion is beyond the scope of this chapter.

5 Mode Switching/Smart Switching

A patch clamp amplifier whose command input is connected to a separate computer interface needs special attention when the experimenter switches from voltage- to current clamp mode. Since the recorded cell is most likely held at a hyperpolarizing potential, typically in the vicinity of the presumable resting potential, the command for that potential would be interpreted as an inward current after switching. To avoid either having to depolarize to 0 mV before the switch, or injecting current after the switch, amplifiers with a separate interface have an "$I = 0$" setting at which the external command input is ignored. Amplifier systems with an integrated interface, such as the HEKA EPC 10 and the Sutter amplifier systems, do not need this setting, since the holding potential in voltage clamp is under entirely independent control from the holding current in current clamp mode. An integrated interface also allows to more accurately control the sequence of events during the switch from voltage to current clamp. Both HEKA's EPC 10 and the Sutter amplifier systems utilize this principle for proprietary features, which are called Gentle Switch and Smart Switch, respectively, to avoid transients that are potentially damaging to the cells.

6 Compensation and Correction Circuitry

In patch clamp recordings, a number of signal modifications are common in order to reveal components of the recorded signal that would otherwise be obscured by passive capacitive or ohmic responses originating from the electrical circuit that consists of the pipette, the pipette holder, and the cell membrane. Several capacitive components and the series resistance R_s cause capacitive transients in voltage clamp mode and form low-pass filters in current clamp mode. It is important to understand that series resistance correction is the only method described in this section which alters the command signal sent out to the cell. Offset and capacitance compensation methods are applied to the recorded signal only and do not affect the command stimulus. They are, therefore, sometimes referred to as "cosmetic" measures. Nevertheless, they constitute effective ways of revealing fast currents, such as voltage-gated Na^+ currents, which might otherwise be hidden in the capacitive transient elicited by a depolarizing step. Capacitance compensation circuitry also prevents the capacitive transient after a voltage

step from driving the headstage circuit into saturation. All compensation and correction methods described in Sects. 6–8 are applied before the signal is stored, and a copy of the untreated signal is not typically retained.

6.1 Offset Compensation

After lowering the recording pipette into the bath in voltage clamp mode, a steady-state offset current is measured. This offset is caused by the sum of offset potentials from several sources. The half-cell potentials of the bath and recording electrodes can be one of the largest sources of an offset potential. Particularly when solutions with large organic anions substituting most of the chloride are used in conjunction with Ag/AgCl electrodes, offset potential amplitudes of well over 100 mV are not unusual.

The current offset is compensated by applying a potential that reduces it to zero. Computer-controlled amplifiers have a function that automatically zeroes the offset potential. The offset potential is not considered part of the command potential, but the potential after offset compensation is defined as 0 mV. While modern patch clamp amplifiers, such as the Sutter Instrument amplifier systems, do keep a record of the offset potential, few researchers routinely pay attention to it.

If a slow drift of the signal is observed, the most likely cause is a deterioration of the chloride coat on the electrode wire or the bath electrode. Another common cause is bath solution that gets in contact with the nonchlorided portion of an electrode wire or the wire portion of an Ag/AgCl pellet. Rechloriding the silver wire should be done in regular intervals. Blank silver portions can be insulated with Sylgard®, epoxy, or glue that remains elastic and does not leach solvents.

Some amplifiers have a feature called Track, which continuously eliminates the offset. This becomes problematic if Track is accidentally left enabled during and after seal formation. Since the reference point for the command potential is lost then, the recording is invalidated. The main reason for enabling Track is a slow drift in the signal, which is preferably addressed by eliminating its root cause (see above).

6.2 Capacitance Compensation

Capacitance compensation is applied at two stages in the process of forming a seal and a whole-cell recording. Depending on the manufacturer of the amplifier the terminology varies. Sutter Instrument and Axon Instruments use the terms Electrode or Pipette Compensation and Cell or Whole-cell Compensation, respectively. Axon Instruments further divides the Pipette Capacitance into its fast and slow component. Both Sutter Instrument and HEKA Elektronik use fixed parameters for the slow component of the electrode capacitance compensation. HEKA Elektronik uses the term *C*-fast to describe the electrode capacitance, while *C*-slow refers to the whole-cell capacitance. These differences in terminology can lead to confusion.

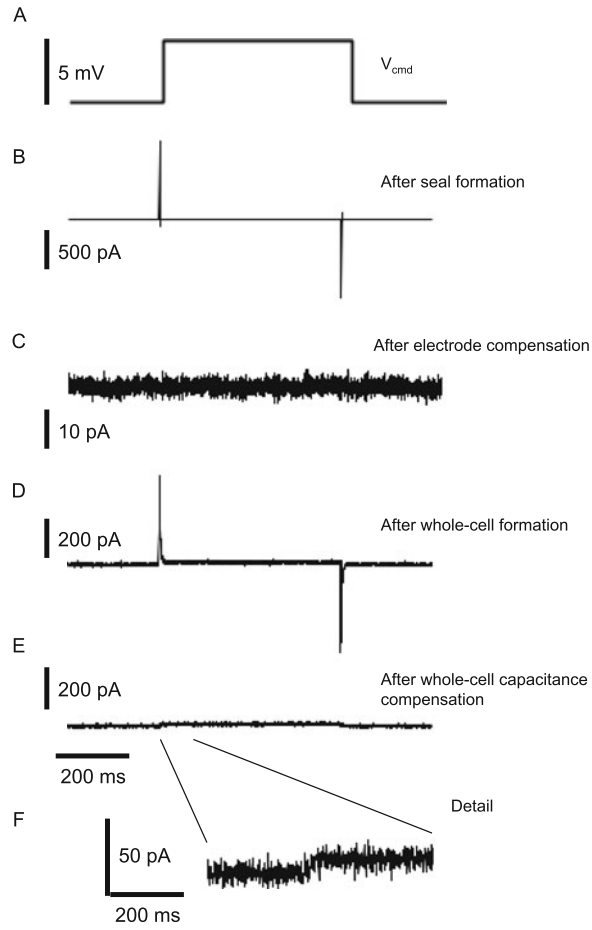

Fig. 3 Capacitance compensation recorded on a Sutter dPatch Amplifier system with a model cell connected. Analog bandwidth: 10 kHz, resistive range ±20 nA. After seal formation, the current response (**b**) to voltage command step (**a**) is a straight line with short capacitive transients. After automatic electrode compensation the capacitive transients virtually disappear in the noise (**c**). The longer capacitive transients after proceeding to whole-cell position (**d**) are compensated by the automatic cell compensation with minimal manual touch-up (**e**). (**f**) Detail of the rising step in **e**. The time scale is identical in **a**–**e**

6.3 Electrode Capacitance

6.3.1 Voltage Clamp Mode

After seal formation (Fig. 3a–c), the capacitance of the recording circuitry is determined by the capacitance of the pipette and the, comparatively small, capacitance of the membrane patch. The latter disappears in comparison to the former. The electrode capacitance consists of two capacitive components, the slower one of which is relatively constant. Many researchers only adjust the faster component of the electrode capacitance, if separate controls are available. Sutter Instrument and HEKA Elektronik amplifier systems use fixed parameters rather than separate controls (see above).

In single-channel recordings and high-bandwidth applications, it is important to physically reduce the electrode capacitance as much as possible rather than compensating for it. This is done to both minimize the capacitive noise and maximize the recording bandwidth. The most commonly used methods are: (1) reducing the area of the capacitor that is formed by the glass pipette in the bath by coating it with wax or Sylgard®, or pouring oil on top of the bath solution, (2) maximizing the distance between the two conductive solutions by coating the pipette and giving it a stubby shape with a short taper, and (3) using quartz capillaries, which have a lower dielectric constant than the commonly used borosilicate. Quartz capillaries cannot be pulled with filament-based pipette pullers, however. Currently, the only commercially available puller that can handle quartz is the Sutter Instrument P-2000 laser-based micropipette puller.

6.3.2 Current Clamp Mode

Most researchers base the capacitance compensation applied in current clamp mode on the value that was determined in voltage clamp. To avoid oscillations that can be triggered by rapid changes in the recorded signal, such as action potentials, the magnitude is commonly reduced by 5–10%. Even though it is the same pipette property that is being compensated, it is not uncommon to refer to this method as Capacitance Neutralization when in current clamp mode.

6.4 Whole-Cell Capacitance

Once the electrode capacitance has been compensated, most experimenters proceed to the whole-cell configuration. This is done by applying stronger suction to the pipette. Some cell types or preparations break in more easily when a "Zap" pulse is applied, a depolarizing voltage step of several hundred mV and typically a duration in the submillisecond range.

The whole-cell configuration is indicated by the presence of much larger capacitive transients on the square membrane test response, since the membrane of the entire cell now contributes to the total capacitance of the system (Fig. 3d–f). All patch clamp amplifiers designed to perform whole-cell recordings have the capability of compensating for the membrane capacitance C_m and series resistance R_s. Note that HEKA Elektronik calls this compensation *C*-slow, which bears the risk of being confused with Axon Instruments' fast and slow components of the electrode capacitance. Sutter Instrument's amplifier systems explicitly refer to Electrode and Cell Compensation, respectively.

The automatic compensation feature, common in computer-controlled amplifiers, should constitute a good starting point for capacitance compensation. Manual touch-up may be required for highly sensitive applications.

7 Series Resistance Correction

The series resistance R_s between the inside of the recording pipette and the lumen of the cell is comprised of the pipette resistance and the resistance of cell membrane lining the inside of the pipette orifice, as well as organelles, vesicles, membrane fragments or other debris that may clog up the opening. Depending on the cell type, there is a variety of strategies to minimize the series resistance, including but not limited to choosing a large pipette opening and applying pressure-suction regimes after whole-cell formation. A rule of thumb is that the best that can be achieved is twice the electrode resistance. But with many preparations, a substantially higher R_s must be accepted.

The series resistance is the R component of a low-pass filter that slows down rapid changes in the command potential. In addition, the voltage drop across R_s causes a reduction of the amplitude of the command potential. Therefore, a command potential step gets both slowed down and reduced in amplitude (Fig. 4).

Two different methods have been applied to mitigate the effect of R_s, commonly referred to as Correction and Prediction. The principle of R_s Correction is adding a scaled version of the recorded current signal to the command potential and thus compensate both the slowing of edges and the voltage drop. Since this circuit constitutes a positive feedback loop, it introduces the risk of oscillation, also referred to as ringing. Series resistance Prediction, also known

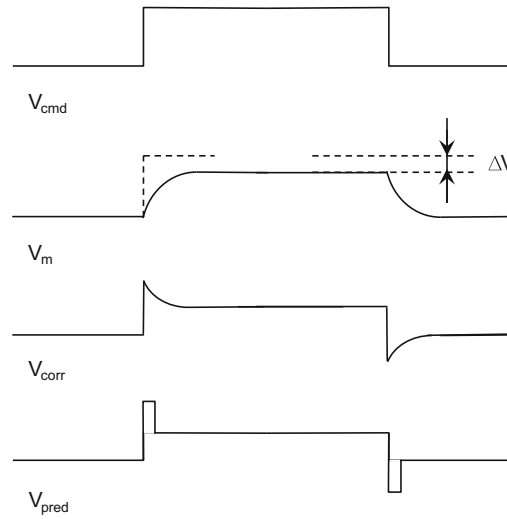

Fig. 4 Series resistance correction and prediction. The command step V_{cmd} is both reduced in amplitude and slowed down by the series Resistance, which results in the membrane potential V_m. Series resistance correction (V_{corr}) and prediction (V_{pred}) mitigate this effect, depending on the percentage applied and square up V_m (not shown)

as Supercharging, adds a scaled and shaped version of the command potential, which is derived from the whole-cell capacitance compensation circuit. Since Prediction is not controlled by a positive feedback loop, it does not introduce the risk of ringing. It also does not correct the voltage drop across R_s. Unlike the other compensation circuitry, series resistance compensation is not applied to the recorded signal only but affects the signal that is applied to the pipette. Therefore, feedback ringing of the Correction circuit applies large command voltage oscillations to the cell membrane, which quickly destroys cells and makes the recording useless.

Both Prediction and Correction are typically combined and applied in a graduated fashion as a percentage. To avoid feedback oscillations and their detrimental effect on the cells, many experimenters keep the Correction under 70%. The Axon Instruments Multiclamp 700B features an oscillation detection feature, which disables R_s Correction as soon as a beginning oscillation is detected. The digital architecture of the Sutter Instrument dPatch system eliminates nonlinearities and tolerances of analog components, which signficantly reduces the occurrence of feedback oscillation and minimizes the detrimental effect. With a model cell connected, R_s Correction of >95% does not cause feedback oscillations, even with a lag of only 2 μs. Provoked oscillations (99% Correction, 1 μs Lag) do not drive the circuit into rail-to-rail operation at an analog bandwidth of 10 kHz. While in theory that should prevent cell damage, this assumption still remains to be tested in wet-lab conditions and with a variety of cell types.

8 Bridge Balance Compensation

In current clamp mode, injected current waveforms cause a voltage drop across the resistance of the recording pipette. Since this voltage drop cannot readily be distinguished from a voltage signal originating from the preparation, bridge balance compensation is used to eliminate the Ohmic component. The name originates from the use of a circuit called a "Wheatstone bridge" in the days of early microelectrode amplifiers. Modern designs use operational amplifiers for this purpose. To adjust bridge balance, a current pulse is applied to the preparation. Then a scaled version of the current waveform is added to the recorded signal and adjusted until the steady-state portion of the voltage response disappears (Fig. 5).

9 Dynamic Holding

In current clamp recordings it is sometimes desirable to compensate for the weakening membrane resting potential of a deteriorating cell and hold it at a target potential by injecting current that

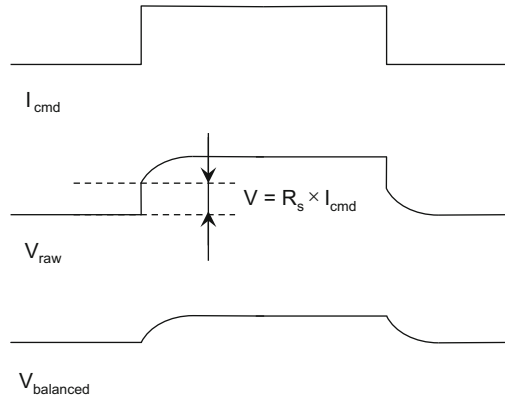

Fig. 5 Bridge balance compensation. A command step applied in current clamp (I_{cmd}) elicits a voltage drop across the pipette resistance, superimposed by the voltage response from the preparation (V_{raw}). Bridge balance compensation eliminates the Ohmic portion and returns the isolated voltage response from the preparation ($V_{balanced}$)

updates with a long time constant. The computer-controlled amplifier systems from the three major providers have features that achieve this. Axon Instruments calls this function Slow Current Injection, HEKA Elektronik uses the term Low-frequency Voltage Clamp (LFVC), and Sutter Instrument calls it Dynamic Holding.

Care must be taken not to accidentally leave this feature enabled, however, when the uninfluenced resting potential of the cell is the subject of the experiment. Continuous current injection also constitutes a net movement of charges, which may lead to depletion or accumulation of ions inside the recorded cell or in its vicinity.

10 Data Acquisition

10.1 Data Acquisition Over the Years

In the early days of the patch clamp technique, it was common to continuously record the current and voltage signals on storage media such as FM tape or, once available, digital audio tape (DAT), using modified recorders. Regular audio recording equipment was not suitable, since it does not preserve DC offsets, either because the storage medium does not support it, or because the signal is purposely highpass-filtered. In addition, it was not uncommon to write the signal to a strip chart recorder for convenient overview. Stimulus waveforms were created using sophisticated stimulus generators, whose signal was recorded along with the elicited signal.

With the advent of more powerful computers and interfaces that had buffering capability and analog-to-digital and digital-to-analog converters (AD/DA boards), data acquisition became much

more streamlined, and stimulus waveforms were often created by software. Commercial amplifiers and data acquisition systems were launched, and the California-based company Axon Instruments Inc., as well as the German HEKA Elektronik Dr. Schulze GmbH established themselves as the market leaders. Both providers offered systems in a three-tier architecture consisting of a patch clamp amplifier, a computer interface, and software packages that controlled data acquisition and provided a certain level of data analysis capability.

It was not uncommon to combine one provider's amplifier with the other's computer interface, or use an amplifier made by one of the smaller manufacturers (see above). Both Axon and HEKA originally used interfaces made by third-party providers, Labmaster and Instrutech, respectively. In the early 1990s, Axon Instruments developed their first in-house interface, the Digidata 1200. HEKA Elektronik continued using Instrutech interfaces until they eventually acquired the company in 2007.

Axon Instruments was sold to Molecular Devices Corp. in 2004, and the company went through two more mergers until 2008. With a focus on high-throughput instrumentation and imaging platforms, not much development has been done for the conventional electrophysiology product line. The only somewhat recent hardware product by Axon Instruments/Molecular Devices is the Digidata 1550 interface. Only incremental updates were made to the pCLAMP software suite in varying intervals. The most recent version upgrade, pCLAMP 11, came with a minimal set of new features. Many long-standing issues and shortcomings have remained unresolved. It is still a 32-bit application, which more and more clearly shows its age.

HEKA Elektronik was acquired by Harvard Bioscience, Inc. in 2015. The established EPC 10 patch clamp amplifier was updated several times until the early 2010s and has since been available in its current revision. Patchmaster Software has continuously undergone development, both for bug fixes and new features. Patchmaster NEXT Software, released in 2018, presents much of the Patchmaster functionality in a more contemporary and intuitive graphical user interface, and on a more modern development platform.

In 2016, Sutter Instrument released the IPA® Integrated Patch Amplifier System, a patch clamp amplifier optimized for whole-cell recordings, with the data acquisition system integrated on the same circuit board, and SutterPatch® Data Acquisition, Management, and Analysis Software being part of the bundle. Over the following few years, the product family was broadened. It currently includes the Double IPA®, a dual-headstage version of the IPA system, the dPatch® Ultra-Fast, Low-Noise Patch Clamp Amplifier System with dynamic clamp capability, available with one or two headstages, and the recently released Dendrite™ Data Acquisition

System. Each product includes SutterPatch Software, which is based on Igor Pro by WaveMetrics and undergoes constant development. A number of research papers about experiments using the IPA Family Amplifier Systems and the included SutterPatch Software have been published [24–32]. Both the dPatch and Dendrite systems are too new to have been used for any publications at the time of this writing.

11 Three-Tier Architecture Evolves into Integrated Systems

In the early days of recording patch clamp data to computers, often with the intermediate step of recording on tape recorders modified to be DC-capable, a three-tier architecture was common (Fig. 6): The amplifier puts out an analog signal, which is routed to the analog-to-digital/digital-to-analog (AD-DA) converter, often referred to as the computer interface, or simply the interface. The interface, under control of software, turns the analog signal into a digital representation, which the software stores in a structured fashion. The software can also generate a command waveform, which the interface turns into an analog signal that is sent to the amplifier. This traditional architecture is still used by Axon Instruments and, for compatibility with others' components, by providers of standalone amplifiers or data acquisition systems.

A contemporary approach integrates the data acquisition hardware with the amplifier. The HEKA Elektronik EPC 10 system combines amplifier and computer interface circuit boards in a single

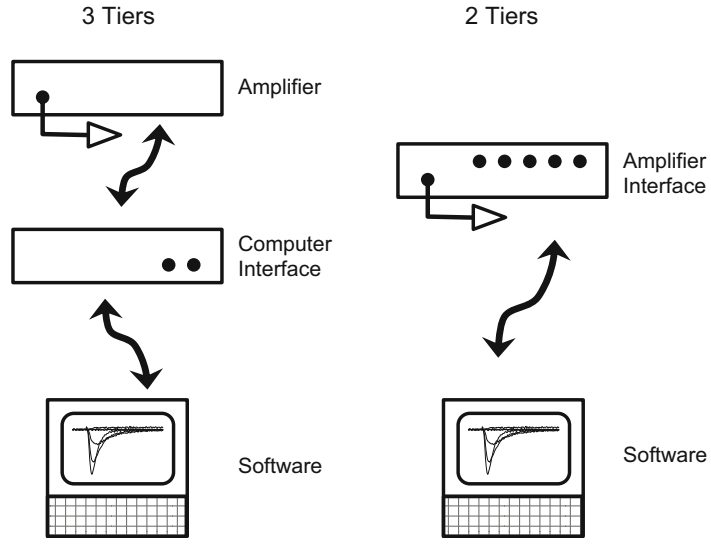

Fig. 6 Comparison of the original three-tier architecture and the more modern two-tier architecture in patch clamp systems

case, with a common power supply. The Sutter Instrument IPA family devices combine both amplifier and interface circuitry on the same board, which reduces power consumption and keeps system cost low. The patented [33, 34] Sutter Instrument dPatch® system even takes it a step further by making the AD-DA converter circuitry part of the headstage assembly and performing all compensation and correction (see above) in the digital realm. Since reliable capacitance compensation requires a very high sampling rate to capture rapidly decaying transients, the dPatch system incorporates the ability to sample at 5 MHz per headstage channel. The analog bandwidth of the headstages lies between 500 kHz and 1 MHz.

Software applications for data acquisition constitute the third tier of the classic architecture. With all three major providers, the acquisition software is tied to the respective computer interface, whether integrated (HEKA, Sutter) or separate (Axon). Axon Instruments offers pCLAMP Software, a package that consists of the data acquisition application Clampex and the data analysis program Clampfit. AxoScope software, a drastically feature-reduced version of Clampex, comes bundled with the Digidata® 1550 interface. pCLAMP Software needs to be acquired separately. Starting with version 11, the Clampfit analysis program requires a separate license protection key.

Most HEKA users purchase Patchmaster Software for data acquisition as a separate item and perform data analysis in third-party software. The most popular third-party analysis platform among HEKA users is Igor Pro by WaveMetrics [35], a technical graphing and analysis software with extensive programming capability.

Sutter Instrument patch clamp systems integrate all three architectural tiers into a single package and provide the Igor Pro-based SutterPatch® data acquisition, management and analysis software as part of the system package.

12 Data Structure Determines Efficiency of Data Analysis

A structured storage format facilitates data management and streamlines analysis. Efficient batch analysis requires that the data structure is consistent between experiments, since it needs to be predictable in which portion of the data a particular family of currents is found, and what its structure is. The three major providers store data in different ways, and certain aspects of the terminology may create confusion.

12.1 Samples, Signals, Sweeps and Series in a Greater Context

The most common form of patch clamp data in voltage clamp mode constitutes a family of currents elicited by a family of voltage steps or more complex waveforms. This data pattern is commonly used, for example, to investigate a current–voltage relationship and create the respective plot, commonly referred to as an I–V curve.

Table 1
Terminology comparison between the three major software packages

Axon Instrument pCLAMP	HEKA Patchmaster	SutterPatch
N/A	Compound Data	Experiment
Sequencing Key Sequence	Protocol	Paradigm
N/A	N/A	Paradigm Data
Protocol	PGF Sequence	Routine
Trial (data file)	Series	Series (Routine Data)
Signal	Signal	Signal
Sweep	Sweep	Sweep
Epoch	Segment	Segment
Sample	Sample	Sample

The data consists of one or multiple input signals, typically with multiple sweeps, between which a portion of the waveform is incremented.

Axon Instruments' pCLAMP software calls the set of instructions that creates this dataset a Protocol. The resulting dataset is called a Trial and constitutes an individual data file (Table 1). Other than data file naming, which includes a prefix and an automatically incrementing numerical index, no context for the dataset is created, and it is up to the experimenter to write down where in the structure of their experiments the data file belongs.

HEKA Elektronik's Patchmaster software refers to the equivalent set of instructions as a Pulse Generator (File) Sequence. The resulting dataset is stored as part of a larger data file, which can contain data from an arbitrary number of executions of Pulse Generator Sequences. The data in a data file can be accessed in a tree-like structure. An infinite number of Pulse Generator Sequences can be stored in a Pulse Generator File.

Sutter Instrument's SutterPatch Software uses the term Routine for the corresponding instruction set and calls the resulting dataset Routine Data or a Series (Fig. 7a). If the recording is started by executing a Routine, a container for the dataset is automatically created, which is called a Paradigm. The Paradigm Data contain various types of information, which is not based on samples, sweeps and signals, called Metadata (see below). Importantly, Paradigm Data also record the temporal context of each Series relative to others, if the same Routine is executed several times, or different Routines are executed successively. The data are organized in a configurable tree structure, represented in the Data Navigator, which provides random access to any Paradigm, Routine, Signal

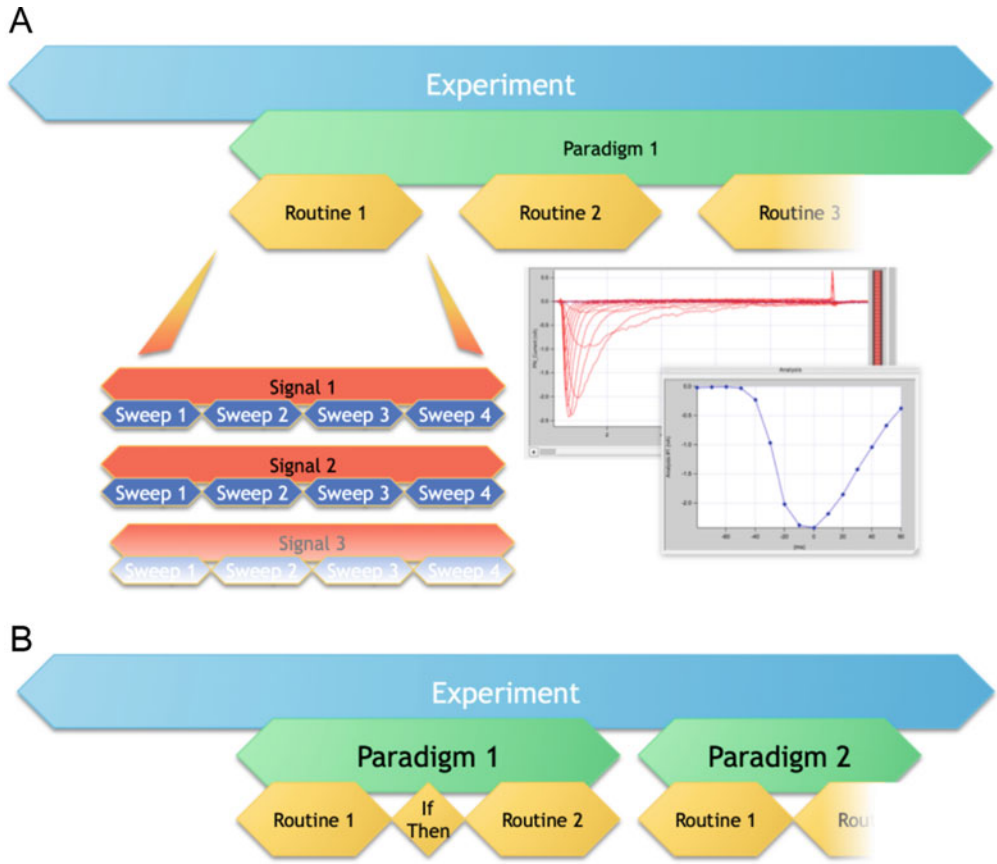

Fig. 7 The data structure in SutterPatch® Software. (**a**) An "Auto-triggered Paradigm" is created by execution of a Routine, in this case a family of voltage steps that elicits voltage-gated sodium (NaV) currents and creates an *I–V* curve in real time. (**b**) An example of two "Planned Paradigms," which can be used to partially automate an experiment. Routine 1 could be the same NaV Routine as in **a**. The flow control element (If-Then) accesses the real-time measurements in Routine 1 and determines whether the peak current is greater than an acceptance threshold. If so, Paradigm 1 proceeds to Routine 2, which is used to determine the half-inactivation potential $V_{1/2}$. Then Paradigm 1 sets the holding potential to $V_{1/2}$ and chains to Paradigm 2, which generates the data for a concentration-response curve. If the acceptance criterion is not met, Paradigm 1 terminates the recording after the If step

or Sweep node. Multiple Series within a Paradigm can be displayed in a Scope Window on a continuous time axis and drilled down to for closer examination and analysis.

All three software packages feature a certain level of automation. The most basic approach is implemented in pCLAMP, where Sequencing Keys let the user load or execute Protocols, change inputs and outputs and link to other Sequences. Flow control is only realized as said links to other keys and Wait steps that block execution for a preset time, or until the user responds to a prompt. Execution of Sequencing Keys is in no way reflected in the data

structure: If a Protocol is executed, a corresponding, but isolated data file is created. If not, execution is only logged in the purely text-based Lab Book.

Patchmaster offers much more sophisticated scripting ability in what is called Protocols. The fact that pCLAMP and Patchmaster use the term Protocol for completely different structural entities leads to considerable confusion among users who switch from one platform to the other (Table 1). Patchmaster Protocols enable control of virtually all hardware settings and, importantly, provide If-Then-Else and Loop steps for advanced flow control and a substantial level of automation of the experiment.

SutterPatch Software also lets the user execute a scripted experiment, as defined by a Paradigm. Other than pCLAMP and Patchmaster, execution of a Paradigm does create a data entity as a subset of the data file, irrespectively of whether it is executed as an "auto-triggered Paradigm," initiated by execution of a Routine (Fig. 7a; see above), or a "planned Paradigm" as a scripted portion of the experiment (Fig. 7b). Not only do SutterPatch Paradigms, much like Patchmaster Protocols, let the user control all hardware functions, provide advanced flow control, give access to real-time measurements and support execution of arbitrary mathematical equations, but they also let the user call Igor Pro commands, including custom code procedures, which enables execution of complex analysis procedures and creation of bespoke graphs or layouts. Last but not least, execution of a planned Paradigm creates an entity within the experiment, whose predictable data structure is the foundation for automated, efficient data analysis.

12.2 Handling Metadata

Besides the electrophysiological recording, which after digital conversion is stored in numerical sample points, the experimenter typically wants to record information about the specimen, instrumentation or environmental parameters. These metadata have traditionally been written down in a lab journal, a separate text document or a text window in the data acquisition software. This unstructured approach results in metadata that are difficult to mine and, in the case of a paper-based journal or a separate file, are at risk of being separated from the associated electrophysiological data. Only SutterPatch Software comes with features that enable storing user-defined metadata in a highly structured fashion.

12.3 Automatically Determined

Most of the metadata that describe the instrumentation used during the recording can be determined automatically. Traditionally, amplifiers transmitted certain settings to dedicated input connectors on a data acquisition interface. This was referred to as Telegraphing and is naturally limited to only a small number of parameters. The only amplifier by a major manufacturer still using this technique is the Axopatch 200B amplifier. All other systems by the major providers either use software telegraphing (MultiClamp

700B), or the amplifier circuitry is directly controlled by the same software that also accomplishes data acquisition (HEKA, Sutter).

But it is not only the amplifier settings that can be stored along with the recorded signals without requiring input from the experimenter. The identity of hardware, such as model and serial numbers, firmware revision, the software configuration that was employed, the identity of the user who was logged into the operating system, and many other parameters can be determined in the background and stored without requiring user intervention.

12.4 User-Defined

Contemporary platforms, such as SutterPatch Software, enable the experimenter to store other information they may find relevant, such as the animal species, genotype, sex, weight or age, as well as parameters of the tissue or cell preparation, such as prep time, dissociation solution or storage conditions. Even parameters that cannot traditionally be recorded with electrophysiology data, such as the recording solutions, or information about the micropipette electrodes, can be stored in a structured way. The user can opt to be prompted to review and confirm parameter values at the beginning of an experiment, or before a data recording commences.

Two aspects are important when it comes to storing metadata in a way that facilitates efficient analysis: (1) Each metadata parameter must be stored in its own, dedicated field. Extracting information from a free-text comment field or a shared space is inefficient and highly error-prone. (2) A measure, such as the animal weight or age, must be recorded separately in two fields, as the numerical quantity and the corresponding unit. Storing the weight of a mouse as "22.3 g" requires parsing of a string parameter for analysis. If the unit is recorded separately, however, the software can prompt for the numerical weight in g at the beginning of an experiment. The unit, "g" in this example, will likely stay the same between experiments.

12.5 Dynamically Updated During Acquisition

While parameters associated with the experimental animal or the preparation do not usually change during an experiment, other metadata parameters may change, and their stored values may need to be dynamically updated. That is naturally the case for amplifier settings if they are changed in the course of long experiments, for example, to account for changes in the series resistance of a whole-cell recording. The amplifier bandwidth and the corresponding sampling rate may be different for different types of acquisition Routines.

Potentially more critical is the capability to record changes in a stimulus, such as the addition of a test compound. The key here is the ability to record a parameter change, with a time stamp, even if it occurs while no signal data is stored to disk. This enables the researcher to more accurately reproduce under which environmental conditions the next dataset was recorded, while accounting for

possible delays in the stimulus delivery system. The most common delays are introduced by switching solutions with a perfusion system, since dead volumes cannot be completely eliminated, the full exchange of the bath solution takes some time, and slow receptor binding may cause additional delays. A time-tagged record of when a solution change was initiated is the most reliable way of ensuring complete solution exchange. Display of a sequence of Routine data sets on a continuous time axis, with tagged events during or between them, provides a convenient overview over an experiment or a portion of it. Among software packages by the major providers, only SutterPatch Software has this ability. Both pCLAMP and Patchmaster software rely on purely text-based tags generated by the user. If they occur outside signal acquisition, they are not in temporal association with the data but merely exist as comments in the respective Lab- or Notebook windows. That makes the analysis unnecessarily inefficient and error-prone, since it involves parsing of plain text entries.

13 Controlling and Monitoring Periphery

A variety of hardware for control or monitoring of environmental parameters, cell identification or stimulus delivery can be associated with a patch clamp setup. From the view of the amplifier and data acquisition system, these devices are considered periphery. Even though a microscope manufacturer will very likely disagree and view their advanced fluorescence lamp as something other than periphery, the term shall be used for the purposes of this chapter.

Peripheral instrumentation is typically controlled by either a graduated, analog voltage signal, or a series of digital pulses. By convention, most commercially available data acquisition systems put out an analog range of ± 10 V. These are typically referred to as Auxiliary Outputs or similar. The same amplitude range of ± 10 V is commonly used for Auxiliary Input channels. Digital Outputs either follow the TTL logic level standard, or the more modern CMOS standard [36]. These standards are designed to be compatible with each other. Most common electrophysiology periphery known to the author accepts CMOS signals as digital input.

An important consideration in the decision of whether to use analog or digital control of peripheral instrumentation is the question whether or not the amplitude of the event generated by the peripheral instrument plays a role for the recorded signal. If, for example, a liquid-filament switch system bathes the cell in either one solution, or the other, only the timing of the signal is relevant, and a digital output line can be used. Similarly, if a bank of pinch valves in a solution switcher is controlled individually. However, if the stimulus amplitude is of relevance for the recorded signal, analog control is preferred. That is also the case if the respective peripheral

instrument provides the capability to trigger sophisticated stimulus waveform patterns through a short digital pulse: Unless that waveform pattern is recorded together with the data, valuable information about the applied stimulus may be irrevocably lost.

One scenario commonly seen in the field is triggering a light stimulus by opening a shutter, while controlling the intensity with a manual dial. Without meticulous documentation, nothing is known about stimulus intensity. Even if the dial setting is diligently written down for each trigger, precision is limited by the dial, and accuracy depends on proper calibration procedures. The record is usually either made in a paper journal or a separate text document. In neither case is a permanent link to the recorded signal created. Most modern light sources accept an analog signal to control the light intensity. An LED light source has short enough switching times to not require a shutter, and the same analog signal also controls the duration. Therefore, an Auxiliary Output should be used to control light intensity, if possible, together with the duration.

Another common scenario is the use of an electric stimulator or stimulus isolator. Some of these devices can create sophisticated waveforms or stimulus trains, triggered by a single, short TTL pulse. If the connection is implemented in that way, the stimulus signal must be recorded on a separate input channel of the data acquisition system. Otherwise, it is virtually impossible to efficiently keep track of a complex stimulus. The much-preferred way is controlling a stimulus isolator through a Digital Output in case the amplitude is irrelevant, or an Auxiliary Analog Output if amplitude does matter.

When a solution switcher is used in pharmacology experiments, it streamlines data analysis if the acquisition software supports mapping a particular configuration of the outputs to a reservoir of the solution switcher and its contents, as characterized by compound identity, concentration and other factors, such as whether it is a control or test compound solution. At present, SutterPatch Software is the only commercially available package that features this ability.

14 Capacitance Measurements/Lock-In Amplifier

Both the Patchmaster and SutterPatch software packages feature software lock-in functionality. For high-resolution capacitance measurements during a voltage clamp recording a sine wave voltage is applied. The phase relation between the stimulus and the elicited current response are used to compute membrane parameters of the three-state model [37–40]. This enables highly sensitive recordings of exo- and endocytotic processes. pCLAMP software does not have this functionality.

15 Dynamic Clamp

The dynamic clamp technique was first introduced in 1993 as a method for simulating ionic conductances in neurons during patch clamp recordings [41, 42] and was later found to be applicable in cardiac and endocrinology research [43], as well as potentially other fields. This method typically involves recording the cell's membrane potential, transmitting the data either to an external computer, or using an interface with the necessary computing power built in, calculating the appropriate current to be injected into the recorded cell, and sending this value back to the amplifier. An external computer needs to run a real-time operating system, or at least an operating system with minimal jitter to ensure timely response to changes in the recorded signal. That precludes simple use of an existing computer interface and a purely software-based addition of dynamic clamp to existing platforms [44]. Communication between the computer and amplifier limits the rate at which the dynamic clamp calculations can be updated. Moreover, the additional hardware and software needed to do dynamic clamp have limited its application.

Unlike conventional platforms, which require either an external computer, or real-time processing power in the computer interface, the patented digital architecture of the Sutter Instrument dPatch amplifier provides an ideal platform for integral dynamic clamp. Utilizing parallel processing across a field programmable gate array (FPGA) and two high-speed ARM core processors, several sophisticated dynamic clamp models are implemented within this architecture. In each model, the update of the applied current values occurs without requiring any communication between the dPatch system and the data acquisition computer. Depending upon the complexity of the model, update rates of up to 500 kHz can thus be achieved.

Simulation of a population of channels within the cell membrane involves modeling the kinetics of the channel's gating mechanisms. The channel kinetics can either be modeled using multiple independent gates as in the Hodgkin–Huxley model [45], or a multistate Markov model [46]. For Hodgkin–Huxley style models, the dPatch system provides up to 16 individual gates per simulated channel, with up to 8 simulated channels running simultaneously per headstage. For Markov models, up to four 14-state models can be run simultaneously, or eight models with 10 or fewer states can be executed.

The values derived from the gating equations are then used to calculate the current applied to the cell. The calculation of current can either be defined in terms of conductance, or permeability. For conductance models, the value defined from the gating calculations is combined with the reversal potential of the ions passing through

the simulated channels, as well as the conductance of the simulated channels. Furthermore, channel conductance values can be voltage-dependent. For permeability models, the value defined from the gating calculations is combined with the intracellular and extracellular concentrations of the ions passing through the simulated channels. These models simulate the Goldman-Hodgkin-Katz equations [47, 48]. A comprehensive overview of the Dynamic Clamp feature in the dPatch amplifier system was given in a 2020 webinar presentation [49]. Since Dynamic Clamp capability is a feature that has been newly introduced to SutterPatch Software at the time of this writing, there is no scientific publication about its use in the laboratory yet.

16 Application Modules

The growing demand for more integrated solutions in patch clamp software has been met with the addition of several application modules to SutterPatch Software. The discussion here shall be limited to two methods of event detection in SutterPatch Software. A 2019 webinar presentation demonstrates the use of the SutterPatch Application modules in everyday laboratory operation [50]. A single-channel analysis module was recently added to SutterPatch Software [51]. Since it is very likely to undergo further development and changes based on user feedback, this application module shall not be discussed here. pCLAMP Software provides similar event detection functionality, while Patchmaster Software does not have event detection features.

16.1 Event Detection

16.1.1 Action Potentials

Action potential detection is typically threshold-based. When applied to patch clamp recordings in current clamp mode, the signal-to-noise ratio is normally good enough to ensure reliable detection of action potentials without false positives or negatives. With juxtacellular in vivo recordings or other extracellular recording techniques, detection may be more involved, and using the event length as an additional detection criterion may be useful. SutterPatch Software puts out a number of standard waveform analysis parameters for each action potential, such as the threshold potential and the time of threshold crossing, the peak amplitude, the action potential duration (APD) at a user-defined level of repolarization, and the greatest afterhyperpolarization as well as its time of occurrence. In addition, a phase plane plot ($\partial V/\partial t$ vs. voltage) for each action potential or the average is generated, and event count and frequency are put out (Fig. 8).

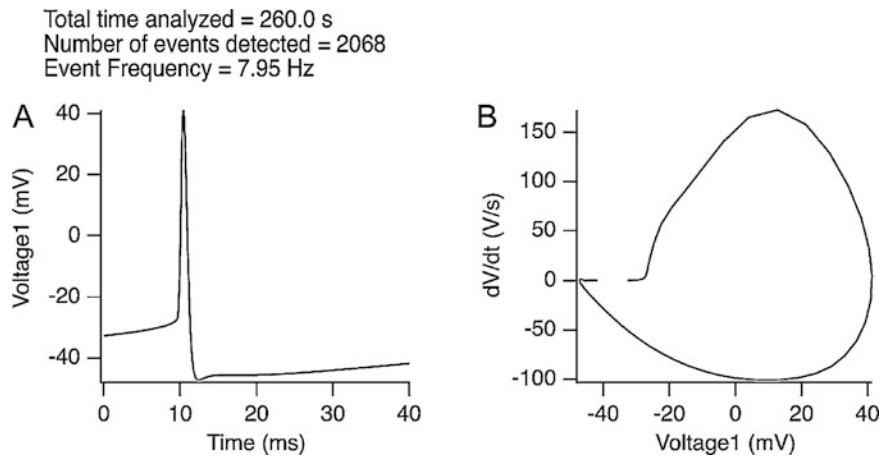

Fig. 8 Graphical output of the SutterPatch Action Potential Analysis Module applied to a recording of spontaneous activity in a brain slice. (**a**) Average action potential waveform across all detected events. (**b**) Phase-plane plot of the averaged action potentials. Above the plots, basic statistics of the analyzed dataset are put out. In addition to the layout, the numerical results for each action potential are compiled and displayed as a table (not shown)

16.1.2 Postsynaptic Events

Depending on the quality of the recording, detection of postsynaptic events can be a lot more demanding than action potential analysis. SutterPatch Software employs a deconvolution algorithm [52, 53], which has proven rather robust to poor signal-to-noise ratio and overlapping events. The event template is defined through rise and decay time, as well as the event polarity. The detection threshold is defined in multiples of the standard deviation and can optionally be further constrained by an amplitude threshold. After event detection, the events can be reviewed individually, and invalid events can be excluded from further analyses. The averaged waveform can be compared to the template for iterative fine tuning if desired. The results are put out in numerical form and as a summary page, which can be used in a workgroup presentation or, with little further editing, be the basis of a publication figure (Fig. 9, [50, 54]).

Other common analysis software for postsynaptic events includes pCLAMP Clampfit, Axograph [14] and Mini Analysis by Synaptosoft Inc. [55]. The latter, although popular, does not seem to have been updated in many years and may not run on modern computer systems. Only the SutterPatch and Axograph software packages run on both Windows and MacOS.

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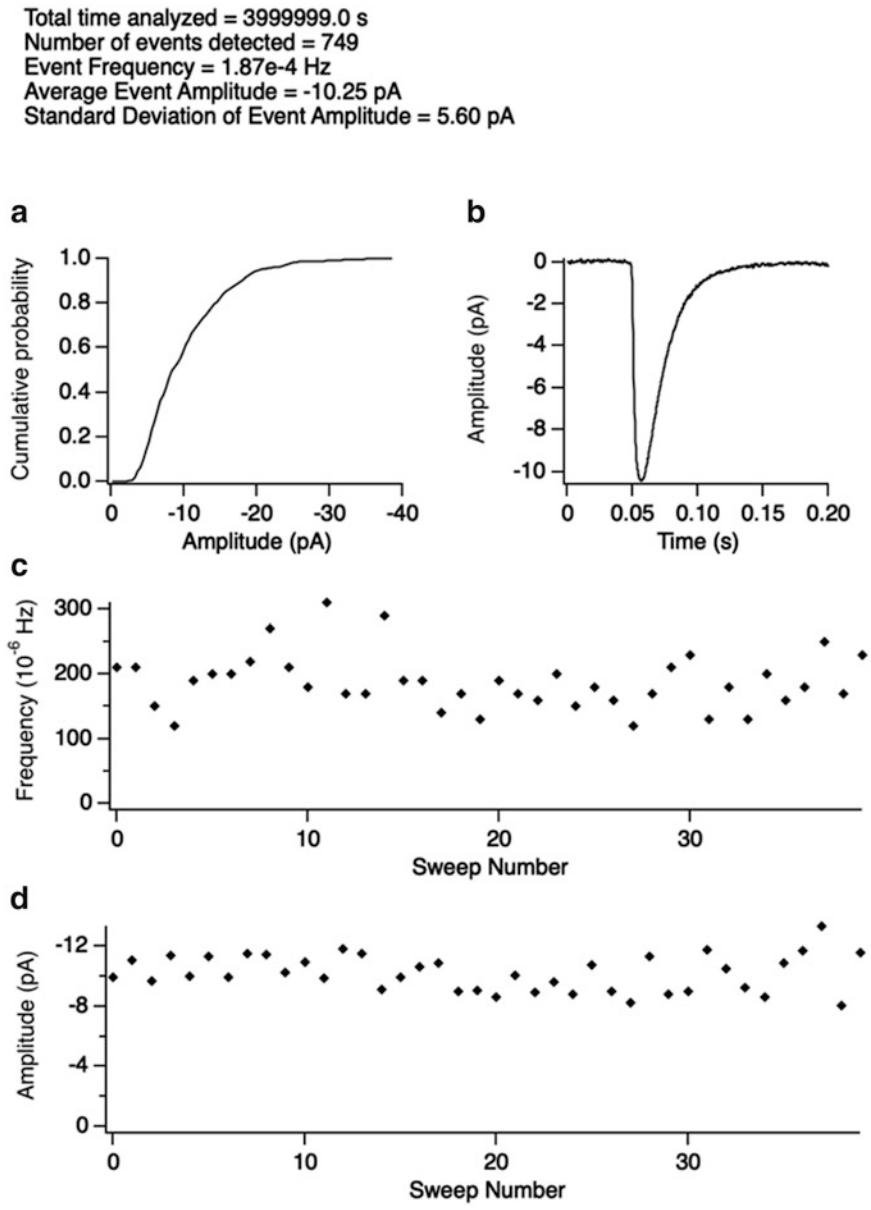

Fig. 9 The graphical results generated by the SutterPatch Event Detection Module from a recording of spontaneous postsynaptic events in a brain slice recording. In addition to basic statistics of the analyzed dataset, four plots are created. (**a**) Amplitude distribution histogram. (**b**) Average waveform of all detected events. (**c**) Frequency distribution plot, showing the average frequency in each 20-s sweep. (**d**) Amplitude distribution plot. **c** and **d** indicate that the recording was stable regarding both frequency and amplitude

contributed to the manuscript with information, short text passages, discussion, and critique. Particular thanks go to Rich Lobdill, Hubert Affolter, Telly Galiatsatos, Greg Hjelmstad, Aaron Best, and Burt Maertz. Last but not least, Dale and Mark Flaming,

owners of Sutter Instrument Company, have provided funding for development of the Patch Clamp Systems, an entirely new product line in the portfolio. This constitutes a substantial financial risk, which will hopefully be mitigated by blowing a fresh breeze into an instrumentation market that has not been particularly innovative over the past 15 years.

Declaration Regarding Potential Conflicts of Interests: The author is the Product Manager for Patch Clamp Systems at Sutter Instrument Company, one of the three providers of complete patch clamp systems. In the past, he worked for both major competitors, Axon Instruments/Molecular Devices LLC and HEKA Elektronik Dr. Schulze GmbH. Every attempt was made to describe features, advantages, and disadvantages of the products made by these three competitors in a factual, fair, and unbiased manner, assuming the point of view of a patch clamp researcher. No insider knowledge that is not in the public domain was revealed in this chapter.

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