Research article

A newly developed free software tool set for averaging electroencephalogram implemented in the Perl programming language

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ABSTRACT

Background: Considering the need for daily activity analysis of older adults, development of easy-to-use, free electroencephalogram (EEG) analysis tools are desired in order to decrease barriers to accessing this technology and increase the entry of a wide range of new researchers.

New method: We describe a newly developed tool set for EEG analysis, enabling import, average, waveform display and iso-potential scalp topographies, utilizing the programming language Perl.

Results: The basic processing, including average, display waveforms, and isopotential scalp topography was implemented in the current system. The validation was examined by making difference waveforms between the results using the current analysis system and a commercial software.

Comparison with Existing Method(s): The current software tool set consists of free software. The scripts are easily editable by any user and there are no black boxes.

Conclusions: The currently reported procedures provide an easy-to-begin, flexible, readable, easy-to-modify basic tool set for EEG analysis and is expected to recruit new EEG researchers.

1. Introduction

In the context of current data science approaches, analysis of human brain activity during daily life requires collecting a wide range of data to guarantee sufficient training data. This is crucial, not only for specialists, who use advanced non-invasive brain function investigation methods, including magnetic resonance imaging (MRI) and positron-emission topography (PET), but also by a much wider range of researchers collecting data from activities in daily life, because most of the methods are used to collect data at specialized facilities and are limited to the laboratory environment, rendering these approaches impractical for analysis of daily life activities. Electroencephalography (EEG), which is a traditional technique used to record brain activity from the scalp in vivo, could be a plausible data collection method for analyzing the real-world daily activities of subjects or patients while in their home, if a mobile recording system can be established (Lau-Zhu et al., 2019; Marini, 2019; Byrom et al., 2018; Ries et al., 2014).

It is challenging to interpret the information processing strategy in the brain by viewing raw EEG data. For the initial extraction of features/characteristics from an EEG during an investigation, event-related potentials (ERPs) are utilized. ERPs are obtained by averaging many trials aligned to stimulus triggers, and there are many reports of this technique being useful in both psychological (Itoh et al., 2005; Suwazono et al., 2000) and clinical investigations (Barceló et al., 2000). An estimate of the number of subjects that should be included in a single ERP experiment to obtain a stable average was reported (Yano et al., 2019), which encouraged researchers to apply ERPs in these investigations at a broad range of facilities. Such advances will facilitate a detailed comprehension of ERPs but will be constrained by the circumstances of the restricted “laboratory environment”.

Recently, an advanced EEG analysis, utilizing artificial intelligence and/or machine learning techniques, reported an up to 74% true-positive rate for detecting seizure waveforms (Emami et al., 2019) or up to 88% correct prediction of antidepressant effects in patients with major depression (Jaworska et al., 2019). Connectivity analysis with a high temporal resolution (milliseconds), utilized with intracranial recording, demonstrated that inter-regional connectivity between the inferior occipital gyrus and the amygdala could be observed during face recognition (Sato et al., 2018).

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raw EEG (binary .EEG file) recorded with Nihon Kohden acquisition system
executed by utility supplied with the acquisition system
tripper data, input to the DC-04 channels while recording, should be included

raw EEG (ascii .txt file) space separated text file with 2 lines of header
spatially, according to any assigned trigger (bin=1-5) with assigned analysis period
artifact rejection of such trial shown as multi-electrode by visual inspection
average according to the trigger
no baseline shifting to the pre-trigger period

averaged (text) file
3 options of artifact rejection: no/assigned voltage/visually inspect each trial
averaged (text) file after low pass filter
optionally, FIR filter (10.6-30 Hz) is applied
display averages
Visualize multichannel waveforms or iso-potential topographical maps

Figure 1. Procedures and data flow utilizing our processing system. Detailed explanations are described in the Methods section.

These observations increased our anticipation of the ability of huge EEG data accumulation to allow analysis of daily activity (Kanda et al., 2014). Such studies require abundant data accumulation to obtain sufficient training data to yield more accurate results.

There are several excellent commercially available software systems, such as EMSE Suite (https://cortechsolutions.com/emse/) or MatLab (https://www.mathworks.com/products/matlab.html), that have proved to be useful for sophisticated and advanced analysis. An example using the latter is EEGlab (Delorme and Makeig, 2004), which utilizes scripting tools to provide a very powerful analysis and visualization system. Nevertheless, there is a paucity of free analysis software in the field of EEG analysis. There are several methods to view raw EEG data using free software, including EDFBrowser (https://www.teuniz.net/edfbrowser/), see also Suwazono, 2017; this minor technical report generated more than 2500 page views, suggesting a clear need for EEG viewer applications. The availability of inexpensive hardware and software systems is essential in order to increase the use of EEG analysis in research, and subsequently to collect huge data as required for data science. One example of free EEG analysis software is MNE-Python (Gramfort et al., 2013, 2014; Jas et al., 2018). This software includes a powerful set of tools for advanced examinations, including connectivity, inverse modeling, and artifact removal by independent component analysis. However, this software lacks flexibility for averaging. Another available candidate tool is the R-based analysis system. The R system is a statistics software tool (www.r-project.org) that also provides impressive visualization tools. However, these tools are not currently commonly used, and commercially available software can be expensive. More options for analysis software could be a valuable resource for users who are not familiar with the above techniques based on the expensive commercial software.

We here report a newly developed free software tool set to epoch/average EEG files aligned with trigger data recorded by the Nihon Kohden EEG acquisition system (Shinjuku, Japan). Every script is a lightweight text file written to work with the programming language Perl (www.perl.org) and there is no blackbox (binary utilities). The software works with all existing operating systems (including Mac OS X, Unix, and Windows). The format of the EEGLab text file is described in the Methods section.

Figure 2. The format of the input EEG file. The first 10 lines of an input EEG file, consisting of 26 channels (including 4 trigger channels), which was exported from the Nihon Kohden EEG machine as a text file. The initial 2 lines describe general information, including the sampling interval, gain (vertical resolution), and channel names. This information is stored in the header hash used by the current analysis system. The DC03 (24th)–DC05 (24th) channels include trigger information (4-bit) from another personal computer used for stimulus (visual or auditory) presentation; these will be analyzed later to judge the onset time of stimulus presentation. Any text data with this format can be imported/analyzed with the current system. These data will be analyzed later to judge the onset time of stimulus presentation. The availability of inexpensive hardware and software systems is essential in order to increase the use of EEG analysis in research, and subsequently to collect huge data as required for data science. One example of free EEG analysis software is MNE-Python (Gramfort et al., 2013, 2014; Jas et al., 2018). This software includes a powerful set of tools for advanced examinations, including connectivity, inverse modeling, and artifact removal by independent component analysis. However, this software lacks flexibility for averaging. Another available candidate tool is the R-based analysis system. The R system is a statistics software tool (www.r-project.org) that also provides impressive visualization tools. However, these tools are not currently commonly used, and commercially available software can be expensive. More options for analysis software could be a valuable resource for users who are not familiar with the above techniques based on the expensive commercial software.

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Table 1. AVERAGER.pl. The Perl script file, describing all steps necessary to make an average file.

| Field name | Description |
|------------|-------------|
| $myFile    | EDF file |
| $samfreq   | Sample frequency |
| $pretrig   | Pre-trigger analysis period |
| $sampint   | Sampling interval |
| $channum   | Number of channels |
| $trigstart | Trigger start channel |
| $trigend   | Trigger end channel |
| $trigint   | Trigger interval |
| $chnum     | Channel number |
| $chnum24   | Channel number 24 |

use erp;

my $trigger_channel_number = $channel_number;
my $trigger_start_channel = $start_channel;
my $trigger_end_channel = $end_channel;

my ($lgth, $nl, $r_pot, $r_header);

my $rtg_channels = $trigger_channels;
my $rtg_start_channels = $trigger_start_channels;
my $rtg_end_channels = $trigger_end_channels;

my $verb;
Windows) that have Perl available. To the best of our knowledge, no EEG analysis software using Perl has been reported to date.

2. Materials and methods

We developed a new script tool set utilizing Perl (www.perl.org) to import and analyze EEG data recorded by a Nihon Kohden EEG machine. An overview of the data flow is given in Figure 1.

Exporting text files from original binary EEG files after the recording is enabled by features available with the EEG machine (Figure 2). The procedures employed thereafter are described in a script file, average.pl (Table 1). The actual command should be typed as “perl input.eeg.filename output.average.filename TriggerNumber artifactRejection_Method,” where the trigger number is a number from 1 to 15 (4-bit). The artifact rejection method indicates the options explained in the previous paragraph, 1 of NAR/Th/Vis. In the case of “Th,” the threshold value for artifact rejection should follow, e.g., 100 for 100 μV, or 80 for 80 μV. If any point within the analysis period exceeds this value, the trial will be rejected and not included in the average file.

The first step is importing the text file, which is executed with the subroutine NihonKohden_READ. If text file export is not implemented in the recording system, the current software systems cannot be utilized. In order to obtain averages, the trigger onset timings need to be determined, which is achieved by the subroutine NK_EXTRACT_TRIGGER_PUREBIN. Since the Nihon Kohden EEG machine can record 4-bit digital triggers simultaneously in EEG channels, this subroutine distinguishes the trigger timing of 4 channels and stores this information in a hash, which is later utilized during averaging.

The next step for obtaining a good average is the artifact rejection procedure. There are 3 options available in the analysis procedure. The stimulus trigger (target stimulus) button press (included in the average) (not included in the average) are displayed in the right panel. Once the user's choice has been entered in Window A (by pressing the “A” or “R” key), the waveforms displayed in Window B immediately changes to those of the next trial. These procedures are repeated for all subsequent trials with the same stimulus triggers.

Users can decide if the trial should be included in the average, by visually inspecting multi-channels' waveforms, trial by trial.

Figure 4. Average multi-channel waveforms obtained. Neurosurgeons' view of the averaged responses (including 3 eye channels), calculated from 47 trials after artifact rejection, following target stimuli in a novel auditory paradigm (70% standard stimuli, 10% novel stimuli, button press task to 20% targets), recorded from a young normal subject. Waveforms on the left panel represent those before filtering, and the low-pass filtered (FIR, 30 Hz) responses (green: standard stimuli, cyan: novel stimuli, red: target stimuli) are superimposed in the right panel. All responses are baseline based on the average of the 100-ms pre-stimulus period.
Figure 5. Iso-potential topographical maps. The panels on the left side show selected target responses at midline electrodes from the data in Figure 4. In the right panels, linearly interpolated iso-potential topographical maps, generated by the current analysis system, are shown at 2 latencies; 116 ms (at which N1 peaks at Fz), and 341 ms (at which P3b peaks at Pz). Color scaling of the maps can be changed according to the maximum/minimum value of each component's voltage.

Figure 6. Validation: Comparison of average waveforms of dummy data. Dummy raw EEG data were generated and averaged by commercial software as well as by the current analysis system. The difference waves between the 2 were completely flat.
first is an average of all trials associated with the triggers of interest, without any rejection, which is executed by using the subroutine NK_NAR23ch, where NAR stands for “no artifact rejection.” The second option, which is most frequently selected in previous studies, is executed with the subroutine NK_TH_AR23ch, which rejects trials that include any sample within the analysis period for any channel exceeding an assigned threshold. For example, 100 μV, which can be changed according to the user’s input. The third option is to inspect individual trial waveforms visually and assign an accept/reject mark, which is executed with the subroutine NK_MANUAL_AR23ch (Figure 3). This procedure is highly effective for excluding trials that present definite artifacts with a low voltage, e.g., eye movement artifacts with a lower voltage than the regularly used threshold, from the final averages. During this step, Window B pauses showing the single trial multichannel waveforms until the user decides to accept or reject the trial by inputting keypress “A”, for accept, or “R”, for reject. Once the user inputs a selection, Window B changes the contents to the waveforms of the next trial among the trials associated with the trigger of interest.

After the trials are selected, averages can finally be obtained using the subroutine NK_AVERAGER, followed by baseline correction for each channel, using the subroutine NK_BASELINE_CORRECTION. The final average file is written as a readable text file by the subroutine NK_WRI-
TOUT_HEADER_DATA2. If filtering is needed, finite response filtering is available by using the library of Perl Data Language (PDL, pdl.perl.org), PDL::DSP::Fir::Simple (https://metacpan.org/pod/PDL::DSP::Fir::Si-
mple), or using a fast Fourier transform algorithm (Math::FFT, https://metacpan.org/pod/Math::FFT), with a flexible filter setting enabled according to those libraries.

Once the averaged data array is exported into a text file, any drawing/graphing tool can be utilized to visualize the data, including some

Figure 7. Flexible band pass filtering is implemented in the current system. Two filters were applied to the original average (A): 1–8 Hz (B) and 20–60 Hz (c), utilizing the fast Fourier transform library (Math::FFT) for Perl. The original averaged data were obtained as target responses from a young normal subject while executing the visual novel P3 paradigm.

Figure 8. Partial averages. In panel A, the average of all 36 artifact-free target trials (green), the averages from odd trials (red), and the average from even trials (blue) are superimposed at 3 channels, recorded from one subject using a visual novelty P3 paradigm. In panel B, partial averages obtained averaging responses to the 1st 10 (green), the 2nd 10 (red), the 3rd 10 (blue), and the last 6 (yellow) stimuli were respectively calculated. The largest amplitude of P3b (mean of 460 ± 5 ms decided by the total average on A) is noticed in the average of the 3rd 10 trials (D). For better visibility, 4 partial averages were low pass filtered (30 Hz) using the fast Fourier transform library (Math::FFT) for Perl (C).
spreadsheets, employing cross-platform utilities:

A) The averaged waveforms can be visualized as images with a multi-channel plot, using the graphics library PGPLOT (http://www.astro.caltech.edu/~tjp/pgplot/) via binding with Perl (https://metacpan.org/pod/PGPLOT) (Figure 4).

B) The linearly interpolated iso-potential scalp topography can be generated as images, using the graphics library GNUPLOT (https://sourceforge.net/projects/gnuplot/), via binding with Perl and PDL (https://metacpan.org/pod/PDL::Graphics::Gnuplot) (Figure 5).

Text exporting of amplitude can be performed at any point or for any latency range at any channel. This also means any single trial can be visualized according to each user’s requirement.

The averaged results were validated by examining the difference between the results obtained by the current analysis system, and the average calculated by using commercial software for EEG analysis (EMSE, https://cortechsolutions.com/emse/). A dummy EEG file (text file) was generated by the current software, and that EEG file was imported and processed to produce averages by both systems. Averaged files were exported as text files, and difference waveforms between these text files were calculated by means of the current software system.

The present study was approved by the institutional review board of the National Hospital Organization Okinawa National Hospital (#2020-4). The scripts used here are uploaded (https://github.com/ShugoSUWAZONO/ERP.pm). Access to this script is available from the author upon request.

3. Results

By using the newly developed EEG analysis system, well-defined clear average waveforms were obtained and visualized as images (Figure 4). Data (single trial or averaged) can be exported to a text file, and the linearly interpolated iso-potential scalp topography was visualized (Figure 5). The correctness of the averaged result generated using our analysis system was confirmed by examining the dummy (simulated) data with triggers. We averaged data using both our analysis system and the commercial software, and the difference waveforms were zero for all channels during the analysis period (Figure 6) (Suwazono and Arao, 2010).

Using this processing system, user-defined filtering of any type (low pass, high pass, or band pass) can be applied to single trial data or averaged data (Figure 7). Very flexible partial averaging can be executed, enabling comparison among selected averages (Figure 8).

4. Discussion

We here report a new analysis tool set for averaging/displaying ERPs utilizing the programming language Perl. Although there is an extensive list of open software for human electrophysiology studies (Donoghue, 2018), the resources on the list do not use the programming language Perl, which allows greater flexibility.

An advisory report about changing laboratory software was recently released (Wessel et al., 2019), which discussed the motivations, merits, and costs of such change. It would be beneficial if researchers had more options for implementation. The current system consists of light-weight, text-based scripts, providing flexible averaging options. Every analysis step is transparent (as it is described by a text script) and no binary utilities are needed. For instance, it is possible to convert variable analysis periods from a long period, including many triggers, to a short analysis period (Figure 9). Other examples of flexible averaging are the ability to make waveforms consist of the initial 10 (20) trials vs the last 10 (20) trials, or odd versus even averages, or user-assigned averages. Another advantage of utilizing the scripting language Perl is that these tools should work on many operating systems. An additional example of the flexible averaging option is the one-by-one (single trial) assessment that allows inclusion/exclusion of each trial via visual inspection. Many EEG analysis systems employ a policy that any trials with a sample displaying an amplitude larger than a threshold (such as 100 μV), are considered contaminated by muscle or eye movement artifacts, and those trials are excluded from the averages. This frequently used method is also implemented in the current analysis system, but we also provide a more detailed analysis method. Eye-related artifacts can occur with lower voltages, such as 20 μV, and these trials would be included in the averages, if inclusion/exclusion is based on a simple amplitude value. During trials, some subjects have a tendency to blink when they feel they have made a mistake. In these cases, detailed discussion is required to determine if those trials should be included or excluded, and flexible trial and error might be needed. It may not be optimal to exclude trials simply based on amplitude criteria, and although it is a time-consuming method, visual inspection of each trial remains one of the best methods to obtain a good average.

There are other analysis systems that could be compared with the current system. MNE-Python (Gramfort et al., 2013, 2014, Jas et al., 2018) provides a graphical user interface-rich environment and detailed analyses, including connectivity, inverse solution, and other

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Figure 9. An example of flexible analysis using the current system. Procedures to make new averages with a shorter analysis period from the original long analysis period by re-splicing. This facilitates “n” back or “n” post analyses, or masking/priming effects.
beneficial features. In contrast, our software tools use mainly command line processing, while maintaining flexibility during the analysis process (Figures 7, 8, and 9). A comprehensive and detailed review of analysis systems/software combinations has been published previously (Michel and Brunet, 2019), which describe advanced generator source analysis. These software systems have contributed to many studies, including a framework for big data analysis (Pedroni et al., 2019).

However, most of these systems are expensive and complicated to use for new researchers.

In summary, our proposed analysis system provides an easy and affordable way to compute ERPs for beginners. This hands-on analysis system can be used in any context without being limited by a laboratory’s existing system or budgets and utilizes a non-blackbox approach for experienced researchers, enabling them to reveal outcomes that may not be apparent when using the default settings in other platforms. Moreover this is an open-source/multi-operating system platform for future development without prerequisite expensive software. In order to encourage the wider use of EEG analyses, establishing easy data collection and pairing it with an easy-to-use analysis software system is essential. While the former will mainly be accomplished by hardware improvement, the latter can be addressed by software development. The current system is expected to impact the latter issue.

Declarations

Author contribution statement

Shugo Suwazono: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Hiroshi Arao: Performed the experiments; Analyzed and interpreted the data.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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