

Non-invasive Electroencephalography Method Development and Validation using Carbamazepine

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Abstract

Context: Recording of neurophysiological properties in a rodent model by stereotaxic surgery is well known, while the non-invasive method used to investigate the neurophysiological effects of studied using carbamazepine (CBZ). **Aims:** The effects of CBZ electrical activity of the brain recorded using a non-invasive technique and validated by an experiment involving stereotaxic surgery procedure are described in this paper. **Settings and Design:** To minimize painful procedures in rodents, an affordable technique was developed to record electroencephalogram (EEG) in the rodent model, and to the test drug molecules screened without giving any anesthetic agent. **Methods and Materials:** The present invention provides a disposable plastic foam EEG leads relates to a EEG component device for recording rat brain physiological changes in the form of EEG. This study was performed to quantify the pharmacodynamic properties of the CBZ. Rat surgery involving stereotaxic experiment used for validation process is described for the non-invasive method in this paper. **Statistical Analysis Used:** Paired t test used to calculate the differences in different EEG bands. **Results:** EEG analysis showed CBZ significantly increased in the delta and theta power and no effects on other the frequencies of the EEG. Similar observations were found in the invasive method of EEG in rats. Clinical and neurophysiological assessment using the simple, inexpensive technique of EEG observed. **Conclusions:** The results have shown CBZ induced a significant increase in slow activity waves represented by delta and theta waves. Administration of the carbamazepine in rats detected major neurological changes in the form of EEG and the technique of non-invasive EEG measurement found to be a useful tool to detect changes in the EEG.

Key words: Bregma, carbamazepine, electroencephalogram, non-invasive, plastic foam, stereotaxic

Key messages: Our research findings demonstrated that carbamazepine enhances slow wave activity on EEG and EEG correlate to the changes observed in both techniques. Non-invasive EEG analysis seems to be simple, fast and reliable method in investigation of the drugs acting on the cognitive functions.

INTRODUCTION

All major anti-epileptic drugs (AED) capable of causing adverse cognitive effects.^[1] With toxic doses the slowing of the electroencephalogram (EEG) documented in epileptic patients.^[2] The study conducted in healthy adults treated with carbamazepine (CBZ) demonstrated mild EEG slowing.^[3] CBZ in addition to its antiepileptic action possesses psychotropic effects. Previously, CBZ investigated for clinical and neurophysiological effects in healthy volunteers, quantitative EEG analysis revealed significant increase in delta-theta power and decreased the activity of alpha band.^[3] In subjects with partial seizures, the

effects of CBZ were observed in extended therapy resulted in increase in the absolute power of low-frequency bands of EEG spectrum and decreased the frequency of alpha waves.^[4] CBZ usually prescribed drug for the treatment of focal seizures and as a mood stabilizer in schizophrenia patients.^[5] Over a

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Received: 03-01-2019

Revised: 19-08-2019

Accepted: 26-08-2019

half a century CBZ included by the practitioner physicians in the 1960s as an AED. Known for its several other properties, CBZ is used as antiepileptic, neurotropic, and psychotropic medication.^[6]

In EEG, electrical activity recorded from the scalp surface after being picked up by metal electrodes and conductive media.^[7] Thus, EEG has been used for diverse reasons including studying the action of drugs in central nervous system.^[8] The studies in rats involve placement of intracranial electrodes.^[9] To determine the stereotactic coordinates and to connect the electrodes, the bregma is used as a reference point.^[10] The surgical method required to place the steel electrodes, prophylaxis, anesthesia, and post-surgical interval is necessary to use the animals for the study.^[11] For recording and processing electrical signals of the brain using a disposable foam plastic patch containing conducting gel, an attempt here made to validate using a drug CBZ. In the past too non-invasive, EEG tool was developed by Silva *et al.*^[12,13] for bovine and modified for rat by Ferrari.^[14] EEG activity is relatively a small signal, measured in microvolt (μV) with the main frequencies of interest up to approximately 30 hertz (HZ). The frequency bands in EEG studied were delta (0.5–4 Hz), theta (4–8 Hz), alpha (8–13 Hz), beta (13–30 Hz), and gamma (>30 Hz). The quantitative analysis of electroencephalographic activity (EEG) is a useful tool for the study of changes in brain electrical activity during cognitive and behavioral functions in numerous investigational circumstances.^[15]

The highly conductive electrode leads made from thin wire, plastic foam, and conductive gel can be applied instantaneously on a small location; additionally, electrode strongly bonds to the scalp in the presence of an adhesive. This application well suits to the awake and freely moving rats. We addressed two questions in this research: First, the development and recording of EEG using cost-effective non-invasive method using CBZ and second, the validation and comparison of the EEG recordings using therapeutic dose of CBZ investigated in normal Wistar rats of either sex.

SUBJECTS AND METHODS

For experiments, 12 healthy Wistar rats of either sex (20–25 weeks of age and 150 ± 10 g weight) used and are purchased from Mahaveer Enterprises, Hyderabad. CBZ was a gift sample from Sun Pharma, India. The experiments were conducted at Jayamukhi College of Pharmacy with permission from the Animal Ethical Committee (JCP/IAEC/2014/01). The rats were caged individually in polypropylene cages until animals used for experimentation and provided pelleted feed (Lipton India Ltd., India) and water provided *ad libitum*. On the day of experiment food and water was withheld. The EEG data recorded and analyzed for all 12 rats after administration of the CBZ.

Procedure for non-invasive recording of EEG

Commercially available very thin wire is connected to the data acquisition system Biopac MP45 (Biopac Systems Inc., USA) and 2 mm of the exposed copper wire inserted through a 3 sq mm of plastic foam. To the tip of all three copper wire electrodes conducting gel is applied. Gel obtained from the commercially available electrocardiograph (ECG) leads, which is used for measuring ECG of the heart. The component of the commercially available ECG lead includes a semisolid gel containing polymer. Conducting gel is dermally non-irritant in nature, cohesive, ionic, and hydrophilic polymer observed to be conductive and soft. Gel remained wet for a prolonged period (>10 h) in a dry environment. Electrode gel contains KCl for ion conductivity and is glycerin based. The dependable plastic foam backing and conductive sticky gel provide intimate skin contact for consistent EEG. Approximately 25 mg gel from ECG lead applied onto the exposed copper wire. The rat comfortably held in between the two palms and the entire setup of plastic foam with wire stucked on the surface of the animal scalp. This is done by applying to the contour of the foam plastic instant glue, a monomer called cyanoacrylate and is applied to fix firmly to shaved head of the rat [Figure 1]. The advantage of this method is that the recording can be done in awaken condition by applying EEG leads on the scalp of the rat without surgery which saves much time, injury, and mortality of rats. The developed EEG leads placed on the depilated scalp of the rat at right and left parietal cortex and reference electrode placed above the bulbus olfactory. EEG source localization is an essential component to reveal the brain activity. Parietal region most relevant brain region related to brain activity.

EEG data acquisition and processing

Bipolar electrodes with a ground electrode used for EEG recording. The recording cable with foam plastic set up placed onto the scalp of the rat and allowed free movement

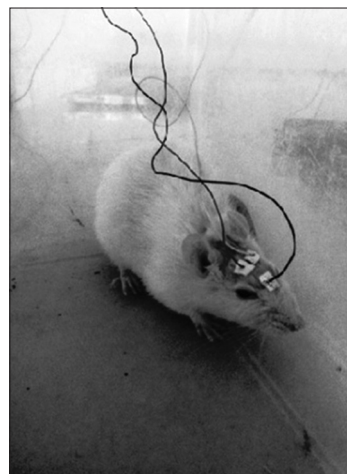


Figure 1: Placement of electroencephalogram electrodes on the scalp of the rat

for the animal in the cage. EEG signals are recorded using 200 sample rate/s and filtered through high pass at 0.5 Hz and low pass at 100 Hz and data recorded using Biopac MP45, Biopac Systems Inc., USA. EEG recording inspected visually and presence of any artifact in EEG discarded before analyzing with *AcqKnowledge* 4.4 software.

EEG recording

On the day of experimentation, animals made to adopt experimental conditions for 15 min. EEG recordings carried out between 9 am and 3 pm. EEG signals generated from the rat brain connected to a microcomputer system coupled with a fast Fourier transformation (FFT). Power spectrum data (PSD) analysis used for EEG data analysis; this involves separating EEG signals into fundamental frequencies. The frequency bands were <4 Hz (delta band), 4–8 Hz (theta band), 8–14 Hz (alpha band), 14–30 Hz (beta band), and >30 Hz (gamma band). The sweeps of 4 s activity averaged as spectra of power density. Further calculations were made to compare pharmacodynamic changes observed in all bands.

Preparation and data collection for surgical procedure and EEG data recording

Animals were surgically prepared for drug-induced pharmacodynamic changes. The animals were anesthetized using ketamine (72 mg/kg, IP) and xylazine (8 mg/kg, IP), to prevent any respiratory distress atropine (8 mg/kg, IP) was given. After anesthetizing the animal, the scalp was shaved with scissor and the rat is fixed in stereotaxic instrument. By making a midline incision on the skinhead skull was exposed. Stainless steel screw used as electrodes (length 2 mm; diameter 1 mm; and head size 2 mm) was fixed on parietal (AP –2 mm; ML: 2 mm; and –2 mm, from bregma) bones to record EEG from the frontal and parietal cortices. One stainless steel screw electrode was fixed laterally to the midline of the nasal bone as ground electrode. Micro drill of 0.7–0.8 mm diameter was used to fixing steel screws in the skull. Three wires cables of 100 nm thickness used to connect nine-pin connector and were fixed on the skull with dental acrylic cement. The animal was removed from stereotaxic equipment and was treated with dexamethasone (1.5 mg/kg) to reduce the brain inflammation and Povidone-iodine solution applied to control post-operative infections. Drugs applied and administered for 4–5 days and animals were treated for 1 week to recover from operation and to engage them in the experiments. Before starting of the experiments, animals were made to habituate to the EEG recording chamber (12" × 12" × 11"). Drug activity on various bands was recorded in between 9 am and 3 pm India standard time through two channels bio amplifier mobile unit (Biopac MP45) processed with the help of software *AcqKnowledge* 4.4 Biopac System Inc., USA. EEG signals were processed with a high pass 5 Hz and low pass 100 Hz filters and samplings rate of 200 samples/s. The recorded

EEG saved for offline analysis. Offline records of EEG for bands were manually scored using 4 s epochs employing the standard criteria for rats. FFT were used to evaluate EEG spectra between 0 and 100 Hz. EEG power in each frequency band expressed as percentage of the baseline recording for quantitative assessment of EEG changes. The EEG data and PSD calculated for the baseline of 30 min of duration and after the administration of the drug CBZ 20 mg/kg body weight, 90 min of duration of recording was done. In each segment of baseline and post-treatment CBZ, epochs of 4 s randomly selected to calculate PSD, PSD is averaged in segment of 30 min and 90 min recording. Power of five EEG frequency bands considered for the statistical evaluation.

The experiment divided into two sessions, first session features the recording of the baseline EEG without administering CBZ and second session, post administration of CBZ and its effects on EEG. Drug-free baseline for 30 min recorded in the animals after administration of the vehicle (2% acacia suspension). After recording the baseline, to the same animal pure drug CBZ (Gift sample from Sun Pharma, Ahmedabad, India) in a dose of 20 mg/kg in 2% acacia suspension was administered orally. After 2 h of post-administration of CBZ, EEG was recorded for 90 min.

Statistical analysis

The student's *t*-test used to analyze the statistical significance in EEG signals. Wilcoxon's test was applied to determine the probabilities in all the groups in power spectra. Each of the frequency bands was analyzed separately. Statistical calculations were performed using MS Excel. For all the analyses, we took the two-tailed significance. EEG data were analyzed by the paired *t*-test. The test is considered to be significant if $P < 0.05$.

RESULTS

The analyses of results have been presented for noninvasive and invasive EEG methods and noted the changes in the EEG spectrum following CBZ administration.

Analysis of changes in EEG

Under the drug-free condition, the behavior of the rats did not fluctuate; animals were remaining active. Rats were kept in a soundproof room and avoided any electrical activity like switching on any electrical gadgets in the EEG recording room to prevent generation of electrical impedance. Post administration of the drug rats was slightly sedated; the activity of the animals decreased compare to previously observed animals. The results clearly indicated that CBZ induced a significant increase in slow activity of the waves. During the recording session of the baseline in non-invasive method, the EEG characterized by the appearance of all five prominent bands, EEG analyzed for any

artifacts or distorted EEG recordings. The non-invasive patch containing electrodes was comfortably carried by the animals, all through experiments and animals were normal with behavior and exhibited motor activity and never slept. Sometimes animals remained motionless with eyes closed. Even in the conventional method of invasive EEG, the administration of the CBZ induced significant changes in EEG and marked by increase in the slow waves. Our results of non-invasive EEG method are in agreement with conventional invasive method under the influence of CBZ EEG characteristics. Most consistent being no differences observed with respect to the gamma, beta, and alpha bands. The steady-state is the acceleration of the slow-wave activities of delta and theta bands, while other frequency band changes were insignificant. The CBZ exerted greater effect in both methods of EEG in particularly theta and delta bands. Low voltage and high-frequency EEG waves associated with increased motor activity and low-frequency EEG waves with noticeable increase in delta waves with decreased motor activity and theta waves correlated with rapid eye movement sleep.

The EEG changes observed before administration of CBZ considered as baseline value for all bands and post-administration of CBZ similar analysis was carried out. The statistical analysis of the data suggested increase in the relative power of the delta and theta bands ($P < 0.05$) following exposure to CBZ in non-invasive quantitative determination of various bands, similarly significant changes ($P < 0.05$) observed in invasive method of EEG analysis of the CBZ. However, interesting to note that the gamma frequency band did not have any effect following CBZ administration and also

the effect of CBZ is insignificant on alpha and beta frequency bands in both methods of EEG. The results demonstrated the non-invasive experiment pattern of various bands recording of the CBZ pharmacodynamic study produces significant effects on the power of EEG frequency spectrum of the rats, while the maximum variations were observed in low-frequency bands delta and theta. Drug effects on EEG power spectra in the post drug CBZ period EEG expressed as changes in corresponding baseline values. The persistent effect of increased delta and theta frequencies observed in both invasive and non-invasive in all cases of treated animals with CBZ. These two effects of CBZ on delta and theta found to be significant ($P < 0.05$) in both methods. We also analyzed potential changes on other frequency bands; we did not find a significant change in both methods of analysis of EEG. EEG data of both methods are shown in Tables 1 and 2.

DISCUSSION

The present research work explains about the effect of the CBZ-induced changes in EEG. Analysis of both invasive and non-invasive methods in rats following administration of CBZ a prominent increase in delta and theta was observed. The present findings suggest that CBZ administration produces alterations in neurophysiological activities by increase in delta and theta bands and no changes observed in remaining bands. The previous studies on the effects of CBZ have reported increase in delta and theta; similar findings were observed in the present studies when drug CBZ attained maximum concentration in the body. The CBZ quantitative electroencephalography

Table 1: Effect of carbamazepine on frequency bands in non-invasive technique method $n=12$, value represents mean \pm SD

EEG band	Before CBZ administration	After CBZ administration	P value	Significance
	Mean \pm SD	Mean \pm SD		
Gamma	6.07 \pm 0.65	6.02 \pm 0.98	1	NS
Delta	4.45 \pm 1.38	5.91 \pm 1.35	0.0313	S ($P < 0.05$)
Theta	5.80 \pm 1.17	6.46 \pm 1.06	0.0355	S ($P < 0.05$)
Beta	5.12 \pm 0.62	5.18 \pm 0.72	0.8438	NS
Alpha	4.96 \pm 0.76	4.89 \pm 0.49	1	NS

EEG: Electroencephalogram, CBZ: Carbamazepine, SD: Standard deviation

Table 2: Effect of carbamazepine on frequency bands in invasive EEG method. $n=12$, value represents mean \pm SD

EEG band	Before CBZ administration	After CBZ administration	P value	Significance
	Mean \pm SD	Mean \pm SD		
Gamma	5.43 \pm 1.80	5.57 \pm 2.03	0.3125	NS
Delta	2.88 \pm 1.26	3.58 \pm 1.09	0.0391	S ($P < 0.05$)
Theta	5.46 \pm 0.56	6.24 \pm 0.46	0.0234	S ($P < 0.05$)
Beta	5.90 \pm 1.50	5.12 \pm 1.66	0.3828	NS
Alpha	6.32 \pm 1.20	6.47 \pm 1.28	0.4609	NS

EEG: Electroencephalogram, CBZ: Carbamazepine, SD: Standard deviation

analysis showed that CBZ significantly increased the delta – theta power like previous studies conducted in a double-blind crossover study in volunteers.^[3] The same neurophysiological changes observed both in non-invasive and invasive methods. Our data of EEG analysis documents about a significant increase in the absolute power of the slowest EEG frequency bands after CBZ administration. In another study, the EEG changes during standard introduction of CBZ in untreated neurological patients revealed increase in the mean values of the relative power of the theta and delta band.^[16]

The results have shown CBZ-induced increase of slow activity of the frequency bands in EEG. The data analysis of the EEG for CBZ disclosed an increase in total power and relative powers of theta and delta, while no significant change was observed in alpha, beta, and gamma bands. Hence, no effect on alpha and gamma bands indicated that CBZ is not having any effect on cognition. The study findings revealed after a single dose of CBZ 20mg/kg, a significant difference in delta and theta band was observed following non-invasive method ($P < 0.05$). The spectral analysis revealed the same significant difference in both delta and theta bands following conventional method. We noted that no prominent effect was observed on gamma activity (30–90 Hz). Hence, our results support the previous theories of CBZ not having any effect on cognitive functions of the animals.^[16] Our finding confirms the developed non-invasive technique may be a potential research tool to monitor EEG.

CONCLUSION

In summary, we observed that non-invasive method provides reliable results to that of invasive method. The influence of CBZ revealed the characteristics of EEG in both methods. The findings are comparable and an increase of absolute power values in low-frequency bands of EEG spectra observed. The result obtained allows us to conclude that non-invasive method and recording of EEG can be accomplished with ease for recording of drugs. Based on our results, an EEG finding of non-invasive method was reliable.

ACKNOWLEDGMENT

This work was funded by All India Council For Technical Education(AICTE) , New Delhi under Research Promotion Scheme (RPS). Authors wish to thank Dr. Sushil K. Jha, School of Life Sciences, JNU, New Delhi and Swetha Tripathi, JNU for their timely help during the project.

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Source of Support: Nil. **Conflict of Interest:** None declared.