A *MATLAB* application for automated H-Reflex measurements and analyses

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Abstract

Objective: H-Reflex is a test that is carried out to measure the relative excitability of reflex pathways. Although reliable, conventional methods consist of performing many small steps, which requires a high level of attentiveness, and thus can carry an elevated risk of human error, despite proper training. Equipment that is available to perform those tests with different levels of automation are typically proprietary, inextensible by the user, and expensive. Here we present a novel MATLAB application that can accurately and reliably perform automated H-Reflex measurements, test the stimulating electrodes, and carry out typical subsequent analyses.

Methods: This application is a Graphical User Interface that works with inexpensive equipment and offers many important features such as measuring electrode impedance in-situ, automating lengthy measurements like recruitment curves and frequency response trials, standardizing electric stimulation properties, automatic exporting of digital data and metadata, and immediately analyzing acquired data with single-click events.

Results: Our new method was validated against conventional H-Reflex measurement methods with 2 anesthetized rats. The difference between acquired data using both methods was negligible (mean difference=0.0038; std=0.0121). Our app also detected electrode impedance with high accuracy (94%).

Conclusion: The method presented here allows reliable and efficient automated H-reflex measurements and can accurately analyze the collected data.

Significance: The features provided by our app can speed up data collection and reduce human error, and unlike conventional methods, allow the user to analyze data immediately after the record. This can result in higher research quality and give broader access to the technique.

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1. Background

The Hoffman Reflex, or H-Reflex, named after Johann Hoffman who first developed the technique in 1910, is an electrophysiological assay of reflex pathway function. The H-reflex test relies on recording electromyogram (EMG) activity of a reflex response following an electrical stimulation (e-stim) [1-6]. Stimulation of a mixed motor and sensory nerve results in both alpha motor neuron activation and afferent neuron excitation. Volleys of spikes will propagate in both directions (orthodromic and antidromic) as a result of this stimulation. The EMG waveform that results from the orthodromic volley in the alpha motor neurons is defined as the M-wave. Orthodromic spikes arising in the Ia afferents will propagate to the spinal cord, where they (amongst other things) activate alpha motor neurons via monosynaptic connections. This induces a later EMG signal in the muscle that is termed the H wave, or sometimes the H-reflex directly [1,7]. The ratio of the maximal H-wave amplitude to the maximal M-wave amplitude (H_{max}/M_{max}; where max is taken across stimulation amplitudes, and stimulus amplitude is typically varied by changing current) is often computed and analyzed instead of the raw H-wave in order to create a quantity that is not susceptible to changes in electrochemical variations in stimulus or recording sites. That is, changes in the strength of stimulus that reaches the nerve or recording site impedance will affect both waves, and thus the ratio should cancel these out. Furthermore, this ratio more closely represents an index of the proportion of available motor neurons that can be recruited via the monosynaptic reflex relative to the accessible motor neuron pool [8]. This ratio is typically thought of as an estimate of spinal excitability [9], but is influenced by the state of all elements of this reflex, including afferents, spinal circuits, and motoneurons [2].

H-Reflex analyses are useful in many scientific and medical contexts. H-Reflex tests can also help understand injuries and neural diseases, such as multiple sclerosis [10], paralysis [11], and spinal cord injury [12-14]. For example, the H_{max}/M_{max} ratio is increased by 70% after transection in rats [15]. Additionally, the H-Reflex can be an important assay that complements locomotor and kinematic analyses. For instance, H-Reflex conditioning changes kinematics on the hip and ankle levels in rat [16]. Locomotor experiments have shown that H-Reflex amplitudes in the human soleus muscle are higher during the stance phase of the gait, and 2.4 times higher during running than in walking [17]. Recently, genetic tools such as chemogenetics are increasingly used to manipulate peripheral neuronal circuitry [18-20] and the H-Reflex test could become an important method with which to assay their function.

H-Reflex tests usually consist of administering a series of electric pulses with increasing amplitude and subsequently measuring the M and H peaks obtained with each amplitude. In many conventional setups, users usually manually adjust the e-stim's amplitude, which necessitates vigilance and meticulous note-taking. This can be time consuming and could

ultimately increase user error, which raises the need for tools that are able to perform automated H-Reflex measurements and analyses. Automatic H-Reflex testing equipment tends to be expensive and is not always customizable; therefore, we sought to develop a user-friendly, open source application that can perform reliable automated H-Reflex measurements and analyses using inexpensive portable equipment.

2. Materials and methods

Many labs develop their own programs for running H-Reflex measurements, providing custom solutions and a learning opportunity. But this can take significant time and effort, and requires a background in programming and electronics. Therefore we sought to create an open source platform that can be widely used and enhanced by the community, as an alternative for those that want to dive in quickly. This app would allow researchers to perform automated H-Reflex measurements and analyses and simplify meta-data collection. The app can be found at https://github.com/georgemoux/h-reflex-app.

2.1. Setup overview

There are two main aspects of gathering H-Reflex data with this app: a data acquisition system must be correctly installed and connected to the computer with the app running on MATLAB, and stimulating and recording electrodes need to be inserted precisely in the animal subject (Fig. 1). Here we cover these aspects of the experiment before moving on to recording and analyzing H-Reflex data with the app.

2.1.1. Data acquisition setup—To stimulate and record from the animal, a data acquisition box (DAQ) is coupled to an analog stimulator and an extracellular amplifier. Analog output waveforms from the DAQ control the stimulator, which in turn stimulates the nerve in the animal, and electromyographic (EMG) waveforms are received by the DAQ's input after amplification (Fig. 1C). The data acquisition box used in this study was an NI-USB-6361 (*National Instruments, TX, USA)* connected to a computer running Windows 10. Stimuli were provided by an AM2200 Analog Stimulus Isolator (A–M Systems, WA, USA), and the extracellular amplifier used to gather EMG signals was an AM1700 Differential AC Amplifier (*A–M Systems, WA, USA*). The **SIGNAL IN** port of the stimulator was connected to the Analog Output of the DAQ. The polarity of the stimulator was set to bipolar, and the range set to 1 mA/V for all measurements. Stimulating electrodes were connected to the **Output** ports of the AM2200 stimulator. EMG electrodes were connected to the AM1700 amplifier (Fig. 1C). The recording gain was typically set to 1000. The notch filter provided was turned on, and the animal ground was supplied by the amplifier.

2.1.2. Animal electrodes setup—Two types of electrodes are used in the animal: stimulating electrodes, which in our case run through the Achilles tendon to stimulate the tibial nerve; and recording, or EMG, electrodes, that record the reflex from the paw of the animal. All animal procedures were approved by the Temple University Institutional Animal Care and Use Committee (IACUC) under Animal Care and Usage Protocol (ACUP) #4675, approved on August 9th, 2019. A naive female Long Evans rat and a naive female Sprague Dawley rat were used for this study. Animals were fully anesthetized with an intraperitoneal

injection of a Ketamine/Xylazine mixture (dosage 92 mg/kg Ketamine; 100 mg/mL solution, Zetamine, Vet One, Boise, ID; Xylazine dosage 9 mg/kg; 100 mg/mL solution, AnaSed, Lloyd Laboratories, Shenandoah, IA), diluted in sterile saline. The plane of anesthesia was monitored at 10-minute intervals and maintained at this level with supplemental doses [21,22]. The stimulating and recording electrodes that were utilized were made of AS631 and AS633 Cooner Wire (*Cooner Wire, CA, USA*) respectively.

Stimulating electrodes were fabricated from thin insulated wire. A knot was first tied at the end of the wire to prevent the wire from slipping out of the heel when inserted. Then, short lengths of wire (~1 mm) were deinsulated, starting approximately 5 mm from the knot. The wire was then threaded onto a curved needle, which was inserted through the thin skin area between the Achilles tendon and tibial bone of the animal. The wire was pulled through until held against the knot, with the deinsulated portion inside the leg. This results in the nearby and parallel tibial nerve being targeted upon stimulation. The electrodes were inserted ~8 mm apart [15,23] (Fig. 1A). Recording electrodes were threaded through 23 G needles and inserted in the paw of the same side of the animal (Fig. 1B). Electrodes were inserted at a shallow, close to 45° angle, with the needles pointing towards the heel. Stimulating and EMG recording electrodes were then connected to the simulator output and EMG amplified input, respectively. Stimulator input and amplified EMG signal output were connected to the appropriate analog output and input, respectively, of the DAQ box. The animal was grounded by placing an 18 G needle through the skin, with an alligator clip on the needle tying it to ground [24].

2.2. Electrode impedance testing: principle of operation

Electrode fabrication is crucial to the animal setup. Hence, to ensure the quality of the electrodes described in the paragraph above, we provided an electrode impedance testing feature in the app. For nerve stimulation, the impedance of the stimulating electrode should typically be less than ~12 kΩ. This is because high impedances would require the stimulator to provide very large voltages (>200 V) to achieve the desired current, and most inexpensive stimulators are not able to provide voltages higher than 50 V in bipolar mode.

Testing the electrode's impedance consists of a measurement of two voltages, one across a 10kΩ resistor and a second across a 1kΩ resistor, while delivering a current ramp ranging from 0 to 1 mA and lasting 1.5 s. Thus the voltage across the 1kΩ resistor mirrors the value of the current that is being delivered in mA, whereas the voltage across the 10kΩ resistor represents 1/100th of the voltage that is being delivered through the stimulator. Although electrode impedances can be non-linear [25, 26], this test is enough to provide a good indication about whether the electrode has a usable impedance.

2.3. App narrative

In this section, we describe the various features implemented in this application, which is a Graphical User Interface (GUI) built in MATLAB's App Designer (Fig. 2). Each sub-section of the following text describes an area of the user interface as seen in Fig. 2.

2.3.1.   Device selection—First, a data acquisition device (or **Simulation mode**) should be selected by the software before any feature can be used. The app will attempt to detect all present devices. If the option "Select Default" is turned on, the app will connect to the first data acquisition device that is available. If no devices are found, the app will automatically go to **Sim** mode, where the desired stimulus pulses are generated and displayed, but a wave consisting of random numbers is generated in the "Response" data. If multiple devices are connected, the proper DAQ can be chosen from the drop-down list. Sample rate is set to 100KHz by default, to avoid aliasing and provide maximum fidelity by default, but this can be modified by the user. The lowest recommended sampling frequency is 10 kHz, as H and M waves are typically 2~3 ms in duration. The default input and output are respectively AI0 and AO0, although they can be selected by the user. After the starting parameters are assigned, **Start Session** should be clicked before proceeding with any testing or stimulation. Once the devices are recognized by MATLAB, the trial number at the bottom of the page is set to 1.

2.3.2.   Impedance testing—This feature allows the user to test the electrode's impedance as described in section II.2. Two additional analog inputs need to be specified for the current and voltage measurements. The value of the impedance is calculated as the slope of the linear fit of voltage against current. The value will be displayed in green if it's below 15kΩ, and in red if it's above 15kΩ.

2.3.3.   Quick Trig—This is a feature that allows the user to search for the intensity where the M wave firsts appears, so that this value can be used as a minimum value later in the automated measurements. When this feature is used, a single 200μs bipolar pulse is delivered. The amplitude of the pulse can be set by adjusting the knob to the preferred value. The response is shown on the "Recorded Data" plot. The acquired data cannot be saved when this feature is used.

2.3.4.   Recruitment Curve—This feature of the GUI allows the user to deliver automated stimulus pulses ranging from a minimum to a maximum amplitude, with a specified increment. This is useful for gathering a standard "recruitment curve," commonly used in H-reflex studies [4,15]. The app generates several bipolar pulses of increasing intensity that are separated by the given interval. The width of the pulses is specified in **Parameters.** The default is a bipolar 200μs pulse (square pulses comprised of a 100μs positive phase followed by a 100μs negative phase; these bipolar pulses separated by a 3 s inter-pulse interval by default).

Ideally, the minimum current value should be below the intensity where the first wave appears, and the maximum value should be above where the H wave saturates. The user also has control over the increment value (**Step Size**) between two consecutive stimulations and the inter-pulse interval between two different stimulations. It is recommended that an inter-pulse interval of 3 s or larger is used in order to avoid frequency adaptation. If the option **Triplicate** is chosen, three different stimulations of the same intensity are delivered before generating to the next intensity. The triplicate option is turned off by default. The **Single Pulse, Pulse Train** and **Recorded Data** plots will show the waveform of the first pulse that

is delivered, the total recruitment curve and the entire record, respectively. If autosave is enabled, data will be saved automatically, and the directory of the saved file will be shown in the bottom right of the app's window.

2.3.5. Frequency adaptation—This feature is used to quantify whether frequency adaptation is occurring during a series of stimuli. Several pulses of the same intensity, separated by a specific time interval, are delivered, and any changes in the amplitude of the H-wave are measured over the course of the pulses. In a naive animal, the H-wave is typically attenuated in subsequent pulses at higher frequencies (typically 10 Hz), whereas it is not at lower frequencies (0.3 Hz; [4,15]), indicating frequency adaptation. Spinal or injured animals may not show frequency adaptation at the higher frequencies [15]. The recommended intensity is the one that is found to have the highest H-wave amplitude. The user can choose between a bipolar, a positive monopolar or a negative monopolar pulse. The default option is a bipolar 200μs pulse. The pulse width (default 200μs), the number of pulses (default of 17), the delay time (default of 1 s) and the inter-pulse interval, which determines the frequency that is being analyzed, can be all manually set by the user. Often, 3 frequency adaptation measurements are taken: 10 Hz (0.1 s inter-pulse), 5 Hz (0.2 s inter-pulse) and 0.3 Hz (3.3 s inter-pulse; [15]). Data is generated and collected after clicking the Start button. The plotting and autosave features function as described below.

2.3.6. Saving—A base filename can be specified in the **filename** field. For repeated measures involving a treatment over time, the treatment state for the current measurement can be chosen from the drop-down list, and will be stored in the filename. The trial number will also be automatically included in the saved file. In the case where a recruitment curve is being taken, the file name will also include the term "*Recruitment_Curve*". After each successful save, the directory of the file will be shown on the bottom left of the app's window. If the autosave feature is turned on, the data will be automatically saved each time the **Start** or **Recruitment Curve** buttons are clicked. Data will not be saved if the user performs a **Quick Trig** or an **Impedance Test.**

Long protocols that involve multiple stimulations can result in unnecessarily large data files. As such the software has a **Minimize Variable Saving** feature that will only save data for a specific period around each stimulation. This feature saves each stimulation as elements of a MATLAB struct array. For instance, if the automated measurement consists of 15 pulses, the struct array will contain 15 different elements, with each element containing the data from 0 to 20 ms for each stimulation. 20 ms of saved data is typically adequate to store both M and H waves for measurements done on most animals, however this is easily extensible with code modifications. The app saves 3 different structs, *Data*, which contains the response acquired from the EMG, *Pulse_Train*, which contains the data generated and sent to the stimulator, and *Time*, which contains the data reflecting the measurements' duration. Another important variable that is saved is the variable "app". The user can use this variable to retrieve metadata that is chosen during the measurements, such as the parameters that are specified by the user prior to performing the measurements (Supplemental Information). By default, both **autosave** and **Minimize Variable Saving** features are turned on.

2.3.7. Generate plot—The app allows the user to generate plots in MATLAB figures that are not attached to the built-in plots. Although the built-in plots are interactive, this feature can be useful in case the user wants to save the figure immediately after a specific stimulation, or perform more customization of an isolated MATLAB figure.

2.3.8. Load data—Users can load previously acquired data, and then run the **Analyze H Reflex** procedure to compute and export H/M ratios and other data. After clicking on the **Load Data** button, a window appears allowing the data to be analyzed to be selected. Upon successful loading, the data's directory will be shown next to this button.

2.3.9. Analyze H-Reflex—The **Analyze H Reflex** feature allows the user to generate figures showing the M and H peaks for each stimulus. The peaks are identified based on latencies. The M/H cutoff in time is specified by the user and will depend on the preparation that is being used for the measurement. For example, in the rat tibial nerve stimulation and intrinsic paw muscle recording preparation presented here, a cutoff of 8 ms is recommended. The M/H cutoff specified by the user is also shown on the plots. Figures showing the variation of M and H peaks as a function of the pulse's amplitude, and the variation of H to M ratio with amplitude are also generated. This feature aids in diagnosing whether the data acquired are reasonable, or whether the measurement may need to be repeated. The code looks for 2 different peaks, the first representing the M peak and the second the H peak. The M peak is the maximum of the absolute value of the acquired data ranging from 2 ms after the beginning of the measurement (to avoid the stimulation artifact) to the cutoff. The retrieval of the H peak depends on the M peak. If the value of the M peak corresponds to the positive peak, then the H peak is the maximum of the acquired data from the cutoff point to the last data point. Likewise, if the detected M peak corresponds to the negative peak, then the H peak is the absolute value of the minimum of the acquired data from the cutoff to the last data point, so that the negative peak is the one selected. This feature can only be used if "**minimize variable saving**" is enabled.

3. Results

Here we present the validation of the features that were described in the App narrative section.

3.1. Reliability of the software

To test the reliability of the software, H-reflex measurements were taken using a conventional manual stimulator (2100 Isolated Pulse Stimulator; A–M Systems, WA, USA) as well as via the GUI, and then compared. A naive Sprague Dawley adult female was used in this study. The animal was anesthetized, and the plane of anesthesia monitored throughout the experiment via toe pinch. All animal welfare guidelines were followed throughout, under Temple Animal Care and Usage Protocol (ACUP) #4675 to AJS.

A pulse train of 200μs biphasic pulses ranging from 1.2–3.8 mA was delivered using the **Recruitment Curve** feature of the software. Results are shown in Fig. 3A. Pulses of the same duration and amplitudes were then delivered using the 2100 Isolated Pulse Stimulator (A–M Systems, WA, USA). Data were then analyzed, and the waveforms corresponding to

the stimulations of the same amplitude from each method were overlaid. As expected, the software and different stimulators accurately recreated the waveforms, and after comparing the values of the M and H peaks of the acquired EMG, it was shown that the app was yielding reliable results compared to a conventional setup with negligible differences (mean = 0.0038; std = 0.0121; [15]; Fig. 3B).

3.2. Impedance testing validation

The circuit shown in Fig. 4A was used to validate this feature. The voltage across R2 and R4 represents the values of the voltage and current sent by the stimulator, respectively. Rx represents the electrode's impedance. Resistors of known value were used as gold standards for Rx. The chosen resistors ranged from 4.6KΩ to 46.6KΩ, simulating realistic electrode impedances. The software showed a high accuracy (94%) in detecting the resistance values (Fig. 4B), with higher impedances yielding slightly less accurate numbers. This likely occurs because the error related to the equipment in use is significantly increased as the tested impedance approaches the amplifier's input impedance. Similarly, the software showed that the AM-2200 delivers current with a high accuracy (99.8%), with higher impedances also yielding slightly less accurate current intensities (95.7% current accuracy for a 46.7kΩ impedance, which would be considered a very high electrode impedance in practice; Fig. 4C).

This feature was then tested in a live animal. The animal was a naive adult female Long Evans rat. The animal was fully anesthetized throughout the entire study, and anesthesia monitored via toe pinch. The app yielded a 5.8 kΩ electrode impedance value and showed a 99.5% stimulation amplitude accuracy (Fig. 4C). We note that this feature will not apply high levels of current and may be used with caution *in situ*.

3.3. H-Reflex data analysis

H-Reflex measurements were conducted on the same animal as III. 1. Using **Quick Trig,** we found that 0.3 mA was the amplitude where the first M and H waves appeared. A recruitment curve of biphasic pulses of 200μs was delivered, ranging from 0.3 mA to 0.95 mA and separated by a 3 s interval. We estimated the noise level as 75% above the maximum of the absolute value of the signal collected before the stimulus artifact. M and H values below the noise level were assigned a value of 0. The M/H ratio returns a Nan (not a number) value when no M peaks are detected.

The **Analyze H-Reflex** feature was then used, and here the software accurately detected the M and H peaks, as confirmed in Fig. 5. Fig. 5 (A, B) shows an example of the computed H and M values. The variation of the H to M ratio with the stimulating current, as well as the variation of the individual M and H peak values, are also displayed on separate plots (Fig. 5 C,D).

To determine whether frequency adaptation is taking place, 17 automated 200μs biphasic pulses of amplitude 1.5 mA were delivered through the stimulator at 3 different frequencies (10 Hz, 5 Hz and 0.3 Hz; these are set in the inter-pulse interval field) through the frequency adaptation feature of the software. By using the **Analyze H-Reflex** feature, it was

determined that frequency adaptation occurred at the 10 Hz and 5 Hz frequencies, but not at the 0.3 Hz frequency, as expected.

4. Discussion

The tests carried out above show that the MATLAB app is reliable in performing automated H-Reflex measurements and analyses and offers several advantages. First, it automates setting and recording of experimental parameters as well as H-reflex data, even when using inexpensive follower-type stimulators and a standard DAQ. Although turning physical knobs for manual stimulus pulse delivery alongside notetaking by hand will always be an important method for conducting H-reflex experiments, and potentially a more satisfying approach for many investigators, our MATLAB application offers a companion approach, especially for long experiments with many recruitment curves. We found that having meta-data saved directly alongside the stimulus and EMG waveforms (Supplemental information) accelerated the analyses as notes did not need to be transcribed to the PC. Furthermore, by running lengthy recruitment curves and frequency adaptation trials in an automated fashion, we had more attention to spare for the animals' plane of anesthesia, a critical factor for H-reflex recordings.

The app may further improve the reliability of recruitment curves because the wait time between each two consecutive pulses is tightly controlled by the software, and not delivered manually, where too rapid stimulation could cause errors due to frequency adaptation [1].

The **Analyze H-Reflex** feature of this app allows the user to immediately interpret their results after a series of stimulations, which can save the trouble of analyzing the data at later timepoints and realizing that measurements have to be repeated. This is especially important in the case of terminal experiments. This feature was tested on various collected data, whether measurements were done on live animals or on previously recorded data with the **Load Data** feature. The detection of the M and H peaks was largely accurate. In one instance, the software had trouble detecting the H peak when an experiment was conducted with a rat that had a slow M-phase recovery, and the H-peak was much smaller compared to the M-peak. Fortunately, this was detectable thanks to the series of plots that appear after clicking on the **Analyze H-Reflex** button. This error was fixed by changing the M/H cutoff from 8 to 10 ms.

The M/H cutoff allows the user to analyze H-Reflex measurements that are done on either larger or smaller animals, since the app analyses the M an H peaks based on their latency [27,28]. After working through any legal approval steps required, this app could potentially be used for clinical work on humans as long as the equipment that are used are also approved for human use. H-Reflex research has been critical in many human studies as it highlights muscle activity during and post exercise and complements clinical gait analyses [17,29].

Given the importance of saving acquired data for future analyses, many labs develop their own GUIs for data acquisition, and while this can offer the user a more personalized interface and overall experience, it can be time consuming and often requires specific

computer science skills. This app is available online and offers a single user-friendly interface that is capable of delivering stimulations, recording the acquired data and analyzing them at once and at a high accuracy (Table 1). The open source nature of the app also enables researchers to use it as a starting point for custom extensions.

5. Conclusion

In conclusion, this MATLAB software was validated in 2 experimental animals and we found that the error between our method and standard H-Reflex measurement methods was negligible when accompanied by standard laboratory equipment (mean error = 0.0038; std = 0.0121). Besides, in addition to reliable performance, this app presents many useful features (Table 1), such as correctly measuring electrode impedance (94% accuracy level) and delivering automatic recruitment curves (Fig. 2,3A). Furthermore, by standardizing inter-stimulation time, as well as saving data and metadata and analyzing recorded data in one-click events, lengthy stimulation protocols are therefore automated via this app which in turn reduces human error and allows for higher quality research.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgment

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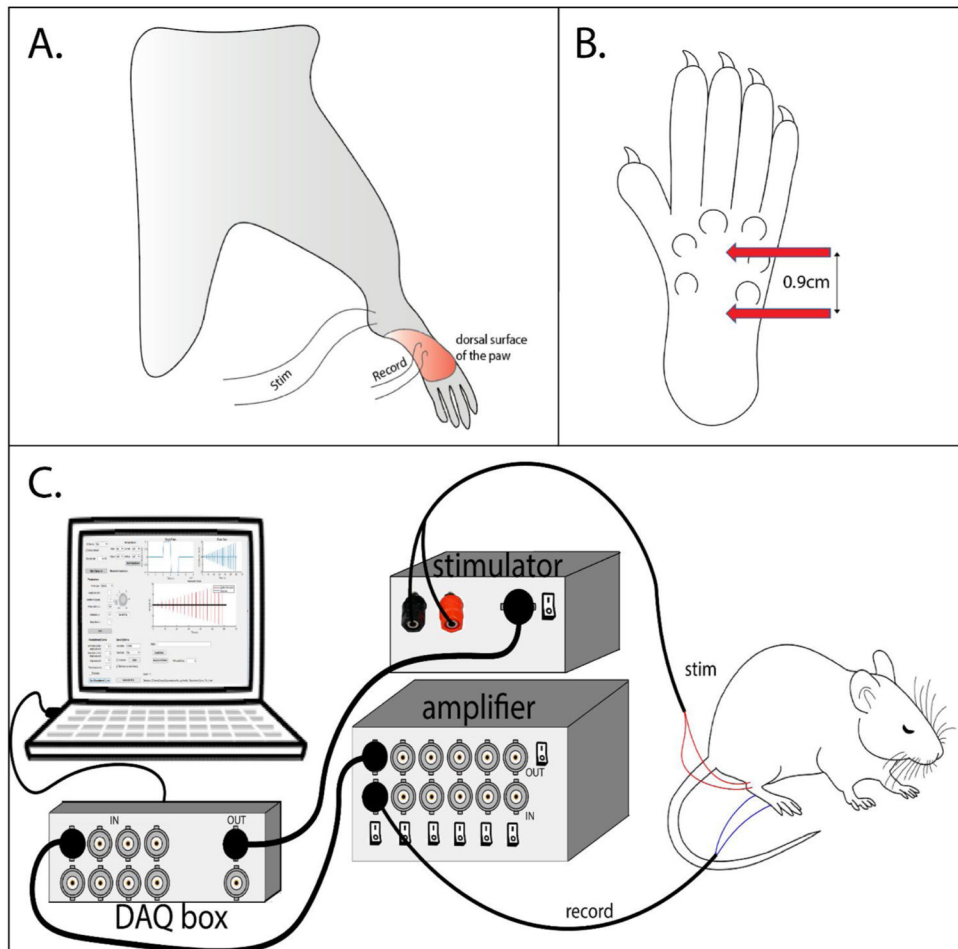
Fig. 1.
Overview of the apparatus. A.Stimulating electrodes were made from fine stainless steel wire (Cooner Wire part #AS631). The electrodes were inserted via a curved suture needle near the rat's Achilles tendon, by pulling wires with a short (~1 mm) deinsulated section followed by a stop-knot through the skin between the tendon and the distal end of the fibula. These were about 0.8 cm apart from each other, such that in this location with this spacing the tibial nerve is reasonably well targeted for electrical stimulation. EMG recording electrodes were also made from fine stainless steel wire (Cooner Wire part# AS632), with the final 2 mm of the wire deinsulated. **B.** Placement of EMG recording electrodes in the rat paw, for recording from interosseous muscle. The electrodes were separated by about 0.9 cm. Electrodes were threaded through a 23 G needle, the needle was inserted into the paw at an approximately 45° angle to a depth of ~2 or 3 mm, and then the needle was withdrawn carefully. A very gentle pull was performed to ensure that the recording wires were embedded in the paw. Noisy or floating EMG signals are indicative of poor EMG wire insertion and require reinsertion. **C.** Schematic of the experimental setup. The GUI was used on a Windows 10 operating system with a USB data acquisition box (National Instruments NI-USB-6361). The stimulator (AM Systems AM2200) was connected to the Analog Output of the DAQ, and set to 1 mA/V. The differential amplifier (AM1700) was connected to the

Analog Input of the DAQ. Stimulating electrodes were connected to the stimulator, and recording electrodes were connected to the amplifier.
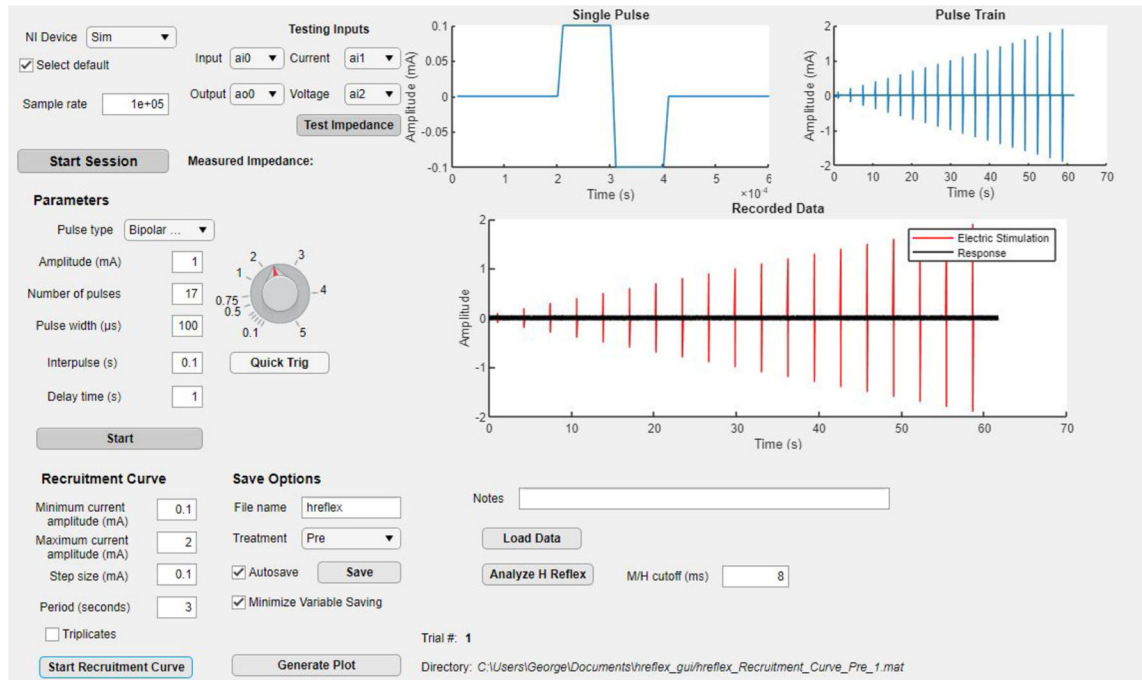
Fig. 2.
The main interface of the H-reflex GUI. This screenshot shows a recruitment curve taken using the app. Each area of the interface is described in detail in the main text in section II.3. This image shows the preset parameters, the single pulse form, the full pulse train and the obtained response. The app was used in Sim mode, and the generated response was a random signal. Sampling frequency used was 100 kHz. The recruitment curve consisted of a series of bipolar 200μs pulses ranging from 0.1 mA to 2 mA with a step size of 0.1 mA. Interpulse interval was set to 3 s. The shape of the single pulse, the entire stimulating signal and the entire record were shown on the 3 interactive graphs shown on the GUI. Autosave was enabled and the directory of the saved mat file was shown at the bottom of the interface.
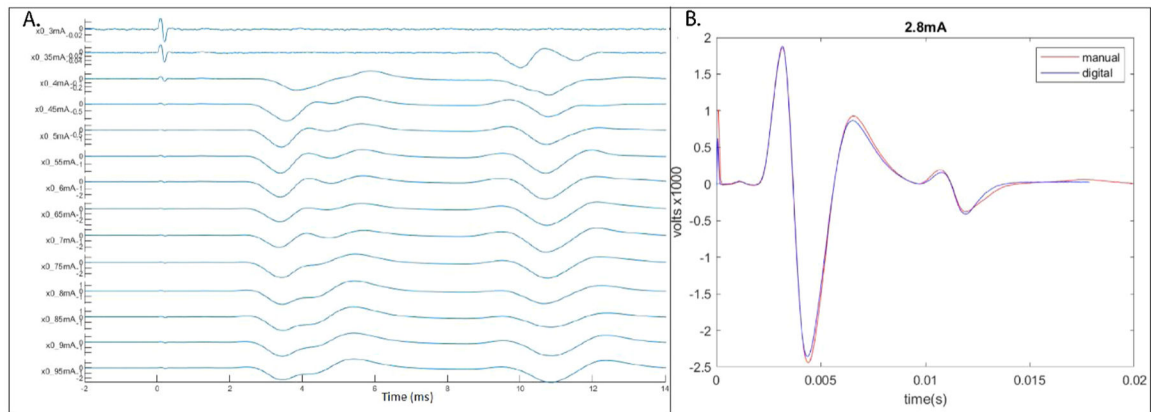
Fig. 3.
Comparison of H-reflex data gathered with the GUI and an inexpensive electrical follower/
stimulator, versus that generated by a combination pulse generator/stimulator. A.Plot
showing the acquired EMG responses after delivering a recruitment curve using the GUI
with an inexpensive stimulator (AM Systems Model 2200). The x-axis represents time in
ms, whereas the y-axis represents the acquired data in V. The recruitment curve ranged from
0.3 mA to 0.95 mA with a step size of 0.05 mA. The interpulse interval was set at 3 s. No
EMG was obtained on the first pulse. At 0.35 mA, only an H-wave was obtained without an
M-response. The plot shows the variation of the M and H wave throughout the recruitment
curve. Data was amplified using a gain of 1000 on the AM 1700 amplifier prior to data
collection. Each graph shown in this image represents the acquired EMG signal at different
stimulating amplitudes (here current), starting with the lowest on the top (0.3 mA) and the
highest on the bottom (0.95 mA).**B.** Plot overlaying EMG data acquired through
conventional method (red curve), and with the use of our GUI coupled with inexpensive
equipment (blue curve). In this example, an electric stimulation of 2.8 mA was delivered to
an anesthetized rat using both methods. Recorded data using both methods yielded very
similar EMG responses on the same rat. The differences between both acquired curves were
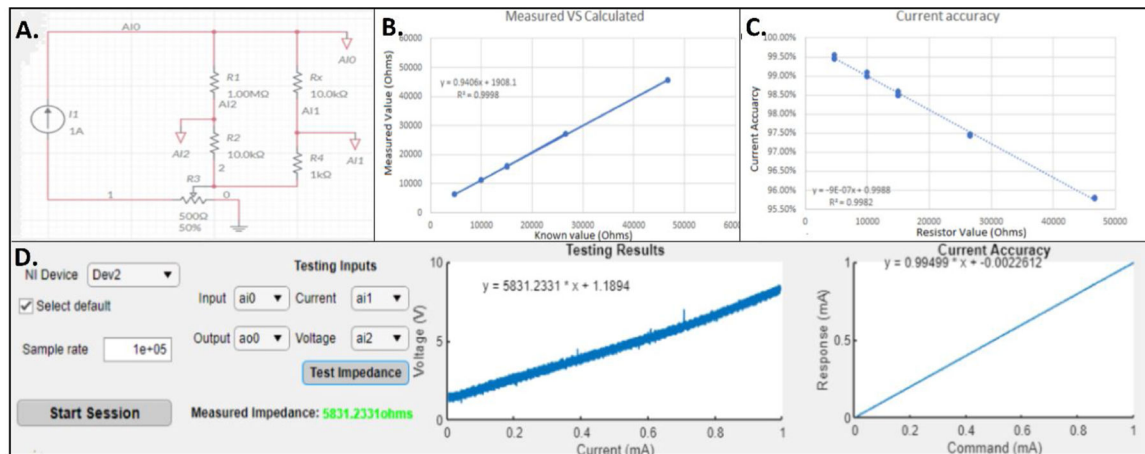negligible (mean = 0.0038; std = 0.0121), as expected.

Fig. 4.

Validation of the impedance testing feature of the GUI. **A.** Schematic of circuit used for the impedance testing feature of the app (Multisim). A 1:100 voltage divider was used to measure the voltage delivered by the stimulator (R1 and R2). Rx corresponds to the measured load. Having a value of 1 kΩ, the value of the voltage across R4 corresponds to the current delivered by the AM2200 stimulator. The voltage across Rx over the calculated current would then correspond to the measured impedance. The outputs of the circuit are readily connected to the DAQ through BNC plug connectors. **B.** Graph showing the difference of known resistor values against measured ones using the GUI. The correlation was linear and gave a high accuracy of impedance measurement detection (94%), with higher resistor values yielding a lower accuracy. The x-axis represents known resistor values in Ohms, and the y-axis shows the valued measured with the GUI in Ohms. **C.** Graph showing the variation of current accuracy as a function of impedance value. The graph shows that current was still delivered accurately, even when a higher impedance was used, which requires a higher voltage administration by the stimulator (average current accuracy of 99.9%, with a value of 95.8% for a 47kΩ load). The limit of the impedance that can be used will be determined by the stimulator chosen. Here the x-axis represents the known resistor value in Ohms, and the y-axis shows the current accuracy as a percentage. An impedance value higher than 15kΩ is not recommended, as it would require the stimulator to go to very high voltages to achieve higher stimulation amplitudes, and it would increase the error and decrease the accuracy of the detected current. **D.** Screenshot showing the GUI's impedance test feature in use. This feature was tested in an anesthetized rat.. The first graph shows data acquired after delivering a slow ramp ranging from 0 to 1 mA, which can provide a usable estimate of the electrode impedance. The x-axis represents the administered current in mA, and the y-axis shows the voltage acquired on R2 of the circuit described in panel A. The graph shows a linear correlation and indicates an impedance electrode of 5.8kΩ. The second graph shows the current accuracy as a function of the stimulating signal. The x-axis represents the commanding signal in mA, and the y-axis represents the voltage acquired on R4 of the circuit described in panel A, which is a direct indication of the actual current that is being delivered with the stimulator. The linear correlation shows a 99.5% accuracy level of the stimulating current.
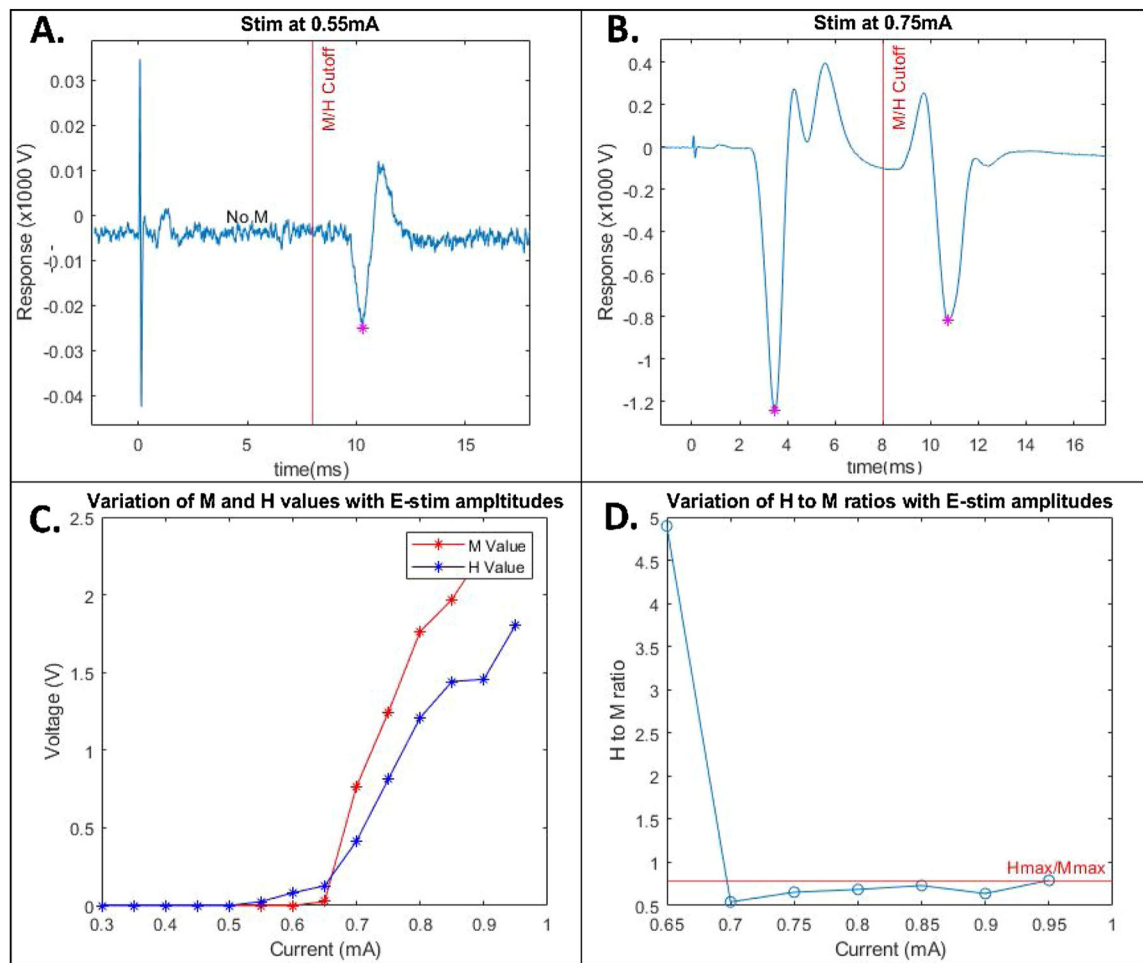
Fig. 5.

H-Reflex data analysis generated from the Analyze H-Reflex function of the GUI. Data was amplified with a gain of 1000 through the AM 1700 amplifier prior to collection. Measurements were done using the GUI. The sampling frequency was set to 100 kHz. A recruitment curve of 200μs bipolar waves, ranging from 0.3 mA to 0.95 mA, with a step size of 0.05 mA and an interpulse interval of 3 s was delivered to an anesthetized rat. The autosave feature, as well as the minimize variable saving feature, were used. The "Analyze H-Reflex" feature was clicked automatically after data collection. M/H cutoff was set at 8 ms. Panels **A** and **B** show the measured peak (in magenta) for both M and H waves 18 ms after the e-stim was delivered. The M/H cutoff, specified by the user, is shown with a vertical red line. The x-axis represents time in ms, and the y-axis represents the acquired voltage in V, after an amplification of a gain of 1000. **A.** M and H peaks shown for a pulse of 0.35 mA. No M-wave was obtained, and the software yielded a value of 0 for the M peak. The H peak was 0.08 V. **B.** M and H peaks obtained after a pulse of 0.95 mA was delivered. The M was at 2.4 V and the H at 2.8 V. **C.** Plot automatically generated by the Analyze H-Reflex function showing the values of the M peaks (red) and the H peaks (blue) for each pulse amplitude. The x-axis represents the different currents in mA, and the y-axis represents the acquired voltage in V, after amplification with a gain of 1000. **D.** Plot

automatically generated by the Analyze H-Reflex feature showing the H/M ratio of each delivered pulse. The x-axis shows the different currents in mA where both an M and an H wave were detected, and the y-axis shows the H/M ratio of each wave acquired at each stimulating current. The Hmax/Mmax value was determined to be 0.8.

Table 1

Comparison of different features using typical H-Reflex methods and the method we describe in this paper. Unlike typical methods, our method allows for a more controlled experiment, with the use of inexpensive equipment and an automation of pulse delivery, data saving and immediate data analysis.

Features	Typical methods	While using our GUI
Possible use of inexpensive equipment	✗	✓
Quick triggering	✓	✓
Testing of electrode impedance	✗	✓
Frequency adaptation	✓	✓
Automatic recruitment curve	✗	✓
Controlling pulse parameters	✓	✓
Control acquisition time	✗	✓
Standardized time between pulses	✗	✓
Overlay pulse and response	✗	✓
Save entire experiment in 1 variable	✗	✓
Automatic saving	✗	✓
Automatic notes saving	✗	✓
Immediate data analysis	✗	✓