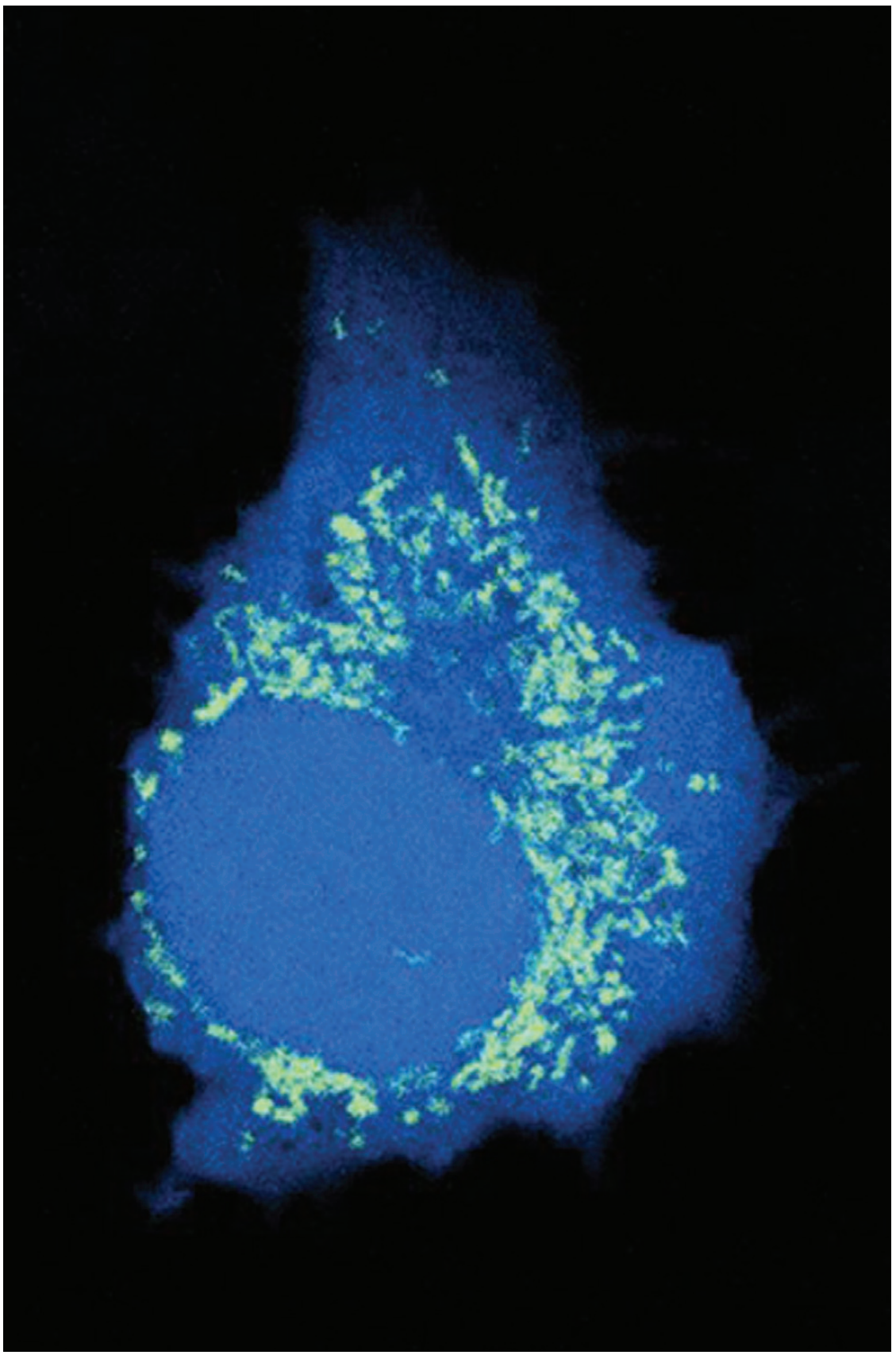
AxoBits

Integrated Solutions for Cellular Neuroscience, Cell-Based Screening and Microarray Analysis • Vol. 39 • November 2003



In This Issue

Axon Instruments

Product News

GenePix 4200A Microarray Scanner

Introductory Pricing Still Available

Don't miss out... Special introductory pricing is still available on the GenePix Professional 4200A. Contact your Sales Representative to schedule a demo and lock in this unprecedented pricing while the offer lasts!

We will be at the following conferences:

Chips to Hits
Boston, MA—October 27-31, 2003

American Society of Human Genetics
Los Angeles, CA—November 4-8, 2003

Society for Neuroscience
New Orleans, LA—November 8-12, 2003

American Society for Cell Biology
San Francisco, CA—December 13-17, 2003

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Acuity 3.1 Microarray Analysis Software

Axon Instruments is committed to making Acuity the premiere platform for advanced microarray data analysis. We recently released Acuity 3.1, which includes important new functionality to enable you to get more done faster:

Significance Statistics
Two-sample t-tests and multiple group comparisons by ANOVA allow you to discover the most significantly changing genes in your experiment. Adjust p-values for multiple hypothesis testing using a number of correction methods, such as Bonferroni, and Benjamini-Hochberg.

Dye Swap
Use the new Dye Swap scriptlet to invert ratios and average dye swapped microarrays with conventionally labeled microarrays.

Import Dataset
If you are working with another database or analysis platform, such as the Stanford Microarray Database or BioConductor, you can easily import a dataset into Acuity and use Acuity's superior analysis and visualization tools to analyze your data.

Import Quicklist
Integrate Acuity with other software environments or with online tools by importing gene lists.

Recycle Bin
Acuity is always careful with your raw data. If you delete a microarray from the Project Tree, it is stored in the Recycle Bin. From the Recycle Bin, you can restore deleted data to the tree, or delete it permanently.

Compact Database
Use the new Compact Database functionality after permanently deleting microarrays from the Recycle Bin to make your Acuity database smaller.

Acuity 3.1 is a free upgrade for all Acuity users.

Cover Photograph

Axoporator 800A, Axon's single-cell electroporator, was used to insert a mitochondrial gene conjugated with the yellow fluorescent protein gene, into an Alpha-TN4 cell from a lens epithelial cell line. On the following day, the Axoporator was again used to deliver Alexa 568 dye into the same cell in order to better visualize the cell containing the mitochodria. This confocal image is courtesy of James Rae, Mayo Clinic, Rochester MN.

Breakthrough to discovery with the Axoporator 800A

Single-cell electroporation is of use to nearly every researcher who works with cells but few have the time or expertise to assemble a system. For those people, the solution lies in the **Axoporator 800A**—*the first commercially available system dedicated to single-cell electroporation.* Our instrument combines two of Axon's time-proven strengths: patch-clamp technology and precise high-voltage waveform generation. **Axoporator 800A** was developed with advice from and tested by the pioneers in single-cell electroporation. These investigators tested prototype units to successfully gather data from two very different preparations—intact tissues and cultured single cells. The list of molecules that have been effectively delivered into cells grows each day as the testers forge ahead with their experiments. Most recently, cutting-edge RNA inhibition experiments have met with success. Proven formulae will be documented in the user manual and application notes that accompany the **Axoporator 800A**.

Why use single-cell electroporation?

Single-cell electroporation delivers molecules into a cell via a micropipette. The mechanism is the same as that used for the bulk electroporation of suspended cells—dielectric breakdown of the membrane by a voltage pulse—however, single-cell electroporation offers distinct advantages over bulk electroporation:

- Individual cells can be targeted for specific modification. Whether you're using isolated cells, tissue slice or an intact preparation, this technique allows you to focus on an individual cell.
- Precise regions of the cell can be selectively targeted. Apical *vs.* basal, neurite *vs.* axon, animal *vs.* vegetal pole—single cell electroporation allows you to focus on very specific regions of the cell under study.
- Very high rates of survivability. Cells better tolerate the intervention because only a small portion of cell membrane is involved with the voltage-delivering micropipette.

What are its features?

Convenient controls and useful readouts make the operation of the **Axoporator 800A** intuitive. An LCD display provides visual information about

- Current and Power readouts
- Pipette resistance
- Pulse and Train settings
- Pulse counts
- Voltage commands
- Three different pulse types are provided, all of which can be run in either polarity and in trains. These include:
    - Rectangular "square"
    - Bipolar
    - Bilevel—a special pulse that has been demonstrated to effectively and efficiently deliver genes as large as 14 kb into the cell.

Once configured, the **Axoporator 800A** can be activated by a single press of a button. Alternatively, a *foot pedal switch* is supplied, so both hands are available to control the microscope and the micromanipulator. Finally, in keeping with Axon's tradition of flexibility and expandability, the device can also be controlled by an external waveform generator, including pCLAMP software with a Digidata 1322A.

To aid in positioning the micropipette, a built-in audio monitor changes tone as you near a cell and make contact. This audio monitor, together with the resistance display, provides a useful tool for blindly searching out cells in tissue slice. Tracking the number of electroporation commands is a snap, as there are both automatic and manual counters.

It has been our experience that researchers are forever finding unconventional and clever ways to use our products—hence Axon's tradition of flexible design. **Axoporator 800A** incorporates very important features in anticipation of future applications:

- a "debug" input is provided for downloading firmware upgrades as the field of discovery evolves.
- The **voltage range extends to 100 V**, because the optimal voltage for single-cell electroporation can be several tens of volts in some preparations.
- An optional headstage is available for those who might wish to use lower resistance pipettes.

If you are currently set up for any microscopic recording or visualization technique, then you already have most of what you will need for single-cell electroporation. For those of you that do not have access to a micropipette puller, we can provide you with the contact information for an established commercial source of the appropriate micropipettes.

Help unravel the cell's mysteries!

Axoporator 800A represents the convergence of biophysics, cell biology and molecular biology. This is your opportunity to participate in groundbreaking work in single-cell electroporation. Studies need not be confined to the intact cell. For instance, large, isolated, and intact organelles might be studied with single-cell electroporation. Seize the opportunity for exploration and let the **Axoporator 800A** pave the way.

Axon is licensed by Cellectricon AB (Sweden) for the use of certain patented technology related to single-cell electroporation.

Suggested reading:

Olofsson, J., Nolkrantz, K., Ryttsen, F. Lambie, B.A., Weber, S.G. and Owar, O. Single-cell electroporation. Current Opinion in Biotech (2003), 14:29-34.

Rae, James L., Levis, Richard A., Single-cell electroporation. Eur J. Physiol. (2002), 443:664-670.

Haas, K., Sin, W-C., Javaherian, Z. L., Cline, H. T., Single-Cell Electroporation for Gene Transfer In Vito. Neuron (2001), Vol. 29, 583-591.

SoftPanel News
Free SoftPanel Winners Selected!

Congratulations to Drs. Kimberle Jacobs and Sandra Kuhlman, each a winner of a **free** SoftPanel ($900 value). Both participated in a survey announced in a recent customer-update email. To enter the drawing, we simply asked MultiClamp 700A users how this amplifier has improved their scientific progress.

Dr. Jacobs (Virginia Commonwealth University) proclaims that the MultiClamp 700A "is one of the easiest amplifiers that I've ever used. The AUTO functions have greatly reduced the time that we would normally spend in achieving correct capacitance and Series Resistance compensation. Altogether, data collection is much more efficient using the MultiClamp 700A."

Dr. Kuhlman (Cold Spring Harbor Laboratory) considers the MultiClamp 700A advantageous because "It is more efficient to switch between current-clamp and voltage clamp…because settings do not have to be changed so much."

We are happy for Drs. Jacobs and Kuhlman, as well as every other MultiClamp 700A customer who benefits from the advanced features of this state-of-the-art amplifier. As always, we appreciate your feedback and suggestions to improve this or any other Axon product. To keep informed about Axon products on a regular basis, sign up for one of our email lists at www.axon.com/mr_Join_Email_Lists.

Using SoftPanel with USB 2.0

When the SoftPanel was originally released, USB version 1.0 was the standard in computers. Since then, USB version 2.0 has been released to allow faster transfer rates with many peripheral computer products. As computers with USB 2.0 ports became available , we discovered problems detecting the SoftPanel via the MultiClamp Commander interface. We have since re-programmed the SoftPanel to be compatible with USB 2.0. If you encounter problems detecting a SoftPanel in a computer with USB 2.0 ports, call Axon Technical Support or email axontech@axon.com. For only the minimal cost of shipping, we will arrange to re-program your SoftPanel to be compatible with the new USB standard.

Questions?

Axon's Knowledge Base has the answers!

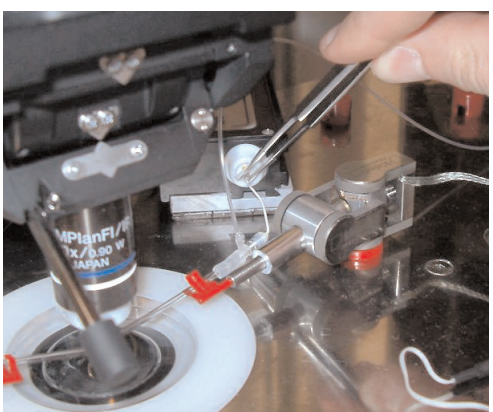
www.support.axon.com

A new drift-free generation of micromanipulators

kleindiek nanotechnik
www.nanotechnik.com

Micropipette drift of the tiniest amount can be disastrous for whole-cell patch recordings. Therefore it is imperative that the micropipette positioning platform is as drift free as possible. An innovative new micromanipulator from Kleindiek Nanotechnik successfully addresses drift, and other problems, for micropipettes.

Typically, the micropipette is inserted into a holder, the holder is mounted to a headstage, and the headstage is mounted to a micromanipulator. Axon Instruments' HL-U micropipette holder, with a threaded collar design, offers time-tested mechanical stability. An alternative employs a quartz rod to secure the portion of the micropipette that extends from a conventional holder[1]. However, the ultimate configuration is one where the micropipette is mounted directly on a precision manipulator through a short-lever arm. In this case a short wire connects the micropipette to the headstage, which is mounted independent of the manipulator. There are several commercial micromanipulators of this sort. Separation between the micropipette and headstage can increase the capacitance and thus the noise of recording, but work to date indicates that this noise is usually the same for conventional whole-cell experiments.[2]



Photograph courtesy of Dr. Jörg Geiger and Dr. Henrik Alle, MPI for Brain Research, Frankfurt, Germany.

The MM3A micromanipulator from Kleindiek Nanotechnik incorporates all of the ideal characteristics of a micromanipulator. Weighing just 30g with short-lever arms (less than 5 cm), the MM3A is virtually immune to vibration. An extremely simple headstage and pressure tube connection fixed directly to the micropipette, and a fork-like holder, ensure that all standard micropipettes are easily, securely, and accurately held in their designated positions. Micropipettes can be exchanged with very little vibration. With an operating range of 100 cm^3, the MM3A is capable of approaching a sample at nearly any angle along the X, Y and Z axes. The micromanipulator can be manually pre-positioned, held in place anywhere on a microscope stage with its strong magnetic base. The MM3A exhibits virtually no drift at all—even during

Axoporator 800A

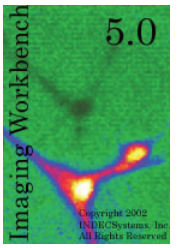
A Revolutionary Single-Cell Electroporator

Coming soon to a lab near you...

long recording sessions—and absolutely no backlash on reversal, or other play, such as is found with conventional micromanipulators. And finally, on top of all these features, the MM3A is essentially immune to temperature fluctuations.

1. Sachs F. A low drift micropipette holder. Pflugers Arch. 1995 429(3):434-5.
2. Communication courtesy of Dr. Matthew Larkum, Max Plank Institute for Medical Research, Cell Physiology, Heidelberg, Germany.

Imaging Workbench 5 Getting Results



INDEC Biosystems' Imaging Workbench 5.0 incorporates many crucial improvements, while maintaining all of the best features of AIW v4. IW 5.0 can acquire images more rapidly, with improved control and synchronization of peripheral devices. IW 5.0 also substantially broadens the control of multiple wavelength-selectors and multiple parallel-ports. This greatly improves control of excitation and emission wavelengths, making IW 5.0 an ideal choice for FRET experiments.

Imaging Workbench remains the tool of choice for simultaneous imaging and electrophysiology, with its coordinated acquisition and analysis, and its interoperability with Axon Instruments pCLAMP, v8 and 9. And of course, Imaging Workbench 5.0 is compatible with Windows 98, 2000, and XP.

Development continues on v5.1, with improvements to IW's analytical tools, along with support for additional hardware.

See IW 5.0 at the upcoming Society for Neurosciences conference (booth 2440). For more information, visit www.imaging-workbench.com or contact michael@imagingworkbench.com for further details, pricing, and availability.

Researcher Contributions

In each issue of AxoBits, we provide at least one Focus on Methods article that discusses a topic of general interest to researchers using one or more of our product groups. We have also dedicated a portion of our web site to profile innovative uses of our products.

To see innovative uses of microelectrode amplifiers, go to: www.axon.com/mr_Innovative_Uses_Amplifiers.html

To see innovative uses of GenePix scanners, go to: www.axon.com/gn_Innovative_Uses_GenePix.html

If you wish to submit an article to AxoBits or the web site, please contact axobits@axon.com.

Liquid Junction Potential Corrections

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Overview

Liquid junction potentials (LJPs) arise between two different solutions when the two solutions:

- are in contact,
- have ions present at different concentrations , and
- contain ions of different mobilities.

This article presents an overview of liquid junction potentials and discusses their measurement and calculation using Clampex's Junction Potential Calculator. In order to qualitatively understand liquid junction potentials, imagine a patch pipette in contact with a bath solution:

- the pipette contains unbuffered 100 mM NaCl solution
- the bath solution is unbuffered 10 mM NaCl solution.

The solutions contain ions at different concentrations, and sodium and chloride have different mobilities (chloride mobility is significantly greater than that of sodium). When the patch pipette comes into contact with the bath solution, sodium and chloride ions move down their concentration gradients—in this case from the pipette into the bath. The chloride ions move faster than the sodium ions. As the negative chloride ions move ahead of the positive sodium ions, a potential difference is set up, making the bath negative with respect to the pipette. The electric field slows the chloride ions and accelerates the sodium ions till they both move at the same rate, but the initial advantage that the chloride ions had is maintained. Provided the pipette is of reasonable volume and its tip not too large, the potential difference between the solutions—the liquid junction potential—remains steady.

By convention (a convention maintained in Clampex's Junction Potential Calculator) LPJs are in the direction of the bath with respect to the pipette. In the example above, the bath is negative with respect to the pipette. Note, however, that when the junction potential is measured by an amplifier attached to the pipette, the pipette would be positive with respect to the bath.

The magnitude of the junction potential depends on the ions present in the pipette and bath solutions, together with their relative concentrations, valencies and mobilities. In some cases, the junction potential is small and can be ignored. In most cases however, command voltages in voltage clamp or measured voltages in current clamp need to be corrected. The confusing part is determining the sign of the correction.

This is where the Junction Potential Calculator is helpful, because it automatically indicates how the correction should be applied.

Most patch clamp experiments need to correct for liquid junction potentials. When the recording pipette is first inserted in the bath, there are voltage offsets that are corrected by the amplifier when the current is zeroed (i.e., in voltage-clamp mode). The offsets consist of liquid junction potentials and potential differences between solid electrodes and the solutions they are in contact with (the "electrode" or "half-cell" potentials). The half-cell potentials of two Ag/AgCl wires can contribute large offsets if they are immersed in solutions with widely different chloride activities. Provided the electrodes are not in contact with the solutions directly (e.g., through an agar bridge), the electrode offsets will be constant and, once the amplifier current is zeroed, will not need any further correction. However, when the Ag/AgCl electrodes are in direct contact with the bath solution, if the bath composition is changed, the change in the ground electrode voltage offset needs to be corrected for (this is accomplished in the Junction Potential Calculator). Regardless of the Ag/AgCl electrode configuration, liquid junction potentials must be corrected for when determining membrane potentials. Note that liquid junction potentials should not be confused with tip potentials. The latter occur with high-resistance electrodes and are thought to be related to the chemical composition of the glass. For more on tip potentials, consult the monograph Purves (1981).

After achieving a high resistance seal ("gigaseal") with the membrane, the pipette solution is effectively no longer in direct contact with the bath solution thus, its liquid junction potential disappears, but its compensating amplifier offset remains.

In summary, using the Pipette Offset control to zero the output (voltage or current) is done with the pipette in the bath solution. At this point, all offsets are balanced by compensatory amplifier offsets. Most of these offsets are constant throughout the experiment and need not be considered further. Remember that the amplifier-zeroing procedure done with the pipette in the bath balances the pipette junction potential with an amplifier offset of opposite sign. After patching (going into the "gigaseal" configuration) the pipette liquid junction potential disappears, so we now have an unbalanced amplifier offset of equal magnitude but opposite sign.

Example:

In the following sections we consider a bath solution consisting of:

- 140 mM NaCl
- 2.8 mM KCl
- 2 mM $MgCl_2$
- 1 mM $CaCl_2$
- 10 mM HEPES
- titrated to pH 7.4 with NaOH

The pipette solution in the experiment contains:

- 145 mM K-gluconate
- 8 mM NaCl
- 1 mM $MgCl_2$
- 10 mM HEPES
- titrated to pH 7.4 with NaOH (this will add about 5 mM free $HEPES^-$ and 5 mM Na^+ to each solution).

From a qualitative standpoint, we need only consider the most abundant ions, NaCl in the bath and K-gluconate in the pipette. Among the anions, Cl^- will be more mobile than gluconate in moving into the pipette and K^+ will move more quickly than Na^+. Both of these mobility discrepancies have the effect of making the bath more positive than the pipette. Quantitatively, there are two ways to determine the value of the LJP. It can be calculated or it can be experimentally determined.

Experimental determination of LJP

Fill a recording pipette with the K-gluconate solution. Fill the bath with the same K-gluconate solution. It is crucial that the reference electrode be a 2-3 M KCl microelectrode or a 2-3 M KCl agar bridge, which you can keep replacing as needed. K^+ and Cl^- ions have similar mobilities, and at such high concentration dominate the LJP at the reference to bath junction. Therefore, this junction has an LJP close to 0 mV. On the amplifier, select I=0 mode (current clamp mode with no commands). Use the pipette offset potentiometer to zero the voltage read on the meter.

Change the bath solution to the NaCl-containing solution. If using a KCl agar bridge, replace the bridge with a fresh one. Read the voltage on the meter. It will be a negative number (around –15 mV). Because, by convention, the LJP is taken as the bath potential relative to pipette, it is the negative of this value, +15 mV (the amplifier measures the pipette potential relative to the bath).

Finally, place the K-gluconate solution in the bath. Use a fresh KCl agar bridge. Now the meter should read 0 mV. We do not generally recommend determining the LJP experimentally because of problems with KCl reference electrodes (Barry and Diamond, 1970), and because even if everything is done carefully there is still a correction at the KCl microelectrode to bath interfaces that needs to be taken into account (Barry and Lynch, 1991). It is true, however, that these corrections are normally relatively small and can be estimated with the Junction Potential Calculator. But in spite of our general recommendation against measuring LJPs, in cases where major ion mobilities are not known or cannot be estimated, or in order to provide a check on the accuracy of a calculated LJP value, experimental measurement is necessary.

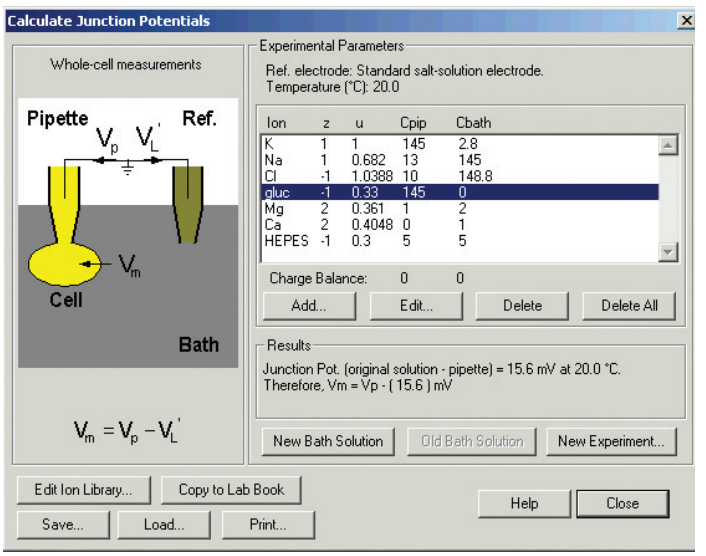
Calculation of LJP using the Clampex Junction Potential Calculator

The second option is to calculate the LJP using a generalized version of the Henderson equation (Barry and Lynch, 1991;

see also Barry, 1994). JPCalc software, developed by Peter Barry (see reference list for details), is one such calculator. Although JPCalc was designed to be stand-alone software, it is incorporated into Clampex (versions 7 and up). The following example uses the Junction Potential Calculator in Clampex 9.

1. Select Tools / Junction Potential.
2. Click on **New Experiment**.
3. Select Patch-clamp measurements. Click **Next**.
4. Select Whole-cell measurements. Click **Next**.
5. Select standard salt-solution electrode. Click **Next**.
6. Enter the temperature (e.g., 20°C). Click **Next** twice, then **Finish**.
7. Click on the **Add** button to add ions to the pipette and bath solutions.

The calculator gives a value of +15.6 mV for the LJP in this situation. This value is always independent of the type of experiment. Because of the large mobility difference among the most abundant ions, the LJP is large. Note that the contribution of the free $HEPES^-$ ion and the Na^+ from the NaOH was taken as about 5 mM each, which is about what is required to get a pH of 7.4 (see note about **Ion Concentrations and Activities** near the end of this article).



Now that the LJP has been determined, we have to decide how to correct for the error. Two methods can be used: the correction is performed either before or after the experiment. For voltage clamp, all commands need to be corrected. For current clamp, all recorded potentials need to be adjusted. *Correction of the LJP before the experiment should only be done in those circumstances where the bath solution is not going to be changed. If you intend to change the ionic composition of the bath solution during the course of the experiment, then the correction for the LJP should always be done after the experiment.* The advantage of postexperiment correction is also that if a calculation or application mistake is made, it is easier to trace and correct than if correction was done before the experiment.

The Junction Potential Calculator in Clampex is very helpful in determining the LJP correction for a variety of experimental setups. The discussion below is designed to provide intuitive recipes to perform voltage correction. Once you are comfortable with the sign conventions, we recommend that the LJP calculator be used to provide the values and signs of the correction.

Correction before the experiment (on-line correction)

Provided the bath solution is not going to be changed, corrections for LJP errors can be done before the experiment begins using either the amplifier or the acquisition software. The aim is to perform all LJP corrections at the start of the experiment so that there is no need for further adjustments to the recorded data. In the following examples we will use techniques that are applicable to Axon products such as the Axopatch and MultiClamp amplifiers in conjunction with Clampex. These techniques should be generally applicable to any combination of hardware and software.

Voltage Clamp

We will consider a few of the many ways that corrections may be done.

Using the amplifier

Using the example above with a NaCl-based bath and a K-gluconate-based pipette solution, we calculate the LJP to be +15.6 mV (because of convention, the sign is positive). With the pipette in the bath, use the pipette offset to null the output as usual (using the Pipette Offset feature on the amplifier). The LJP is then corrected for by applying a constant holding command of +15.6 mV (on an Axopatch by using the Holding Command potentiometer on the front panel; in the MultiClamp Commander by typing the value in the Holding field in the V-Clamp tab). Note that this command may be applied at any time, before or after gigaseal formation. The holding command of +15.6 mV is maintained throughout the experiment. This scenario, therefore, assumes that all subsequent voltage commands will be delivered using the software, so that the software command and the amplifier's holding command will be added together.

NOTE: Command voltages are often monitored by using a dedicated output on the amplifier. The drawback of the procedure described above for on-line LJP correction is that if the voltage command is monitored using these outputs from the amplifiers, the monitored command value will be offset incorrectly by +15.6 mV, since all commands are additive on Axon amplifier outputs. The command voltage specified in the software should be taken as the accurate Vm command.

Alternatively, the LJP can be corrected using the Pipette Offset potentiometer of the amplifier. With the pipette in the bath, the pipette offset is nulled as usual (see above).
In voltage-clamp mode, a holding command of –15.6 mV is applied. This will give rise to an offset current. The Pipette

Offset potentiometer is adjusted again to zero the resulting offset. Now reset the Holding Command on the amplifier back to zero. We are essentially using the Pipette Offset to deliver the –15.6 mV offset necessary to correct for the LJP. Once this adjustment is done, the voltage or current commands can be delivered using either the Holding Command potentiometer on the amplifier or by using the acquisition software.

The methods just described apply constant commands to the pipette. If the resistance of the electrode is low, these commands can generate large current offsets that in some situations saturate the output of the amplifier. If you plan to use low-resistance electrodes, you should consider an alternate method for LJP correction.

Using Clampex

This technique is very similar to the Pipette Offset strategy described above. Use Clampex to apply a holding command of –15.6 mV with the amplifier in voltage clamp mode. This can be achieved by setting the command potential from the Real Time Controls panel or by setting a constant command in the Protocol Editor. On an Axopatch, use the Pipette Offset potentiometer to null the resulting current. Using the MultiClamp, zero the output current by using the Auto Pipette Offset button. Switch off the –15.6 mV command from Clampex and load any protocol. From this point forward, no further corrections are necessary in the software since the LJP correction is delivered by the Pipette Offset circuitry of the amplifier.

Another, but more cumbersome, approach involves manually correcting all step commands in a protocol by adding +15.6 mV to their value.

Current Clamp

In Current Clamp mode, the zeroing performed with the pipette in the bath affects the recorded voltages (not the command current). In this case, the best approaches are to use the Pipette Offset method or to offset the output by the LJP. Both are described in the discussion that follows.

Using the amplifier

After zeroing any offsets with the pipette in the bath, adjust the pipette offset until a value of +15.6 mV is read on the meter. On the Axopatch, turn the Pipette Offset potentiometer until the meter reads +15.6 mV; on the MultiClamp, use the Pipette Offset slider. This value will be automatically subtracted from the recorded voltage (from the Scaled Output).

Using Clampex

We need to perform the correction in the Lab Bench dialog box that has been set up for a current clamp experiment. First, note the signal name in Clampex that is recording the membrane voltage. Then open the Lab Bench and with that signal selected, enter +15.6 mV in the Offset text box.

The experimental configuration does not matter. All examples above apply whether the experiment involves cell-attached or whole-cell patches. From the amplifier's perspective, the orientation of the membrane is irrelevant. However, it is important to restate that LJP corrections made before the experiment are only valid if there are no changes in the ionic composition of the bathing solution during the course of the experiment.

Correction after the experiment (a posteriori correction)

If the bathing solution is to be changed during the experiment, then the correction for LJP **must** be done **after** the experiment. When correcting for the LJP after the experiment, it is necessary to take into account the experimental configuration. This is because of the sign convention for membrane potential, which is expressed relative to the inside of the cell. For example, outside-out and inside-out patches at the same membrane potential require command potentials from the headstage that are opposite in sign. *The Junction Potential Calculator takes information from the user about which experimental configuration is being used and indicates in each case exactly how to correct for the LJP.*

Voltage clamp

Outside-out and whole-cell configurations

In both of these cases, the correction is the same. The value of the LJP needs to be subtracted from all command voltages:

$Vm = Vcmd - LJP$

($V_m = V_p - V_L$) in the Junction Potential Calculator where Vcmd represents the voltage command delivered by the software. For example, if the software was delivering a constant holding command of –50 mV, the true holding command using the solutions from our example is –65.6 mV.

Inside-out configuration

In this configuration, the membrane orientation is inverted and so the signs are inverted:

$Vm = -Vcmd + LJP$

[$V_m = -(V_p - V_L) = -V_p + V_L$ in the Junction Potential Calculator].

Cell-attached configuration

With the pipette attached to the cell, we are in a situation that is topologically identical to the inside-out configuration. However, because the intracellular solution is of unknown ionic composition, it will contribute an additional unknown quantity—the cell's own resting potential (RMP):

$Vm = -Vcmd + LJP + RMP$

($V_m = -V_p + V_L + V_c$ in the Junction Potential Calculator).

Currentclamp

Current clamping is typically performed in whole-cell configurations. In this case, the LJP needs to be subtracted from the measured voltages:
Vm = Vrec – LJP
($V_m = V_p - V_L$ in the Junction Potential Calculator) where Vrec is the measured voltage, typically from the Scaled Output of the amplifier.

Further reading:

Every book on practical electrophysiology contains at least a few paragraphs regarding LJPs. The best reviews on the subject are from Erwin Neher and Peter Barry.

Barry, Peter H., and Lynch, Joseph W., Topical review. Liquid junction potentials and small cell effects in patch-clamp analysis, J. Membr. Biol. 121, 101-117, 1991.

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Purves, R.D. Microelectrode Methods for Intracellular Recording and Ionophoresis, Academic Press, London, 1981.

Ion Concentrations and Activities

It should be noted that only the free ionic concentration (or activity) values should be used in the program to calculate liquid junction potentials. This is especially important for ions which are not fully dissociated in the solution. Ideally, activity values should be used, but provided the total ionic strength of the solutions is about the same, concentration values are quite adequate. For further details, see website below.

Additional Mobility Values

For additional mobility values and other information about liquid junction potential corrections, see website: http://www.med.unsw.edu.au/PHBSoft/mobility_listings.htm. These values are periodically updated, as information about new ions becomes available.

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A History of the Glass Micropipette Electrode

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It is astonishing how the fine glass micropipettes used for single cell electrical measurement and stimulation—invented to prove the theories of Robert Koch and Louis Pasteur in relation to the microbial origins of disease—have become a universally accepted, but generally unacknowledged, mainstay of modern biological science. Micropipettes can now be found in use in such diverse fields as:

- micropuncture analysis of glandular secretions and of kidney function
- microperfusion of kidney tubules and of single cells
- unravelling the chemistry of synaptic transmission by iontophoresis
- microinjection of mRNA and heterologous protein expression
- embryogenesis
- *in vitro* fertilisation, cloning and stem cell research
- drug development through observation of membrane currents in such voltage-clamped cells as ganglion cells of the autonomic nervous system, neurones of the central nervous system, *Xenopus* oocytes, and any number of cultured cell lines expressing receptors of interest
- fluorescent labelling of cytoplasmic and membrane components of single cells by microinjection
- intracellular recordings from deep brain neurones correlated with behaviour
- analysis of protein function in relation to structure using site-directed mutagenesis of ion channels, single channel currents and the patch-clamp.

This article presents a brief history of the micropipette and its use in electrophysiology.

Although these micropipettes are today often termed "Ling-Gerard" microelectrodes, they have a history that significantly predates Ling and Gerard's 1949 measurements of frog skeletal muscle fibre resting potentials. The idea of using electrolyte-filled glass micropipettes as the active electrode for stimulating excitable tissues occurred prior to 1920. By this time, elegant techniques for the production of glass capillaries—with pores no more than a few micrometres in diameter—had been developed, and micromanipulators were available to position them close to single cells. These enabled Frederick Pratt (1917) and Ida Hyde (1921) to observe, respectively, the characteristics of contraction in individual frog skeletal muscle cells (an "all or none" response) and the stalk of *Vorticella* (not an "all or none" response).

It is to University of Kansas bacteriologist, Marshall Barber,

that the initial credit must go for the invention, around 1902, of the glass micropipette. Barber also created the first precision three-dimensional micromanipulator—an essential corequisite for the isolation of single microorganisms and inoculation of substances into single cells. Following several brief preliminary accounts, Barber described his method for preparing micropipettes (Figs. 1 and 2) and his micromanipulators (e.g., Fig. 2) in considerable detail in his landmark paper in the Philippine Journal of Science (Barber, 1914). He had, at this time, turned his attention to tropical diseases and moved to Manila (Korzh and Strähle, 2002).

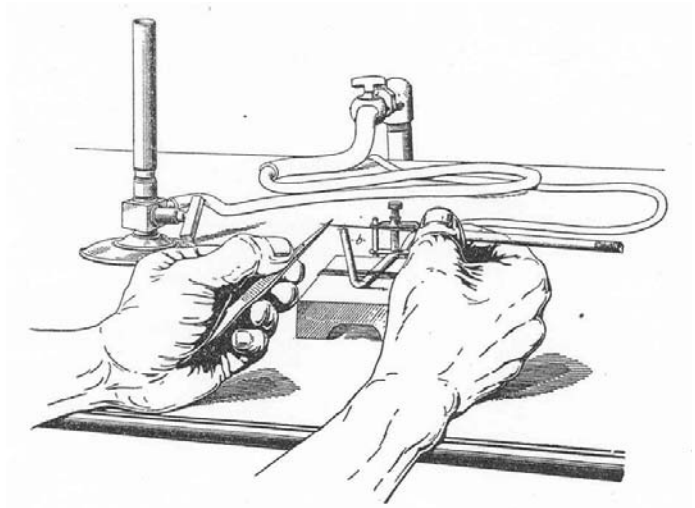

Figure 1. Fig. 1. Method of pulling micropipette over microburner, *b*, from Barber, 1914.

Barber's work and its potential for use in other biological research fields were noticed immediately. German Nobel Laureate Robert Koch himself visited the University of Kansas for a demonstration of Barber's method in 1908 (Terreros and Grantham, 1982). Then, around 1912, Lester Kite was sent by Albert Matthews, Chairman of the Department of Physiology at the University of Chicago, for training in Barber's laboratory (Terreros and Grantham, 1982; Korzh and Strähle, 2002). As we shall see, this is not the last time the University of Chicago Physiology Department features in the micropipette story. Kite subsequently moved to the Marine Biological Laboratory in Woods Hole where he collaborated with Robert Chambers. Chambers championed Barber's technique, extending it and making substantial improvements both to pipetting methods and micromanipulator reliability (Chambers, 1922).

By 1920, Charles Taylor from Johns Hopkins University was able to describe his employment of a Barber pipette with a lumen diameter of about 5 micrometres. Taylor manipulated the pipette with sufficient accuracy to extract the micronucleus from *Euplotes* and inject minute quantities of substances into its cytoplasm and macronucleus (Taylor, 1920).

With hindsight it seems that, having come this far—penetrating into the cell interior—a natural progression would have been to pass current through the pipette. In this regard, there is some misunderstanding over the contribution of Ida Hyde (1921). Despite suggestions to the contrary (e.g., Tucker,

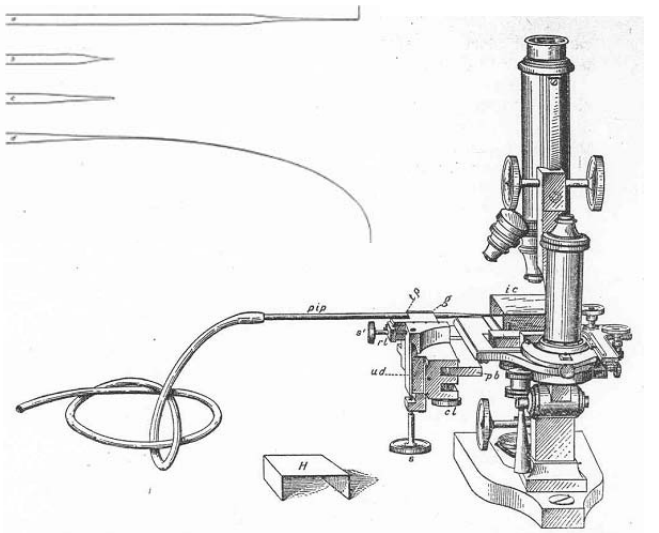

Figure 2. Micropipettes (magnified) and micromanipulator, from Barber, 1914.

1981), it is certain that she did not use her Barber pipettes to stimulate the stalk of *Vorticella* intracellularly. Firstly, she describes her micropipette electrodes as having a lumen diameter of three or more micrometres. This would give them an external diameter similar to that of a *Vorticella* stalk, making penetration impossible. Secondly, she claimed no more than that she could stimulate any part of a *Vorticella* that was "near contact with the active electrode." But Hyde does seem to have been the first to use a Barber pipette for extracellular electrical stimulation confined to a single cell. Perhaps intracellular stimulation was not considered worthwhile, because it was so easy to stimulate cells using extracellular electrodes. Nevertheless, it seems odd that the first definite use of intracellular, current-passing, capillary electrodes was a full nine years later, by Blinks (1930) in *Valonia*. It was much later still, that Graham and Gerard (1946) first employed intracellular micropipettes for the determination of threshold stimulating voltages and strength-duration curves in individual muscle fibres (although, see comments on Péterfi, below).

By contrast to the delay in the use of intracellular electrodes for passing current, intracellular electrical measurement soon became an obsession for researchers in a number of laboratories, in spite of the fact that initial success was very limited. Osterhout attempted to use a glass capillary electrode in *Valonia* (Osterhout, 1925) in experiments that began in 1923 (Osterhout, 1931); Cole says that he "chased paramecia with a pipette at the end of a Compton electrometer" (Cole, 1957), in a 1924 endeavour (Cole, 1968); Peterfi, by 1925, was using micropipettes fine enough to be inserted into animal cells, although his results from *Amoeba* failed to persuade him of the existence of a membrane potential (Gicklhorn and Umrath, 1928). Taylor and Whitaker (1926) described a tiny and variable potential difference, averaging about –1 mV, in *Clypeaster* eggs.

Eventually, in the late 1920s and early 1930s, giant plant cells provided the first real successes. These included the studies of Taylor and Whitaker (1927), who used their now sophisti-

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cated—less than one micrometre lumen diameter (Taylor, 1925)—Barber micropipettes, in conjunction with platinum-black microelectrodes, for potentiometric pH measurements in *Nitella*. Gicklhorn and Umrath (1928) used pipettes of about eight micrometres diameter to measure potentials averaging –15 mV in *Tulipa* pollen sprouts and –3 mV to –19mV in *Nitella*. After this, Blinks (1930), Damon (1930) and others were able to measure potential differences in *Valonia*, *Chara*, *Nitella* and *Halicystis* under a variety of experimental conditions (reviewed in Osterhout, 1931). On the other hand, further microelectrode studies with large echinoderm eggs (Gelfan, 1931; Rothschild, 1938) served only to reinforce the view that these had zero membrane potential. It was not until the work of Tyler et al. (1956) that these results were accepted to be wrong. Tyler's team found typical potentials of –10 mV to –60 mV, and suggested that earlier results were consistent with failure to penetrate the plasma membrane, a proposition now generally accepted (Cole, 1968).

Of course, the next major advance was the application of the capillary electrode technique to the squid giant axon, by Hodgkin and Huxley (1939). This led, in due course, to a complete analysis of the nerve action potential and its propagation. Between times, in an unlucky attempt at impaling cells of more normal size, and with precocious results that were not believable at the time (Cole, 1968), pipettes of around two micrometres diameter were used by Hogg, Goss and Cole (1934) on embryonic rat cardiac myocytes in tissue culture.

Out of the work from this period it is my opinion that that of Taylor and Whitaker (1927) presents us with the definitive invention of the intracellular glass micropipette electrode. Taylor and Whitaker used an agar-saturated KCl-filled Barber micropipette as the reference electrode for the determination of pH in *Nitella*. Amazingly, neither they, nor others, seem to have used anything similar for accurate transmembrane potential measurements until the work of Nastuk and Hodgkin more than 20 years later.

Meanwhile, the trail now returns to the University of Chicago. By the late 1930s, Ralph Gerard, in Matthews' former department, had developed a keen interest in excitable cells and membrane potentials. In about 1940 he charged his PhD student, Judith Graham, with the manufacture of sufficiently fine micropipettes to make reliable measurements of membrane potentials in frog skeletal muscle fibres.

According to Florey (1966), Gerard had learned the technique of hand pulling micropipettes during a visit to Umrath's laboratory in Graz, Austria. Umrath had collaborated with Gicklhorn (from the German University in Prague) on the first successful measurement of potential differences in plant cells (Gicklhorn and Umrath, 1928). In this paper they gave a detailed account of how they modified the preparation and filling of their micropipette electrodes after initially following the technique of the Hungarian, Tibor Péterfi, who had moved to the German University in Prague in 1919 (Chambers and Maskar, 1953). Péterfi not only seems to have been the first to measure a cellular membrane potential using a micropipette electrode, but also to have made improvements to the design of Barber's micromanipulator, based on an example from Jacobus Janse, Professor of Botany in Leiden (Péterfi, 1923). Carl Zeiss manufactured a micromanipulator according to Péterfi's instructions while Péterfi was an adjunct staff member at the Zeiss laboratories in Jena in 1921 (Chambers and Maskar, 1953). This equipment, along with an indication that he had, possibly, already inserted a current-passing microelectrode into *Amoeba*, is described in detail in his major work, "Mikrurgische Methodik" (Péterfi, 1923). Publications from this time indicate mutual respect between Barber, Chambers and Taylor in the United States and Péterfi in Europe, and examples of equipment passed each way between them. The perfected Péterfi-Micromanipulator, manufactured by Zeiss, became the most widespread instrument of its kind in the world (Chambers and Maskar, 1953).

In Chicago, with instruction from Gerard in microelectrode preparation, Graham succeeded in obtaining the first reasonable estimates of muscle fibre resting potentials, reporting averages of –62 mV (Graham and Gerard, 1946). Though a significant advance, this value was later shown to be too small, due probably to damage caused by the still relatively large diameter pipettes (two to five micrometres) that Graham used.

At this time Gilbert Ling came to Gerard's laboratory from Chungking, China. Ling set about perfecting the art of hand pulling micropipettes, a skill which had not really advanced, and had perhaps even regressed, since the original examples of Barber, Taylor and others. Ling's major contribution was to establish a source of stable heat. All previous workers had pulled their micropipettes over small gas burners—down to 1 mm in diameter. As Ling realised, these were too unstable in drafts of any kind, leading to inconsistencies in microelectrode characteristics. Ling pulled his micropipettes after heating his glass tubing in the edge of a large diameter (1 cm) air-gas blowtorch, with the air supplied by a reliable air pump (Ling, personal communication). It is noteworthy that Barber, himself, although he advocated the use of a micro-burner with the lowest possible flame, also recommended that the preparation area "be free from drafts of air" (Barber, 1914). The result was that Ling could consistently obtain pipettes of less than one micrometre in tip diameter. These he filled with isotonic KCl by boiling under intermittent pressure. Using the resultant microelectrodes, Ling and Gerard (1949) published membrane potentials averaging –85 mV. In an ultimate renaissance of Taylor and Whitaker's 1920s technology, Nastuk and Hodgkin (1950) filled micropipettes, still pulled by hand, with 3M KCl, thereby reducing pipette resistance, eliminating a junction potential between pipette and cytoplasm, and obtaining resting potentials in frog muscle of about –90 mV. In a more recent corollary to these advances, Peter Barry (1994) designed a computer program, "JPCalc," that calculates and allows correction of junction potentials for most experimental conditions.

Glass microelectrodes are no longer restricted to their original use in measuring macroscopic transmembrane potentials and currents. Their reincarnation as fire-polished, patch-clamp electrodes (cf., the stimulating electrode of Pratt, 1917) now sees them used for simultaneously clamping voltage and monitoring currents in various membrane configurations, including, most conspicuously, in single channels (e.g., Hamill et al., 1981). Coevolution of the glass microelectrode and micromanipulator with high input impedance amplifiers, to their final flowering as the contemporary patch-clamp amplifier, is another interesting tale, but outside the scope of the present account.

With respect to the consistent pulling of micropipettes, automated electromechanical pullers have long since removed the variability introduced by manual pulling (Brown and Flaming, 1986). Laser-based heating avoids the problems inherent in flames and heater filaments. Absolutely true and invariable commercial glass capillaries have eliminated further difficulties, and integral glass fibres, as in omega dot capillary tubing, have removed the frustrations previously associated with pipette filling.

I now look back with amusement (not felt at the time) on my own early encounters with microelectrodes. I learned through bitter experience the need to use callipers to choose consistent glass capillary stock, to monitor the mains voltage and to keep the supply to my homemade puller constant with a variable transformer. And this is not to mention the need to hold my breath during heating and pulling (to avoid drafts) and to check on the room temperature, atmospheric pressure and phase of the moon (I became paranoid about anything that might influence success). On those days when nothing seemed to work, I could be caught muttering incantations (like the witches in Macbeth) watching over my pipettes as they filled, first with boiling methanol and then with cold 3M KCl. How much more difficult it must have been for the pioneers.

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