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Command-line cellular electrophysiology for conventional and real-time closed-loop experiments

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HIGHLIGHTS

- We developed a new software toolbox for performing electrophysiology experiments.
- The toolbox is composed of a suite of applications, operated by the command-line.
- The toolbox allows to perform standard as well as closed-loop real-time experiments.
- Scripts allow integrating experiments with data analysis and computational modelling.
- We present cellular electrophysiology experiments to show the features of the system.

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ABSTRACT

Background: Current software tools for electrophysiological experiments are limited in flexibility and rarely offer adequate support for advanced techniques such as dynamic clamp and hybrid experiments, which are therefore limited to laboratories with a significant expertise in neuroinformatics.
New method: We have developed LCG, a software suite based on a command-line interface (CLI) that allows performing both standard and advanced electrophysiological experiments. Stimulation protocols for classical voltage and current clamp experiments are defined by a concise and flexible meta description that allows representing complex waveforms as a piece-wise parametric decomposition of elementary sub-waveforms, abstracting the stimulation hardware. To perform complex experiments LCG provides a set of elementary building blocks that can be interconnected to yield a large variety of experimental paradigms.
Results: We present various cellular electrophysiological experiments in which LCG has been employed, ranging from the automated application of current clamp protocols for characterizing basic electrophysiological properties of neurons, to dynamic clamp, response clamp, and hybrid experiments. We finally show how the scripting capabilities behind a CLI are suited for integrating experimental trials into complex workflows, where actual experiment, online data analysis and computational modeling seamlessly integrate.
Comparison with existing methods: We compare LCG with two open source toolboxes, RTXI and *RELACS*.
Conclusions: We believe that LCG will greatly contribute to the standardization and reproducibility of both simple and complex experiments. Additionally, on the long run the increased efficiency due to a CLI will prove a great benefit for the experimental community.

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1. Introduction

A number of experimental paradigms are available for studying brain (dys)function at the cellular and microcircuit levels. One of the most successful and broadly applied is electrophysiology, which comprises intracellular experimental techniques such as whole-cell patch clamp (Hamill et al., 1981; Edwards et al., 1989; Stuart et al., 1993), and extracellular techniques such as substrate-integrated arrays of microelectrodes (Potter, 2001; Heuschkel et al., 2002), tetrodes (O'Keefe and Recce, 1993; Wilson et al., 1994; Gray et al., 1995) and silicon probes (Bragin et al., 2000). These latest techniques, in particular, allow recording the simultaneous activity of an ever increasing number of cells (Buzsáki, 2004), possibly over very long periods of time (Gal et al., 2010).

In the context of cellular electrophysiology, routine application of advanced methods such as conductance and dynamic clamp (see Robinson and Kawai, 1993; Sharp et al., 1993 and Prinz et al., 2004 for a review), the reactive clamp (Fellous and Sejnowski, 2003), the active electrode compensation (Brette et al., 2008) and the response clamp (Wallach et al., 2011) remains limited to only a few laboratories around the world with in-house computer programming proficiency and expensive hardware. Similarly, sophisticated real-time search and optimization of input stimulation parameters (Edin et al., 2004; Benda et al., 2007; Arsiero et al., 2007) and automated application of cell characterization protocols (Wang et al., 2002; Druckmann et al., 2007) have been explored only by laboratories with specific interests in neuroinformatics.

In order to provide the community with simple and inexpensive access to high quality implementations of the above techniques, we developed a software toolbox, called LCG, whose main features are the following:

- simple implementation of non-real-time protocols, i.e., voltage and current clamp as well as extracellular recordings;
- compact description of stimulation waveforms;
- command-line operability;
- ease of automation by scripting;
- seamless integration with on-the-fly data analysis;
- dynamic clamp capabilities, with built-in active electrode compensation;
- flexible design and implementation of closed-loop and hybrid experiments.

Our effort falls in the same category as recently proposed general purpose tools for performing closed-loop real-time experiments (Bettencourt et al., 2008; Lin et al., 2010; Zrenner et al., 2010), but with an important difference: LCG is operated by a command-line interface (CLI), which, despite a steep initial learning curve, offers several advantages over a graphical user interface (GUI), namely operation speed, scripting capabilities and interface stability across software versions.

One of the main reasons behind the adoption of a CLI is that, observing patterns of operation of commercial or custom-built software environments during experimental sessions in our laboratory, we found that average users waste a significant amount of time in repeatedly interacting with the software GUIs. This is particularly severe in invasive cellular electrophysiology, such as patch-clamp recordings, where the stability of an experiment can quickly change and progressively deteriorate, so that time saved ultimately means better data and higher throughput. We thus wondered whether a CLI could be more effective to control and perform standard experiments and developed LCG to validate our hypothesis.

One of the key advantages of CLIs over GUIs is the ease of automation of standardized protocols, such as those required to characterize a cell in terms of its electrophysiological response code, or e-code (Wang et al., 2002, 2004), which comprises properties such as input resistance, membrane time constant, action potential threshold and duration and so forth. Indeed, describing and repeating precise sequences of commands is a trivial task with LCG. This feature is very important, as stimulation protocols often need to be repeated several times at precise intervals, in order to compute, for instance, the average response to a particular stimulus, or to study activity-dependent plasticity and refractoriness.

Most importantly, LCG also supports natively real-time experiments, which allow, for instance, constantly monitoring the membrane potential of the recorded cell(s) and injecting a current that is a function of the voltage and of a given conductance waveform. This is the concept behind conductance and dynamic clamp (Robinson and Kawai, 1993; Sharp et al., 1993), which has allowed studying, among other things, the impact of channel noise (White et al., 1998) and of synthetic ion channels (see Vervaeke et al., 2006 for an example) on neuronal excitability and the recreation of *in vivo*-like synaptic background activity *in vitro* (Destexhe et al., 2001).

More in general, recent works have focused on developing closed-loop experimental paradigms, which involve monitoring a certain quantity during an experiment and reacting in an appropriate time. Dynamic clamp is a particular kind of closed-loop paradigm, but many others have been proposed. For instance, (Fellous and Sejnowski, 2003) have suggested how to recreate recurrent persistent activity *in vitro* by using a so-called reactive clamp. More recently, (Gal et al., 2010; Wallach et al., 2011) introduced the response clamp paradigm, generalizing the concept of voltage clamp introduced by (Hodgkin and Huxley, 1952) to any measurable experimental quantity, and showing its relevance for neuronal response variability. We show how to employ this paradigm for clamping the firing frequency of a neuron in Sec 2.3, both to constant (Section 2.3.1) and time-varying values (Section 2.3.2), while computing the corresponding instantaneous current clamp command.

Finally, scripting offers the possibility of easily embedding sequences of stimulation–response trials into highly versatile and complex workflows that seamlessly integrate experimental recordings, data analysis and computational modeling. As an example, in Section 2.5 we show how to optimize the strength of a synaptic connection in a hybrid microcircuit.

2. Results

LCG is a suite of command-line programs, called *commands*, running on UNIX-based operating systems and designed to easily perform electrophysiological experiments. The entry point of all LCG commands is the program called `lcg`: its purpose is merely to parse its arguments and call the appropriate command with the arguments with which it was invoked.

LCG supports two main operating modes: the first is suited for "standard" voltage and current clamp experiments, performed in *open loop*, i.e., when it is not required to monitor in real time any of the recorded quantities. For this type of experiments, described in Section 2.1, LCG does not require a real-time operating system, but simply a data acquisition card supported by COMEDI, the open source library for data acquisition used by LCG.[1]

The second working mode is suited for real-time experiments that require a form of closed-loop operation: this mode spans a wide variety of paradigms, ranging from dynamic and conductance clamp experiments, in which the injected current depends

[1] The choice of COMEDI has been dictated by the large number of data acquisition cards that it supports (at the time of this writing, over 400 cards by 25 vendors, but see http://www.comedi.org for an up-to-date list).