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Dynamic clamp with StdpC software

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Abstract

Dynamic clamp is a powerful method that allows the introduction of artificial electrical components into target cells to simulate ionic conductances and synaptic inputs. This method is based on a fast cycle of measuring the membrane potential of a cell, calculating the current of a desired simulated component using an appropriate model and injecting this current into the cell. Here, we present a dynamic clamp protocol using free, fully integrated, open-source software (StdpC, Spike timing dependent plasticity Clamp). Use of this protocol does not require specialist hardware, costly commercial software, experience in real time operating systems or a strong programming background. The software enables the configuration and operation of a wide range of complex and fully automated dynamic clamp experiments via an intuitive and powerful interface with a minimal initial lead-time of a few hours. After initial configuration, experimental results can be generated within minutes of cell impalement.

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Keywords

Neuroscience; electrophysiology; ion channels; synapses; pattern clamp; hybrid system; current clamp; voltage clamp; brain-computer interface; closed loop interaction; real time control

Introduction

The dynamic clamp method was introduced independently by Robinson and Kawai[1] and Sharp et al.[2] Initially it was used to introduce artificial conductances into neurons to mimic the effect of voltage-gated ion channels and synaptic inputs arising from other neurons[3,4]. More recently, with further developments in hardware and software, it has become an important tool for a broad range of applications, including the full control of cell activity (pattern clamp)[5], the simulation of forms of synaptic plasticity[6,7,8], the connection of living neurons to simulated[9] and electronic[10] neuron models and neuron model populations[11,12] and even the connection of preparations from different animals through simulated synapses[13]. In addition to a range of central neurons, dynamic clamp has also been used to study sensory cells[14,15], motoneurons[16], endocrine cells[17,18], cardiac cells[19,20,21] and the peripheral nervous system[22,23]. Other researchers have even extended the principles of dynamic clamp to interact with the nervous system through mechanical stimulation[24] or micro-injection of drugs[25].

All dynamic clamp methods are based on the same general principle. Central to this is a fast “dynamic clamp cycle”, comprised of three main components. First, the membrane potential of a cell is measured, then a computer calculates the current that would flow through a simulated electrical component of the cell at the measured potential, and finally, this current is then injected into the cell. If the cycle is executed fast enough (typical implementations exceed 10 kHz), the membrane potential of the cell will be indistinguishable from a situation in which the simulated electrical component is actually present in the membrane. See Fig. 1 for an illustration of this general dynamic clamp principle.

Since the introduction of the principal method a number of different implementations have been developed, each with particular advantages and disadvantages. While some approaches invested considerable effort on precisely fulfilling real-time constraints (i.e., fixed length time step intervals in the dynamic clamp cycle) such as real-time Linux-based, digital signal processing-based, and early analog solutions, others emphasized the importance of ease of installation and use. Some of the most common dynamic clamp implementations are listed in Table 1 (see[26-29] for more detailed reviews of dynamic clamp and its implementations).

Although many studies have used dynamic clamp methods (for examples, see[26-30]), they have never quite become a standard tool in electrophysiology in spite of their huge potential for actively probing neuronal systems. We can identify at least two reasons for this: 1) most implementations require a high initial investment into specialized hardware and software and/or a lot of technological expertise that is not available in a typical electrophysiology group. 2) the design of dynamic clamp experiments is different from standard electrophysiology and is not typically part of standard electrophysiology training.

The protocol based on StdpC, presented here, addresses these problems in the following ways: 1) StdpC can be downloaded for free at http://sourceforge.net/projects/stdpc/ and is released under the GNU General Public Licence (GPL). It runs on standard Windows platforms and requires only a data acquisition board in addition to a standard electrophysiology setup. It supports the traditional Digidata 1200/1200A boards (Axon Instruments, part of Danaher, Washington DC) as well as any NIDAQ-enabled National

Instruments board (National Instruments, Austin, Texas), devices that are commonly already present in most electrophysiology laboratories. 2) The system requires minimal expertise for setting up dynamic clamp experiments and can be used by any electrophysiologist without extended additional training.

StdpC

StdpC[7] is based on software originally developed by Pinto et al.[31]. In its current form, the software has a modern graphical interface based on QT (Nokia) and is suitable for general public use. It permits the definition of up to six voltage-gated conductances and/or up to six synapses using built-in standard models for gap junctions and chemical synapses as well as two standard formalisms for Hodgkin-Huxley type voltage-dependent ionic conductances. The synapses can additionally be specified to be plastic according to two common formalisms (additive spike-timing dependent plasticity (STDP) rule[32], or ordinary differential equations (ODE) based STDP formalism[33]). Additionally, a configurable artificial neuron can be connected to the preparation through StdpC's versatile spike generator facility.

Besides the fully integrated graphical user interface, the software also includes a basic but powerful scripting mechanism for automatically adjusting all aspects of an introduced dynamic clamp object (e.g., maximal conductance or time constants of synapses and ion channels) during the experiment according to pre-defined experimental protocols. This allows complex experimental designs that can be run semi-automatically on many preparations.

Practical considerations and limitations

When considering the use of a dynamic clamp protocol it is important to be aware of the limitations of the approach. One principal limitation is that dynamic clamp only simulates the effect of an electrical component in the cell membrane, not the actual flow of specific ions. Thus, the effects of an ion acting as a second messenger (e.g., Ca^{2+} activating calcium-calmodulin dependent kinase enzymes), cannot be simulated by dynamic clamp. Similarly, so far it has not been possible to simulate ligand-gated ion channels using dynamic clamp.

With modern computers and interface boards, hardware limitations are unlikely to be a major concern for the most typical dynamic clamp applications. StdpC allows the simultaneous use of up to six ion channels and six synapses. We have found that using biologically realistic time scales, the variable soft real-time dynamic clamp cycle with a typical >20 kHz mean cycle frequency[7] does not present a major limitation. Finally, the setup we outline here involves two separate computers, one for dynamic clamp and another for recording. In theory, there are no key reasons why a single computer cannot be used to perform both tasks although this has not been exhaustively tested with StdpC.

Experimental Design

The common design principle of dynamic clamp experimentation is the so called dynamic clamp cycle. The membrane potential of one or several neurons is measured and model equations, which depend on the measured membrane potential(s), are evaluated on the dynamic clamp computer resulting in (a) current amplitude value(s). The corresponding current(s) is (are) then injected into the target cell(s).

In the procedures detailed below we outline three typical dynamic clamp experiments: 1. the simulation of a chemical synapse, 2. the introduction of an ionic current and 3. a pattern clamp experiment.

1. When introducing an artificial synapse, the current entering the post-synaptic cell depends on the membrane potential of the pre-synaptic neuron by virtue of the voltage-dependent transmitter release and on the membrane potential of the post-synaptic target cell through Ohm's law. Accordingly, in this experiment, two neurons are monitored intracellularly and one of them, the designated post-synaptic cell of the simulated synapse receives current injections. The current through the simulated synapse is calculated from the measured pre- and post-synaptic membrane potentials using a computational model. StdpC provides two common computational models for chemical synapses; the one used for our example dataset (see below) is illustrated in Box 1.
2. When introducing a simulated ion channel into a target neuron, only the target neuron's membrane potential needs to be measured and current injections occur into the same cell. The computational model in this case is based on Hodgkin-Huxley-type equations. StdpC provides two common formalisms to parameterise the equations. The formalism used in the anticipated results (below) is illustrated in the supplementary equations.
3. In pattern clamp, both a simulated neuron and a simulated strong gap junction are used. A target cell is monitored and its membrane potential is clamped to the activity pattern of the membrane potential of the simulated neuron by the simulated gap junction.

In the anticipated results section we show examples of these three types of experiments using either neurons from a classical molluscan preparation or neurons from dissociated hippocampal cultures.

For designing new dynamic clamp experiments, or adapting existing ones, the experimenter needs to identify which measurements need to be taken, which neurons need to receive current injections and what the magnitudes of the measured signals and injected currents are. When using a single recording/injecting electrode, it is important to reduce any artefact in the readout of the voltage that may be caused by the simultaneous current injections (see step 4 in the procedure for a list of options). The voltage drop across an uncompensated electrode is proportional to the electrode resistance and the amplitude of the injected current and becomes problematic in procedures that require large current injections such as pattern clamp. The voltage artefact is absent when no current is injected; for this reason no compensation is required when using two different electrodes for voltage recording and current injection. On the other hand, injection artefacts can typically be ignored when the resistance of the electrode is low (e.g., in patch clamp) and the current injection amplitudes are small.

Other parameters that need to be taken into account when designing experiments are the maximum number of electrodes and manipulators that can be used at once on a preparation and the number of input and output channels available on the data acquisition board used for dynamic clamp. For example, the Digidata 1200/A boards have two analog outputs whereas the NI PCI-6229 card we used here has four. The number of analog inputs is typically less problematic. The Digidata 1200/A has 16 while the NI PCI-6229 board has 32. Along with the practical experimental considerations, another important consideration in designing dynamic clamp experiments is to formulate or obtain an *accurate* computational model of the simulated electrical component. For example, for simulating ion channels, a Hodgkin-Huxley type model with accurate parameter values is required or, for simulating a synapse, a full description including conductance, time scale, activation characteristics and reversal potential is necessary.

Unlike in other experimental paradigms there is typically no need for elaborate negative controls in dynamic clamp other than measuring the activity with and without the simulated component repeatedly and preferably in several preparations. It is good practice, however, to inspect the data offline and re-calculate on a computer that the current injection, as measured on the independent electrophysiology workstation (see Figure 1), was correct according to the computational model used.

EQUIPMENT

Standard electrophysiology rig

The rig needs to be capable of intracellular recordings in current clamp mode and current injection into neurons. Typically, the necessary equipment would be:

- Microelectrodes
- Headstages and a microelectrode amplifier. StdpC does not impose any restriction on the amplifier used; even a combination of different types of amplifiers is possible. In the results presented below we have used an Axoclamp 2 amplifier (Axon Instruments, part of Danaher, Washington, DC)
- Appropriate cables to connect the amplifier with the data acquisition board (usually standard BNC cables)
- A computer with electrophysiology software. Below we have used a Pentium 4, 2.8 GHz PC with 1 GB of RAM, Windows 2000 operating system and Spike2 software (CED, Cambridge).

Data acquisition board

We recommend the following data acquisition boards:

- Digidata 1200/1200A (Axon Instruments, part of Danaher, Washington, DC). Note that modern Axon Instruments boards could not be supported because the technical specifications of their interfaces have not been disclosed. With a recent merger this situation has now changed and we may be able to develop hardware drivers for these boards in the future.

OR

- Any National Instrument board (National Instruments, Austin, Texas) that supports the NIDAQ device interface and has at least two analog inputs and two analog outputs (e.g. the National Instruments (NI) “E” or “M” series boards). For the results below we have used the NI M-series PCI-6229 board with 4 analog outputs (833 kS/s) and 32 analog inputs (250 kS/s). It is recommended that you choose a board with >100 kS/s sampling frequencies.

Dynamic clamp PC

Any recent PC with Windows 2000, Windows XP (both Digidata and NI boards) or Windows 7 (NI boards only). StdpC has been tested and confirmed to work for these operating systems, although we have only trialled Windows 7 Professional 64 bit. As StdpC runs in the 32 bit compatibility mode in Windows 7 ×64, we anticipate that Windows Vista and other versions of Windows 7 will work with NI boards as well. The old Digidata 1200/A boards are not recognised in Windows 7 according to our tests. The Digidata board requires an ISA bus slot, commonly not available in modern PCs. If a lab does not have a previously installed Digidata 1200/A setup, it is recommended to use the National Instruments option. Alternatively, specialist industrial PCs with legacy ISA bus support are available, e.g. from

Spectra Computer Systeme GmbH, Leinfelden, Germany. For the results presented below, we have used a 1.8 GHz Intel Core 2 CPU 6320 based PC with 1GB RAM and Windows XP.

For advanced users, who wish to modify the source code of the original StdpC software in order to alter or extend parts of it to their specific needs, another requirement is to have the QT software framework and development environment installed for compiling the software from its source code (QT is available under the GNU public license at http://qt.nokia.com/products free of charge).

EQUIPMENT SETUP

Standard electrophysiology rig

Typically, dynamic clamp is used with either two or more sharp electrodes or one or more low resistance patch electrodes. Each electrode should be connected via the electrode headstage to the appropriate electrode socket of the amplifier, and the amplifier voltage output (typically labeled V1, V2) should be connected to the data acquisition board used for recording. The data acquisition board should be connected to a recording computer with electrophysiology software installed (e.g. Clampex, WinEDR, WinWCP, Spike2). This is a standard setup for any electrophysiological experiment. Special care should be taken to choose the amplifier's output channels to obtain the highest possible signal-to-noise ratio; the highest amplification which does not exceed the input range of the data acquisition board for expected signal amplitudes is usually best. It is recommended to filter the membrane potential with hardware filters because, unlike in a normal electrophysiological recording, noise in the membrane potential recording feeds back into the system through the dynamic clamp.

PROCEDURE

StdpC and hardware installation

TIMING 2-3 h—1 Download StdpC. Direct your browser to http://sourceforge.net/projects/stdpc/ and click the "Download now!" button. Choose "save" in the dialog box that appears and save the file StdpC2010.zip in a convenient location (e.g. your desktop). Unzip the file. It will create its own sub-directory StdpC2010-bin. This completes the software installation.

TROUBLESHOOTING

PAUSE POINT: The installation is only required once and does not need to be repeated in subsequent uses of the protocol.

2 Install the data acquisition board in the dynamic clamp computer according to the manufacturer's instructions. In the case of NI devices, install the manufacturer-provided windows drivers. Calibrate the board with the manufacturer's tools.

PAUSE POINT: The device installation is only necessary once.

3 Connect the dynamic clamp acquisition board to the amplifier. For a two-electrode configuration, you will be using two electrodes in every cell that receives input from the dynamic clamp. One is the "recording electrode". Connect the voltage output of this electrode on the amplifier to an input channel of the dynamic clamp acquisition board. The other electrode is the "injection electrode". Care should be taken to choose the correct gain for current injection. Connect an analog output channel of the dynamic clamp acquisition board to the current command input for this electrode on the amplifier. Other cells can be

used as pre-synaptic cells for simulated synapses. For these cells use and connect only one "recording electrode". The separate electrodes for injection and recording serve the purpose of avoiding current injection artifacts in the recorded membrane potential. Alternatively, single low resistance electrodes, with artifact compensation (e.g. bridge balance, discontinuous current injection, or the recently introduced active electrode compensation (AEC)[34,35]. The most recent version of StdpC includes an AEC feature.) can be used for both recording and current injection.

4 Adjust the amplifier settings. Perform all experiments with StdpC in current clamp mode.

StdpC configuration

TIMING 1 h—5 Start StdpC. There are two alternative options for this step depending on whether you are using a National Instruments board or a Digidata 1200/1200A board.

A If you are using a National Instruments board, double-click StdpC2010-Nidaq.exe.

B If you are using a Digidata board, double-click StdpC2010-Basic.exe.

If you have both boards installed you should use option A.

TROUBLESHOOTING—6 Choose your dynamic clamp acquisition board from the dropdown menu on the top of the main window of StdpC. The message window will show a message "Good news: <board name> found and opened successfully".

TROUBLESHOOTING—7 Set up the input channel configuration in StdpC.

Click "Config->Input Channels" (marker 1 in Fig. 2) and choose a data acquisition range for each channel that you are using (the channels you connected the amplifier to in step 3). Choose the smallest range that includes your expected signal amplitudes for best results.

Enter the overall amplifier gain for each input channel. This is the product of the headstage gain, the amplifier gain and the gain of any other pre-amplifiers or filters. The gain is entered as the ratio between the actual voltage at the electrode and the corresponding voltage present at the dynamic clamp acquisition board. For example, if there is 10x amplification in the amplifier and no other amplification, the conversion factor would be 1/10= 0.1.

Choose to save a channel on disk by clicking on the "Save Channel" checkbox.

CRITICAL STEP—All input channels are denoted in a "StdpC-centric" way, i.e. the input channels are the recorded membrane potential of neurons (output of the neurons but inputs to the dynamic clamp) and the output channels are the current commands for the injection of currents into neurons (input to the neurons but outputs from the dynamic clamp).

CRITICAL STEP—Setting the most appropriate acquisition ranges and *correct* gain factors is an essential step when using StdpC. An acquisition range that is too small will lead to clipped signals and associated errors in the injected current. If the acquisition range is too large, this will lead to unnecessary digitization noise and degrade the quality of the dynamic clamp. If the conversion factor is incorrect, all inputs can appear to be 0 or always saturated, similar to what you might observe during a hardware failure.

TROUBLESHOOTING—8 Set up the output channel configuration in StdpC.

Click “Config->Output Channels” and choose output ranges and conversion factors as for the input channels in the previous step. Here, the conversion factor is entered as the ratio between the command voltage that is generated at the dynamic clamp acquisition board (in V) and the resulting current injected at the electrode (in nA). For example, if the current command of the amplifier generates 10 nA / V the conversion factor is 0.1. Choose whether to save a channel on disk with the “Save Channel” checkbox.

CRITICAL STEP—As with the input channels, all output channels are denoted in a “StdpC-centric” way.

CRITICAL STEP—Setting the most appropriate output ranges and *correct* conversion factors is an essential step when using StdpC. Too small output range will lead to clipped signals and associated errors in the injected current. An output range that is too large will lead to unnecessary digitization noise and degrade the quality of the dynamic clamp. Incorrect conversion factors will lead to inappropriate current commands that may even damage the biological preparation.

TROUBLESHOOTING—9 Test the input and output channel settings.

The best way to test the correctness of acquisition/ output range and conversion factor settings is to directly test each input and output channels that is used. To test an input channel, activate one of StdpC’s graphical display windows (marker 8 in Fig. 2) by clicking the “Settings” button next to it and select a channel to test from the dropdown menu in the dialog window that appears. Choose a minimum and maximum limit for the y axis of the display and the unit (mV for input channels and nA for output channels). Hit the Start button of the main window (marker 2 in Fig. 2) and check, whether the displayed voltage is correct. You can manipulate the voltage on the amplifier (amplifier offset) to establish this. When this is satisfactory, hit the Stop button (marker 3 in Fig. 2) and disable the display by choosing “None” in the Data dropdown box of the Data display dialog. The graphical displays compete with the core dynamic clamp software for CPU and memory resources and, if left active, can degrade the quality of dynamic clamp. We, therefore, recommend using the data displays for testing only.

In order to test an output channel, open the Config->Output Channels dialog box and enter a “Bias current” for the channel. Click the Start button and check on your amplifier display, your recording computer or an external oscilloscope that the voltage generated on the dynamic clamp data acquisition board matches the voltage command that is necessary to generate the chosen bias current. When this is satisfactory, stop the dynamic clamp with the Stop button and reset the bias current to 0.

Defining the simulated electrical component(s)

TIMING 10-30 min—10 Depending on the scientific enquiry there are many options for this step. Here, we describe three classical examples: a simulated synapse, a simulated Hodgkin-Huxley type ionic conductance, and a pattern clamp experiment.

A Simulated chemical synapse.

- **i.** Click on one of the Synapse drop-down boxes (marker 5 in Fig. 2) and choose “Chemical”.
- **ii.** Click the “Parameters” button below.

iii. In the dialog box that appears choose the Presynaptic Channel, i.e. the number of the analog input channel of the dynamic clamp acquisition board to which the recording electrode of the pre-synaptic cell is connected.

iv. Choose the Postsynaptic Channel, i.e. the number of the analog input channel of the dynamic clamp acquisition board to which the recording electrode of the post-synaptic cell is connected.

v. Choose the Output Channel, i.e. the number of the analog output channel of the dynamic clamp acquisition board which is connected to the current command socket of the current injection electrode in the post-synaptic cell.

vi. Enter parameters of the simulated synapse. The main parameters ("Standard") are explained in Box 1. All possible advanced options and parameters are explained in the StdpC manual that is distributed with the StdpC software.

B Simulated Hodgkin-Huxley conductance.

i. Choose the formalism of your model for the conductance from one of the Ionic conductance dropdown boxes (marker 6 in Fig. 2). This activates the ionic conductance model.

ii. Click the "Parameters" button below.

iii. In the dialog box that appears, choose "V inChannel" as the analog input channel of the dynamic clamp data acquisition board that is connected to the recording electrode in the target cell.

iv. Choose "I outChannel" as the analog output channel of the dynamic clamp data acquisition board that is connected to the current command input of the amplifier for the current injection electrode.

v. Enter the maximal conductance of the channel and the reversal potential.

vi. Enter the parameters of your model for activation and inactivation. A detailed explanation of the supported Hodgkin-Huxley formalisms and the corresponding parameters is given in the StdpC manual. The equations for the α-β formalism used for the anticipated results below are given in the supplementary equations.

C Pattern clamp.

i. In the "Spike generator" sub-panel (marker 7 in Fig. 2), choose "Explicit Spike times" from the Spike Method dropdown box.

ii. Enter desired properties of the pre-synaptic spikes: Vspike = desired spike amplitude; Width = desired spike duration, VRest = desired resting potential.

iii. Enter the number of spikes per burst and a bursting period.

iv. Click the "Spike Times" button and enter the relative spike times of spikes within each burst with respect to the start of the burst. The default settings produce a somewhat irregular burst with 10 spikes and spike deceleration in the second half of the burst. Other options for specifying presynaptic spiking are explained in the StdpC manual.

v. Choose "Gap Junction" in one of the Synapses drop-down boxes (marker 5 in Fig. 2).

vi. Click the "Parameters" button below it.

vii. In the dialog box that appears choose the spike generator “SG” for the Presynaptic input Channel.

viii. Choose the channel number of the dynamic clamp acquisition board’s input channel connected to the controlled cell’s recording electrode as the Postsynaptic input Channel.

ix. Choose “None” for the presynaptic output channel.

x. Choose the channel number of the output channel connected to the current command of the controlled cell’s current injection electrode as the Postsynaptic output Channel.

xi. Enter a high conductance on the order of 50 nS for the parameter gSyn.

CRITICAL STEP—It is important to find the right synaptic conductance gSyn by experimentation. If it is too low, the target cell will not be well controlled, if it is too high, there will be strong ringing noise. Compare the anticipated results in Fig. 3 below that show examples of successful and unsuccessful pattern clamp (Fig. 3 C-E).

TROUBLE SHOOTING

PAUSE POINT—Once the hardware and software configurations are complete, the whole configuration can be saved by clicking “File->Save Protocol” and choosing a file location and name. In subsequent experiments, steps 7-10 can be replaced by step 11.

CRITICAL STEP—Enter a file name ending on “.cpr” (“**c**lamp **pr**otocol”) for the saved configuration to retrieve it easily in the future.

11 Load a previously saved configuration.

Click “File->Load Protocol”, navigate to the previously saved protocol file, select it by mouse click and then click “Open”.

TROUBLESHOOTING

Experimental preparation

TIMING 30 min – 4 h—12 Set up the biological preparation.

The details of this experiment-specific step are independent of the general protocol described here. StdpC can be used for dynamic clamp in electrophysiological experiments based on both intracellular and whole-cell patch-recordings.

Dynamic clamp

TIMING 40 min - 12 h—13 Prepare electrodes.

The characteristics of the electrodes used for dynamic clamp depends mostly on the preparation in use. In general, low impedance electrodes are advised for injection of large currents. Insert the electrodes into the head stages and immerse them into the bathing solution appropriate for the preparation under study.

14 Assess properties of the electrodes in solution.

When your electrodes are in the saline, measure their characteristics, paying particular attention to the resistance. If the observed resistance is much higher than expected for the

type of electrode you are using, the success of the dynamic clamp experiment may be compromised.

If you have not already done so, set your amplifier in current clamp mode and adjust the amplifier offset on each channel so that the measured potential is 0.

15 Insert electrodes into target cells or perform whole-cell patch clamp recording. There are different configurations for this depending on whether you are simulating a chemical synapse, introduce a simulated Hodgkin-Huxley conductance, or performing pattern clamp:

A Chemical synapse simulation:

Obtain voltage recordings from the designated pre- and post-synaptic cells. Current will need to be injected into the post-synaptic cell, either using a second electrode, or the voltage recording electrode with appropriate compensations, as outlined in point 4.

B Simulated Hodgkin-Huxley conductance:

Obtain voltage recording from the target cell. Current will be injected into the same target cell, either using a second electrode, or the voltage recording electrode with appropriate compensations, as outlined in point 4.

C Pattern clamp:

Obtain voltage recording from the target cell. The same electrode used to record voltage can be used to inject current, but due to the large amount of current that is typically necessary to impose a pattern onto a cell, the use of a second “injecting” electrode is preferred in this configuration.

Insert recording and injection electrode into the neuron that was chosen to be controlled.

16 Assess the properties of each electrode in the cell. Pay particular attention to the stability of the recording, that the recorded signal reasonably reflects the actual (anticipated) membrane potential and that the cell has reasonable membrane resistance. These characteristics vary depending on the cell type. In the preparations used below one observes typical resistances of 15-45 MΩ in the CGC cell and 5-10 MΩ in the hippocampal cells.

17 Start recording on the separate recording computer and click the “Start” button (marker 2 in Fig. 2) in StdpC. This starts the dynamic clamp cycle and the simulated electrical component is added to the target cell until the “Stop” button (marker 3 in Fig.2) is pressed. Parameters in StdpC can be manipulated while the dynamic clamp is running but changes will only take effect at the next time when “Start” is pressed.

TROUBLESHOOTING

Data Processing

TIMING 1 h – 1 day—18 Process experimental data off line. During the experiment, all the relevant data (time, membrane potential, injected currents) are being saved according to the data saving configuration set in steps 7 and 8. Independently, all signals are recorded and saved on the recording workstation. Post-processing involves the analysis and visualization of the data using any statistical platform of the user’s preference (Origin, Matlab, Octave, Scilab, R, Python (Pylab/SciPy), etc).

TIMING

Steps 1- 4, StdpC and hardware installation: 2-3 h

Steps 5-9, StdpC Configuration: 1h

Step 10, Defining the simulated electrical component(s): 10-30 min

Steps 12, Experimental preparation: Depends strongly on the preparation, 30 min – 4 h

Step 13-17, Dynamic clamp: Depends on the experimental design and longevity of the preparation, 40 min – 12 h.

Step 18, Data analysis: Depends on the scientific problem, 1 h – 1 day

TROUBLESHOOTING

Step	Problem	Possible reason	Solution
1	unzipping "StdpC.zip" fails with an error message "zip file empty" or "zip file broken"	In rare cases the download can be interrupted, leading to a damaged archive file.	Refresh the StdpC webpage in your browser and try downloading again.
5	Starting StdpC-Nidaq fails with the error message "The program can't start because nicaiu.dll is missing from your computer … "	You either do not have a National Instruments driver software installed or this installation is broken.	(Re)install National Instruments driver software or use StdpC-Basic with a Digidata board.
6	You see the error message "Bad news: <board name> not found or not opened successfully!" in the StdpC mesage window.	There is a problem with your data acquisition board installation. If you are using a Digidata board this may be due to an I/O address conflict. If you are trying to use a National Instruments board, this error can occur due to a mismatch in the device name.	The StdpC manual explains in detail how to solve I/O address conflicts. In case of a National Instruments board, StdpC expects to use the device "Devi" by default. If your NI device has a different name, open Config->DAQ and enter the correct device name in the dialog window that appears.
7,8	The signal-to-noise ratio (SNR) on an input or output channel is too low.	Excessive noise can be caused by sources of electro-magnetic radiation in proximity to the electrodes, pre-amplifiers or amplifier, inappropriately chosen channels on the amplifier(s) or inappropriate digitalization ranges on the input/output channel configuration.	Besides eliminating classical noise sources (line noise, PC monitor noise, pump noise, lighting, etc, see e.g. *The Axon Guide*[45] or other electro-physiology manuals for detailed discussions), there are two key points of consideration to increase SNR: 1, Prioritize the electrodes and assign higher quality equipment (electrode headstage, amplifier channel, any intermediate amplifier, etc.) to more important electrodes. 2, Always make sure, that the most appropriate digitalization range is used

Step	Problem	Possible reason	Solution
			on the DAQ I/O channels both for data acquisition and current injection. Too wide ranges (eg. +−10V for recording or +−30 nA for injection) increase SNR unnecessarily, while too low ranges do not allow recording and/or injecting the required signal amplitudes. StdpC warns the user about possible signal clipping whenever the value of any voltage or current channel gets close to its upper or lower limit.
11	You navigated to a directory containing a saved configuration but cannot find it in the “open protocol” dialog.	The file is named incorrectly. Only files ending on “.cpr” will appear as possible choices in the “open protocol” dialog.	Rename the file in a Windows file browser. Alternatively, differently named files can still be opened by typing their file name explicitly.
17	Pattern clamp appears unsuccessful (the target neuron does not show the intended spiking pattern) or a ringing noise appears.	The synaptic conductance of the simulated gap junction is too low or too high. The target neuron is too large.	Adjust the gSyn parameter in the gap junction parameter dialog. Enter a small (10 nS) synaptic conductance and start the dynamic clamp only for a short time. Observe on the recording computer whether the target neuron follows the “command waveform” of the spike generator closely. If not, increase the synaptic conductance in increments (e.g. doubling it) until the match becomes better. Stop immediately and down-regulate if you observe a ringing noise. Large cells can be hard to pattern clamp and require very large current injections. If you are in doubt what the pattern waveform should look like you can display it following instructions in Step 9 and choosing to display “SG” (“**S**pike **G**enerator”).
17	There are no ouputs from the dynamic clamp system (flat line).	There are many possible reasons, the most common: **1** Conversion factors are set wrong. **2** The dynamic clamp acquisition board is not installed correctly **3** The connections from the dynamic clamp acquisition board to the amplifier are not correct **4** The current injection function on the	**1** Repeat and test the configuration of input and output channels using the instructions in Steps 7-9 **2** Test the acquisition board with the manufacturer’s tools. Make sure the message “Good news: <board name> found …” did appear in the StdpC message window. **3** Use the recording data acquisition board or an oscilloscope to monitor the output from the dynamic clamp acquisition board’s output channel that normally connects to the amplifier. Complementary to this, use a different command source (e.g. a signal generator) to try giving current commands to the amplifier.

Step	Problem	Possible reason	Solution
		amplifier is switched off.	**4** Toggle the switch for current injection for the channel of the current injection electrode.
17	There is an offset to the command voltage/ current injection.	There are two common reasons: **1** A direct current injection is added by the amplifier through settings on the amplifier. **2** There is a problem with the calibration of the output channel of the dynamic clamp acquisition board.	To decide which problem applies, visualize the output of the dynamic clamp acquisition board channel on the recording computer or an oscilloscope. If the offset is visible, perform a recalibration of the dynamic clamp acquisition board with the manufacturer's tools. Otherwise the source of the offset is the amplifier. Turn explicit current commands to 0 on the amplifier interface. Consult the amplifier's manual on how to adjust these settings.
17	The characteristics of the recorded voltage trace are (slowly) changing without any apparent reason.	There is a problem with the electrophysiological recording.	Adjust the electrodes. Possible steps to be taken may include adjusting electrode position, reinserting electrodes, changing electrodes, using different cells or even a fresh preparation. It may also be worth checking/ changing the reference electrode.

ANTICIPATED RESULTS

Simulated chemical synapse

A chemical synapse was simulated between a large (~100 μm diameter) tonically firing neuron (Cerebral Giant Cell, CGC[46]), which plays a modulatory role in the nervous system of the mollusc *Lymnaea stagnalis*, and another, silent, neuron (B1), see[46] for a detailed description of the general preparation. The CGC was impaled with a single electrode to record the "pre-synaptic" potential, while two electrodes were inserted into the "post-synaptic" neuron B1, one to record voltage changes and another one for current injection. The saline and electrode solution used in this experiment are described in the Supplementary Methods. StdpC was configured as described in Step 10A with an excitatory reversal potential (VSyn= 0 mV) and standard threshold (VThresh= −20 mV) and slope of synaptic activation (VSlope= 25 mV). Fig. 3A illustrates the resulting EPSPs (middle sub-panel) and EPSCs (bottom sub-panel) for tauSyn= 10 ms (blue and red) and tauSyn= 40 ms (cyan and magenta). For small maximal conductance (30 nS), the current injection could be replaced by a simple waveform-injection of an alpha-function. However, once voltage-dependent ion channels are activated in the post-synaptic neuron, the shape of the injected current changes markedly, almost inverting during a post-synaptic spike (arrowhead, for a more detailed view see Supplementary Figure 1).

Injecting a simulated Hodgkin-Huxley conductance

As well as simulating artificial synapses between cells, dynamic clamp can also be used to modify neuron dynamics by injecting simulated voltage-dependent ionic currents. The results shown in Fig. 3B were obtained by injecting a simulated potassium current (from[47]) and described in the supplementary equations) into a cultured hippocampal neuron[48, 49] using whole-cell patch clamp[50] (the bath and electrode solution used in this experiment are

described in the Supplementary Methods). StdpC was configured according to Step 10B. Then, depolarising current steps were introduced into the hippocampal cell to trigger action potentials. The spike shapes shown in Fig. 3B (top) were observed for an increasing series of values for the maximum conductance of the simulated current. The amplitude and duration of action potentials (Fig. 3B, top) decreased monotonically with increasing maximal conductance for the artificial potassium current. The injected currents are shown in Fig. 3B, bottom.

Pattern clamp

The CGC was impaled with two electrodes and a pattern clamp protocol was implemented according to Step 10C. The results are illustrated in Fig. 3 C-E. The pattern specified in StdpC's spike generator was an accelerating-decelerating burst which normally would not be observed in the tonically spiking CGC neuron. Fig. 3C shows two examples of attempted pattern clamp with gap junction conductance 300 nS (middle sub-panel) and 2 μS (bottom sub-panel). Due to the large size of the CGC neuron, the 300 nS conductance only achieved a partial pattern clamp: some of the intended spikes were elicited but others were not. Furthermore, the pattern clamp was unable to suppress intrinsic spiking of the CGC. In contrast, at a conductance of 2 μS, the CGC generated every spike of the target pattern faithfully and did not exhibit any spontaneous spikes. As the enlargements in Fig. 3 D and E show, the membrane potential of the CGC slightly exceeds the target potential at the top of the spikes, indicating that actual spikes were induced in the neuron. Furthermore, when comparing the current injections in D and E (blue traces, bottom sub-panels) we note that the more than 6 times larger conductance in E leads to only 2-3 times larger current injections. This is because the CGC membrane potential in E matches better with the target pattern. It is the ability to deliver sufficiently strong control signals that makes the pattern clamp successful for the larger conductance, not necessarily the overall amount of injected current.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Box 1: Chemical synapse equation and parameters

The synaptic current I is modeled by Ohm's law with a conductance that depends on the pre-synaptic membrane potential (h(t) models short term depression, which is not considered here).

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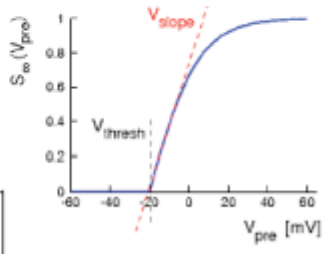
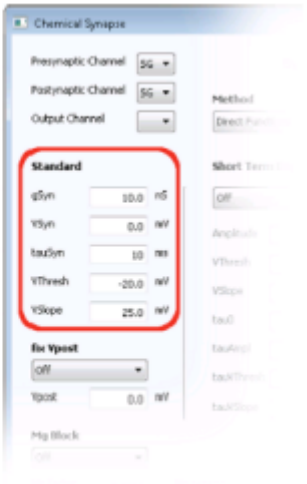
The synaptic current I is modeled by Ohm's law with a conductance that depends on the pre-synaptic membrane potential (h(t) models short term depression, which is not considered here).

$$I_{Syn} = g_{Syn} S(t) h(t) (V_{Syn} - V_{post}(t))$$

$$\frac{dS}{dt} = \frac{1}{\tau_{Syn}} \frac{S_\infty(V_{pre}) - S(t)}{1 - S_\infty(V_{pre})}$$



Parameter	Description	Typical values
gSyn	Maximal synaptic conductance	1 nS to 100 nS
VSyn	Reversal potential	0 mV (excitatory) -80 mV (inhibitory)
tauSyn	Time scale	5-100 ms
VThresh	Pre-synaptic activation threshold	-20 mV
VSlope	Speed of activation	5-50 mV



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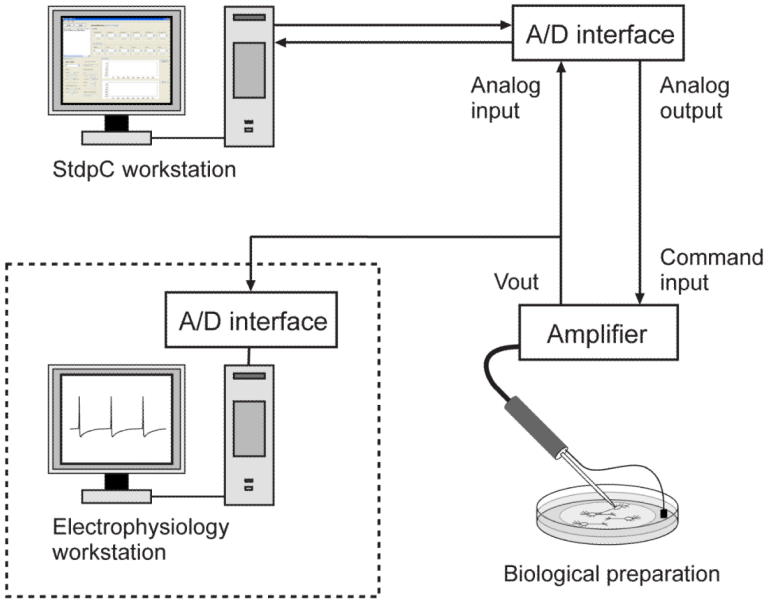

Figure 1. The general dynamic clamp setup using StdpC

The dynamic clamp system forms a closed observation-stimulus loop in which the measured and amplified membrane potential (Vout) is input to the StdpC software (StdpC workstation), which calculates a corresponding trans-membrane current according to a model specified by the user. The software then issues an appropriate current command, which is converted into a physical current injection by the amplifier. A second, independent, analog to digital converter and PC with standard electrophysiology recording software monitors and saves all aspects of the experiment (Electrophysiology workstation). The measurement-injection loop is repeated at 10-20 kHz making the interaction essentially instantaneous for the target neuron.

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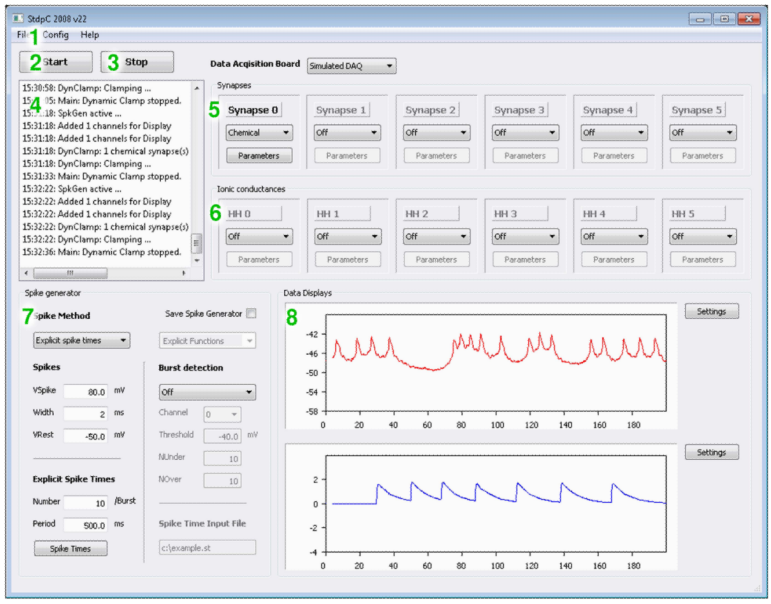

Figure 2. The main window of the StdpC graphical interface

The main control elements are marked by 8 markers:

1. General menus for file control (loading/saving protocols and scripts) and configuration.
2. Start and Stop button to control execution of the dynamic clamp
3. Dropdown choice of the used hardware driver
4. Message window displaying status information and a log of user actions
5. Panel for configuring of up to 6 different simulated synapses
6. Panel for configuring up to 6 ionic conductances
7. Spike generator module for configuring computer generated neuronal activity.
8. Data displays for debugging the dynamic clamp configuration.

Many of the configuration buttons open separate dialog windows like the one illustrated in Box 1.

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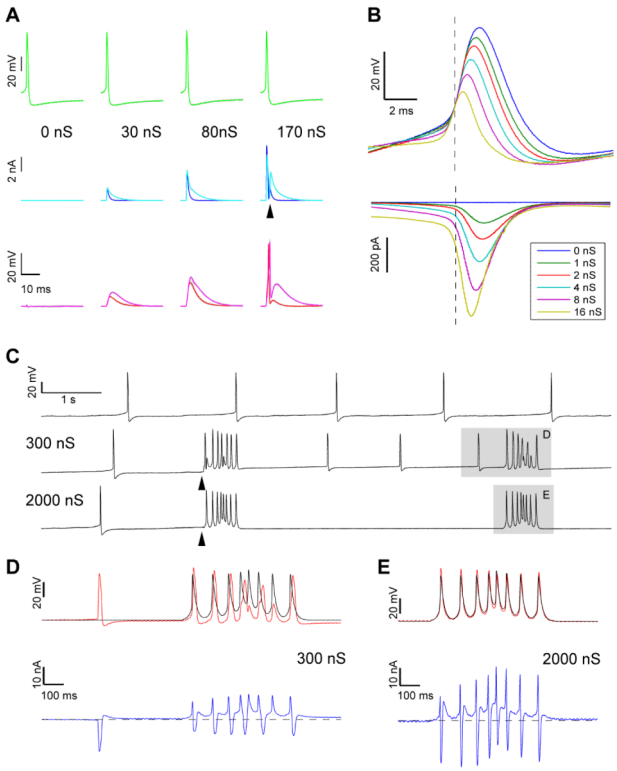

Figure 3. Anticipated results

Example results of dynamic clamp. A) Results of a simulated chemical synapse between a tonically spiking cell (top) and a silent post-synaptic cell (bottom). The middle sub-panel shows the injected dynamic clamp current for timescales of 10 ms (blue) and 40 ms (cyan). The injected current resulted in the EPSPs shown in red and magenta respectively (bottom). Note how the current through the simulated channels reverts during a post-synaptic spike (arrow). B) A simulated voltage-activated potassium current was introduced into a cultured hippocampal neuron. Action potentials were generated by applying 100 pA current steps of 50 ms duration. The effect of the simulated current can be observed as a reduction in amplitude and duration of the action potentials (top) as the conductance, and therefore the injected current (bottom), increased. The action potentials were aligned at the maximum slope of the rising phase as indicated by the dashed line. C) Results of a pattern clamp experiment. The target cell spikes tonically when the pattern clamp is off (top). When the gap junction coupling in pattern clamp is not strong enough, the target neuron will only be clamped partially (middle) but sufficient conductance leads to a complete clamp (bottom). Pattern clamp is switched on at the arrowheads. Panels D and E are detailed views of the episodes shaded in grey in panel C. The red trace was recorded from the target neuron, the black trace shows the desired pattern. Currents injected are shown in blue in the bottom panels. With 300 nS conductance, the pattern clamp is not able to prevent intrinsic spiking (C, arrowhead). This is readily achieved at 2 μS maximal conductance even though the currents injected for this > 6 times larger conductance are only 2-3 times larger owing to the better match achieved. Animal care and use protocols complied with Home Office (UK) guidelines. The compositions of the saline and internal solutions used in these experiments are described in the Supplementary Methods.

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Table 1

Commonly used dynamic clamp systems. The real time Linux based systems offer hard real time performance, i.e., guaranteed constant cycle times in the dynamic clamp cycle. The developers have reported operating frequencies in the 20-50 kHz range. The necessity to install a real time Linux operating system has been a major impediment for the use of these systems (RTLDC, MRCI, RTLab) by non-experts in the past. However, the recent development of a live CD based on the Ubuntu Linux distribution for RTXI has made this approach much more accessible. The Comedi libraries support a wide range of hardware. The systems are very flexible and designed for advanced or expert users with an emphasis on extendibility.

The hardware based systems offer hard real time performance at even more competitive rates (in the >100 kHz range) but are often costly to buy and update. They are usually less flexible and extendible.

The Windows based solutions Dynclamp 2/4 and StdpC have been developed for novice users and come with predefined models. They allow users to work within a familiar Windows environment and are ready to use out of the box. However, they are less flexible than RTXI and do not comply with hard real time constraints. Typical clamping speeds are observed in the 10-30 kHz range and this speed scales automatically with the capability of the dynamic clamp computer. The scripting function in StdpC has reintroduced some flexibility and the release of the software under the GPL allows full extendibility for the expert user.

QuB is similar in that it is not a hard real time system but it is more flexible in its model definitions while also being somewhat more demanding on the expertise of the user. Using high-end hardware the author reports cycle frequencies of up to 150 kHz. The system does not work with Windows Vista and compatibility with later versions of Windows is unclear.

The new CED dynamic clamp is similar to StdpC in its predefined models and will come as a welcome addition for current owners of CED hardware and CED “Signal” software. Preliminary reports indicate hard real time update frequencies in the >100 kHz range.

The RTBiomanager, once fully developed, will be a promising system for expert users as it is the first to also combine other stimulation modalities (mechanical, chemical) into dynamic clamp like protocols.

Name	Technology	URL	Comments
RTLDC (Real Time Linux Dynamic Controller)[36]	Based on Real Time Linux and Comedi hardware drivers	http://www.bu.edu/ndl/dynamicclamp.html	superseded by RTXI
MRCI[37]	Real Time Linux, Comedi	http://www.neuro.gatech.edu/mrci/	superseded by RTXI
RTLab[38]	Real Time Linux, Comedi	withdrawn	superseded by RTXI
RTXI (Real Time eXperiment Interface)[39,40]	Real Time Linux, Comedi	http://www.rtxi.org/	based on RTLDC and MRCI and RTlab
DynClamp2/4[31]	Windows, custom driver for DigiData 1200	http://inls.ucsd.edu/~rpinto/dynclamp.html	Partially superseded by StdpC
StdpC[7]	Windows-based, custom DigiData driver, NIDAQmx	http://sourceforge.net/projects/stdpc/	Partially based on DynClamp2/4

Name	Technology	URL	Comments
G-clamp[41]	Labview RT, NIDAQmx, embedded RT hardware subsystem	http://www.hornlab.neurobio.pitt.edu/	
SM-1, SM-2 amplifiers by Cambridge Conductance[1,42]	Custom analog hardware and DSP board.	-	
Dynamic Clamp in CED signal	Uses DSP capabilities of CED DAQs	http://www.ced.co.uk/	Offers a subset of models in StdpC; will be released fall 2010
QuB[43]	Windows based, NIDAQmx	http://www.qub.buffalo.edu/wiki/index.php/Dynamic_Clamp	
RTBiomanager[44]	Real Time Linux, Comedi	http://www.ii.uam.es/~gnb	

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