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A novel computational method for rodent electrographic recording and analysis using off-the-shelf intracerebral depth electrodes ☆,☆☆

Cora Helton [a,c,#], Nicole Rodgers [a,#], Payton Klosa [a], Erik Van Newenhizen [b], Matt Hodges [d], Matt Jones [e], Kunal Gupta [a,b,c,*]

[a] Medical College of Wisconsin, Department of Neurosurgery, 8701 Watertown Plank Road, Milwaukee, WI, 53226
[b] Medical College of Wisconsin, Neuroscience Research Center, 8701 Watertown Plank Road, Milwaukee, WI, 53226
[c] Medical College of Wisconsin, Department of Cell biology, Neurobiology and Anatomy, 8701 Watertown Plank Road, Milwaukee, WI, 53226
[d] Medical College of Wisconsin, Department of Physiology, 8701 Watertown Plank Road, Milwaukee, WI, 53226
[e] University of Wisconsin-Madison, Department of Neuroscience, 5531 WIMR, 1111 Highland Ave, Madison, WI, 53705

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Placement of hippocampal depth electrodes for long term recording of hippocampal electrocorticography (ECOG) and computational analysis of epileptiform activity

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ABSTRACT

Electrographic recording of brain activity through either surface electrodes (electroencephalography, EEG) or implanted electrodes (electrocorticography, ECOG) are valuable research tools in neuroscience across many disciplines, including epilepsy, sleep science and more. Research techniques to perform recordings in rodents are wide-ranging and often require custom parts that may not be readily available. Moreover, the information required to connect individual components is often limited and can therefore be challenging to implement. The quantity of data obtained can also be large and therefore difficult to analyze manually, and existing software detection tools are often task specific and require extensive coding experience to use. In this methods paper, we provide step-by-step instructions using off-the-shelf parts for electrographic recording in mice using intracerebral depth electrodes. We also provide a novel software-based detection tool that requires limited prior coding knowledge to use and with detection parameters that can be easily customized. The method is summarized as follows:

- The electrode unit is assembled and implanted;
- Recordings are obtained and analyzed using the novel software tool;
- This method was validated using recordings taken during status epilepticus and chronic epilepsy in the intrahippocampal kainate mouse model of temporal lobe epilepsy

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* Corresponding author.
E-mail address: kugupta@mcw.edu (K. Gupta).
Social media: @kunalguptalab (K. Gupta)
# denotes authors contributed equally to the work.

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Resource availability:	Code housed at: https://github.com/kg244/SpikeDetector. Equipment list available in Table 1

Background

Electroencephalography (EEG) and electrocorticography (ECOG) are techniques used to record the electrical activity of the brain. These techniques have been used as a mainstay to study epilepsy [1,2], as well as sleep-wake cycles [3], traumatic brain injuries [4], and pharmaceutical effects [5]. Electrographic recordings can be performed using different methodologies, including scalp-based electrodes or epidural screws placed through the skull (EEG), or brain surface grids or depth electrodes inserted into the brain (ECOG). Several techniques have been described to perform these, including subdermal needle electrodes [6], cortical electrode arrays [7], and skull based microelectrode arrays [1]. Minimally invasive needle electrodes can reduce procedure and sedation times and improve post-implantation mobility, especially in combination with wireless transmission technologies [6]. Recording systems can also be combined with intravenous access to allow for intravenous anesthetic administration through the external jugular vein and anesthesia monitoring by EEG [7]. Minimally invasive methods that do not penetrate the brain leave tissue intact for *post hoc* histology, however, may have lower signal-to-noise ratio and specificity of recording localization. Intracerebral recordings through intracranial depth electrodes, while more invasive, potentially offer higher signal-to-noise ratio and improved recording localization.

Previously described techniques for research electrographic recording, however, are often challenging to replicate due to custom parts that require fabrication, are obsolete or only available in limited markets, such as custom gold wire electrodes [7] or recording cages [1]. Data interpretation can also be challenging due to the duration of recordings involved and myriad recording systems. As a result, there have been efforts made to generate automated data interpretation software pipelines that can reduce analysis time

Table 1
Equipment list

General equipment			
Small animal stereotax and drill Gas anesthesia: vaporizer, oxygen supply Basic surgical tools Fume hood access Construction tools: drill vise, wire cutters (Hakko CHP-170), soldering iron (Hakko FX888-D), sandpaper (100-200 grit)			
Electrode construction and implantation			
Item	Item code	Vendor	Source
Bipolar teflon coated steel twist electrode	E363/3-2TW/SPC ELEC .005/.125MM 55 2TW 20mm length	Protech International	https://protechinternational.com
26AWG copper wire	ECW26AWG025LB	BNTechGo	Amazon.com
Female brass socket	E363/0	Protech International	https://protechinternational.com
0.015" Diameter Rosin Flux Core Lead-Free Solder	EST-0.4LDF50	EnerSystec	Amazon.com
6 Channel Delrin pedestal	MS363	Protech International	https://protechinternational.com
6 Channel pedestal mounting holder	MH363	Protech International	https://protechinternational.com
Stereotax probe holder	51643	Stoelting Co	https://stoeltingco.com
Cyanoacrylate glue	102433	The Gorilla Glue Co.	Amazon.com
Glue setting accelerant	Insta-Set™ CA Accelerator BSI-151	Bob Smith Inc	Amazon.com
Dental cement	51458	Stoelting Co	https://stoeltingco.com
Surgical implantation and connection			
Item	Item code	Vendor	Source
Electrode implant / commutator to touchproof 363-441 recording cable	363441/6XXXX002	Protech International	https://protechinternational.com
Electrode implant to commutator 363-363 recording cable	363363XXXXCM001-100CM	Protech International	https://protechinternational.com
6 Channel commutator	8BSL6CXC0MMT	Protech International	https://protechinternational.com
Commutator holder	PT/CH-001	Texas Scientific Instruments, LLC	https://protechinternational.com
EEG Amplifier	EEG100C	Biopac Systems Inc	https://www.biopac.com
Touchproof jump cable	JUMP100C	Biopac Systems Inc	https://www.biopac.com
Data acquisition system	MP160WSW	Biopac Systems Inc	https://www.biopac.com
37-pin Male/Female D-Sub cable	CS-DSSMDB37MF-005	Amphenol	Amazon.com

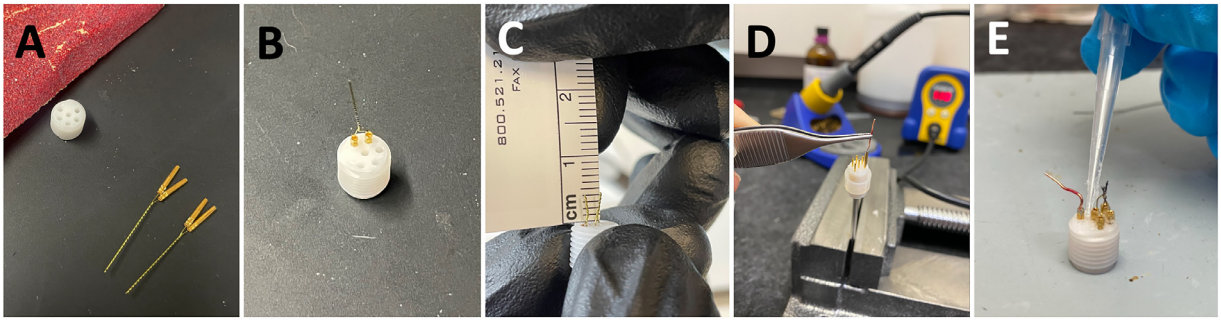

Fig. 1. Preparation of electrode implant. A) The skull-facing surface of the pedestal is abraded with sandpaper to enhance adhesion. B) The bipolar twist electrodes are placed in the appropriate holes. C) The electrodes are trimmed to length. D) The monopolar ground electrode is prepared. E). The electrodes are secured in the pedestal with cyanoacrylate glue.

and standardize outcomes. Many of these software scripts are highly customized, using advanced MatLab algorithms for seizure interpretation in epilepsy models for example [2,8]. These tools, however, can be challenging to implement without a high-level of technical expertise and may offer limited potential for customization to different paradigms (e.g. status epilepticus versus chronic epilepsy). In the context of epilepsy, epilepsy-related spike frequencies and burst patterns are well defined [9,10], and these definitions can be used to identify epileptiform activity in seizure models and after potential therapeutic interventions. While these data can be interpreted manually through trained pattern recognition and parameter validation [11], these same parameters could also be used to identify epochs of epileptiform activity to increase the speed and sensitivity of electrographic data interpretation using semi-automated detection algorithms.

To address these issues, we describe an ambulatory *in vivo* ECOG setup that uses off-the-shelf commercially available supplies, with step-by-step instructions from electrode preparation to recording electrical brain activity in mice. We also provide a novel semi-automated computational tool for extracting epochs of epileptiform activity from large datasets, with user-defined parameters that can be altered as needed.

Method details

Animal husbandry and ethics

C57Bl6/J mice (Jax #000664) underwent injection of intrahippocampal kainate (IHK) and bilateral hippocampal depth electrode placement at 6-8 weeks old. The IHK procedure was performed as described previously [11–13]. Mice were housed singly for electrographic recordings at constant temperature and humidity in a 10hr dark/14hr light cycle with food and water *ad libitum*. All animal procedures were performed in accordance with the NIH *Guide for the care and use of laboratory animals* and were approved by the local Institutional Animal Care and Use Committee.

Overview

In this section, we describe the implantation of bipolar depth electrodes into the bilateral hippocampus for ECOG recording. For a comprehensive method describing EEG recording with skull-based epidural screw electrodes, we direct the reader to Medlej et al [14]. Herein, we describe the equipment required, electrode preparation steps and nuances, surgical implantation steps and nuances, and methodology to connect implanted depth electrodes to the Epoch BioPac data-acquisition system (DACs) and initiate recording. We also provide a novel data analysis tool with instruction and validation. We found that these individual steps were previously insufficiently described, and, to our knowledge, there is no existing single description of all steps required to perform ECOG recording with this methodology. This methodology represents an extensive modification of a previously described technique [11], now utilizing wired recording and a novel semi-automated seizure detection software tool. The techniques and tools can be adapted as needed to other variations, such as different electrodes or DACs and different analysis parameters.

Preparation of depth electrodes

In this section, we describe the preparation of the depth electrodes and implant. For preparation of the recording implant, this requires a 6-channel Delrin pedestal (MS-363, Protech Int) to accommodate the electrodes, a bipolar twist electrode (E363/3-2TW/SPC .005"/.125MM 2TW, Protech Int) for each recording target, sandpaper (100-200 grit) and cyanoacrylate liquid glue (102433, The Gorilla Glue Company).

The electrodes and pedestal are obtained and the animal facing surface of the pedestal is abraded using the sandpaper (Fig. 1A); this improves adhesion to the glue and dental cement used to affix the electrodes and pedestal to the head. This surface can be identified easily as the holes are larger to accommodate the brass sockets of the implanted electrodes. The bipolar twist electrodes are then placed into the pedestal openings (Fig. 1B). For hippocampal recordings, the electrode sockets are placed in adjacent paired

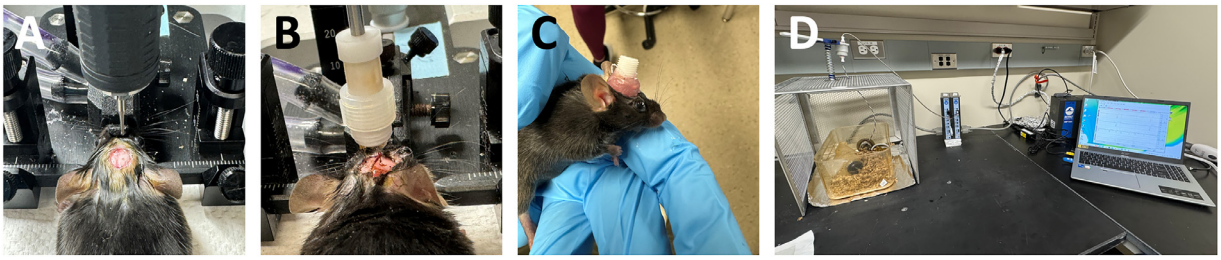

Fig. 2. Surgical placement of the electrode implant. A) The scalp is excised as an ellipse and burr holes are placed for passage of the electrodes into the brain. B) The electrode implant is mounted onto the holder, which is secured in the stereotax instrument holder. The electrodes are lowered into the brain through the burr holes. C) The electrode implant is secured to the skull with dental cement. D) The mouse is connected to the apparatus for electrographic recordings.

holes to provide approximately 3mm distance between the electrodes; this can be adjusted for different targets. Each wire is trimmed to 4mm using a ruler and wire cutters (Fig. 1C), and approximately 1mm of the distal end of the wire (implanted into the brain) is stripped of the Teflon insulation using a razor blade. Each animal also requires a ground electrode in addition to the bipolar twist electrodes; only a single ground per animal is required as this can be bridged between recording electrodes. The ground electrode is prepared from 10mm of 26Ga copper wire (Bntechgo, Amazon.com), 1-2mm of insulation is stripped from each end using a razor blade, and one end is soldered into a brass socket (E363/0, Protech Int), opposite the side which is inserted into the recording cable (Fig. 1D). Securing the socket/wire in a 6-channel mounting holder (MH363, Protech Int) can help facilitate soldering, and using a small amount of solder will prevent any runoff. The ends of the brass socket that will be inserted into the recording wire are widened slightly with a 16Ga hypodermic needle to facilitate connection / disconnection, these are tested on the mounting holder prior to implantation for fit. The ground wire brass socket is inserted into the appropriate channel on the pedestal and the wire angled towards the nuchal musculature of the mouse. The electrodes are secured to the pedestal using cyanoacrylate glue placed carefully with a pipette tip around each socket on the surface of the pedestal (Fig. 1E). Glue setting accelerant (Insta-Set CA Accelerator BSI-151, Bob Smith Inc) can be used. This stage completes preparation of the electrode recording unit and can be performed in advance. The electrode tips can be sterilized in 70% ethanol prior to implantation.

Depth electrode implantation surgery

The mouse is maintained under anesthesia, the head is shaved and the scalp prepared for sterility per IACUC-approved protocols. The scalp is excised in an ellipse of approximately the same area as the pedestal. Burr holes are drilled for passage of the depth electrodes, for dentate gyrus the coordinates used are ML±1.0, AP-1.8 relative to bregma (Fig. 2A). The assembled electrode implant is loosely secured to the mounting holder (MH363, Protech International), which is mounted on the stereotax electrode holder (51634, Stoelting Co.). The electrodes are lowered to the surface of the brain at the burr hole, the Z axis on the digital stereotax is zeroed, and the electrodes advanced 2.0mm into the brain to lower the tip of the electrodes into the dentate gyrus (Fig. 2B). The ground wire is positioned and placed subcutaneously at the neck. Dental cement (51458, Stoelting Co.) is then prepared. Appropriate safety equipment, including eye coverings, should be worn and cement preparation should be performed in a well-ventilated area. The liquid component is shaken prior to use, and the powder and liquid are mixed 1:1 in a 3ml syringe using the back end of a sterile-cotton tip applicator. To do this, the syringe plunger is removed, and the powder/liquid are backfilled; when replacing the plunger, cover the opening of the syringe to prevent inadvertent spraying of cement. The syringe is tilted back and forth to mix, the viscosity is monitored by observing an air bubble retained in the syringe. When the cement is sufficiently viscous to slow the bubble's movements, a small amount is extruded onto a paper towel. Once the extruded cement can retain its form, the space between the skull and the pedestal is filled with cement until the electrodes and wires are covered, up to the first 2 turns of the screw helix at the base of the pedestal (Fig. 2C). The cement is allowed to set for 5 minutes before carefully detaching the pedestal from the mounting holder. The mouse is then recovered in a warm chamber with food and water *ad libitum*. Analgesia is provided as recommended by IACUC-approved protocols, injectable analgesics can be administered under anesthesia prior to completion of surgery to reduce handling.

Connecting the circuit

Making the connections between the implanted electrodes and the DAC carries some nuances, and having a working knowledge of the required circuit connections greatly facilitates connectivity and troubleshooting. In the Epoch BioPac system, the head implant is connected by a recording cable to an intermediate amplifier module (EEG100C, Epoch BioPac), which is then connected to the data acquisition unit (MP160, Epoch BioPac); the MP160 is then connected to a computer by ethernet (via an ethernet-usb adaptor if needed) running the proprietary software package Acquisition (Epoch BioPac) (Fig. 2D). The head implant can be connected directly to the EEG100C amplifier unit or via a 6-channel commutator (SL6C, Protech Int), and the EEG100C amplifier unit can be connected directly into the MP160 DAC or using shielded 37-pin D-sub cables (Amphenol, Amazon.com). Two (or multiple) EEG100C amplifiers can be connected to create a functional recording unit for a single animal, with one unit used for each recording electrode. These can then be connected directly into the MP160 DAC or using 37-pin D-Sub cables for simultaneous recording of multiple animals (Fig. 3).

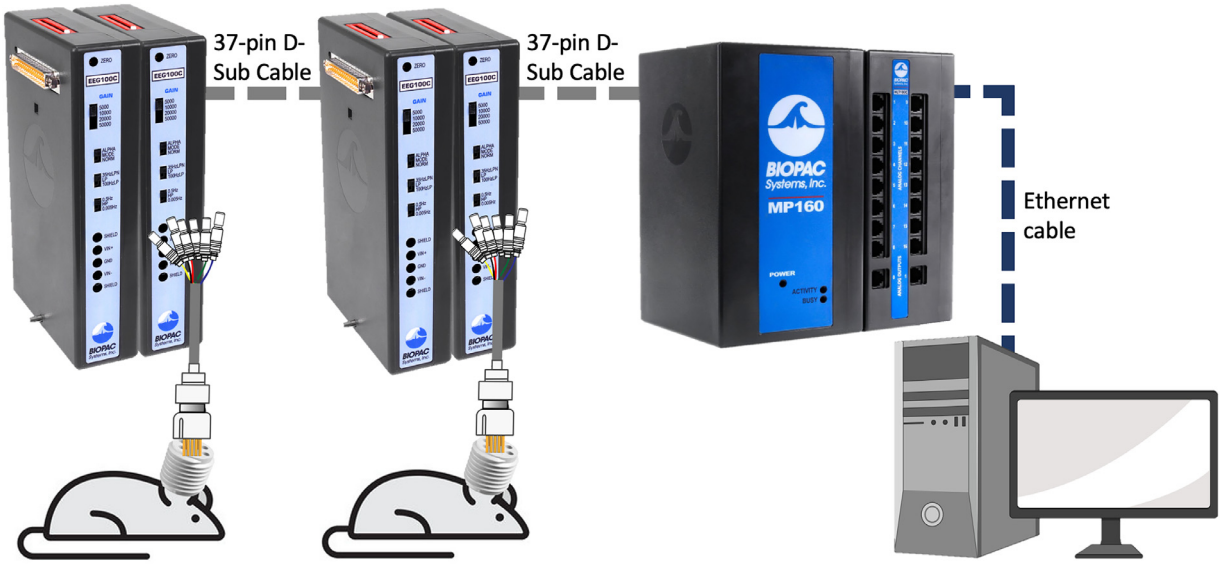

Fig. 3. Connection overview. Each electrode is connected to an EEG100C amplifier, which is then connected serially to the MP160 data acquisition unit. The MP160 is connected to a computer running the Acquisition software program for data recording.

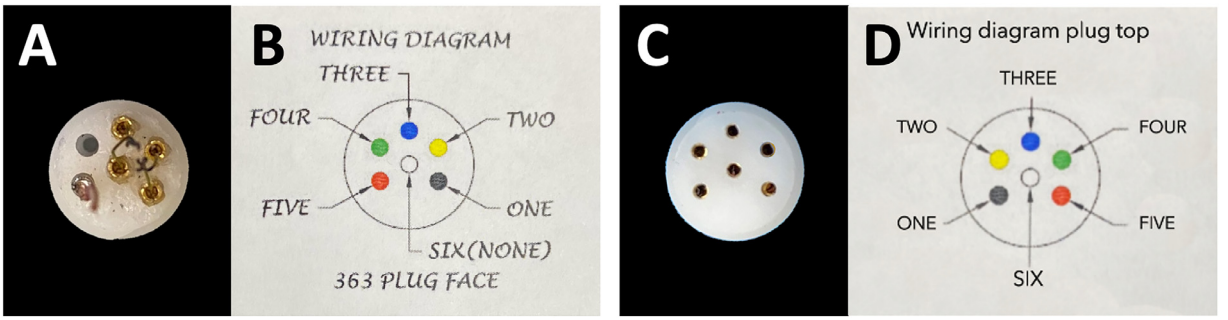

Fig. 4. Implant wiring diagrams for connection to the amplifier. A) The face of the implant when viewing the implanted electrodes. B) The wiring diagram supplied with the recording cable describes the plug face, which corresponds with the animal facing electrodes implanted into the brain. C) After implantation, the upper aspect of the implant is visualized on the top of the mouse's head. D) The wiring diagram is then reversed to match the implanted electrodes viewed from above with the corresponding-colored touch-proof wires on the connection cable.

The recording wire required to connect the electrode implant pedestal to the EEG100C amplifier has a proprietary connection for the pedestal, which carries the Protech moniker 363, and a touchproof connection for the EEG100C amplifier, which carries the Protech moniker 441. Therefore, the connection requires a 363-441 connecting cable. This cable can be requested with a wire sheath to prevent chewing damage but increases the weight of the cable. In our system, we use an intermediate unshielded 363-363 connecting cable attached to a commutator, which is then subsequently attached to the amplifier with a wire-shielded 363-441 connecting cable (Fig. 2D). We found that stripping the enrobing plastic sheath from the 363-363 cable attached to the animal reduces the weight further and increases the flexibility of the cable, improving animal mobility. The pattern of the recording head (363 end) is mapped to colored touchproof connectors (441 end); the image supplied by the vendor is of the plug face (Fig. 4B). When implanted on the mouse's head, this pattern must be reversed (Fig. 4D) to better understand the configuration of the electrodes as they pertain to the head of the connecting cable and the touchproof connectors.

For example, in the transmitter shown, there are two bipolar electrodes and one monopolar ground wire (Fig. 4A). Each of the brass sockets corresponds to a different colored touchproof connection on the recording wire depending on its location in the pedestal (Fig. 4B). The right sided recording electrode is located in the middle two blue and white sockets (Fig. 4A), which are marked as "three" (blue) and "six" (white) (Fig. 4B). When placed on the head, the blue and white corresponding sockets are on the right (Fig. 4C), so these wires are connected to VIN- and VIN+ on the EEG100C amplifier for the right hemisphere. The left sided recording electrode is located in the yellow and black sockets (Fig. 4A), which are marked as "two" (yellow) and "one" (black) (Fig. 3B).

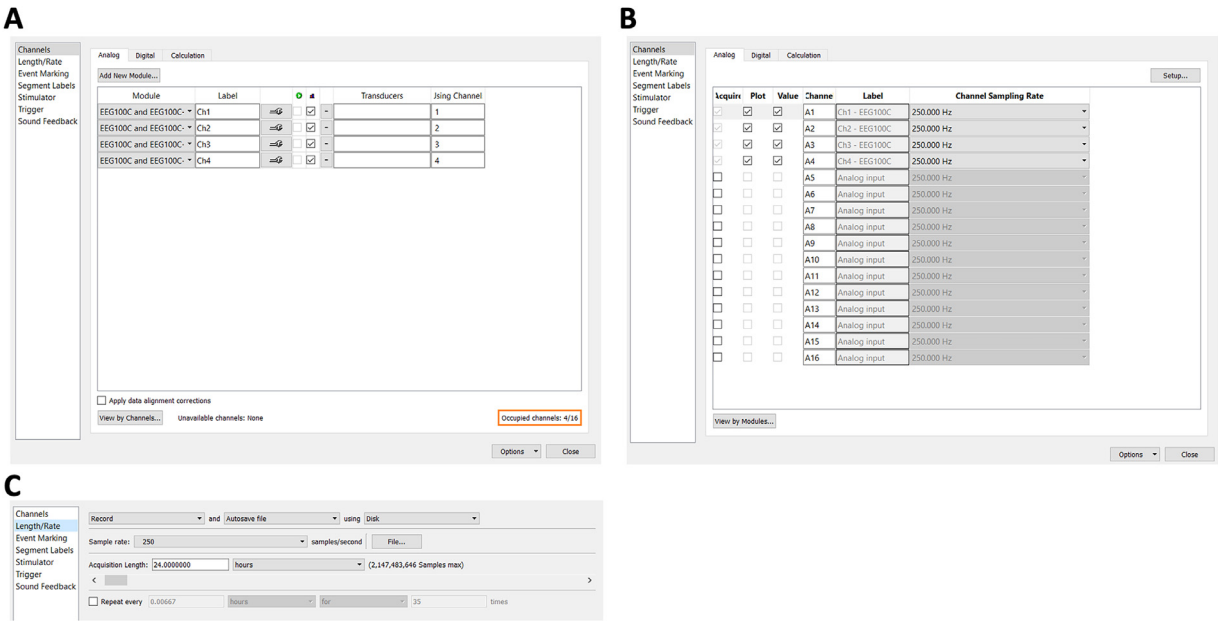

Fig. 5. Initiating recordings in BioPac Acquistion. A) Each EEG100C amplifier is added in the channels / modules window and labeled. B) Sampling rates are allocated for each amplifier. C) Recording length, save locations and repeats are set in the length/rate window.

When placed on the head, the yellow and black corresponding sockets are on the left (Fig. 4C), so these wires are connected to VIN- and VIN+ on the EEG100C amplifier for the left hemisphere. The ground wire is recorded by the red cable (Fig. 4A), labeled “five” (Fig. 4B). The red cable is then plugged into the GND port on the left amplifier. Remapping the pattern to represent the plug top facilitates connections and troubleshooting (Fig. 4D).

Having connected the recording wire to the implant and mapped the implanted electrodes to the correctly colored touchproof connections, these need to be correctly connected into the EEG100C amplifier. Each EEG100C amplifier module manages a single input channel; recordings are performed in a bipolar configuration and therefore require bipolar electrode connections to record a single channel. The recording channel is connected into the VIN+ channel and the reference into the VIN- channel. The ground electrode is connected into the GND channel. The two GND connections are bridged with a touchproof jump cable (JUMP100C, Epoch BioPac, also commercially available) as only one ground is needed per animal. Bridging the ground electrode is not required for recording a single animal, as the ground will be shared between the EEG100C units automatically. If multiple animals are being recorded simultaneously, each animal requires a ground electrode. The EEG100C amplifiers are then connected to the input channels in the MP160 DAC. Our EEG100C units are set to Gain 10000, normal mode, high pass filter 0.5Hz to eliminate baseline variability from slow waves, low pass filter 35Hz to eliminate mains electrical frequencies; selective notch filters can also be applied for this purpose.

Acquisition of electrographic recordings

With the animal connected to the recording system, the DAC is powered on and the Acquisition program initiated on the computer. To set up the initial recording, a new template is opened. “Set-up” and “data acquisition” are selected from the menu to open the set-up window. The new window is toggled to modules and each EEG100C amplifier is registered to an input channel and labeled (Fig. 5A), with each input channel corresponding to an implanted electrode (Fig. 5B). The sampling rate is set (we use 250Hz, which, with a low pass filter of 35Hz, exceeds the Nyquist rate), and the duration of recording can be modified for duration and repeat recordings, for example to create hourly files for 24-hours, or 24-hour files for 21 days (Fig. 5C). These settings can be saved as a template, which can facilitate repeated recordings if the same experimental set-up is reused. The window is closed and recordings begun by clicking start, and selecting a file save location.

Creation of the semi-automated seizure detection tool

A custom analysis script was developed in Python (version 3.11.7) to analyze EEG recordings and detect potential epileptiform activity. The script leveraged the xlwings library (Open Source) to enable user-defined parameter input through a Microsoft Excel interface, allowing flexible adjustments for key parameters. The script extracts channel-specific EEG data from the Biopac .acq files

using the bioread module (developed by the Board of Regents of the University of Wisconsin System). Extracted data are then imported into a Pandas DataFrame for efficient processing and organization by channel.

Seizure Detection Algorithm

An automated seizure detection algorithm was implemented based on a sliding window approach. Using custom length windows advanced at user-defined fractional increments across the dataset, the algorithm analyzes each window to identify potential epileptiform activity. The software is designed to flag waveforms that meet user-defined frequency and amplitude criteria. An iterative algorithm reviews adjacent flagged windows to capture seizure activity that may span multiple windows without individually meeting the threshold criteria in each. When two or more adjacent windows collectively indicate a pattern consistent with continuous epileptiform activity, they are merged into a single seizure event. This approach enables comprehensive identification of seizure episodes while avoiding duplicate counts.

Output and Visualization

For each data file, an Excel output is generated with separate data logs for each EEG channel. Each log provides a table summarizing detected epileptiform events, with columns for the start time, end time, and duration (in seconds) of each waveform that meet the criteria for epileptiform activity. Additionally, individual plots are generated for each channel using Matplotlib and Plotly (Plotly Technologies, Inc.). These plots represent time (seconds) on the x-axis and amplitude (mV) on the y-axis. Waveforms identified as meeting epileptiform criteria are highlighted in red on the plots to facilitate further review and, if necessary, exclusion. Individual data ranges can be expanded easily for review and images exported for reporting.

Instructions for use of the software tool

The installation files are hosted at GitHub and require Python and the xlwings add-on for Microsoft Excel to run the graphical user interface (GUI). Data files are placed in the 'data' subfolder, the file list is refreshed in the associated Excel GUI; individual files can then be selected or the whole folder can be batch processed. The principal feature of this software tool is the ability to adjust detection settings as needed. Window size is set for event detection and the slide size interval defines the overlap in detection between frames; reducing intervals improves resolution but requires increased processing time. For our analyses we used a 2 second window size and 0.5 second sliding window. The sampling rate of the file, set previously in BioPac Acquisition during recording acquisition, is provided, as are the desired frequency range and amplitude of spikes. Downsample factor is solely for graphics creation and can be adjusted for data size and computing power; for example, 1 will graph all data and 4 will graph 25% of the data.

Method validation

We developed these methods to efficiently capture epileptiform activity from the mouse hippocampus using readily accessible materials. To validate our methods, we utilized the well-established intrahippocampal kainate (IHK) mouse model of mesial temporal lobe epilepsy. In this model, injection of kainate into hippocampus results in an acute period of status epilepticus, defined as convulsive seizures (Racine 4-6) with <5min interval and without recovery for at least 30 mins [15,16]. After recovery, mice then enter a latent period for 10-14 days, after which they develop spontaneous recurrent seizures [10,17,18]. IHK surgery was performed on 8-week-old mice as described previously [11,13]. Mice were then implanted with bilateral intrahippocampal depth electrodes for ECOG recording as described herein. All data analyses were applied to the ipsilateral hippocampus.

We were able to effectively capture electrographic brain activity using this methodology. We focused our analyses on 2 important epochs during epileptogenesis, the initial period of status epilepticus immediately after IHK and the onset of spontaneous seizures 14 days after IHK. Epileptiform activity was defined using previously established criteria; status epilepticus (SE) was defined by established criteria as periods of epileptiform activity exceeding 12 seconds in duration with at least 0.8Hz spike rate and 0.5mV amplitude [15], and epileptiform activity during the chronic epilepsy phase was defined by the presence of hippocampal high voltage spike waves defined as at least 5 seconds of 2-5Hz spike rate and 0.5mV amplitude (at least 3x baseline) based on established criteria [9,10].

In the immediate 24-hours following IHK, we identified rhythmic spiking epileptiform activity consistent with status epilepticus (Fig. 6A). When analyzed manually, the duration of electrographic activity meeting criteria for SE was approximately 23 hours in duration. We repeated the analysis with the semi-automated software tool, utilizing the same parameters. Spikes were readily identified (Fig. 6B), and the duration of electrographic activity meeting criteria for SE was calculated as approximately 21.6 hours, an error margin of 6%. From review of the manual and semi-automated software-based detection datasets, it was apparent that within prolonged periods considered manually to meet criteria for status epilepticus, the software tool excluded focal areas where electrographic spike rates fell transiently below the detection threshold, periods that were otherwise included in the manual review. We also analyzed epileptiform activity at the onset of spontaneous recurrent seizures, 14 days after IHK (Fig. 7A). The software tool detection algorithm identified several epochs of epileptiform activity meeting detection criteria (Fig. 7B), and omitted periods

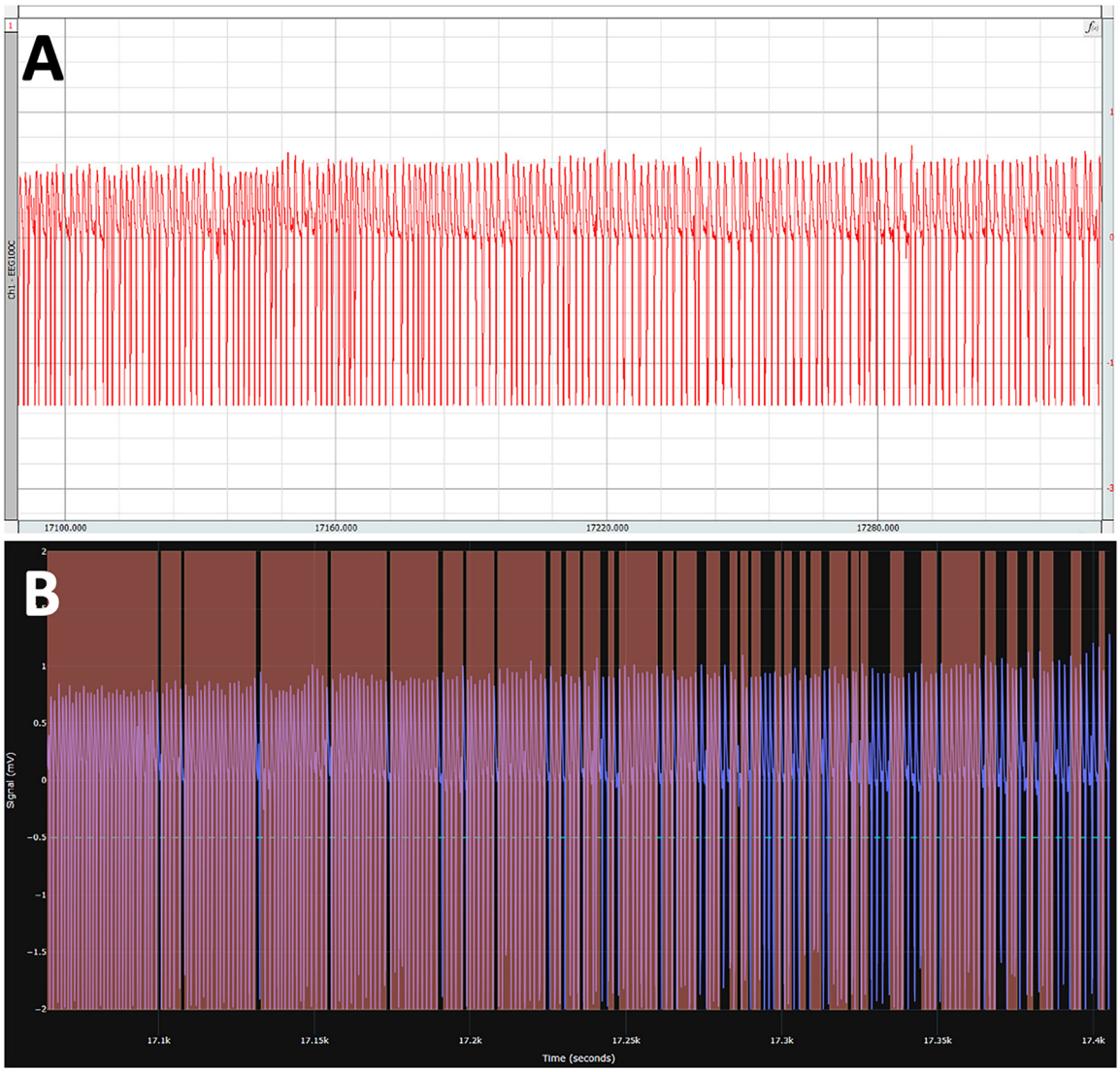

Fig. 6. Semi-automated computerized detection of epileptiform activity during status epilepticus. A) During manual review, >1Hz frequency spikes are observed during status epilepticus after IHK. Each individual spike must be quantified for frequency to determine if the observed activity meets defined criteria. B) The same period of this recording is evaluated using the seizure detection software tool to identify electrographic activity that meets the input criteria for status epilepticus (areas shaded red). The areas without red shading show electrographic spikes that fall below the required frequency, demonstrating a gradual reduction in overall firing rate during this period which was not readily apparent through manual review alone.

consistent with interictal discharges (Fig. 7C). We manually evaluated the same recording period and compared data collection modalities (Fig. 7D); most event periods (9 of 11) were correctly identified by the detection tool with <5% omission of total detected epileptiform activity by duration.

These methods were established to capture epileptiform activity but are broadly applicable for monitoring electrical activity of the mouse brain. The placement of intrahippocampal depth electrodes, while invasive, improves recording localization and potentially signal-to-noise ratio. Previously published methods utilize custom and expensive materials that can be difficult to obtain; the present method utilizes readily accessible and comparatively less expensive materials. Our novel seizure detection algorithm requires minimal technical knowledge to implement, utilizes open-source tools, and detection settings can be readily modified by the user. It provides an output visualization interface that can be manipulated for spike review and quantified data output in Excel worksheets.

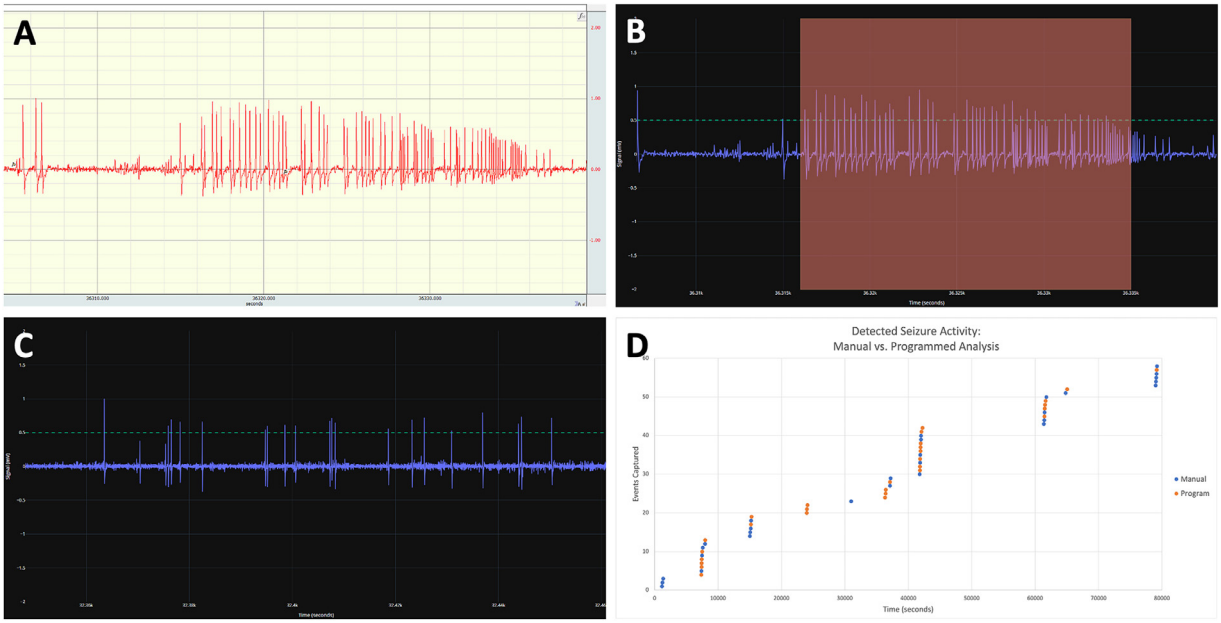

Fig. 7. Semi-automated computerized detection of epileptiform activity during chronic epilepsy. A) During manual review, spikes consistent with epileptiform activity were flagged. B) Using the seizure detection software tool, this same flagged region was automatically detected and delineated by the red box. C) Regions consistent with interictal spikes were, appropriately, not flagged using the software tool. D) Periods consistent with epileptiform activity were identified by manual review and compared with epochs identified by the seizure detection software tool. The semi-automated tool correctly identified 9 of 11 activity periods identified on manual review.

Limitations

There are several limitations to these methods. The methods utilized in this study require the manual production of transmitters, which can be time consuming. Changes in availability of these materials over time may also require revisions in technique. The surgical placement of intrahippocampal depth electrodes carries the risk of mortality, tissue trauma and infection. Additionally, the pedestal which holds the electrodes in place is secured with dental cement, which does not always guarantee secure placement for the duration of each experiment, especially during active seizures. Despite this, in our experience the implants do typically remain securely in place for prolonged recordings. The method also utilizes a tethered recording system, which can limit the animals' ambulatory experience. There are also artifacts and recording noise events captured by the system. The size of the implant limits the density of the array, these limitations are also inherent to the recording system. There are higher channel count implants and systems available, however at considerably higher complexity and expense. Our novel software tool, though customizable, does not provide advanced pattern recognition nor AI learning capabilities. The graphics interface also does not currently offer tools to quantify individual spike ranges; these could be added in future updates.

Ethics statements

Experiments complied with the ARRIVE guidelines and were carried out in accordance with *the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978) and* were approved by the local Institutional Animal Care and Use Committees (MCW #AUA0008221). Male and female mice were used equally with no impact on methods.

CRediT author statement

Cora Helton writing, investigation. **Nicole Rodgers:** writing, investigation. **Payton Klosa:** analysis. **Erik VanNewenhizen:** software. **Matt Hodges:** writing review and editing. **Matt Jones:** writing review and editing. **Kunal Gupta:** Conceptualization, methodology, investigation, analysis, resources, writing, supervision, funding.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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