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Original Article

Fabrication of a Cost-Effective in-House Electrode Assembly for Recording EEG and Behavioral Seizures in Unrestrained Rats

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ABSTRACT

Background: Electroencephalography (EEG) is a valuable tool in neuroscience research for analyzing epileptic seizures in animal models. However, the cost that comes with commercial electrode assemblies is a critical challenge, particularly in low- and limited-resource settings. Thus, developing an inexpensive but dependable substitute is critical for improving access to epilepsy pre-clinical research. **Materials and Methods:** The research article here discusses a systematic methodology for designing and developing a low-cost electrode system for EEG recording and behavioral seizure monitoring in freely moving rats. The electrode assembly utilizes readily available materials significantly reducing the costs without compromising the data integrity. The electrode fixation ensures stable signal acquisition with reduced signal loss in experiments. **Results:** The stability and performance of internally assembled electrode setup was evaluated during spontaneous and induced seizures in rat models of epilepsy. Our research findings demonstrate that these electrodes were reliable for repeatedly detecting the epileptiform activity, in regards to signal quality and seizure evaluation accuracy. **Conclusion:** The proposed low-cost electrode structure offers a viable alternative to costly commercial arrangements, making EEG-based seizure analysis more accessible to researchers working within budget restrictions or in limited resource facilities. By providing high-quality EEG recordings and behavioural tests, this method contributes to a better understanding of seizure pathophysiology and supports preclinical research on epilepsy employing animal models.

Keywords: Electroencephalography (EEG), Seizures, Electrode Configuration, Wired EEG, Low-Cost Electrode.

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INTRODUCTION

Aberrant highly synchronous electrical activity in the brain cells, leading to seizures, is a hallmark of neurological disorders, including epilepsy. Accurate diagnosis is essential for effective treatment. Various neuroimaging and electrophysiological techniques, such as functional Magnetic Resonance Imaging (fMRI), Computed Tomography (CT) scan, Magnetoencephalography (MEG), and Electroencephalography (EEG), can be employed to detect brain disorders.

EEG is one of the major diagnostic techniques for seizure disorders. It captures the summation of the excitatory and inhibitory potentials in the post-synaptic neurons firing synchronously. The method operates by capturing differences in potentials from the point of electrodes to produce EEG waveforms, hence becoming a major tool in the evaluation of dynamic cerebral function.[1,2] It facilitates seizure monitoring, differential diagnosis, and determination of seizure type and frequency. EEG also has various applications, such as the determination of syndrome-specific alterations, the probability of recurrence following an unprovoked seizure, the detection of convulsive and non-convulsive status, selection of antiepileptic medication.[3] EEG is commonly used in preclinical studies to investigate seizure patterns in rodent models of human epilepsy, brain injuries and encephalopathies.[4,5]

Currently, there are wired (or tethered) and wireless EEG systems available for rodent models, each with its pros and cons. The wireless systems limit cable disruption but are costly and support a limited number of electrodes, making it challenging for large-scale studies. On the contrary, wired systems are more affordable and widely used, but are prone to cable disconnections due to animal mobility, leading to the loss of data and termination of experiments.[6] The low signal-to-noise ratio, which is brought

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on by environmental disturbances and motion artefacts, presents another technical difficulty. Due to the tethered EEG system being inexpensive, it remains the most widely employed technique to record EEG in rodents.[4]

This study provides a detailed protocol for fabricating and assembling a cost-effective electrode setup for recording EEG in freely moving rats using readily available materials. The assembled electrodes were developed prioritizing the animal's comfort.[7] Use of completely insulated recording wires minimizes the signal loss. In comparison to the screw setup, direct implantation of wired electrodes minimizes surgical challenges such as tissue damage and bleeding.[7] The use of computer pins aided in reducing the total weight of the assembly to 2 g, resulting in comfort and signal stability.[8] Our study proposed an approach that provides high-quality EEG recordings with accuracy and reliability, and provides a viable and cost-efficient option for preclinical epilepsy studies.[8]

While EEG is very important in epilepsy research, commercially available electrode systems present significant hindrances due to their high cost, technical limitations and logistical inconvenience. Although wireless EEG systems are free from cable disruption, but their high costs and limited electrode capacity, make them less preferable for large scale studies. On the other hand, wired EEG systems have low-cost but results in issues like signal loss, motion artifact, and animal-related cable disconnection leading to compromised data quality and significant experimental inefficiencies. The present research addresses these gaps by providing a low-cost, stable, and minimally invasive EEG electrode setup that improves data reliability, and maximizes the application value of preclinical epilepsy models.

MATERIALS AND METHODS

Male Sprague-Dawley rats (180-315 g) were procured from Institutional Animal facility of ACBR, University of Delhi. Ethical approval to conduct the study was obtained from Animal Ethics Committee, ACBR (File No: 09/IAEC/ACBR/December 2022). Animals were housed in separate cages with 12-hr light/dark cycle and temperature maintained at 22-25°C. The adequate food and water were provided to all the animals *ad libitum*.

A total of 12 male SD rats were utilized and randomly divided into 2 groups (n=6/group): a control and a Temporal Lobe Epilepsy (TLE) group. The control group were given saline and TLE rats were treated with appropriate dose of lithium-pilocarpine to induce Status Epilepticus (SE). The same 6-pin electrode assembly design, implantation coordinates, and recording configuration were used for both control and TLE groups to ensure uniformity and eliminate hardware-related variability.

Electrode Assembly Construction

A 6-channel electrode assembly (merged into three channels) was built using male-to-female jumper cables with tin-plated annealed copper wire pins.[9] The assembled electrodes were autoclaved and UV-sterilized and were stored under sterile conditions. Electrode implantation involved stereotaxic surgery: two electrodes in the parietal area, one on each hemisphere (2 mm anterior to bregma), two in the temporal bone (3 mm posterior to bregma), a ground electrode in the occipital area, and a reference electrode in the ipsilateral corpus callosum (Figure 1A).[10]

The electrodes were made using the Jumper wires (male to female) with a breadboard and a 3-mm pin. The male connectors plugged into standard 0.1-inch (2.54 mm) female sockets, while the female connectors accommodate standard 0.1-inch (2.54 mm) male headers. Male connectors and female receptors were connected securely. By the use of surgical tweezers, electrodes were aligned and glued together using superglue, to create a stable three pin sets. A group of 3 similar pins was attached on top of these 3 pins. A wire was attached to each pin, leading to a female output, with careful soldering to avoid signal loss. To achieve the optimal electrode length, male-to-male and female-to-female jumpers were employed. The final construction was light in weight (2 g) and constructed for stability during recording EEG.

EEG electrode assembly implantation by stereotaxic surgery

All procedures were conducted in a sterile environment.

All procedures were conducted in a sterile environment. Rats were anesthetized using ketamine (80 mg/kg) and xylazine (10 mg/kg) injected intraperitoneally. A thermostat was used to maintain the body temperature. A sterilized trimmer was used to shave the scalp and rat was mounted onto the stereotaxic frame (Stoelting CO., USA). To minimize discomfort, lignocaine was applied to the ear bars and ophthalmic ointment (Carboxymethylcellulose) was applied over the rat's eyes to prevent eye irritation. The scalp was sterilized using 70% ethanol, followed by 10% betadine and 4% chlorhexidine. A 3-cm midline incision was made using aseptic surgical blade (No. 22) from the nasion to the occipital region, exposing bregma and lambda. Coordinates for electrode placement were marked for drill[11,12] and a surgical high-speed drill was used for electrode insertion, ensuring minimal tissue damage. The skull surface was made rough using dental drill with a 1 mm crosscut burr, enhancing adhesion for dental cement (DPI RR Cold Cure, India) fixation. The electrode assembly was carefully positioned, with each pin placed 2 mm deep—just enough to contact but not penetrate the brain surface (Figure 2).[10] The electrodes were secured using glass ionomer cement. After cement solidification (5 min), the incision was closed with sutures. Rats were removed from stereotaxic frame and were placed in pre warmed recovery cages. Post-operative care included antibiotic (amoxicillin) administration for three days, subcutaneous

Ringer's lactate, and oral sucrose solution for hydration. Softened food was provided to facilitate feeding during recovery. Full recovery, marked by normal feeding, drinking, and activity levels, was achieved within 24 hr. Animals were monitored for general health, wound healing, and normal feeding behavior for 48 hr post-surgery prior to initiation of EEG recordings.

Acute TLE Model Generation

Post surgical implantation of electrodes, animals were allowed to recover for 48 hr. Li-Pilocarpine TLE model was generated, wherein Lithium Chloride was administered (127 mg/Kg, i.p.) 24 hr before pilocarpine treatment. Pilocarpine administered at a dose of 240 mg/Kg, i.p. to induce SE. Methyl Scopolamine (1 mg/Kg, i.p.) was injected 30 min before pilocarpine. Seizures were recorded.

Recording Integrated EEG

Since the Biopac leads cannot be connected directly to the fabricated electrode as the lead has a clip-on system. Therefore, the output wire from the electrode assembly was connected to disposable point electrodes (Cat # 2200 Series, B00037603, 3M India Ltd.) mounted on a wooden insulated board. The wires were entwined around the base of the surface electrodes pasted on the wooden board and soldered on the point electrode using solder flux to improve connectivity. The lead SS2L (Biopac Inc., USA) was clipped onto this electrode. The lead was connected to the Channel 1 of the MP36 data acquisition unit. The positive, negative, and ground wires were all interconnected identically. To record the integrated EEG from the rat, the Biopac equipment (MP36) was activated and the BSL4.2 software was launched. The lesson L03-EEG was chosen and the file name was entered. Meanwhile, the rat was placed inside a cage that had feed, a water bottle, and bedding on the floor (Figure 3).

The female output on the electrode assembly implanted on the head of the rat was connected to the male connecter relayed on the electrode on the wooden board. Once, the connections were made, the connectivity of the electrode was assessed using Electrode Check in the software.

To record EEG from freely moving rats, the software was calibrated using the Calibrate button, once the calibration was done successfully, the EEG recording was started. Integrated EEG along with alpha, beta, delta and theta waves was recorded for 3 hr. All recordings were conducted inside a soundproof room in a 12:12 light: dark cycle. We used a separate computer for EEG to avoid recording data overflow. For each recording session, the rat was transferred to the recording room and we waited for at least 30 min before recording EEG.

Analysis of the EEG data

The EEG data files were opened on Biopac Student Lab 4.0 software. The EEG trace was selected using the I cursor. As soon as the wave is selected the average frequency of the selected region is shown in the SC channel of the software, whereas the Amplitude of the Alpha waves in CH40, beta waves in CH41, delta waves in CH42 and theta waves in CH43. The recorded values were an average from 3 different time intervals (Figure 4). Minor motion artefacts were occasionally observed during active grooming or exploratory behavior. These segments were manually excluded from analysis using visual inspection within the Biopac software prior to frequency and amplitude quantification.

RESULTS

The electrode assembly remained stable throughout the 3-hr continuous recording sessions without significant signal attenuation or detachment. In animals monitored for up to 7

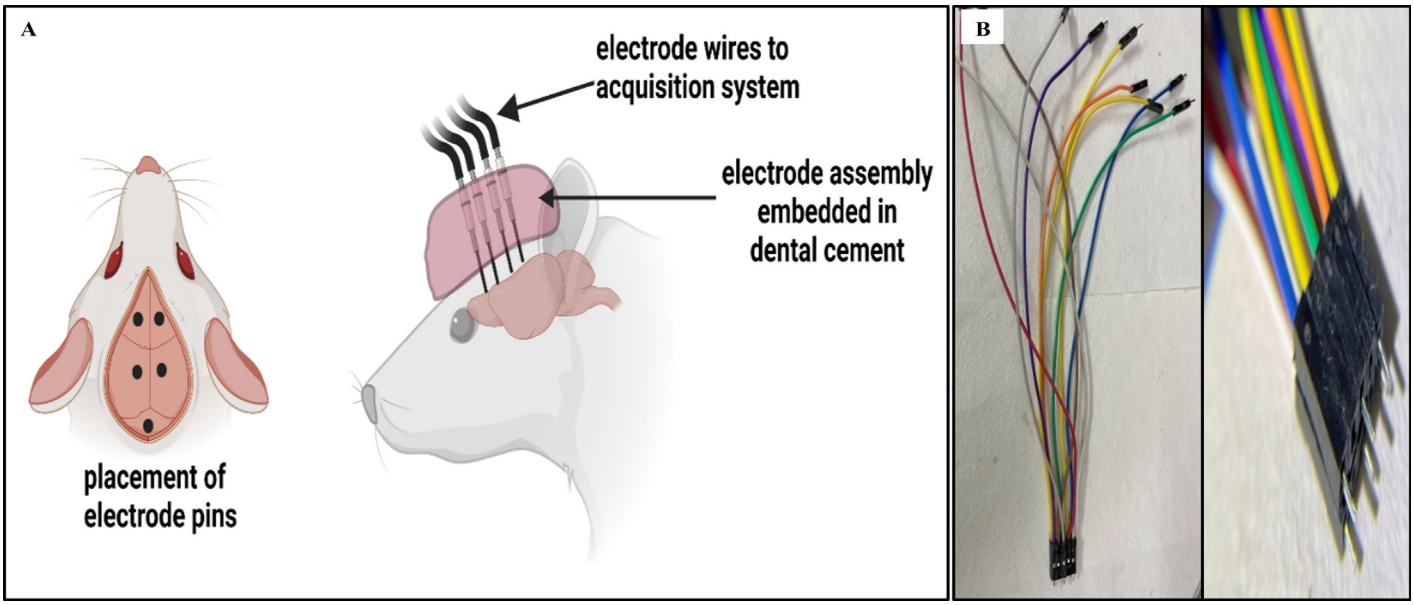


Figure 1: A: Placement of the electrode assembly on the head of the rat by stereotaxic surgery. B: Assembled 6-pin electrode assembly from jumper cables.

days post-implantation, the assembly-maintained signal integrity without evidence of structural loosening. No animals were excluded from analysis due to electrode failure, detachment, or excessive signal artefacts. EEG recordings were successfully obtained from both control and TLE animals using the in-house electrode assembly. Control rats exhibited normal baseline EEG rhythms with stable alpha, beta, delta, and theta activity. In contrast, pilocarpine-treated rats demonstrated characteristic epileptiform discharges including high-amplitude spike-and-wave activity and increased delta frequency power during seizure episodes (Figure 4). The signal-to-noise ratio was sufficient to clearly distinguish physiological rhythms from epileptiform activity. The qualitative characteristics of recorded signals were comparable to previously reported rodent EEG recordings using commercially available systems.

DISCUSSION

The present manuscript provides a detailed procedure for curation of the electrode assembly, implantation and recording of wired EEG from freely moving adult rats. Similar approaches have been used previously, but we present a novel material, that is computer jumper cables for fabrication of EEG electrodes. All the materials used in the procedure are readily available at e-commerce or regular computer stores. This method is cost-effective, reliable and at par with all other methods in terms of data output which are used for recording EEG in rodents. The electrode assembly is recommended for single-animal use to maintain sterility and optimal signal quality. Although certain non-implantable components may be reused after sterilization, the implanted electrode unit itself is intended for single use. Although optimized in adult Sprague-Dawley rats (180-315 g), the electrode assembly can be adapted for mice or neonatal rat pups by reducing pin length, assembly weight, and stereotaxic coordinates appropriate for species and age. The minimal invasiveness of the pin also improves mortality as we observed less than 10% mortality during or post-electrode implantation. The electrode assembly design allows adaptation for chronic recordings extending beyond the acute phase, provided regular inspection of cement stability and connector integrity is performed. Although this is a

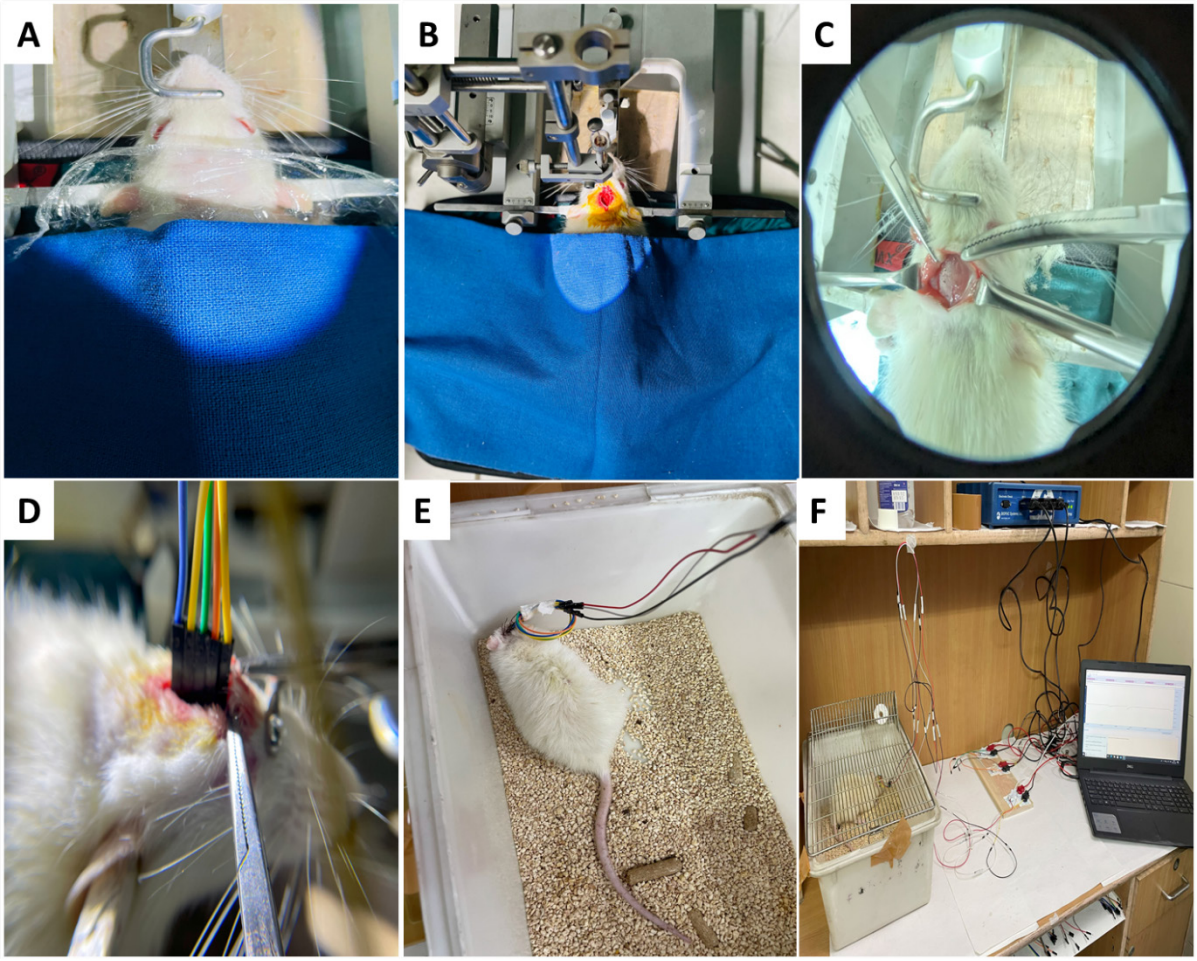


Figure 2: Schematic representation of electrode implantation by stereotaxic surgery and EEG recording; A: Rat's head fixed in stereotaxic frame; B: Midline incision to expose bregma and lambda; C: Marking the position of holes for electrode pins; D: Wired electrode implanted at the desired location; E: A freely moving rat in a recording cage connected to the recording cables; F: Wired EEG recording setup.

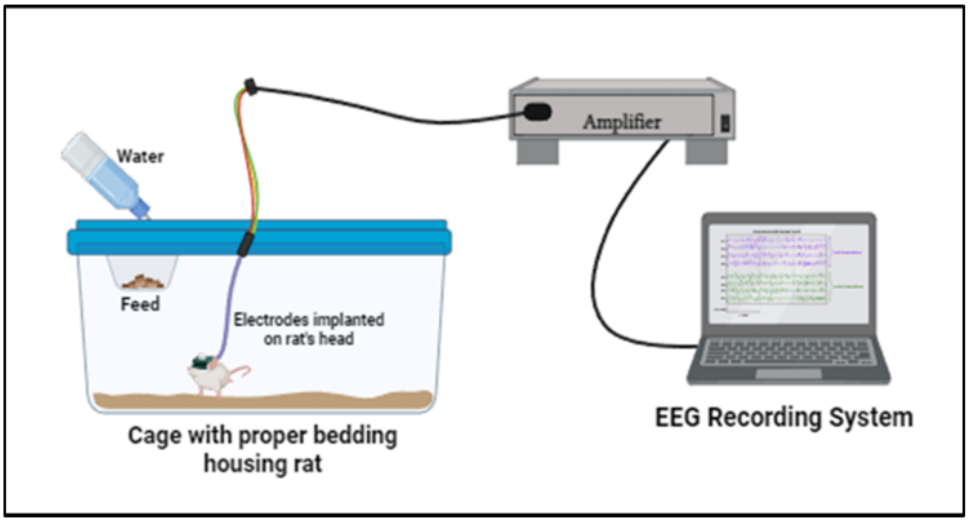
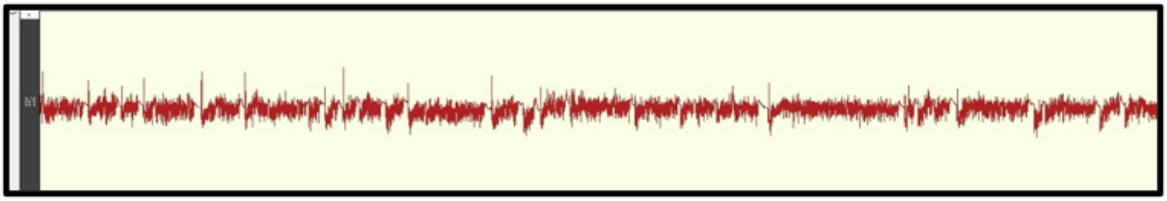
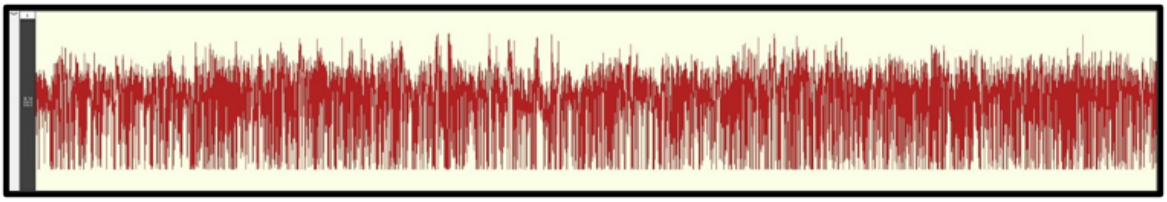


Figure 3: Schematic representation of EEG being recorded from a rat.



A



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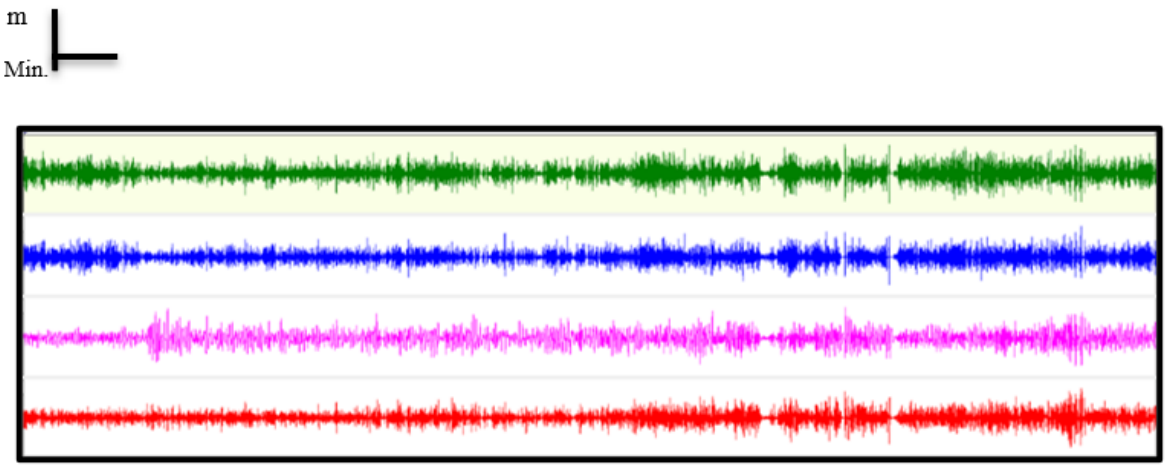

C

Figure 4: Representative traces of EEG recorded from the control (A) and Pilocarpine treated rat (B). All signals recorded were analyzable. (C): Traces of alpha (green), beta (blue), delta (pink) and theta (red) waves.

robust electrode assembly which can be kept in place for a longer duration it has its share of limitations.

Scope for Improvement

A wired EEG system is believed to be more stable due to its continued connectivity, and because of the increased data throughput in a particular period as compared to the wireless system. The connectivity in the wireless system is not ensured and there might be a loss of data, hence even though wireless EEG systems are emerging these days, wired EEG recording systems are more reliable.[13] By making little amendments in several areas, the performance of wired EEG systems for rodents may be enhanced. One such way is by reducing the size (or weight) of the electrode implanted, which would contribute to lowering the burden on the animal's head and hence would add to its comfort.[14] The high-durability electrodes can be curated in such a way that they are less intrusive, thereby minimising the damage to brain tissue during penetration.[15] For better conductivity, electrodes made up of silver material may be used, as it is a good conductor, but adds to the cost of setup. The resolution in the EEG recording system may also be enhanced by increasing the number of channels in the system, enabling the investigation of the complex regions of the brain. With the increase in period for monitoring the neural activity through EEG, chronic pathologies are well assessed, by simultaneously assessing the changes in the electrical activity of the brain.[16] Real-time analysis of signal processing would provide a more accurate picture of the neuronal activity. Integration of the EEG recording with imaging techniques such as fMRI would couple the information of one with the other, contributing to a more comprehensive study, and reliable findings. For artefact-free EEG tracings, correct electrode grounding and reference are critical for an enhanced signal-to-noise ratio.[4]

CONCLUSION

This study illustrates the effective design and production of an economical, in-house electrode assembly for recording EEG and behavioral seizures in freely moving rats. This electrode system employs accessible and cost-effective materials, offering a dependable and robust alternative to commercial assemblies without sacrificing data quality. The internal configuration facilitated precise identification of epileptiform activity, preserved signal stability across extended recordings, and reduced surgical difficulties and animal distress. This cost-effective technology enhances accessibility to preclinical epilepsy research in resource-constrained environments and provides a viable model that may be extended and enhanced for wider uses in neuroscience research. The created method serves as a practical and sustainable instrument for facilitating high-quality EEG-based research in rodent models.

ACKNOWLEDGEMENT

None.

ABBREVIATIONS

EEG: Electroencephalography; **fMRI:** Functional Magnetic Resonance Imaging; **CT:** Computed Tomography; **MEG:** Magnetoencephalography; **TLE:** Temporal Lobe Epilepsy; **SE:** Status Epilepticus; **SD:** Sprague-Dawley; **i.p.:** Intraperitoneal; **IAEC:** Institutional Animal Ethics Committee.

CONFLICT OF INTEREST STATEMENT

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

FUNDING

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ICMJE STATEMENT

All authors meet the ICMJE criteria for authorship. Specifically, the contributions of each author are as follows:

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

NY: Conceived and designed the study, conducted experiments, analyzed data, made figures and wrote the manuscript. **RM:** assisted in the data collection and wrote the manuscript. **PP:** assisted in the experimental design and data collection and made figures. **JB:** critically reviewed the manuscript, **AC:** Assisted in the experimental design and data collection. **ABD:** Supervised the research project, secured funding, provided resources, critically revised the manuscript and provided intellectual input. All authors read and approved the manuscript.

ETHICAL STATEMENT

All procedures were approved by the Institutional Animal Ethics Committee (IAEC) of ACBR, University of Delhi. (File No. IAEC/ACBR/March2024/AD/03).

SUMMARY

This paper presents the development of an economical, in-house EEG electrode assembly utilizing accessible materials for seizure monitoring in freely moving rats. The technology delivered consistent recordings and precise identification of epileptiform activity in a temporal lobe epilepsy model. This cost-effective and dependable configuration provides a viable alternative to commercial systems for preclinical neuroscience research.

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