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Wireless EEG Recording of Audiogenic Seizure Activity in Freely Moving Krushinsky-Molodkina Rats

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Abstract: This study evaluates audiogenic epilepsy in Krushinsky-Molodkina rats using a wireless EEG system to overcome the limitations of traditional methods that restrict animal movement. Nine male KM rats, prone to audiogenic seizures, were implanted with electrodes in areas critical for seizure activity (inferior colliculus, ventrolateral periaqueductal gray, dorsal area of the secondary auditory cortex, and motor cortex). This setup allowed for the observation of seizures without movement restriction. Results showed that targeted neural intervention via electrode implantation reduced the intensity of convulsive seizures in half of the subjects, suggesting a potential therapeutic approach. The severity of seizures was influenced by the pre-phonostimulation EEG background activity in specific brain regions, identifying them as key in the genesis and generalization of seizures. Observations of dual generalized seizure waves and induced hyperkinesis challenge conventional epilepsy models and suggest new directions for research and therapy development.

Keywords: audiogenic epilepsy; behavioral neurology; ecological validity; free movement recording; krushinsky-molodkina rats; neurogenetics; seizure mechanisms; wireless electroencephalogram (EEG)

1. Introduction

Epilepsy is a complex neurological disorder characterized by an enduring predisposition to generate seizures, which are recurrent paroxysmal events that induce stereotyped behavioral alterations [1,2]. These seizures are underpinned by distinct neural mechanisms, and their differential diagnosis often includes various clinical conditions involving transient alterations in awareness and/or behavior [3].

Genetic rat epilepsy models have been instrumental in elucidating the underlying mechanisms of the disorder. The Krushinsky–Molodkina (KM) inbred rat strain was the first among strains selected for audiogenic epilepsy, and it holds significance along with other inbred strains such as the GAERS and WAG/Rij strains [4,5]. Furthermore, rats with clinically relevant mutations provide a crucial source of comparative data [6].

Although electroencephalogram (EEG) setups, both tethered [7] and wireless [8], have been used to record brain activity in these models, methodological limitations persist. Specifically, the requirement for animal immobilization during EEG recordings significantly compromises the ecological validity of acquired data [9–11]. Furthermore, the effects of immobilization stress on both brain activity and external behavioral manifestations of seizures have not been adequately addressed [11–15].

The aim of this study is to develop and introduce a wireless EEG recording system for Krushinsky-Molodkina rats, designed to allow free movement during recording sessions. This methodology is expected to not only enhance the reliability and validity of the data, but also allow simultaneous measurements of neural activity and behavior, thus serving as a tool for elucidating causal relationships [9].

The study was conducted at the Institute of Immunology and Physiology of the Russian Academy of Sciences and aims at both rodent models and broader applications in the research of human epilepsy [16]. The null hypothesis states that the wireless electroencephalogram (EEG) recording methodology does not offer practical advantages over traditional wired systems in terms of the completeness of the data extracted, as well as the potential for their in-depth interpretation [9].

2. Materials and Methods

2.1. Animal Husbandry and Ethical Approvals

This study employed nine male Krushinsky-Molodkina rats, aged between 6-7 months and weighing 250 ± 30g [17]. The animals were purchased in a cooperative agreement with the Laboratory of Physiology and Genetics of Behavior, Department of Higher Nervous Activity, Faculty of Biology, Moscow State University named after MV. Lomonosov. The eligibility to be included in the study was based on seizure susceptibility; only rats exhibited a classical 4-point seizure at the first phonostimulation were included [19].

Rats were kept in a vivarium under controlled conditions: single cages, complete diet, ad libitum access to water, and a 12-hour light-dark cycle. All experimental procedures complied with both the Russian Federation's current legislation and the European Convention for the Protection of Vertebrate Animals Used for Experiments and Other Scientific Purposes (2007/526/EC). Ethical approval was obtained from the Bioethics Commission of IIP UrB RAS (Act No 17.22.06.23).

2.2. Experimental Design and Surgical Procedures

The animals were implanted with stainless steel insulated sterile needle electrodes (length: 12 mm; diameter: 0.3 mm; conical working part: 0.4 mm). Electrode resistance was predetermined. Surgeries were performed using a Drive stereotactic system (Neurostar, Germany), and anesthesia was administered by inhalation of isoflurane. Intraoperative monitoring included ECG, spirometry, thermometry, and assessment of unconditioned reflexes, such as the corneal reflex.

2.2.1. Electrode Placement, Orientation and Stabilization

The working parts of the electrodes were precisely positioned relative to the Bregma point, in accordance with established protocols [20]. Five working electrodes were introduced into each animal in specific brain zones responsible for the initiation (Figure 1), development and external manifestations of audiogenic seizures, including EC IC – external cortex of the caudal colliculus (2.0; -8.5; 4.0) of the brain, VLPAG – ventrolateral periaqueductal gray matter (-0.8; -7.8; 6.0), PnO – olivary complex (1.2 ; -8.0; 8.0), AuD – dorsal area of the secondary auditory cortex (6.5; -3.1; 5.4), and M1 – motor cortex (3.7; -3.5; 2.5). Additionally, a reference electrode was implanted into the cortex of the fifth lobe of the cerebellum (0.0; -11.3; 4.0). Brain atlas scaling, based on preliminary craniometry data, was used to improve the accuracy of electrode placement.
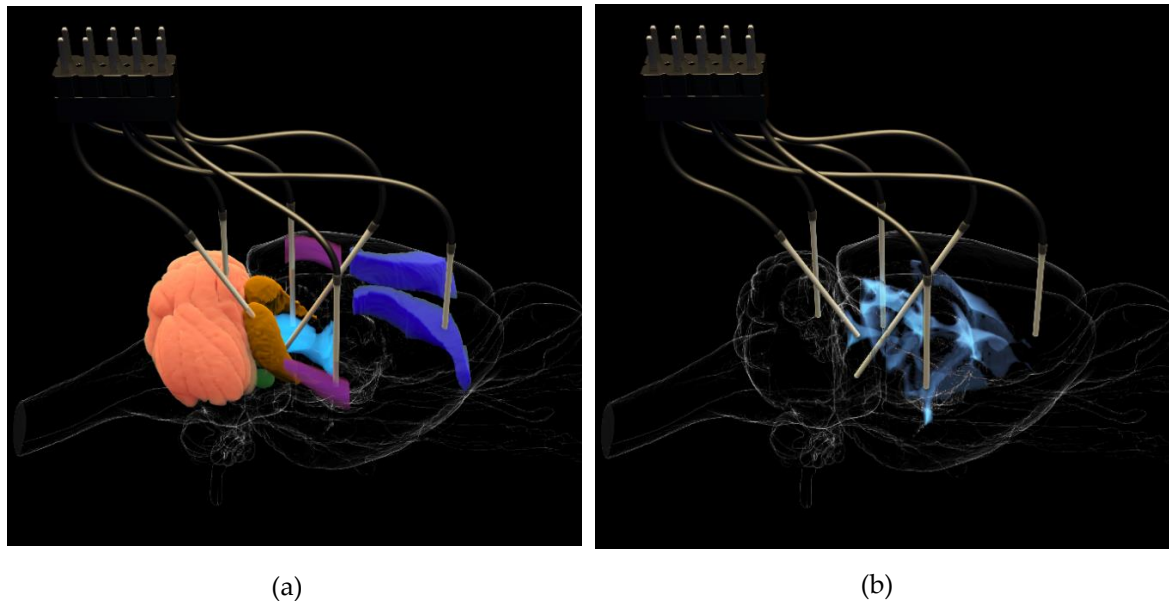
Figure 1. Electrode placement in the brain of K-M rats. (a), relative to the investigated zones (EC IC - brown, VLPAG - light blue, PnO - green, AuD - violet, M1 - dark blue) and cerebellum; (b), relative to the ventricular system.

Electrode angles were theoretically determined to minimize distance to target zones while avoiding critical structures. Electrodes were introduced through 0.9-mm holes drilled into the skull, at coordinates corresponding to the targeted brain regions. The electrodes were stabilized with fast-hardening epoxy materials, fortified with crystalline Nitrofural (furatsilin). Four cortical screws (M1.5, stainless steel), positioned bilaterally in the frontal and parietal bones, served as anchor points and were connected to form a ground circuit (Figure 2).
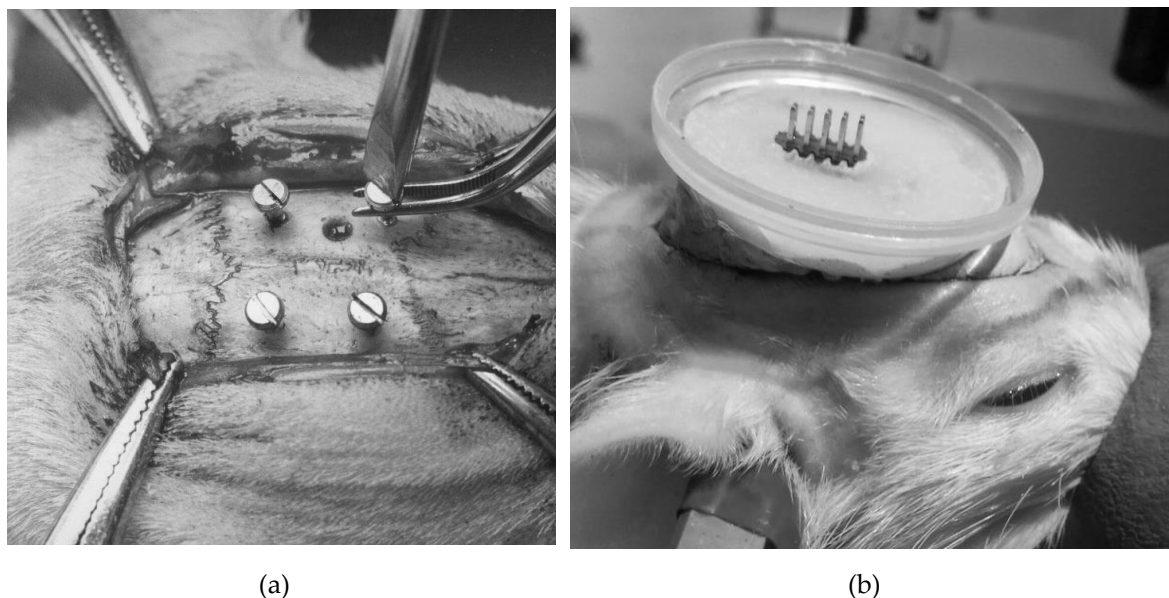
Figure 2. Stereotactic surgery stages: (a) installation of cortical screws; (b), fixation of the connector within the protective housing base.

2.2.2. Transmitter and Connector Installation and Postoperative Care

A plastic protective housing was implemented to protect the transmitter and connector from potential damage during induced seizures. To ensure reliable detachable electrical connections, a specialized adapter consisting of enlarged connecting elements was employed. A lighter alternative

battery (LIR 2032 3.6 V) was substituted for the standard battery, reducing the transmitter's weight by 2.5g. This configuration allowed the placement of the transmitting device within the protective housing above the transmitter, allowing complete freedom of movement of the head and obviating the need for separate body fixation.

Following surgical procedures, the animals were relocated to a vivarium for a two-week convalescence period under veterinary supervision (Figure 3). Animals that showed symptoms such as wound suppuration, neurological depression, or refusal to eat or drink were promptly removed from the experiment.

Figure 3. Appearance of the head-mounted transmitter in a rat during the preliminary stages of technique evaluation.

2.2.3. EEG Recording and Signal Processing

EEG signals were acquired through a multichannel digital polygraph PowerLab 8/35 – PL3508 (ADinstruments, Germany), utilized in conjunction with LabChart software v7.3.7 and a five-channel wireless transmitter and receiver (TBSI 5 Ch Wireless Neural Headstage – TB5653/F-LED; TBSI 5 Ch Wireless Neural Receiver – TB5663/FV). A total of 38 seizures were studied in 9 animals. LabChart and Neuron-Spectr.Net Omega v1.5.9.0 were utilized for the processing of electroencephalograms, encompassing visual analysis, phase arrangement, amplitude calculation, and evaluation of the characteristic frequency wave.

2.2.4. Experimental Protocol

Post-recovery, the animals were individually placed in a one-meter diameter "Open Field" arena (Open Science, Russia) for simultaneous EEG and behavioral recording. Background neural activity was recorded for an initial ten minutes, followed by phonostimulation to induce convulsive seizures. Audiogenic seizures were triggered via a multifrequency sound stimulus emitted by an electric bell (sound pressure at 1m: 100 dB).

Seizure intensities were evaluated according to the Krushinsky scale [17], where scores ranged from 1 (limited to motor excitation) to 4 (tonic and clonic convulsions with opisthotonus). Several distinct phases, as described by us earlier [18], of a four-point seizure were identified and coded.

To investigate the potential impact of repetitive seizures on EEG activity, serial phonostimulations were performed with 15-minute intervals, during which the background EEG was continuously recorded. The sequence persisted until the animals ceased responding to auditory stimuli.

2.2.5. Statistical Analysis

Data were analyzed using Statistica 10 software (v 10.0.1011.0), using nonparametric methods, including Friedman rank analysis of variance and the Mann-Whitney U test.

3. Results

### 3.1. Electroencephalographic Recordings

Electroencephalograms obtained using the presented method are virtually devoid of artifacts, allowing for unambiguous visual evaluation across all channels. Specifically, sleep spindles are easily identifiable against a backdrop of stage 2 slow wave sleep (Figure 4).
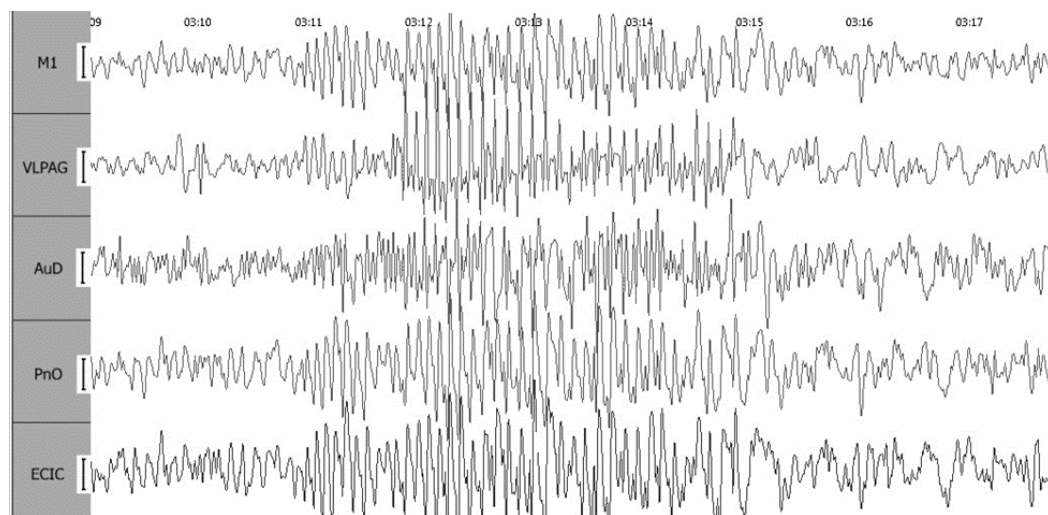
Figure 4. Sleep spindles during background recording.

3.1.2. Phases of 4-Point Seizures

The EEG allows visual determination of the convulsive and postictal periods of a 4-point seizure, which are usually defined externally. The latent period (L) shows a significant increase in amplitude in the caudal colliculus (ECIC). During the phase of locomotor excitation (WR), a sharp increase in signal amplitude occurs, subsequently decreasing with the onset of clonic-tonic convulsions (S1). The convulsive period is characterized by rhythmic peak-wave complexes in the ECIC, PnO, and M1 leads, eventually transitioning to synchronized oscillations of similar amplitude (Figure 5).
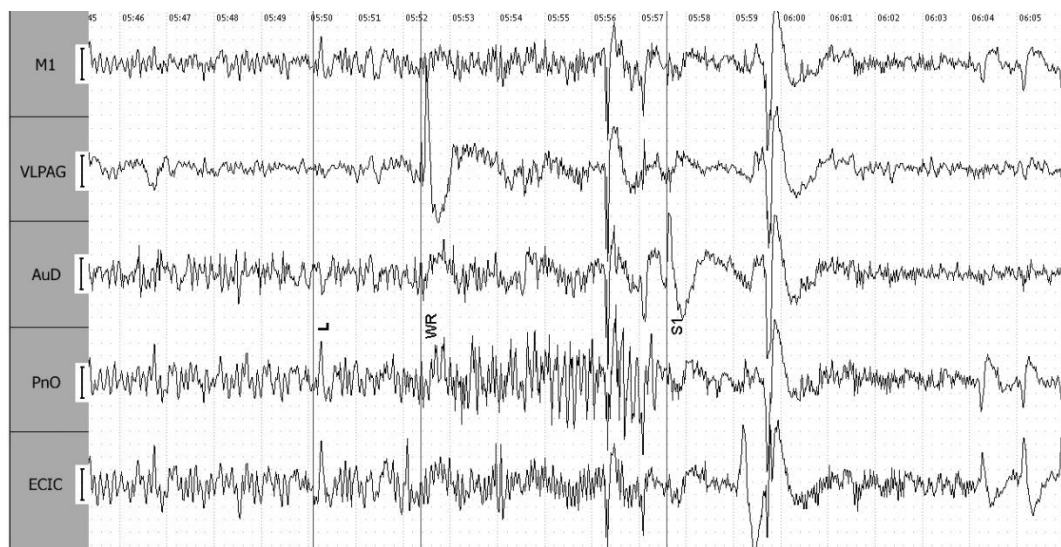
Figure 5. Start of a single wave 4-point epileptiform seizure, with delineated phases. L – the latent period, WR – locomotor excitation, S1 – phase of clonicotonic convulsions, vertical lines – the boundaries between them, established by external manifestations.

3.1.3. Postoperative Effects

Implantation of an electrode alters the nature of seizures in experimental subjects. The initial postoperative stimulus reveals a heterogeneity of the test group: only 55.6% of rats exhibit a classic 4-point seizure. The remaining subjects manifest weaker convulsions, some cessing immediately following the WR phase. This corresponds to a Krushinsky scale rating of 1-2 points. In particular, no 3-point seizures were observed during the first postoperative test.

3.1.4. Amplitude Analysis

The postoperative decline in seizure strength requires an independent amplitude evaluation for seizures of varying magnitudes and postictal states. Background EEG becomes critical in this context, as the response to sound stimulus is influenced by the initial neural state.

3.1.5. Analysis of 4-Point Seizures

For animals experiencing 4-point seizures, the highest amplitude is recorded in the ECIC. Lower values (at 80% of the ECIC level) are noted in PnO and AuD, and the remaining leads show voltages around 45% of the ECIC level. The L period features a pronounced (15-20% relative to the baseline) increase in amplitude in both the ECIC and PnO leads. The WR phase displays moderate increases in ECIC and VLPAG, a sharp jump in PnO (to 140% of the L phase values), and synchronized amplitude values across the cerebral cortical areas. The S1 phase experiences a drastic amplitude drop in all leads (45-65% relative to the baseline recording), followed by a transient apnea phase (A) with minimal signal amplitudes (15-25% of baseline values).

After the A phase, another convulsive phenomenon was observed – the clonic seizure phase (S3), in which a 4-point seizure was characterized by a multiple increase in amplitude in all leads relative to the A values. However, such an increase was short-lived, and the recorded values did not reach baseline magnitudes, remaining at 50-75% of them.

Restoring a stable respiratory rhythm in the breathing recovery phase (R1) of the postictal period of a 4-point attack is accompanied by a new decrease in AuD amplitude to the level recorded in the S1 phase and pronounced rhythm synchronization in all leads (Figure 6).
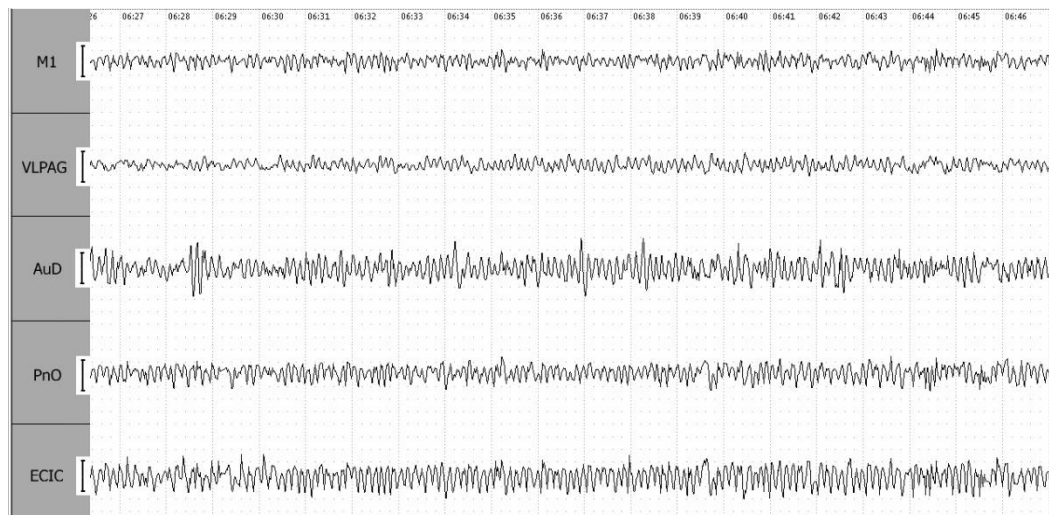
Figure 6. EEG during the breathing recovery phase (R1) of a 4-point seizure.

3.1.6. Subsequent Phases

During the shortness-of-breath phase (R2), rhythm synchronization persists between leads ECIC, PnO, and M1, without significant amplitude deviation. The cataleptoid state (C) at the end of the postictal period is marked by a 40% increase in amplitude in all leads, except for ECIC (Figure 7).
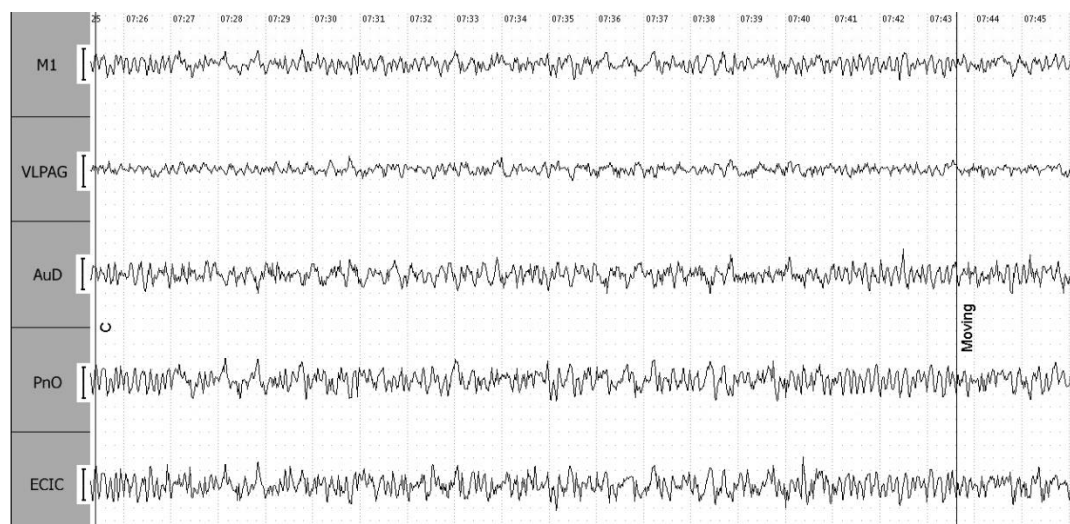
Figure 7. End-of-postictal cataleptoid state (C) in a 4-grade epileptiform seizure.

3.2. Electroencephalographic Characteristics Across Seizure Types

3.2.1. 4-Point vs 2-Point Seizures

Compared to 4-point seizures, 2-point seizures electroencephalograms (EEG) display distinct amplitude profiles in various neural regions. Specifically, lower background amplitudes are observed in the ECIC and PnO regions, while higher amplitudes are recorded in the M1 region. This ECIC-PnO amplitude decrease pattern persists through the latent period, the phase of locomotor excitation (WR), and a consecutive increase in amplitude is observed in the VLPAG and AuD areas. With the development of the clonic-tonic phase (S1), higher signal values compared to 4-point seizures are already registered in all leads without exception. Relatively high values in the VLPAG and M1 leads continue into the phase of clonic seizures (S3). A nuanced pattern emerges during the breathing recovery phase (R1) and the cataleptoid state (C), characterized by varied amplitudes in the aforementioned regions.

3.2.2. 1-Point Seizures

EEG amplitudes during 1-point seizures largely resemble those of 2-point seizures, although with certain exceptions. For example, in the background recording, higher amplitude values are observed in the AuD region instead of M1. Furthermore, the postictal period of 1-point seizures features amplitudes that are indistinguishable from background levels, casting doubt on the development of a cataleptoid state in these animals.

3.2.3. Effects of Repeated Phonostimulation

A series of phonostimulations resulted in significant changes in the nature of seizures. Specifically, rats that initially experienced 4-point seizures exhibited weakened seizures upon the third or fourth phonostimulation, mitigating to 1-point seizures without entering the cataleptoid state. Subsequent phonostimulations initially intensified the seizures to 2 points and induced a novel postictal state of hyperkinesis, and then led to a complete loss of sensitivity to sound (after 6-7 repetitions).

3.2.4. Postictal Periods: Hyperkinesis versus Cataleptoid State

The development of hyperkinesis in the postictal period is accompanied by lower amplitudes in the PnO region and higher values in VLPAG, AuD, and M1. Interestingly, spike-wave complexes are most noticeable in the ECIC region and exhibit a frequency of up to 2 Hz, fading out within seconds in VLPAG and PnO.

3.2.5. Induced Hyperkinesis and Electrode Implantation

The phenomenon of induced hyperkinesis is conspicuously absent in animals where electrode implantation diminished the seizure strength to 1-2 points. In these cases, phonostimulation initially increases the strength of the seizures temporarily before stopping to elicit any response by the third or fourth repetition.

3.3. *Correlation Analysis of Amplitude Characteristics*

Despite these varying effects, electroencephalograms of seizures of equivalent strength exhibited a high degree of similarity in amplitude characteristics in all leads and animals. These findings substantiate the feasibility of employing cumulative statistical analyses, such as correlation analysis (upon meeting its formal requirements), to evaluate the interactive dynamics of the neural structures under investigation.

3.3.1. Correlation Analysis of EEG Signal Amplitudes across Neural Regions

An initial assessment of the background amplitude correlations across different neural regions revealed two distinct blocks of strongly correlated structures. Specifically, ECIC and PnO were very strongly correlated ($r = 0.98$), as were AuD and M1 ($r = 0.95$). The latter structures also demonstrated a strong correlation with VLPAG ($r = 0.86$ and $r = 0.81$, respectively). In particular, there existed an antagonistic relationship between these two blocks, characterized by negative correlation coefficients ranging from -0.55 to -0.74.

3.3.2. Impact of Sound Stimulus

The presentation of an auditory stimulus did not alter the correlation relationships within the VLPAG, AuD, and M1 block. However, during the latent period, the negative correlation between ECIC on one side, and VLPAG and AuD intensified ($r = -0.71$ and $r = -0.83$, respectively), while the correlation between PnO and ECIC ($r = 0.69$), and PnO and M1 ($r = -0.14$) weakened.

Locomotor excitation phase (WR). The hallmark of this phase was the complete independence of amplitude in the ECIC from VLPAG, AuD, and PnO. Coupled with the reestablishment of a strong

negative correlation between PnO and M1 (r = -0.83), against the backdrop of the integrity of the "block" VLPAG-AuD-M1, this observation suggests the potential development of a focal seizure.

Convulsive phases (S1, S2). During these phases, all leads showed only positive correlations, both within and between blocks. These correlations, which ranged from 0.64 to 0.95, occurred against a backdrop of decreasing signal amplitude across all leads, indicating the generalization of a convulsive seizure.

Apnea Phase (A). The ECIC lead showed weak positive correlations, while negative correlations emerged between VLPAG on one side, and PnO, AuD, and M1 on the other (r = -0.71, r = -0.73, and r = -0.72, respectively). However, positive correlations between these latter structures remained robust.

Respiratory Recovery Phase. The onset of individual respiratory movements reinstated both intra-block and inter-block correlations, albeit at weaker levels compared to the background state.

Clonic Convulsions (S3). The correlation pattern in this phase mimicked that of S1 and S2, with coefficients greater than r = 0.83, confirming the interpretation of observed clonic convulsions as a second wave of a generalized seizure.

Dyspnea and Postictal Cataleptoid State. During the dyspnea phase, antagonistic relationships between ECIC and the VLPAG-AuD-M1 block were reestablished. PnO was positively correlated not only with ECIC (r = 0.43) but also with VLPAG and M1 (r = 0.43 and r = 0.21, respectively). At the same time, a weaker correlation than in the background was observed in the cortex between AuD and M1 (r = 0.57).

The cataleptoid state in the postictal period demonstrated a landscape of correlation closely mirroring the background, with increased negative correlations between ECIC and PnO with VLPAG (r = -0.79), and reduced negative correlations with AuD and M1 (r = 0.60 and r = 0.55, respectively).

The observed patterns of correlation between different neural areas and seizure phases, particularly in the context of a 4-point seizure, provide substantial insights into the underlying neural dynamics. The data garnered from this study could form the basis for further exploration into the mechanisms of seizure onset, propagation, and generalization, as well as the development of related postictal states.

4. Discussion

Encephalographic studies in animals are crucial in advancing our understanding of neural mechanisms. A significant limitation of most animal studies, however, is the utilization of tethered EEG systems and the restrained condition of the subjects, which complicates the correlation between EEG data and clinical seizure manifestations [15–21]. We have introduced a wireless EEG method for use in Krushinsky-Molodkina rats with genetically determined audiogenic seizures. This approach in unrestrained animals has illuminated patterns that are unobservable with traditional methods.

Evaluation of the null hypothesis. The null hypothesis posited that the wireless EEG system would not produce data of quality or interpretability significantly different from those of traditional tethered EEG methods [9]. Our findings reject this hypothesis, as wireless EEG not only preserved data quality, but also facilitated the observation of new patterns in unrestrained animals, potentially due to the lack of restraint and associated stress in the animals [9–15,21,22].

Therapeutic Implications of Electrode Implantation. Electrode implantation has been shown to reduce the clinical severity of seizures in a significant proportion of cases [23]. This is consistent with the surgical treatment literature in epilepsy [24] and suggests a theranostic effect, where diagnostic procedures yield therapeutic benefits. The variation in impact between subjects is likely influenced by brain lateralization and electrode placement asymmetry: The implants were inserted into ECIC, VLPAG, AuD, and M1 on the right side and PnO on the left. Since all the listed structures (brain areas) are paired, and the ratio of animals in which the surgical intervention weakened the seizure is close to the known ratio of "right-handed" and "left-handed" rats (50/50), there is reason to believe that the lateralization of the rat brain modulates not only the sphere of cognitive functions but is also significantly related to the concept of seizure readiness of the brain.

Neurophysiological Factors. Background brain activity serves as a determinant of seizure strength and clinical presentation [25]. In particular, the path of seizure propagation remains

consistent between episodes. Hyperkinesis can also be induced through a regimen of interrupted phonostimulations, thereby offering the animals an additional 15 minutes for recovery after exiting the cataleptoid state [26]. This methodology seems to increase the convulsive readiness of specific brain regions, thus enhancing the intensity of seizures in some animals [27]. The consequent rapid depletion of nervous processes manifests in a prolonged postictal state, indicating a deficiency in compensatory mechanisms after repeated seizures.

Individual variability and structural roles. The study notes individual variability in the exhaustion of nervous processes, which explains the absence of hyperkinesis in certain rats. A focal point of sufficient epileptic activity might not form in the cortex, while it weakens in the caudal tuberosity of the quadrigemina. The EEG data further reveal a dynamic interplay between the cortical and subcortical structures. Specifically, the signal amplitude in the inferior colliculus (Ic) and PnO is reciprocally correlated with that in M1 and the auditory cortex (AuD). The Olivary complex (PnO) likely modulates the intensity of seizures, especially when the divergence of amplitude between the cortical and subcortical regions is maximized.

Interpretation of the locomotor phase. The locomotor excitation phase, characterized by both EEG patterns and observable behavior, aligns more closely with background activity and latent period than with clonic-tonic seizure phases. This may parallel the phase of human aura in epilepsy [28]

Overall, our wireless EEG approach not only refutes the initial null hypothesis but also provides a more nuanced understanding of seizure mechanisms, thus offering valuable insights for future research and therapeutic interventions.

5. Conclusions

Implantation of electrodes in specific auditory and motor cortical regions, including Ic, PnO, VLPAG, AuD and M1, attenuates convulsive seizures in approximately half of the KM rats studied. Furthermore, the amplitude characteristics of brain activity in the ECIC, PnO, and AuD regions at the time of stimulus presentation significantly influence the severity and nature of phonostimulation-induced seizures in KM rats. Seizures, which vary in strength from 1 to 4 points, predominantly start in the caudal tuberosities of the quadrigeminal tract and serve as focal points for the propagation of epileptic activity. In severe 4-point seizures among KM rats, dual waves of generalized seizures are evident.

In the context of epileptic activity, two stable reciprocal interrelationships can be delineated: one between M1 and VLPAG and another between ECIC and PnO. These interrelationships are temporarily disrupted during the generalization of convulsive activity. Induced hyperkinesis in KM rats can be achieved through a series of phonostimulations separated by intervals sufficient for the completion of the postictal cataleptoid state and the emergence of orienting-exploratory behavioral responses. The onset of induced hyperkinesis is associated with the activation of a secondary epileptic focus. Specifically, against a backdrop of high amplitude in the AuD region, the first structure to respond with increased amplitude to an auditory stimulus is M1. The remaining regions studied are engaged only during the phase of locomotor excitation.

Locomotor excitation, in terms of both EEG amplitude characteristics and observable behavior, exhibits greater congruence with background activity, latent periods, and postictal catalepsy than with clonic-tonic convulsions. Therefore, this state should be considered an analog of the human aura in epilepsy. Lastly, the postictal cataleptic state in KM rats shows amplitude characteristics in all regions examined that closely resemble those preceding the initiation of the stimulus. By refuting the initial null hypothesis and providing nuanced insights into seizure mechanisms and neural interrelationships, our study contributes valuable findings for future research and potential therapeutic interventions.

6. Study limitations

Certainly, the limitations of this study warrant meticulous delineation to offer a nuanced understanding of its results. A principal constraint is the small and homogeneous sample size of male

Krushinsky-Molodkina rats, which hampers the study's generalizability to other rat strains or, more broadly, to other animal models and human populations [29]. Furthermore, the study's cross-sectional design restricts any longitudinal analysis, limiting insights into the temporal progression of seizure mechanisms or the long-term implications of electrode implantation.

Regarding the technology employed, while the wireless EEG system has its merits in enhancing data collection in unrestrained animals, it also poses questions concerning signal integrity, such as potential signal loss or interference. Importantly, during the method validation phase, the study included a comparative assessment of the signals obtained through wired and wireless methods on the same animals. This comparison demonstrated complete equivalence in the recorded data and a reduced number of artifacts in the background recording.

The asymmetric placement of electrodes, particularly with PnO on the left side, could introduce biases in the assessment of the theranostic effects of electrode implantation. Furthermore, the study primarily focused on encephalographic data and did not adequately integrate other behavioral or physiological metrics that could have offered a comprehensive understanding of seizure activity.

The specific regimen of interrupted phonostimulations used in this study may have limited external validity, as it may not adequately represent natural, real-world stimuli inducing seizures. While our findings might be of some clinical relevance, particularly in terms of their theranostic implications, the translational applicability of these results to human clinical settings remains an open question. Concerns related to statistical power and operational definitions, such as the definitions used for seizure severity and intensity, also need to be considered carefully.

Lastly, although the study adhered to ethical guidelines, the ethical implications of animal studies are an ever-present consideration that should not be overlooked. By comprehensively discussing these limitations, the aim is to refine interpretations of the current findings and to guide the direction of subsequent research efforts.

Appendix A

Table A1. The results of correlation analysis.

Phase	IC	PnO	VLPAG	AuD	M1	Lead
	1	0,98	-0,55	-0,64	-0,69	IC
	0,98	1	-0,57	-0,71	-0,74	PnO
B	-0,55	-0,57	1	0,86	0,81	PAG
	-0,64	-0,71	0,86	1	0,95	AuD
	-0,69	-0,74	0,81	0,95	1	M1
	1	0,69	-0,71	-0,83	-0,62	IC
	0,69	1	-0,69	-0,52	-0,14	PnO
L	-0,71	-0,69	1	0,83	0,76	PAG
	-0,83	-0,52	0,83	1	0,81	AuD
	-0,62	-0,14	0,76	0,81	1	M1
	1	0,05	0,1	-0,07	0,33	IC
	0,05	1	-0,93	-0,6	-0,83	PnO
WR	0,1	-0,93	1	0,71	0,9	PAG
	-0,07	-0,6	0,71	1	0,76	AuD
	0,33	-0,83	0,9	0,76	1	M1
	1	0,86	0,79	0,81	0,86	IC
	0,86	1	0,93	0,76	0,71	PnO
S1	0,79	0,93	1	0,74	0,64	PAG
	0,81	0,76	0,74	1	0,95	AuD
	0,86	0,71	0,64	0,95	1	M1
	1	1	1	1	1	IC
	1	1	1	1	1	PnO
S2	1	1	1	1	1	PAG
	1	1	1	1	1	AuD
	1	1	1	1	1	M1
	1	0,23	0,43	-0,13	0,24	IC
	0,23	1	-0,71	0,62	1	PnO
A	0,43	-0,71	1	-0,73	-0,72	PAG
	-0,13	0,62	-0,73	1	0,62	AuD
	0,24	1	-0,72	0,62	1	M1
	1	0,74	-0,24	-0,57	-0,52	IC
	0,74	1	-0,21	-0,69	-0,31	PnO
R1	-0,24	-0,21	1	0,57	0,76	PAG
	-0,52	-0,31	0,76	0,76	1	AuD
	-0,57	-0,69	0,57	1	0,76	M1
	1	0,93	0,98	0,9	1	IC
	0,93	1	0,9	0,83	0,93	PnO
S3	0,98	0,9	1	0,93	0,98	PAG
	0,9	0,83	0,93	1	0,9	AuD
	1	0,93	0,98	0,9	1	M1

Phase	IC	PnO	VLPAG	AuD	M1	Lead
	1	0,43	-0,52	-0,74	-0,74	IC
	0,43	1	0,43	-0,17	0,21	PnO
R2	-0,52	0,43	1	0,79	0,79	PAG
	-0,74	-0,17	0,79	1	0,57	AuD
	-0,74	0,21	0,79	0,57	1	M1
	1	1	-0,79	-0,6	-0,55	IC
	1	1	-0,79	-0,6	-0,55	PnO
C	-0,79	-0,79	1	0,83	0,8	PAG
	-0,6	-0,6	0,83	1	0,9	AuD
	-0,55	-0,55	0,8	0,9	1	M1

Table A1. Average amplitude of background EEGs in animals with different seizure strengths, mV.

Phase or period	4-point seizures	2-point seizures	1-point seizures	Hyperkinesis	Lead
	36,27 ± 0,34	24,95 ± 0,33[4]	23,21 ± 1,29 [4]	22,96 ± 0,07 [4 2]	ECIC
	29,43 ± 0,40[I]	20,83 ± 0,47 [I 4]	19,73 ± 1,22 [4]	17,21 ± 0,56 [I 4 2]	PnO
B	15,41 ± 0,24 [I P]	18,98 ± 2,05 [I]	17,31 ± 1,39 [I]	22,97 ± 1,08 [4 1]	VLPAG
	28,45 ± 0,30 [I V]	28,99 ± 3,10 [P V]	22,32 ± 2,03 [4]	43,10 ± 0,28 [I P V 4 2 1]	AuD
	17,72 ± 0,27 [I P V A]	22,63 ± 1,49 [4]	18,51 ± 0,85 [I]	23,99 ± 0,60 [P A 4 1]	M1
	43,44 ± 0,32	30,99 ± 1,65 [4]	28,55 ± 2,32 [4]	22,22 ± 0,34 [4 2 1]	ECIC
	33,57 ± 2,13 [I]	22,66 ± 1,07 [I 4]	22,08 ± 1,55 [4]	17,80 ± 0,68 [I 4 2 1]	PnO
L	15,29 ± 0,74 [I P]	19,56 ± 1,85 [I 4]	19,48 ± 1,62 [I 4]	25,43 ± 1,40 [P 4 2 1]	VLPAG
	27,51 ± 3,86 [I V]	28,73 ± 2,80 [V]	26,91 ± 2,65	43,13 ± 1,11 [I P V 4 2 1]	AuD
	21,23 ± 2,45 [I P]	24,03 ± 1,46 [I]	20,91 ± 0,92 [I]	38,03 ± 2,14 [I P V 4 2 1]	M1
	47,75 ± 1,96	38,59 ± 0,89 [4]	41,92 ± 3,63	45,37 ± 1,53 [2]	ECIC
	88,13 ± 1,45 [I]	40,43 ± 1,52 [4]	54,27 ± 4,58 [4 2]	32,46 ± 0,80 [I 4 2 1]	PnO
WR	22,33 ± 1,81 [I P]	36,05 ± 3,94 [4]	28,45 ± 2,05 [I P]	47,19 ± 1,24 [P 4 2 1]	VLPAG
	25,58 ± 1,72 [I P]	49,05 ± 4,62 [4]	33,08 ± 3,45 [P 2]	63,74 ± 2,07 [I P V 4 2 1]	AuD
	23,10 ± 1,14 [I P]	40,24 ± 2,72 [4]	31,10 ± 2,20 [P 4]	61,98 ± 1,86 [I P V 4 2 1]	M1
	19,56 ± 0,21	29,15 ± 2,29 [4]		28,10 ± 0,68 [4]	ECIC
	12,54 ± 0,10 [I]	29,26 ± 2,43 [4]		20,80 ± 0,22 [I 4 2]	PnO
S1	5,89 ± 0,33 [I P]	29,15 ± 2,05 [4]		30,95 ± 0,42 [I P 4]	VLPAG
	15,07 ± 0,38 [I P V]	36,90 ± 4,32 [4]		51,31 ± 3,87 [I P V 4]	AuD
	9,28 ± 0,65 [I P V A]	25,47 ± 1,42 [A 4]		40,15 ± 3,14 [I P V 4 2]	M1
		23,74 ± 1,43			ECIC
		22,57 ± 0,90			PnO
S2		20,32 ± 2,22			VLPAG
		31,79 ± 4,19			AuD
		19,63 ± 1,62 [A]			M1
A	4,26 ± 1,92	3,66 ± 1,61			ECIC
	3,95 ± 0,80	3,24 ± 1,43			PnO

Phase or period	4-point seizures	2-point seizures	1-point seizures	Hyperkinesis	Lead
	5,15 ± 0,94	5,53 ± 2,44			VLPAG
	6,85 ± 0,48 [P]	5,09 ± 2,24			AuD
	3,62 ± 1,29	3,71 ± 1,64			M1
R1	21,59 ± 0,59	8,1 ± 2,07 [4]			ECIC
	14,09 ± 0,67 [I]	7,36 ± 1,88 [4]			PnO
	6,57 ± 0,72 [I P]	8,38 ± 2,6			VLPAG
	15,95 ± 2,26 [I V]	8,29 ± 2,41			AuD
	7,39 ± 1,81 [I P A]	7,63 ± 2,09			M1
S3	24,64 ± 2,06	21,23 ± 0,60			ECIC
	18,44 ± 2,43	17,47 ± 0,32 [I]			PnO
	9,60 ± 0,88 [I P]	16,35 ± 2,00 [4]			VLPAG
	21,05 ± 0,64 [V]	27,18 ± 2,97 [P]			AuD
	10,99 ± 1,90 [I A]	16,70 ± 1,01 [I A 4]			M1
R2	21,75 ± 0,18				ECIC
	14,63 ± 1,17 [I]				PnO
	6,41 ± 0,29 [I P]				VLPAG
	12,2 ± 0,27 [I V]				AuD
	8,62 ± 0,68 [I P V A]				M1
PP	24,72 ± 1,63	19,17 ± 0,59 [4]	23,06 ± 1,30 [1]	22,09 ± 0,43 [2]	ECIC
	19,87 ± 1,65	17,37 ± 0,76	19,45 ± 1,35	15,61 ± 0,31 [I 4 2]	PnO
	9,22 ± 1,35 [I P]	15,61 ± 1,25 [4]	18,02 ± 1,47 [4]	21,75 ± 0,29 [P 4 2 1]	VLPAG
	17,05 ± 2,98	26,25 ± 3,15 [I P V]	18,56 ± 1,08	46,94 ± 1,24 [I P V 4 2 1]	AuD
	11,99 ± 1,17 [I P]	17,89 ± 1,11 [A 4]	18,65 ± 1,05 [4]	32,29 ± 2,17 [I P V A 4 2 1]	M1

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