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Biophysical essentials – A full stack open-source software framework for conserved and advanced analysis of patch-clamp recordings

David Zimmermann [a,*], Michaela Kress [a], Maximilian Zeidler [a,b]

[a] Institute of Physiology, Medical University of Innsbruck, Innsbruck, Austria
[b] Omiqa Bioinformatics GmbH, Berlin, Germany

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ABSTRACT

Background and Objectives: Patch-Clamp recordings allow for in depth electrophysiological characterization of single cells, their general biophysical properties as well as characteristics of voltage- and ligand-gated ionic currents. Different acquisition modes, such as whole-cell patch-clamp recordings in the current or voltage clamp configuration, capacitance measurements or single channel recordings from cultured cells as well as acute brain slices are routinely performed for these purposes. Nevertheless, multipurpose transparent and adaptable software tools to perform reproducible state-of-the-art analysis of multiple experiment types and to manage larger sets of experimental data are currently unavailable.

Methods: Biophysical Essentials (BPE) was developed as an open-source full stack python software for transparent and reproducible analysis of electrophysiological recordings. For validation, BPE results were compared with manually analyzed single-cell patch-clamp data acquired from a human in vitro nociceptor-model and mouse dorsal root ganglia neurons.

Results: While initially designed to improve time consuming and repetitive analysis steps, BPE was further optimized as a technical software solution for entire workflow processing including data acquisition, data preprocessing, normalization and visualization and of single recordings up to stacked calculations and statistics of multiple experiments. BPE can operate with different file formats from different amplifier systems and producers. An in-process database logs all analysis steps reproducible review and serves as a central storage point for recordings. Statistical testing as well as advanced analysis functions like Boltzmann-fitting and dimensional reduction methods further support the researchers' needs in projects involving electrophysiology techniques.

Conclusions: BPE extends beyond available patch-clamp specific, open source – and commercial analysis tools in particular because of reproducible and sharable analysis workflows. BPE enables full analysis from raw data acquisition to publication ready result visualizations – all within one single program. Thereby, BPE significantly enhances transparency in the analytical process of patch-clamp data analysis. BPEs function scope is completely accessible through an easy-to-use graphical user interface eliminating the need for programing language proficiency as required by many community patch-clamp analysis frameworks and algorithms.

1. Introduction

Since the first amplifiers were developed [25] the single electrode patch-clamp technique has developed into a widely used routine method in neuroscience and electrophysiology [11]. Starting from the characterization and pharmacology of nicotinic acetylcholine receptor, pharmacological interventions targeting specific ion channels including voltage gated Na^+, K^+ or Ca^{2+} channels, NMDA and AMPA glutamate receptors, GABA and glycine activated ion channels, and ryanodine-, inositol1,4,5-trisphosphate (IP3) or transient receptor potential (TRP) channel family members have been systematically examined with conventional patch-clamp recordings [16,32]. The classical patch technique offers broad experimental flexibility and therefore represents the gold standard in basic research today. In order to speed up the pace in drug discovery and development, high throughput automated patch-clamp (APC) approaches are emerging, such as Flyscreen [20], AutoPatch and RobotPatch [38] or Synchropatch (Nanion Technologies GmbH) which allow to record from multiple cells at the same time for example by using a planar recording substrate instead of a classical patch electrode [5]. Such high throughput experiments reveal valuable insights

* Corresponding author.
E-mail address: david.zimmermann@i-med.ac.at (D. Zimmermann).

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into biological variability in well-defined experimental flows [34].

Both, classical and APC technology, require detailed analyses of current and voltage traces to provide large amounts of high precision biophysical data. The analyses of these data requires significant expertise in signal processing, curve fitting and statistics which issupported by various existing commercial and open-source software tools that are optximised and tuned for specific amplifier systems and data formats such as Patchmaster and Fitmaster (current version PatchmasterNext [12] pClamp (current version is pClamp11 with Clampex, Clampfit and Clampfit Advanced) [8] IgorPro ([15]), OpenEphys [4], Matlab [3], Neo [26], Elephant [6] and multiple more [7]

In an initial software requirement analysis (Section 2), we first compared the feature stack of different state-of-the-art software solutions within the context of representative neuroscience patch-clamp scenarios. We found that none of the existing software solutions addressed all of the identified requirements indicating that there is an unmet need for good-practice, transparent, universally applicable and reproducible data analysis of patch-clamp recordings' data flows. To tackle this issue, we developed the Biophysical Essentials (BPE) full stack python [28] software with an additional web interface to store, share and compare research data and findings. BPE allows for fast and conserved patch-clamp data analysis for multiple recordings simultaneously. The BPE framework eliminates time consuming, error prone and repetitive analysis steps and is contributed open-source and online to the entire scientific community in a format that can be modified to meet individual research questions and experimental needs.

2. Software requirement analysis

We investigated relevant criteria derived from two typical use cases in neuroscience and patch-clamp research. Scenario (A) resembled the characterization of electrophysiological excitability of cell populations e.g., with or without a specific gene knockout or mutation. Scenario (B) described research studying alterations in electrophysiological properties of a cell before and after the application of specific substance. Both scenarios would incorporate recordings of a cell's response to various stimulation protocols, e.g. I-V relationship in the voltage-clamp configuration, rheobase determination, action potential (AP) fitting or a firing pattern analysis in the current clamp mode. This exemplary selection of stimulation protocols illustrated specific analysis needs with respect to a step or ramp protocol, normalization procedures dependent on the operation modes like voltage- or current clamp configuration, and fitting functions. These needs, summarized as patch-clamp specific analysis functions criterion (1), were fully met by the three most referenced patch-clamp analysis tools Patch/Fitmaster, pCLAMP/ClampFit and IgorPro. Despite the broad heterogeneous analysis functions offered by these software tools, only Fitmaster partially satisfied criterion (2), extendable for new analysis functions since user defined functions and fitting equations can be entered individually. In contrast, open source packages like Neo or the commercial programming and computing platform Matlab provided high degree of freedom options to append and integrate individual analysis functions into the data analysis process with existing/provided functions, however, these also require proficiency in programming as a prerequisite. Criterion (3) the transparency and reproducibility of analysis functions is satisfied by Patch/Fitmaster, pMaster/ClampFit and IgorPro for single files but not an entire file set with multiple recordings.

Experiments as described in scenarios (A) and (B) would require a large number of repetitions. Therefore, the analysis of multiple cells with conserved parametrizations resembles a fundamental need in patch-clamp analysis which was summarized as criterion (4), *analysis of multiple cells in parallel*. Criterion (4) was found partially available for a channel-specific online analysis in Patch/Fitmaster. The result presentation and the calculation of potential mean traces requires appropriate handling of the meta data of the cells. This need is resembled by criterion (5), *analysis and result visualization according to cell-specific meta data*. None of the tools Patch/Fitmaster, pMaster/ClampFit or IgorPro satisfied criteria (4) and (5). Since none of the state-of-the-art software tools satisfied all 5 criteria, we aimed to develop a new software addressing these criteria to better meet the needs of many electrophysiologists. To release electrophysiological research from technical limitations and to improve reproducibility and transparency, we added criterion (6), *cross-platform functionality* including Linux, Windows, MacOS and criterion (7), *interoperability between different file formats*, such as .dat and .abf files and open file formats such as neurodata without borders [35] Patch/-Fitmaster, pMaster/ClampFit or IgorPro are available for Windows and MacOs and Patch/Fitmaster allow data export into an IgorPro supported file format. Igor Pro itself supports multiple file formats including HDF and HDF5, Matlab files and multiple more (Supplementary Table 1).

3. Methods

3.1. BPE technical solutions and hardware requirements

The source code of BPE was implemented in Python (current version 3.11) and uses PySide6 (version 6.5.1.1) as the official Python module from the QT for Python project, which provided complete access to the QT 6.0+ framework. Button images were selected from ICONS8 [14]. Graphics were generated using the python packages *Matplotlib, PyQt-Graph*, and *Seaborn*. Process and error logging is handled by *picologging*. *Pandas* and *Numpy* were selected for fast in-built data processing. The background database uses *duckdb*. Automated tests are executed using *pytest* in respective github actions upon each main-branch pull request. All software packages were selected to enable BPE execution on Linux (Ubuntu, 23.04), MacOS (macOS 13) and Windows (Windows 10/11) as well as on x86 and ARM architecture. Generally, the minimum hardware requirements include 4 GB RAM and a 2.5 GHz CPU. Memory usage depends on the amount and the size of data stored in the local database. The import of data from the recording files into the database as well as the application of analysis functions support multithreading to speed up the most time-consuming procedures. Current package versions were provided within the respective .toml file in the github repository https://github.com/ZiDa20/Biophysical_Essentials.

3.2. Amplifier communication and experimental setup

For BPE communication with the HEKA amplifier (EPC 10), a valid version of HEKAs control software Patchmaster (tested with Patchmaster v2×90) is required via the batch communication (BCOM) feature. BCOM allows Patchmaster to read and execute control commands from an "in-out file" that we manipulate with BPE by updating the command id and the command to be executed. The entire list of available batch communication commands can be found in HEKAs documentation.

3.3. Data import and local database management system

Either live recorded data or previously acquired recordings can be imported into BPE framework from HEKA amplifiers (.dat file format) and amplifiers from Molecular Devices (*.abf file format). To read *.dat files we used a modified version of the publicly available heka_reader package with pgf file reading support (github campagnola/heka reader) adapted from the official matlab heka dat reader. To enable *.abf support, the pyabf-package was used [29]. Files are imported into an in-process SQL online analytical processing (OLAP) database management system based on DuckDB [30] because of its high-performance design to process large amounts of data. BPEs offline analysis module operates on the permanent and locally stored database while during online analysis, files are imported into an in-memory database. As one measure to keep the permanent database at a minimum dataspace level and to avoid sources of inconsistency it is not accessible after the restart of the software. Recordings viewed in the online analysis module can be

transferred into the permanent local database. Further additions in terms of metadata and image data can be added to the database.

3.4. BPE's signal processing procedures

BPE includes basic analysis functions like maximum, minimum- or mean determination which are performed on the raw signals without pre-processing except of signal interval selection by user defined cursor bounds. For action potential fitting, the first derivative is calculated using NumPy, smoothed by a moving averaging filter with a window length of 20 data points (tested in signals sampled with a default frequency of 20 kHz). Within the smoothened first derivative, the time-point where the depolarization speed continuously exceeds a user-defined speed (per default 10 mV/ms) is considered as the action

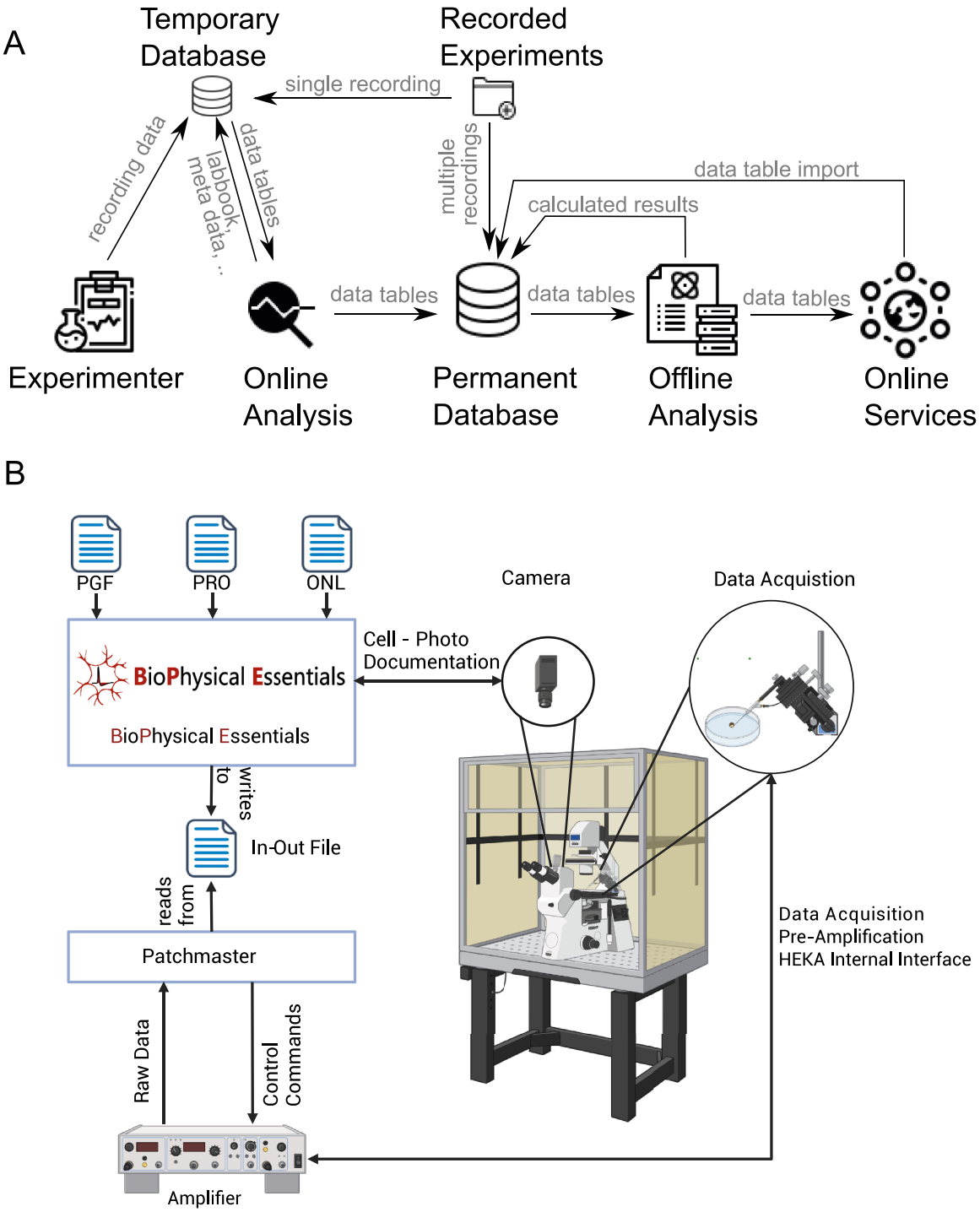

Fig. 1. Modular Composition of BPE and data flow. A) Five different modules allow BPE to provide a full stack analysis pipeline represented by independently functioning modules experimenter, online analysis, offline analysis, database and online analysis. As soon as recording data have been imported to the permanent database, all downstream analysis will be performed on the data tables. The database scheme including existing tables and detailed parameters are visualized in Fig. 2. B) The experimenter module requires the input of a pulse generator file (.pgf), a protocol file (.pro) and an online analysis (.onl) file generated by Patchmaster. To establish a batch communication, Patchmaster needs be set as a receiver and will therefore search for a specific in-out-file in a determined directory. BPE writes commands to this in-out-file according to HEKA's provided documentation of the batch communication interface. Created with BioRender.com.

potential threshold. The first derivative is further considered for determining the maximum depolarization and repolarization speed. Afterhyperpolarization is calculated by identifying the minimum of the smoothened first derivative and locating the subsequent point where the derivative becomes positive. The AP-half-width is calculated by determining the time points where the signal crosses half of its maximum amplitude based on the previously determined threshold voltage. Firing pattern analysis as well as peak detection and rheobase detection functions incorporate the "find_peaks" function from the scipy.signals package. Curve fitting from the scipy.optimize package was used to perform second order Hill function and Boltzmann fittings. For principal component analysis (PCA), CPA and StandardScaler from the sklearn. preprocessing and sklearn.decomposition package were included.

3.5. Statistics

The statistics module compromises *t*-test, Welchs-test, Mann-Whitney-U test, paired-*t*-test, Wilcoxon-signed-rank-test, Kruskal-Wallis test and ANOVA. Therefore, result data distribution is automatically tested using Shapiro-Wilk test, data variance is tested using Levene-test. The appropriate statistical test will be suggested according to the number of specified meta data groups, data distribution, variance and data dependency. All the tests were performed using *scipy stats* package.

3.6. Patch-Clamp recordings

DRG neurons were harvested as described [18], plated and incubated in culture medium at 37 °C and 5 % CO_2 for 24 h. Neurons derived from induced pluripotent stem cells were differentiated as described in [40]. Whole cell patch-clamp recordings were performed using a HEKA EPC 10 amplifier. The cells were kept in extracellular solution (ECS) containing NaCl (145 mM), HEPES (10 mM), d-Glucose (10 mM), KCl (5 mM), $CaCl_2$ (2 mM) and $MgCl_2$ (1 mM) at a pH of 7.3 adjusted with NaOH. Intracellular solution (ICS) was used to fill borosilicate glass pipettes pulled with a horizontal puller (P-1000, Sutter Instrument Company), with a pipette resistance between 3–5 MΩ. The ICS contained potassium d-gluconate (98 mM), EGTA (5 mM), HEPES (10 mM), KCL (45 mM), $MgCl_2$ (2 mM), Na_2GTP (0.2 mM), MgATP (2 mM) and $CaCl_2$ (0.5 mM), with a pH adjusted to 7.3 using KOH.

4. Results

Biophysical Essentials compromises five modules: an experimenter module, an online analysis module, an offline analysis module, a database viewer module and a BPE online service module. These modules function independently from each other but can be also concatenated to an analysis pipeline depending on individual demands as illustrated in Fig. 1A to satisfy introduced criteria 1–7 (Supplementary Table S1) to provide an optimal data analysis workflow in electrophysiology research

4.1. Experimenter module

BPE can be integrated into the patch-clamp experiment workflow from the very beginning of the data collection, starting at raw data acquisition. Currently, BPE supports communication with the HEKA EPC 10 amplifier and requires a running Patchmaster software with enabled batch communication (details are described in the methods section). The experimenter module (EM) was designed to select and execute electrical stimulation protocols, establish the hardware connection with the amplifier and an optional camera device (Fig. 1B). By integrating live cell microscopy images with the biomedical patch-clamp signals BPE provides additional relevant information about the state, morphology, size and environment of the cell contributing to experiment reproducibility. EM offers to add recording-relevant additional information, such as the composition of intra-, extra- and optional stimulation solution. For manual parametrization of amplifier input, such as adjusting the injected current or voltage within a protocol, execution of protocols via Patchmaster remains crucial. During raw data acquisition, EM operates in parallel to the existing hardware and software pipeline from HEKA, by fetching the recorded data from the Patchmaster notebook via the opened batch connection. If the EM executing computer is connected with a camera attached to the microscope, photo documentation of the patched cells can be activated. Once the execution of a stimulation protocol is finished, the recorded data, as well as the applied protocol, are displayed in BPE's online analysis module. From here, the recorded data can be imported into the BPE local database while the original recording file remains unaltered. All provided metadata information of the experiment are added to the database too. This allows for in-depth metadata analysis, batch correction as well as normalization methods in later analysis steps as we will show in the offline analysis module.

4.2. Online analysis module

The online analysis (OA) module is designed to serve as a viewer module of individual recording traces, provides a digital lab book to enter additional experiment information and allows to transfer single traces into the BPEs database (described in the next section). In BPE, recordings are generally interpreted as a structure with three levels: experiment, series, and sweeps. Experiments refer to a single recorded cell, series represent a specific stimulation/recording protocol that was performed on this cell, and sweeps represent individual voltage or current steps of the individual stimulation protocols. Within the graphical user interface (GUI), stimulation and recording data are displayed simultaneously which allows a good overview and evaluation of the cell specific response to changes in current, voltage or other stimulation types. The digital lab book allows for manipulation of existing metadata or entering new data and comments in a table- like structure. If combined with the EM, intra- and extracellular solutions, pipette resistance, performed compensation, and additional required parameters or comments are transferred from the EM into the lab book automatically. Also, recorded videos and images of the cell throughout the experiment are appended to the lab book. Recordings, their annotated meta data and the digital lab book can be transferred from the OA module into BPE's local database to become available for further data analysis.

4.3. Database module

BPE stores all data and analysis steps and details within an online analytical processing (OLAP) database (DB) that is only stored locally and always operates in the background as soon as BPE is started. To achieve data format interoperability (criterion 7), individual manufacturer specific data import functions and the database structure were designed accordingly. Once the data are imported into the database, the downstream analysis is intended to process data independent of data format – as far as required parameters are genuinely provided within the original data. The main structure of the database distinguishes between raw data and analysis data, as indicated in the entity-relationship diagram in Fig. 2. To keep the database as small as possible, all experiments are constrained to be unique in the database by the experiment name and a series identifier. Via mapping tables, an experiment can be assigned to multiple different analyses and each analysis is identified by a unique identifier. The entire offline analysis process, which will be described in the next section, is stored in the database and can be reconstructed at any later time point from the database entries. The database content is represented within a dashboard as shown in Supplementary Figure S3. Performed analysis within BPE can be exported from one computer and imported onto another computer via implemented import and export functions.

4.4. Offline analysis module

The core of BPE is its offline analysis (OFA) module, which enables

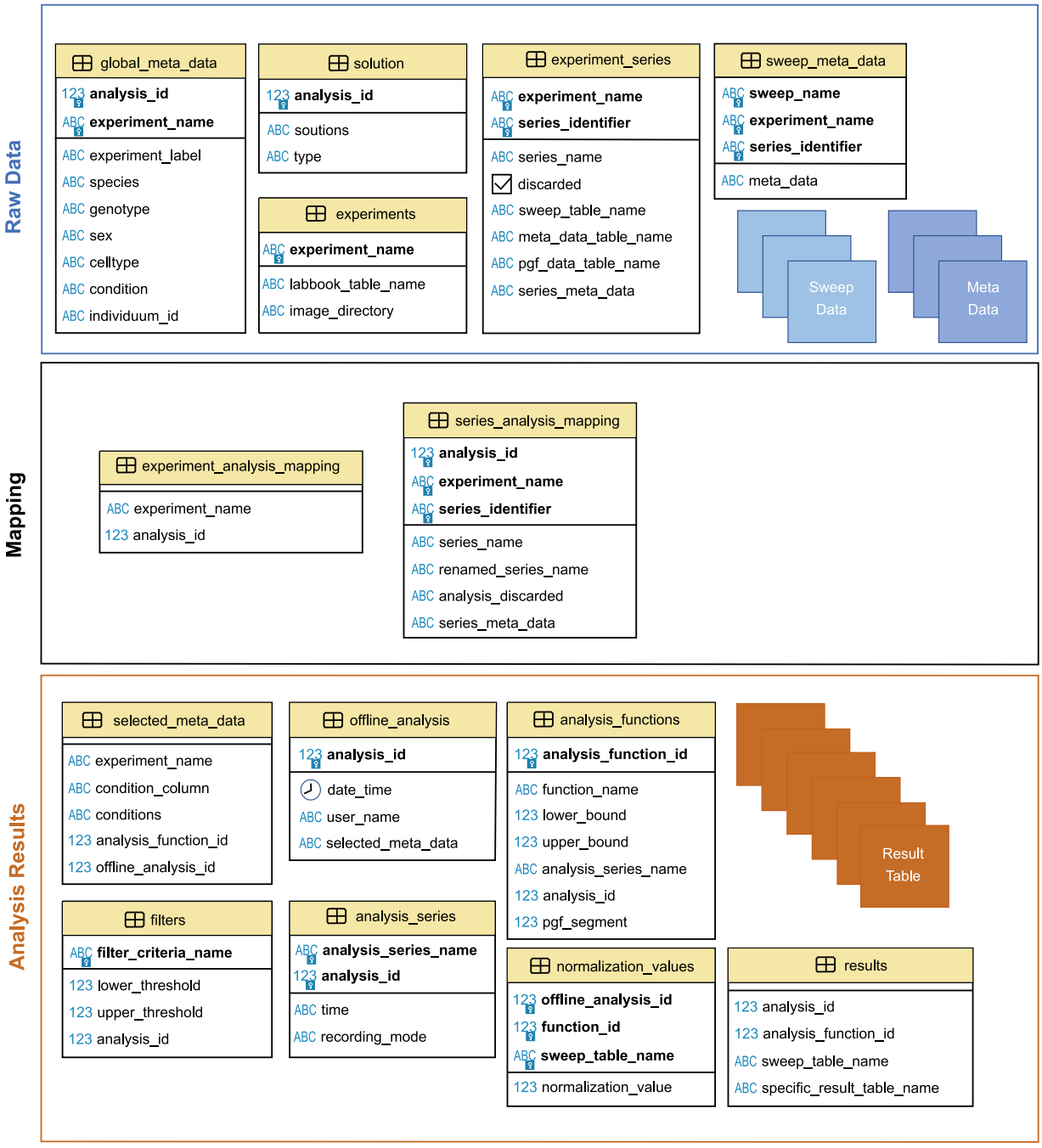

Fig. 2. Entity Relationship Diagram of the local database structure: BPE organizes its internal data structure within a local database based on the DuckDB architecture. Raw data must be imported once into the DB, the recording file itself will be never modified. All further processes are performed on the database data only. To keep the size of the database small, raw recording data are unique within the DB while they can be mapped to multiple analysis via the introduced mapping tables.

the analysis of multiple patch-clamp recordings in parallel (criterion 4) and according to annotated meta data (criterion 5). The analysis process is composed of three steps, which compromise (1) (manual) data control, (2) analysis setup and (3) result visualization. Each analysis is given a unique identifier and once performed, analysis can be reopened from the database quickly by collecting all data associated with this id (criterion 3). To start a new analysis, new recordings can be imported from the original recording files into the database as well as already imported recordings can be selected from the DB via the DB dashboard (Supplementary Figure S1). The analysis was designed to cover comparisons between different cells or within the same cells. According to given meta-data, experiments and recordings can be selected for analysis from the data dashboard (Figure S2) which shows all the collected data from the database. For SQL-experienced users, advanced data selection options are available.

The first panel of the OFA shows the structural composition of each single cell and performed protocols within in a hierarchical tree view as shown Figure S3. The structural view issupported by a graphical visualization for cell specific stimulation protocols and the electrical responses. This aggregated and comprehensive data assembly and graphical visualization allows an easy and manual control of signal traces and electrical properties. Experiments and series that do not fulfill quality criteria, such as low leakage or noise levels, can be manually removed from the analysis. Graphical visualization and individual data selection allow OFA to be applied in different biological context, such as peripheral neurons, pancreas cells or myocardial cells. The manual

filtering process can be extended and mimicked by automatic filter options, which allow thresholding of relevant parameters, such as the capacitance of the cell (as a measure of the cell size) or serial resistance (as a measure of the series resistance between the pipette and the cell) throughout the experiment. Experiments can be further separated according to annotated metadata, such as genotype, sex, or condition. This first panel of the OFA includes manual data screening and filtering of the input data to gain a maximum of control over performed patch-clamp-recordings and the downstream analysis.

In the second panel, patch-clamp specific analysis functions (criterion 1) can be selected and parametrized related to the respective protocol, e.g. analysis for all IV recordings or analysis for all rheobase recordings respectively. Therefore, OFA provides various default and protocol-specific 1st level analysis functions listed in Table 1. Multiple independent functions can be applied to one specific protocol. Also, the setup of multi-interval analysis functions, such as subtracting the maximum value from interval *x* and the mean value from an interval *y* are supported by the GUI. The respective intervals can be selected by movable cursor bounds. For voltage-clamp protocols, normalization of the recorded currents to the cell membrane size (CSLOW-parameter) [17] or a manual normalization parameter are supported. Both methods are implemented in OFA and can be selected from the front end. The python-code provides an abstract class template which can be inherited to create new individual analysis functions (criterion 2) and provides easy access to entering specific calculations and specific result-plot visualizations. This interface was developed to ease the contribution of new analysis functions and to reduce the amount of interference with the remaining code of the complex framework.

Once a 1st order analysis was set up respective to the specific protocol and data quality, existing meta data groups, normalization method and analysis functions, the results will be determined, stored in the DB and result visualizations will be displayed immediately. Graphics are displayed as interactive objects where e.g. outliers can be selected by mouse click which empowers users to delve into potential underlying factors contributing to the identified outliers for each recording individually. In addition to the graphical representation, all results are accessible and extractable in numerical format. Various 2nd order functions (Table 1) are available and can be selected for further analyses like dimensional reduction, cell clustering or IV curve fitting. For quantitative assessment of differences between experimental groups, a statistics module was implemented and provides statistical tests to be applied to the generated results. The number of the comparison groups, data distribution (via Wilcoxon-Rank-Sum test) and variance (via Levene-test) are accessed automatically by OFA and suggest appropriate statistical tests. However, tests can be also selected manually.

The full documentation of BPEs workflows with additional videos and further descriptions is available at https://biophysical-essentials.i-med.ac.at/.

4.5. Validation

To ensure correctness of the implemented analysis functions and workflows in BPE, all 1st order analysis functions listed in table 1 were carried out on a dataset of ten cells using Patchmaster v2×90.5 “manually”. Numerical values were compared individually for each recording and analysis function as summarized in Supplementary Table 3. To ensure that BPEs meta data handling and meta data specific workflows result in the same values as manual meta data specific analysis, the recordings were separated into two arbitrary groups of five cells. The mean values of the respective groups were compared for each analysis function as described before. Eventually, to validate proper function of the normalization algorithms, additional analysis with and without normalization to the C-slow parameter was carried out. As demonstrated in Supplementary Table 3, manual as well as BPE performed calculations resulted in the same numerical values for not normalized, normalized and meta data grouped results. To ensure that further implementations and adoptions to BPE will not interfere with the validated result calculations, these validations will be performed prior to every update to the main code.

4.6. Classification and characterization of neurons using bpe

Translating knowledge and research findings from animal into human model systems represents a major task in neuroscience and medical research. Nociceptors derived from induced pluripotent stem cells (iNocs) are emerging as promising preclinical model to study human nociception [33,40]. Since BPEs analysis functions are not restricted to specific biological context, it can be used to process patch-clamp recordings from both, iNocs as well as murine sensory neurons obtained from dorsal root ganglia (DRG). As a first experiment to document BPE’s versatile functionalities, DRG neurons’ and iNocs’ specific electrical characteristics were accessed by conducting patch-clamp recordings and action potential (AP) properties were compared (Fig. 3A-D). Following the workflow illustrated in Fig. 1A, raw data were imported from the recording file into the DB of BPE with the respective meta data annotation (iNoc or DRG), a new offline analysis was started and action potential fitting, peak detection analysis and PCA were performed for five action potentials ($n = 9$, Fig. 3A). Characteristic AP mean traces for iNocs and DRG neurons were determined and aligned along the AP-maximum to qualitatively compare the mean AP signal shapes. In Fig. 3B, representative mean traces are shown within a 10 ms window before and after the AP maximum. For quantitative comparison, AP-fitting was performed for 18 parameters of interest and for each recorded cell individually, followed by mean value determination (Fig. 3C, Supplementary Table 2). Based on the 18 AP-fitting parameters, principal component analysis (PCA) was performed and revealed a dimensional separation between iNocs and DRG neurons (Fig. 3D). The BPEs statistics module was used to identify statistical differences between the AP characteristics of the two cell

Table 1
Summary of implemented analysis functions and brief description.

	Analysis Function	Description
1st order	Max, Min, Mean Amplitude	Within a given interval, determination of maximum- or minimum amplitude, or calculation of the mean amplitude within the interval
	Time to Max, Min	Time to the maximum or minimum of a signal
	Action Potential Fitting	Determination and calculation of action potential characteristics, e.g. threshold, maximum, half width and 15 other parameters (Supplementary Table 2)
	Rheobase Detection	Detection of the minimum current required to initiate an action potential in 3 consecutive pulses
	Peak Detection	Detection of peaks and alignment of the signals at the peak position
	Input Resistance	Linear regression to determine the input resistance of a cell
	Rheoramp-Detection	Determination of action potentials per sweep, results are either presented as individual parameters or z-scored in a parameter heatmap
	Firing Pattern Analysis	Analysis of the spike pattern considering AP count, frequency and bursting, tonic or phasic-properties
2nd order	PCA	Linear dimensional reduction method: Principle Component Analysis
	IV-curve Fitting	Boltzmann fit
	Hill-Function-Fitting	Determination of ligand-receptor binding relationship

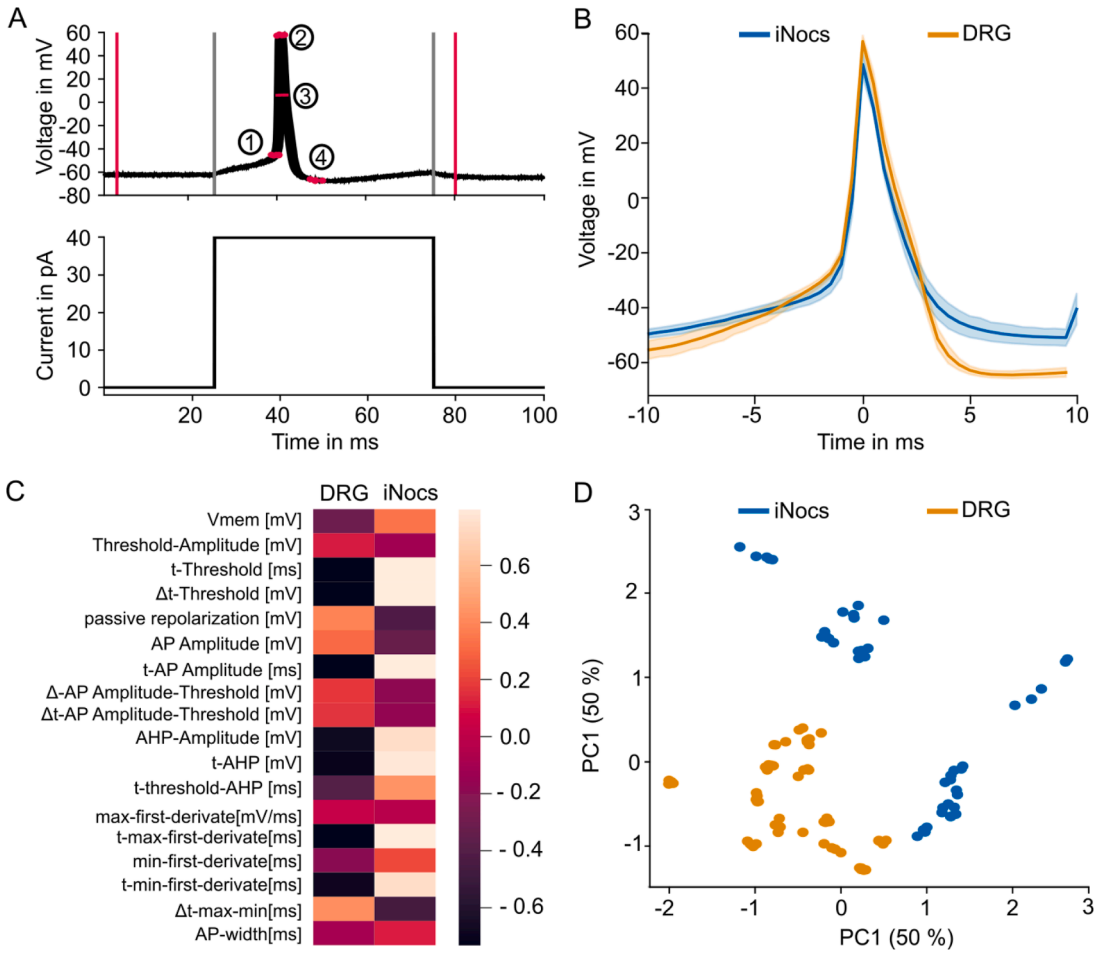

Fig. 3. Comparison of Action potential characteristics of mouse DRG neurons and human iNocs. (A) Representative stimulation protocol and the recorded action potential of the cell. The protocol was repeated 5 times. The red vertical lines indicate the analysis interval, the light grey horizontal lines separate the 3 intervals defined in the pulse generation file. Markers 1–4 illustrate the result of the enabled live analysis feature. For action potential fitting, V_{thresh} (1), V_{max} (2), $t_{half\ width}$ (3) and V_{AHP} (4) are displayed as subsitutes. (B) Comparison of the mean trajectory between 9 iNocs and 9 DRG neurons. Detected action potentials were aligned by their maximum peak V_{max} .(C) Action potential fitting was performed for 18 parameters and the mean for each cell type was scaled and visualized. Mean data and standard deviation are summarized in supplementary Table 2. (D) PCA was applied to the AP fitting parameters of each single AP and principal components 1 and 2 were plotted accordingly.

populations. Independent *t*-test analysis between AP parameters of DRG neurons and iNocs revealed significant differences between most AP parameters. Detailed mean values, standard deviation and statistical significance are summarized in Supplementary Table 2.

5. Discussion

Patch-clamp electrophysiology plays a pivotal role in drug discovery and biomedical research [2]. Developments in automated patch-clamp technique have proven suitability and advantages of high throughput approaches, however, not all conventional patch-clamp experiments can be transferred to automated devices [27]. Regardless whether acquired manually or with an automated device, single cell patch-clamp data require in detail data analysis and evaluation. Current workflows in single-cell patch-clamp data analysis incorporate state-of the software like PatchMaster, ClampFit or IgorPro but require additional programs to cover a full analysis workflow including data acquisition, data processing, result visualization and statistical testing. Results generated by copying outputs between different programs require input specific manual formatting or pre-processing, are difficult to reproduce and workflows are therefore not transparent [23]. Moreover, such analysis is susceptible to errors due to the lack of standardized evaluation procedures causing inefficiencies as the experiment sizes increase.

In this work, we have introduced BPE, a new full stack Python software tool for patch-clamp experiment data analysis which provides all relevant patch-clamp analysis functionalities within one single program. This single-program-analysis-approach introduces new levels of efficacy, reproducibility, standardization and transparency to electrophysiology research and data analysis workflows in patch clamp analysis. Furthermore, BPE compensates for lacking features identified in state-of-the-art patch-clamp analysis software (Supplementary Table 1) and introduces novel features relevant for good-practice patch-clamp data analysis, trustworthy results and removal of technical limitations. These include the ability to incorporate new and individual analysis functions, conduct analysis of multiple recordings in parallel or perform analysis while considering meta data (full feature list in Supplementary Table 1). In BPE, basic analysis functions from signal processing are accompanied by patch-clamp specific analysis functions like action potential fitting, firing pattern analysis or Boltzmann- and Hill-fittings (Table 1) regularly found in experiments assessing neuron excitability [22,39]. Compared to similar available patch clamp specific, open source analysis tools such as the python tool PatchView [13] or the C++ based software StimFit [10] BPE stands out due to reproducible analysis workflows. User-operations are logged in an OLAP-background database [30] and allowing to review the entire analysis workflow of performed analysis on a single mouse-click. This analysis-reopen feature

provides genuinely new value in terms of workflow transparency and reproducibility in electrophysiology. Moreover, as recorded data from different setups and manufacturer brands can be equally imported into the same database, BPEs database represents a central storage point for data management and patch-clamp data collection of an entire lab. In addition to the currently supported data formats (.dat and .abf) further data formats will be supported in future releases.

To enable buildup of comprehensive electrophysiological data sets, data reanalysis and maximum exploitation of already available and new data, an online platform has been launched at https://biophysical-essentials.i-med.ac.at/. This platform allows to share data and results generated within the BPE environment with the scientific community, independent from the patch-clamp amplifier system format. The web-platform aims to foster reproducibility, transparency and enables comparison of data from different laboratories, species and model systems and can be incorporated in computational modelling approaches [19,24,37]. BPEs ability to keep track of intra-and extracellular recording solution as well as extracting meta data information about stimulation protocols and further patch-clamp analysis relevant measures like CSlow Capacitance or RSeries resistance enables data comparison taking into account different recording conditions such as ionic strength and will be further developed to correct for these differences relevant for data integration.

To document BPE's applicability for reliable and rapid analysis of patch-clamp recordings and performance of respective experiments, we have compared electrical AP characteristics between mouse and from human pluripotent stem cell derived nociceptors (iNocs) (Fig. 3), as introduced in various translational pain research studies [1,40,41]. Dimensional reduction analysis using PCA revealed the highest similarities of AP characteristics within signals from the same neuron but also within the respective iNoc or DRG neuron category (Fig. 3D). However, the parameter-specific analysis revealed significant differences for most of the AP parameters between human and mouse sensory neurons. Integration of our own data with other patch-clamp data sets e.g. from human primary sensory neurons [36,41] offers great potential to elucidate functional species dependent differences which are already demonstrated at the RNA and protein level [31].

BPEs limitations are in general minor. The software tool can operate with recording data from different brands and tissues. BPEs open-source property and python skeleton allow for unlimited adoptions to the code in an individualised configuration of the platform. Users without programming experience aresupported by GUI-operated combination of existing analysis functions. However, integration of completely new analysis functions will require basic coding skills. As visualized in Supplementary Figure S12, template classes were designed to reduce the amount of required coding skills and effort to implement individual analysis functions which is further facilitating criterion 2. We offer to support users with personalized support and function integration. As BPE will be a community tool, implementation requests of new analysis functions can be additionally submitted in the public git repository as so called "issue" which can be solved or answered by the developers or any member of the community.

Taken together, BPE processes data from different input file formats and integrates analysis functions and graphical visualizations at high velocity by making optimal use of available hardware resources. Currently, the HEKA (.dat) as well as the AXON file formats are supported. The current version of BPE satisfies all the criteria (1–7) and needs of time-effective, standardized, transparent and reproducible patch-clamp analysis. It is important to note that the function scope is still in its nascent stage with many additional implementations anticipated in subsequent updates. Based on its versatile philosophy, BPE is suitable for implementing new datatypes, including data emerging from high throughput or automated recording systems such as the Syncropatch environment. Also, BPE will be equipped with more complex statistical tools like generalized linear mixed model, linear regression model and permutation testing [21]. New analysis functions such as ePSC/iPSC signal detection as well as additional normalization methods, such as t- normalization will further improve BPE's function stack [9].

Impact statement

Biophysical Essentials provides fundamental support for transparent patch-clamp analysis to improve reproducibility in electrophysiology research. The innovation of our paper and the BPE software is represented by its ability to process biomedical patch-clamp signals including data acquisition, data filtering, data analysis, result visualization, result statistic calculation and data sharing steps within one single program where all the relevant processing steps are stored within a database allowing to reproduce study results in seconds. BPE provides, for the first time, a full range software allowing to perform state-of-the art signal processing from raw data to result statistics without the need of extra software tools. BPE furthermore allows to connect to a camera software and supports the integration of biomedical current or voltage traces with the morphology of a particular cell providing relevant information about the state, morphology, size and cellular environment. Our proposed BPE analysis framework provides a platform for collecting standardized and comparable patch-clamp raw and processed data for more complex supervised and unsupervised biomedical signal integration and processing.

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CRediT authorship contribution statement

David Zimmermann: Writing – original draft, Visualization, Validation, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Michaela Kress:** Writing – original draft, Methodology, Funding acquisition. **Maximilian Zeidler:** Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Conceptualization.

Declaration of competing interest

No conflicts of interest have to be declared.

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The source code of BPE is available on Github: https://github.com/ZiDa20/Biophysical_Essentials. The website for documentation, and online data access or sharing is accessible via https://biophysical-essentials.i-med.ac.at/

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cmpb.2024.108328.

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