Ultra Fast Solution Applications for Prolonged Gap-free Recordings: Controlling a Burleigh Piezo-Electric Positioner with Clampex 7

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Introduction

Typically, the voltage command signals used to control a piezo-electric device such as the Burleigh LSS-3200 solution switcher have to be programmed as a series of voltage ramp epochs in order to control motion, as well as to protect the piezo-electric crystal from sharp voltage jumps. In the pCLAMP 6 environment this involves using the waveform output of Clampex and the episodic acquisition mode. Using Clampex 7 and an inexpensive RC filter circuit (Figure 1) to suitably condition voltage step commands, new Clampex 7 features permit the coordination of piezo-electric solution exchange with other Clampex acquisition modes such as gap-free and event-driven acquisition.

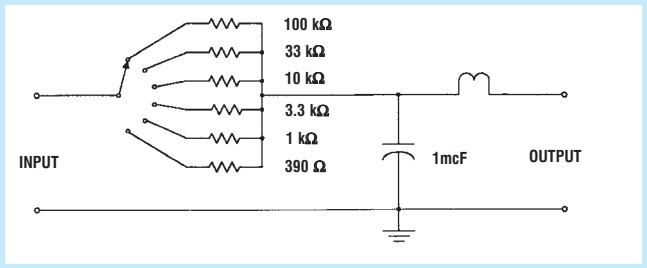

Figure 1. Schematic for the signal conditioning circuit (SCC).

Methods

Signal Conditioning Circuit (SCC)

Switchable RC filters in a signal conditioning circuit (SCC, Figure 1) were used to transform a voltage step command into a waveform suitable to move a Burleigh LSS-3200. The circuit shown permits an RC time constant to be selected over the range from 0.4 ms to 100 ms. The inductance in the SCC schematic represents a coil of approximately 20 loops, 3-4 mm in diameter with ferro-magnetic core. The inductive element served to decrease the initial rise rate of the conditioned signal.

Solution Application

Theta tubing may be used for rapid switching between two solutions. However, our experiments were conducted using a internally perfused pipette, essentially a modified U-tube[1], so that we could select any of several experimental drug solutions. The solution selected for application enters the upper port of a 2 port pipette holder (E.W. Wright, part number A179-2mm), via PE10 tubing and is carried to near the tip of the glass application pipette (2.0 mm O.D.). As long as there is gravity flow from the lower port, solution will pass back up the barrel of the glass pipette (along the outside of the PE10 tube) and to waste. When flow through the waste tube is cut off with an electronic valve, perfusion solution will flow out the tip. Solutions can be changed, usually in about one minute, while the flow is off the patch, and then the piezo-electric device can be used to rapidly move the new solution onto the recording pipette. This system has the advantage that there is no change in positioning when different solutions are used.

Experimental Chamber

Prior to the electrophysiological experiments, a sterile reusable Teflon insert was placed into either a 60 mm Petri dish (outside-out oocyte patch experiments) or 35 mm tissue culture dish (for whole-cell patch-clamp experiments, Figure 2). This insert had a cut channel which provides a pathway for perfusion solution. At either end of the channel are relatively large reservoirs (400 µl for the 35 mm inserts) for solution in-flow and out-flow. Solution flows from the reservoirs through 1 mm constrictions (which decrease surface tension effects) and then into a linear portion of the channel, where a laminar flow of solution exists.

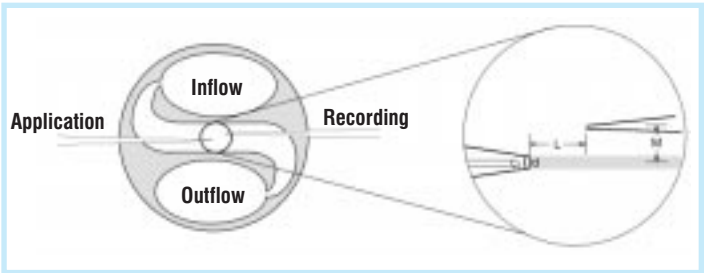

Figure 2. Experimental chamber and pipette configuration (see Table 1). Note the PE10 tubing inside the application pipette for internal perfusion and solution exchange.

Software Control

Experiments were conducted using the continuous (*i.e.*, gap-free) recording mode that is suitable for the acquisition of data for single channel recording. Files acquired in this mode may be opened by Fetchan 6 for the creation of idealized records. A new feature in Clampex 7 permits external

trigger signals to be used to start gap-free acquisition so that an external pulse generator can be used to generate both a trigger pulse for the digidata and a voltage step command to be conditioned by the SCC. The SCC output is then used to control movement of the solution switcher. The output of the SCC may also be sent in parallel to one channel of the Digidata to confirm the synchronization of agonist application and data acquisition.

The Clampex 7 real time controls for the V-out channels provide another way to integrate the use of the SCC and a piezoelectric positioner into gap-free recording. Specifically, the voltage command signals to the SCC can be generated directly from the scope window of Clampex 7 during an acquisition by typing directly into the screen field for the V-out value. The timing of the V-out signal is then controlled by hitting the enter key. To control movement of the piezoelectric positioner, the voltage steps generated by the V-out channel are modified by the SCC and the SCC output sent to the piezo device. Synchronization of the data with the V-out signal can be evaluated by acquisition of the SCC channel. Alternatively, the option can be set in Clampex 7 to automatically tag changes in V-out.

Further flexibility in setting up experimental protocols can come from using the programmable "sequencing" keys in Clampex 7. The sequencing keys function like linked macro commands that can define a series of protocols and parameter changes, implementing different V-out settings, or toggling between view and record modes.

In addition to being used for gap-free recordings, these methods can also be employed for event driven data acquisition; either variable-length event driven for single channel data or fixed length event driven for whole cell currents. Data can be selectively written to disk based on the channel activity, or the commands sent to the SCC can be acquired on a separate channel and selected in the protocol edit dialog as the trigger source. The step commands to the SCC can come from an external pulse generator or the real time controls of Clampex itself.

Results

Figure 3A illustrates our application system as evaluated with an open tip recording from a patch electrode immersed into the flow of the bath Ringer's solution. The SCC time constants used effectively were in the range of 1-10 ms. However, it should be noted that solution exchange is determined not by the RC value but by the transit time of the interface across the pipette (or cell) and therefore is significantly faster than the RC time constant of the SCC. Figure 3B illustrates the response to the application of 10 µM ACh, obtained with these methods from a outside-out patch of a *Xenopus* oocyte expressing mouse muscle AChR.

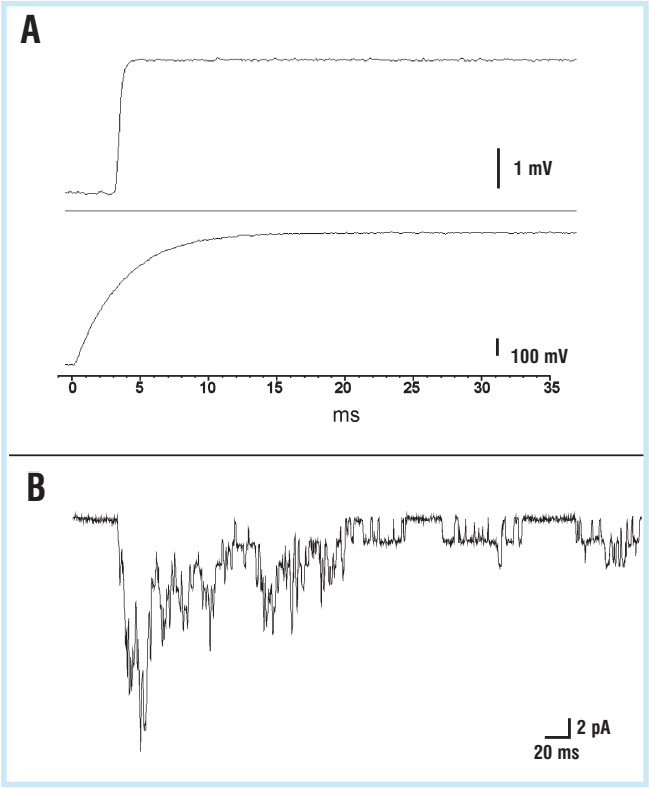

Figure 3. A) The upper trace is an open tip recording made when the application pipette was filled with 50% diluted Ringer solution. The application rate was ≤ 0.7 ms. In reference to Table 1: D = 35, L = 30, H = 9, M = 20, P = 20, RC = 2, V = 18. The lower trace is the SCC output. Note that the delay between the SCC signal and the open tip record is associated with the flow rate and the distance L. B) Response of mouse muscle-type AChR to the application of a 10 μM ACh solution at a holding potential of -40 mV.

Note that the optimal application of these methods requires careful empirical determination of performance through the tuning of several factors which will vary for the specific application. Some of these factors are listed in Table I. The optimal parameters are unique for each application pipette and therefore should be empirically determined for each pipette. However, once optimally configured, the same application pipette may be used for multiple experiments.

Caveats and Trouble Shooting

Oscillations

Application of the voltage to the piezoelectric manipulator not only causes movement in accordance with the applied voltage, but also may produce unavoidable mechanical oscillation of the pipette tip. It is important to synchronize these two types of movements during the open tip transition through the solution interface, so that this transition should occur only once.

Oscillations may occur when the stream diameter is too small, the initial position of the application pipette tip or the

agonist stream is too close to the electrode tip (as defined in Table 1, values for L or H too small), or total movement of the application pipette (M) is too large. Also, the thin shank of the application pipette is an elastic element that should be as short as possible to avoid oscillations.

Table 1. Description of Parameters

d	The diameter (µm) of the application pipette tip
L	Distance (µm) between the application pipette tip and the electrode tip in the horizontal direction (see Figure 2)
H	Distance (µm) between the edge of the application solution stream and the electrode tip, in the direction of pipette movement
M	Movement (µm) of the electrode tip during application in the direction perpendicular to bulk solution flow (see Figure 2)
P	Pressure (cm H_2O) in the application solution
RC	Time constant (ms) of the low pass filter of the SCC
T	Time (ms) when the electrode tip potential changes by 90%
V	Voltage (V) input to SCC, proportionate to M

Long Rising Time of the Open Tip Current

If the application pipette has relatively thick walls (> 1 µm), it may produce an apparent enlargement of the solution interface, slowing the solution change rate. Also, low pressure in the application pipette results in an apparent increase of the interface thickness due to dilution. We found optimal pressures to be around 10-20 cm H_2O when the flow rate through the chamber was 1 mm/s, resulting in a flow rate for the agonist stream of 2.5 mm/s.

Note that for setting up your system, the best fidelity of open tip recording is obtained with fresh electrodes. Repeated application to the same patch electrode appeared to dilute the electrode solution with the Ringerís solution and slow the detection of interface transition.

Conclusion

Through the integration of the hardware and software elements described above, we can extend the application of a piezo-electric solution switcher such as the Burleigh LSS 3200 to satisfy a wide range of the experimental needs, going beyond previous limitations presented by the use of software controls alone.

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Reference

[1]Magazanik, L.G., and Vyskocil, F. Dependence of acetylcholine desensitization on membrane potential. *J. Physiology*. **210:**507-518, 1970