SOPHION BIOSCIENCE

Application Report

Fast Series Resistance Compensation on Qube 384 and QPatch

Use of series resistance compensation for fast activating ion channels remove voltage clamp errors and increase voltage clamp accuracy

Summary

Series resistance compensation for fast activating ion channels can help remove voltage clamp errors and increase clamp accuracy. All Sophions patch clamp amplifiers utilize a patented technique enabling up to 100% R_S compensation. In addition, Sophion amplifiers are equipped with automatic clip detection to avoid loss of cells due to fatal oscillations, thereby increasing data throughput while maintaining high quality recordings.

Here we show that voltage clamp errors can be up to 20-30 mV when uncompensated and in general that errors occur if not using R_S compensation. However, we also show that the increase of accuracy due to use of R_S compensation comes with a price of a lower success rate, and that the settings are critical to maximize success rates while avoiding or decreasing errors. Last we demonstrate the importance of automated clip detection.

Introduction

When performing whole cell voltage clamp measurements, the membrane potential is controlled or clamped to a user specified potential. The command potential does not translate directly to membrane potential due to the so-called access resistance. The access resistance is made up of the resistance from the electrode through the orifice in the substrate and the whole in the cell membrane. These resistances combined are electrically in series with the membrane resistance, hence the name series resistance (R_S), and they constitute an "obstruction" to obtaining precise membrane potential. The series resistance has several effects when performing whole cell recordings. Firstly the time constant at which one can perform the compensation, can be limiting how good and how fast the compensation is. Will you record the true nature of your ion channel target, or are you being limiting by the amplifier setting or limited by your amplifiers capability? If a fast channel e.g. sodium channel, is being recorded the amplifier needs to be equally fast. The electronic of the amplifier have a speed of a few µs so this might not be the limiting factor, but the R_S circuit loop of the amplifier might be significant slower > 50 µs.

The Sophion amplifier utilizes a patented technique invented by Adam Sherman (US Pat. 6163719, US6700427B1) characterized by its unique algorithm. In addition, it is equipped with automatic clip detection allowing R_S to be temporarily turned off and thus avoid loss of cells due to fatal oscillations. The R_S compensation is re-enabled in the following sweeps and clipped sweeps can automatically be removed in the analysis process. Automatic clip detection thereby increases the data throughput, while maintaining high quality recordings.

Sophion QPatch amplifiers are equipped with two different series resistance compensations algorithms, regular and fast series resistance compensation.

Sophion Qube amplifiers are equipped with fast series resistance compensation.

Regular series resistance compensation is able to compensate for series resistance as well as simultaneously compensating for cell capacitance. This however has a down side, namely that the speed of the R_S compensation time constant is limited to 400 µs and slower.

Fast series resistance compensation is able to compensate for series resistance a lot faster (in theory down to a time constant of 2 µs), but with the cost of cell capacitance compensation. It should be noted that leak compensation will remove any cell capacitance which would otherwise interfere with the ion channel measurements.

In this study using QPatch we have looked at voltage-gated sodium channels and since they have so fast kinetics we have only used the fast R_S compensation. We used it with either 80% or 100% compensation (100 to 200 µs).

The cell membrane constitute a capacitor and the time constant for charging a capacitor is given by multiplying the series resistance with the cell capacitance. In case of a 10 MΩ series resistance and 15 pF cell, the time constant is thus 150 µs. Using the rule of thumb that one needs 6-7 time constants to reach equilibrium this becomes far too slow to compete with the kinetics of the sodium channel. On the other hand, if charging with a time constant much faster there is risk of oscillations in the current response. To prevent these oscillations the fraction of compensation must be lowered to e.g. 80% compensation.

Another dimension of series resistance is that the voltage error due to Rs increases with current. In case of a series resistance of 10 MΩ and a current of 3 nA, it will give rise to a voltage clamp error of 30 mV (Sakmann, Bert and Neher, Erwin, Single channel recording, 2nd ed. New York, 1995).

The Sophion R_S compensation is designed to be able to handle voltage errors in the range up to 100 mV (10 nA current with a R_S of 10 MΩ)

Results

All data from all four sodium sub-types were analyzed in the same way. Data was grouped by sodium ion channel sub-types and filtered for oscillations, current level and clip detection (table 1).

Fig. 1B indicates that the current reverses at +60 mV. The calculated Nernst equilibrium potential for sodium was +67 mV, and the junction potential was 13 mV, i.e. the expected reversal potential of +54 mV is close to what was obtained.

Table 1: Success rates from four QPlates (64 cells). *The majority of failed data was found where R_S was compensated to its maximum (100%@100µs)

Full data	100% (64 cells) or 47.392 sweeps
Sealing (>100 MΩ)	90% (58 cells)
Current level (>200 pA)	86% (55 cells) or 82% (liquid periods)
Oscillation sweeps	80% (37.931 sweeps)
Clip detection	85% (40.392 sweeps)
Overall*	58% (437 liquid periods)

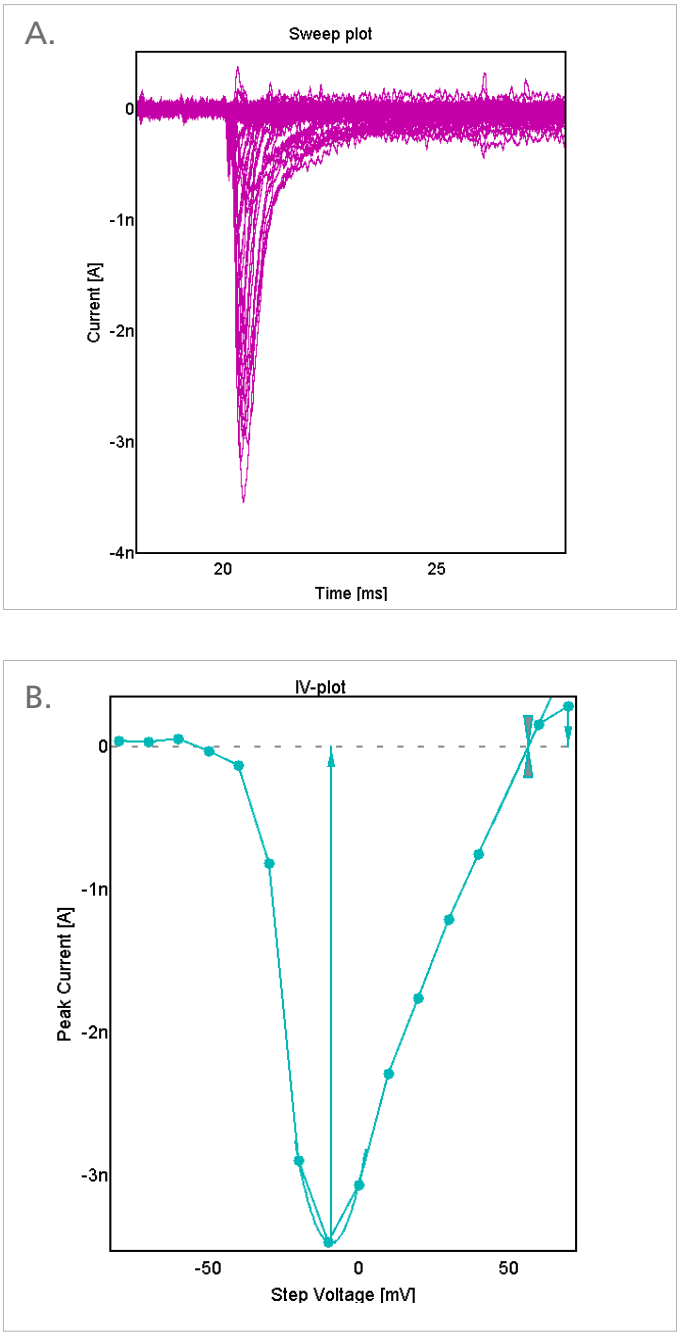


Fig. 1: A) Example of raw data current trace(s) from HEK293-$Na_V1.6$. B) Peak current in every trace in A) versus step potential.

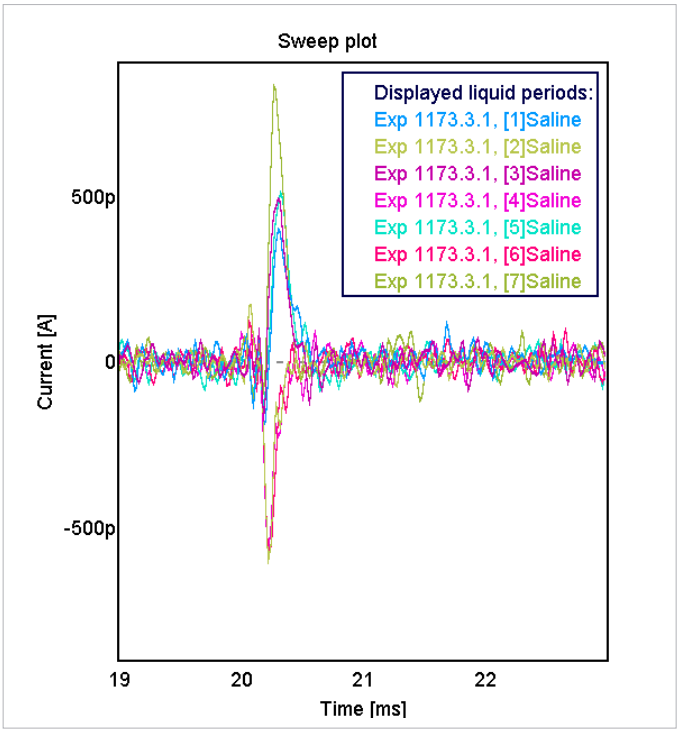
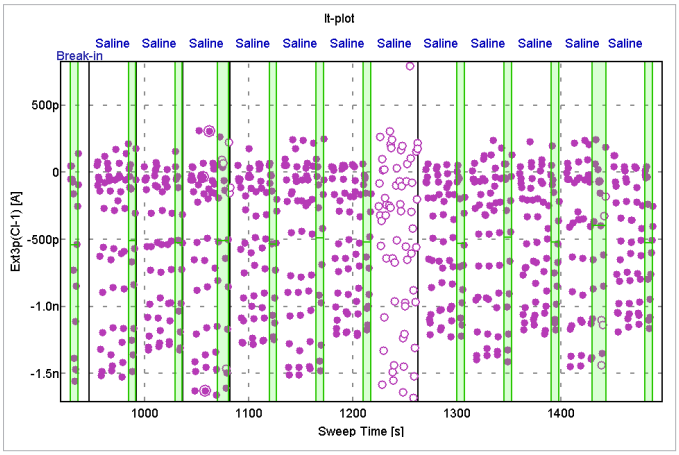


Fig. 2: Zoom of $Na_v1.4$ current @ +70 mV with alternating series resistance compensation. Even numbered sweeps (denoted "Liquid period") are without series resistance compensation [2, 4 and 6], whereas odd numbered sweeps are with different Rs compensation settings [1, 3, 5 and 7]. Since current are going the wrong way, the inward current is clearly wrong. The series resistance was 3.8 MΩ throughout the experiment.

Comparing the current at +70 mV not all currents are outward (Fig. 2). Since the clamped potential (+70 mV) is more positive than the observed reversal potential (E_{revNa} = 60 mV), it would be expected that all current was outward. It is only when using series resistance compensation the current runs as expected. By looking at Fig. 6 it can be seen that the clamp error are 20-30 mV, when the data are uncompensated and by calculating the clamp error, an error of only 2 mV give rise to current running in the wrong direction.

Clip detection was activated for all sweeps and is online active in the amplifiers. The clip detection is a unique Sophion feature, where the amplifiers automatically detects instability and disable the series resistance compensation immediately, so the cell is rescued.



Break-in; 80% & 200µs
Saline 1; 80% & 200µs
Saline 2; None
Saline 3; 80% & 100µs
Saline 4; None
Saline 5; 100% & 200µs
Saline 6; None
Saline 7; 100% & 100µs
Saline 8; None
Saline 9; 80% & 200µs
Saline 10; None
Saline 11; 100% & 200µs
Saline 12; None

Fig. 3: Current plotted in a time manner over all 13 different liquid periods, to show clip detection. The break-in and odd numbered liquid periods are with the series resistance compensation enabled (green bars), whereas the even liquid periods are without R_S compensation. Each data point represents peak current at all clamped potentials. Open data points represent data where the amplifier have detected instability and disabled the series resistance compensation.

Another problem when not compensating for R_S are clamp errors (Fig. 4). It can be seen from the raw current traces when the amplifier cannot clamp the membrane sufficiently fast Fig. 1. Clamp errors are completely gone with any setting when using R_S compensation.

In order to adjust for different cells with different current levels, all sweeps were normalized. By performing this normalization, the data could be grouped into the four different recording regiments and plotted together (Fig. 5 - Fig. 8).

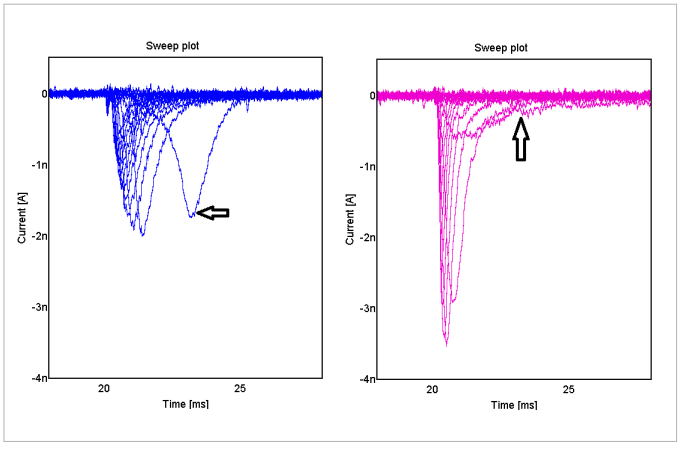


Fig. 4: Current response to a step protocol. Examples of classical clamp errors due to missing R_S compensation.

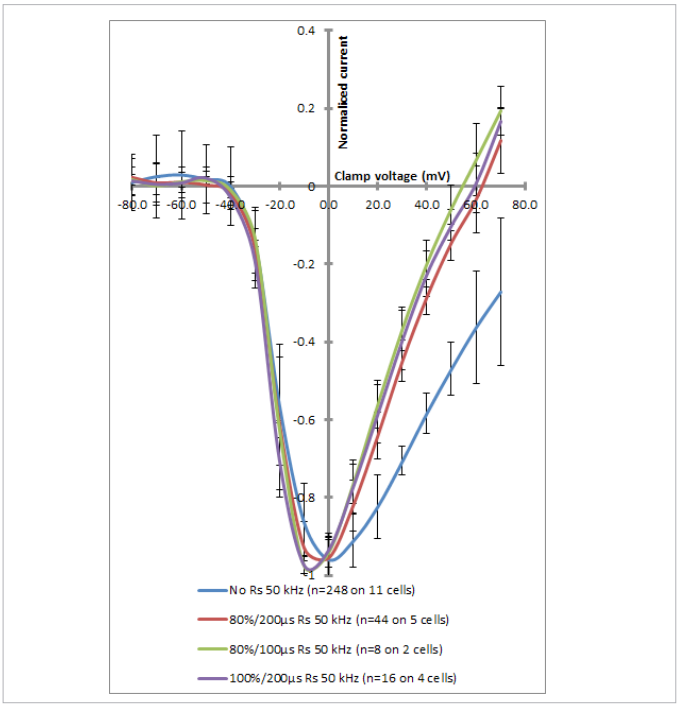


Fig. 5: Normalized current vs. clamp voltage plot for HEK293 cells expressing $Na_V1.3$. Different series resistance settings were used, see the legend for details. Error bars are SD on the normalized current. One full QPlate16 was used and the success rate for each R_S setting can be calculated by using the number of cells for "No R_S 50 kHz". E.g. 5 cell @ 80%/200µs=45% success rate (data from 5 cells in red data, compared with the 11 cells in the blue data).

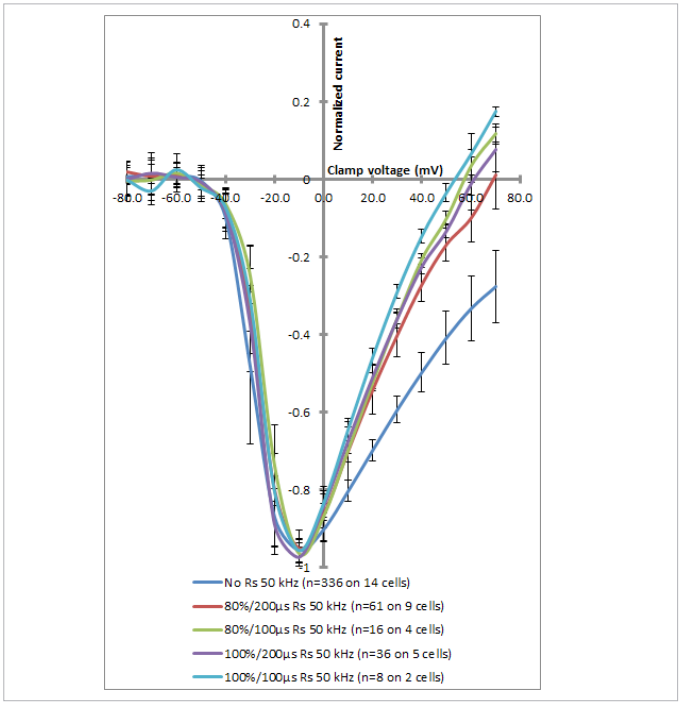


Fig. 6: Normalized current vs. clamp potential plot for TE671 cells endogenously expressing $Na_V1.4$. Different series resistance settings were used, see the legend for details. Error bars are SD on the normalized current. One full QPlate16 was used and the success rate for each R_S setting can be calculated by using the number of cells for "No R_S 50 kHz". E.g. 9 cell @ 80%/200µs=64% success rate (data from 9 cells in red data, compared with the 14 cells in the blue data).

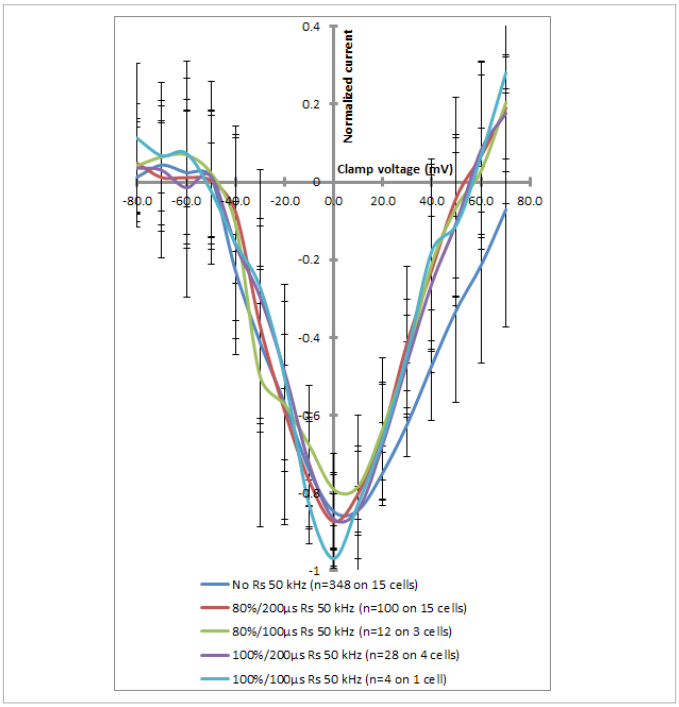


Fig. 7: Normalized current vs. clamp voltage plot for CHO cells expressing $Na_V1.5$. Different series resistance settings were used, see the legend for details. Error bars are SD on the normalized current. One full QPlate16 was used and the success rate for each R_S setting can be calculated by using the number of cells for "No R_S 50 kHz". E.g. 15 cell @ 80%/200µs=100% success rate (data from 15 cells, both in blue and red data).

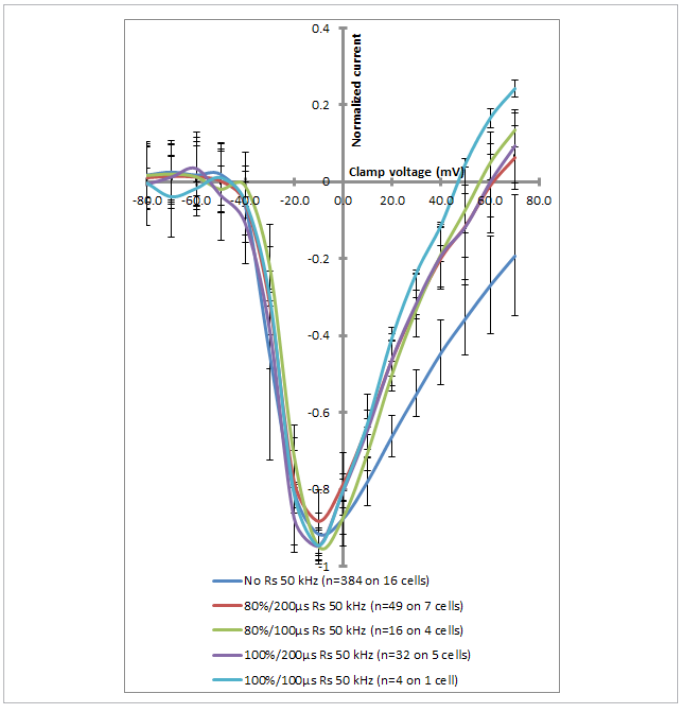


Fig. 8: Normalized current vs. potential plot for HEK293 cells expressing $Na_V1.6$. Different series resistance settings were used, see the legend for details. Error bars are SD on the normalized current. One full QPlate16 was used and the success rate for each R_S setting can be calculated by using the number of cells for "No R_S 50 kHz". E.g. 7 cell @ 80%/200µs=44% success rate (data from 7 cells in red data, compared with the 16 cells in the blue data).

Discussion and conclusion

When performing electro-physiological measurements it's key that the cell is clamped as intended at all times. Having a potential drop over a series resistance, the membrane is not clamped correct and the scientist might in worst case be concluding wrongly.

The obvious thing to do would be to compensate for R_S in all experiments, but R_S compensation comes at a price of lowering success rate. By looking at the overall success rate in Table 1 and calculating the individual success rates from the legends in Fig. 5-8, it is clear that compensation for series resistance lower the success rate. For $Na_V1.5$ experiments in this study, all cells could be compensated without errors (Fig. 7). However, for the $Na_V1.3$ experiments the success rate are down to 45% for compensation 80% at a time constant of 200 µs (Fig. 5). But all in all, success rates are reduced when turning on the Rs compensation.

The activation speed of the sodium current is fast, but still dependent on the temperature. The classical biophysics of the Squid Giant Axon were mostly done at 4 to 16°C (Hille 2001), where most sodium activation time constants are over 1 ms. The experiments in this study have been made at ambient temperature when sodium channels are activated on the sub-ms scale. The $Na_V1.2$ subtype is one of the fastest subtypes, with an activation time constant of 100-150 µs (positive potentials), and $Na_V1.4$ to be significant slower with an activation time constant around 400-500 µs (Lacrois et. al 2013). Why would one have to think about activation speed when performing experiments with series resistance compensation? The answers to this would be that the clamp, and thereby the speed of the Rs compensation needs to match the ion channel activation speed. So when performing experiments on e.g. the $Na_V1.4$ subtype, the speed of the R_S compensation need to be faster than 400 µs in order to get the correct clamp and measure the correct peak.

Classical errors from missing clamp is proportional to the current and R_S, and will in some cases give rise to wrong conclusions and wrong biophysical characters (see Fig. 4).

The reversal potential (E_{rev}) is not possible to estimate directly from the data presented here, and even when extrapolating the reversal potential, the E_{rev} is wrongly estimated without R_S compensation (Fig. 5-8, current never crosses 0 current). Theoretically the reversal potential should be the same with all four targets, since the solutions are the same and the four targets all conduct sodium. The calculated E_{rev}(Na) is +67 mV, whereas the measured are close to +60 mV. These numbers are within the same range and when correcting for liquid junction potential (13 mV), the measured E_{rev} are similar.

Well-functioning R_S compensation is critical to obtain precise patch clamp measurements and to avoid errors not only when studying fast activated ion channels. Also ion channels that are perceived as slow can have fast transitions, e.g. deactivation of tail-currents in many K-channels. With the patented R_S compensation algorithms on the Sophion amplifiers it is possible to achieve 100% R_S compensation. Additionally, oscillations can cause significant data loss when using traditional patch clamp amplifiers. Cell loss caused by oscillations can be avoided with the automatic clip detection system, so a high throughput can be maintained. R_S compensation is important for many applications, since it increases accuracy and removes errors.

Materials and Methods

Cell lines

- HEK293-hNa$_V$1.3
- TE671-hNa$_V$1.4
- CHO-hNa$_V$1.5
- HEK293-hNa$_V$1.6

A series of experiments were performed with a standard step protocol for sodium channels: 60 ms long protocol (step = 20 ms) with 10 mV step increment (Fig. 9). The protocol was applied at 0.1 Hz with 500 ms between each sweep in protocol. Data was sampled at 50 kHz and filtered to 10 kHz with an 8 order Bessel filter.

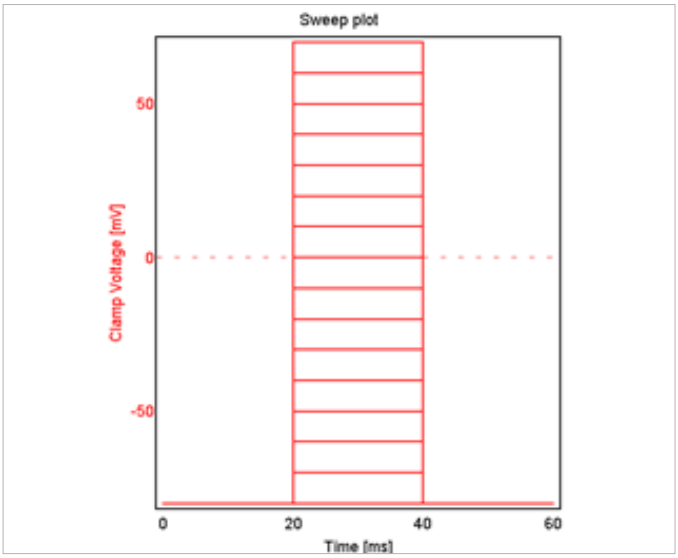


Fig. 9: Voltage protocol used for activation the voltage-gated sodium current.

References:

Sakmann B and Neher E 1995. Single channel recording, 2nd ed. New York, 1995.

Hille B 2001. Ion channels of Excitable Membranes, 3rd ed, Sunderland.

Lacrois JJ, Campos FV, Frezza L and Bezanilla F 2013. Molecular bases for the asynchronous activation of sodium and potassium channels required for nerve impulse generation. Neuron. 79(4)

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