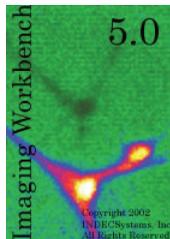


long recording sessions—and absolutely no backlash on reversal, or other play, such as is found with conventional micromanipulators. And finally, on top of all these features, the MM3A is essentially immune to temperature fluctuations.

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

INDIRECT 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.

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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) LJPs 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 MgCl₂
- 1 mM CaCl₂
- 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 MgCl₂
- 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;

FOCUS ON METHODS

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).

Calculate Junction Potentials

Whole-cell measurements

Pipette Ref.

Cell Bath

Experimental Parameters

Ref. electrode: Standard salt-solution electrode.
Temperature (°C): 20.0

Ion	z	u	Cpip	Cbath
K	1	1	145	2.8
Na	1	0.682	13	145
Cl	-1	1.0388	10	148.8
gluc	-1	0.33	145	0
Mg	2	0.361	1	2
Ca	2	0.4048	0	1
HEPES	-1	0.3	5	5

Charge Balance: 0 0

Results

Junction Pot. (original solution - pipette) = 15.6 mV at 20.0 °C.
Therefore, $V_m = V_p - 15.6$ mV

$V_m = V_p - V_L$

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 V_m 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:

$$V_m = V_{cmd} - LJP$$

($V_m = V_p - V_L$) in the Junction Potential Calculator where V_{cmd} 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:

$$V_m = -V_{cmd} + LJP$$

[$V_m = -(V_p - V_L) = -V_p + V_L$ 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):

$$V_m = -V_{cmd} + LJP + RMP$$

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

Current clamp

Current clamping is typically performed in whole-cell configurations. In this case, the LJP needs to be subtracted from

the measured voltages:

$$V_m = V_{rec} - LJP$$

($V_m = V_p - V_L$ in the Junction Potential Calculator) where

V_{rec} 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.

Barry, Peter H., and Diamond, Jared M., Junction potentials, electrode standard potentials, and other problems in interpreting electrical properties in membranes, *J. Membr. Biol.* 3, 93-122, 1970.

Barry, Peter H. JPCalc - a software package for calculating liquid junction potential corrections in patch-clamp, intracellular, epithelial and bilayer measurements and for correcting junction potential measurements. *J. Neurosci. Methods*, 51: 107-116, 1994.

Neher, Erwin, Correction for liquid junction potentials in patch clamp experiments, *Methods in Enzymology*, Bernardo Rudy and Linda Iverson, eds., Academic Press, San Diego, 207, 123-131, 1992.

Neher, Erwin, Voltage Offsets in Patch-Clamp Experiments, in *Single-Channel Recording*, 2nd ed., Bert Sakmann and Erwin Neher, eds., Plenum Press, New York, 147-153, 1995.

Kenyon, James L., *Primer on Junction Potentials for the Patchologist*, Third Revised Edition (Winter 2002) at <http://134.197.54.225/department/kenyon/default.html>

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,