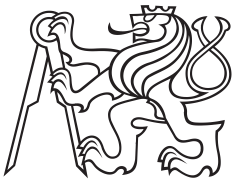
Master Thesis



Czech Technical University in Prague

F3

Faculty of Electrical Engineering
Department of Circuit Theory

Analysis of long-term video recordings of laboratory mice with epilepsy

Jonáš Fér

Supervisor: Ing. Jan Kudláček, DiS., Ph.D.
Supervisor–specialist: prof. Ing. Roman Čmejla, CSc.
Field of study: Medical Electronics and bioinformatics
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MASTER‘S THESIS ASSIGNMENT

I. Personal and study details

Student's name: **Fér Jonáš** Personal ID number: **492046**

Faculty / Institute: **Faculty of Electrical Engineering**

Department / Institute: **Department of Circuit Theory**

Study program: **Medical Electronics and Bioinformatics**

Specialisation: **Signal processing**

II. Master’s thesis details

Master’s thesis title in English:

Analysis of Long-Term Video Recordings of Laboratory Mice with Epilepsy

Master’s thesis title in Czech:

Analýza dlouhodobých video záznamů laboratorních myší s epilepsií

Guidelines:

Epilepsy is a serious chronic neurological disorder affecting 0.5 - 1 % of population in developed countries. Although the defining feature of epilepsy are epileptic seizures, an important disabling factor of the condition are psychiatric comorbidities such as mood disorders or cognitive deficit [1, 2]. Some of the comorbidities can be studied also in animal models of epilepsy [3]. Long-term video recording of laboratory mice with experimentally induced epilepsy is an excellent tool for studiyng both seizures and comorbidities. The student will explore possibilities of automatic tracking of the mouse and its pose in the video recordings [4, 5]. The first goal will be identifying epileptic seizures which will be confirmed by concomitant EEG recordings [6]. The second goal will be to analyze long-term fluctuations in the behavior, such as amount of locomotor activity and correlate it to the seizure probability [7]. The results of this ambicious project will enable automatic detection of seizures in video recordings and automatic analysis of features of interictal behavior. The knowledge on long-term fluctuation of behavioral comorbidities can guide the development of chronotherapy, i.e. application of the treatment at the times when it is most effective and has least adverse effects.

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[7] Kudlacek et al.: Long-term seizure dynamics are determined by the nature of seizures and the mutual interactions between them. Neurobiol Dis 2021. doi: 10.1016/j.nbd.2021.105347

Name and workplace of master's thesis supervisor:

Ing. Jan Kudláček, DiS., Ph.D. Second Faculty of Medicine, Charles University

Name and workplace of second master's thesis supervisor or consultant:

prof. Ing. Roman Čmejla, CSc. Department of Circuit Theory FEE

Date of master's thesis assignment: **04.01.2024** Deadline for master's thesis submission: **24.05.2024**

Assignment valid until: **21.09.2025**

Ing. Jan Kudláček, DiS., Ph.D. Supervisor's signature	doc. Ing. Radoslav Bortel, Ph.D. Head of department's signature	prof. Mgr. Petr Páta, Ph.D. Dean's signature

III. Assignment receipt

The student acknowledges that the master's thesis is an individual work. The student must produce his thesis without the assistance of others, with the exception of provided consultations. Within the master's thesis, the author must state the names of consultants and include a list of references.

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Date of assignment receipt	Student's signature

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Declaration

I declare that this work was developed independently and that I have listed all sources of information used within it in accordance with the methodical instructions for observing the ethical principles in the preparation of university theses. In Prague, 24. May 2024

Abstract

Epilepsy is a neurological disorder affecting about 50 million patients worldwide. While seizures are the defining feature of epilepsy, behavioral comorbidities such as depression or hyperactivity are common and constitute significant burden to the patients. Multiple studies demonstrated slow fluctuations in seizure risk over days to weeks in both patients and mouse models of epilepsy. We hypothesized that these fluctuations were accompanied by changes in the behavior (quantified by locomotor activity), which would potentially enable better estimation of current or future level of seizure risk. We analysed this hypothesis using video-EEG recordings from a mouse model of neocortical epilepsy. Since manual analysis of several weeks-long recordings is prohibitively laborious, we used automatic detection of the locomotor activity of the mouse. We tested two algorithms, DeepLabCut (DLC) and PixelCount (PIX). Since the analysis was still highly time consuming, we also tested various levels of frame rate reduction. Reducing the frame rate from 25 FPS to 5 FPS yielded the best trade off between the accuracy of the analysis and speed of the computation. The locomotor activity fluctuated in a relation to the seizure rate. However, due to the high variability of this relationship and insufficient amount of data, this relationship could not be fully characterized. Importantly, we showed that the both DLC and PIX algorithms are usable for this type of analysis which opens great prospects for the future work.

Keywords: Epilepsy, Behavior monitoring, Seizure forecasting

Supervisor: Ing. Jan Kudláček, DiS., Ph.D.
Department of Physiology, Second Faculty of Medicine, Charles University

Abstrakt

Epilepsie je neurologické onemocnění postihující přibližně 50 milionů pacientů po celém světě. Zatímco záchvaty jsou definujícím rysem epilepsie, komorbidity jako je deprese nebo hyperaktivita, jsou běžné a představují významnou zátěž pro pacienty. Několik studií prokázalo pomalé fluktuace záchvatů v průběhu dnů až týdnů u pacientů i v myších modelech epilepsie. Předpokládali jsme, že tyto fluktuace jsou doprovázeny změnami v chování (kvantifikované pohybovou aktivitou), což by potenciálně umožnilo lepší odhad aktuálního nebo budoucího rizika záchvatu. Tuto hypotézu jsme testovali pomocí video-EEG záznamů z myšího modelu neokortikální epilepsie. Protože vizuální analýza několik týdnů dlouhých záznamů je nesmírně pracná, využili jsme automatickou detekci pohybové aktivity myši. Otestovali jsme dva algoritmy, DeepLabCut (DLC) a PixelCount (PIX). Protože analýza byla stále velmi časově náročná, otestovali jsme také různé úrovně snížení snímkové frekvence. Snížení snímkové frekvence z 25 snímků za sekundu na 5 snímků za sekundu poskytlo nejlepší kompromis mezi přesností analýzy a rychlostí výpočtu. Pohybová aktivita fluktuovala v souvislosti s rizikem záchvatu. Nicméně, kvůli vysoké variabilitě tohoto vztahu a nedostatečnému množství dat nebylo možné tento vztah plně charakterizovat. Důležitým poznatkem však je, že oba algoritmy - DLC i PIX - jsou použitelné pro tento typ analýzy, což otevírá možnosti pro budoucí práci.

Klíčová slova: Epilepsie, Behaviorální monitoring, Předpověď záchvatů

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Chapter 1

Introduction

1.1 Epilepsy

Epilepsy is a chronic disease of the brain with about 50 million patients (around 0.5 to 1 percent of population) worldwide [1]. It is spread around the world, occurring at all ages [2]. Around 80-90% of epilepsy patients come from developing countries [2] [1]. The disease is characterized by recurring interruptions of normal brain function called epileptic seizures. In clinics, at least one seizure is needed to establish the diagnosis of epilepsy [3]. According to WHO, up to 70% patients with epilepsy can become seizure free with proper use of antiseizure medicine [1]. The rest of the patients remain refractory to antiepileptic drugs, this accounts to about 15 million people worldwide [4].

People who have had a seizure may fear having another, even if inter-seizure interval is long [5]. Seizures can impair patient's ability to work, to drive, and to develop social relationships [5]. Fear and uncertainty were labeled as the worst aspects when living with epilepsy [5]. These aspects originate from unpredictability of seizures.

Epilepsy patients not only suffer from seizures, but some of them also encounter behavioral disturbances. These disturbances, comorbidities, include psychiatric disorders and certain pain disorders [6]. The most commonly reported comorbidities are mood and anxiety disorders [4]. In childhood epilepsy, the most common comorbidities are depression, anxiety, autism spectrum disorders, sleep disorders, attention deficits, cognitive impairment, and migraine [7]. Other psychiatric disorders associated with epilepsy are bipolar disorder, ADHD and sleep disorders, asthma or apnea, which have been shown to have higher prevalence in epilepsy patients than in general population [6] [8]. There has also been reported higher prevalence of pain comorbidities in epilepsy patients, mainly migraine, chronic pain and fibromyalgia (chronic widespread pain, accompanied by fatigue) [6]. Memory loss in form of newly acquired memories fading over and also loss of events from past was also described in patients with epilepsy [9].

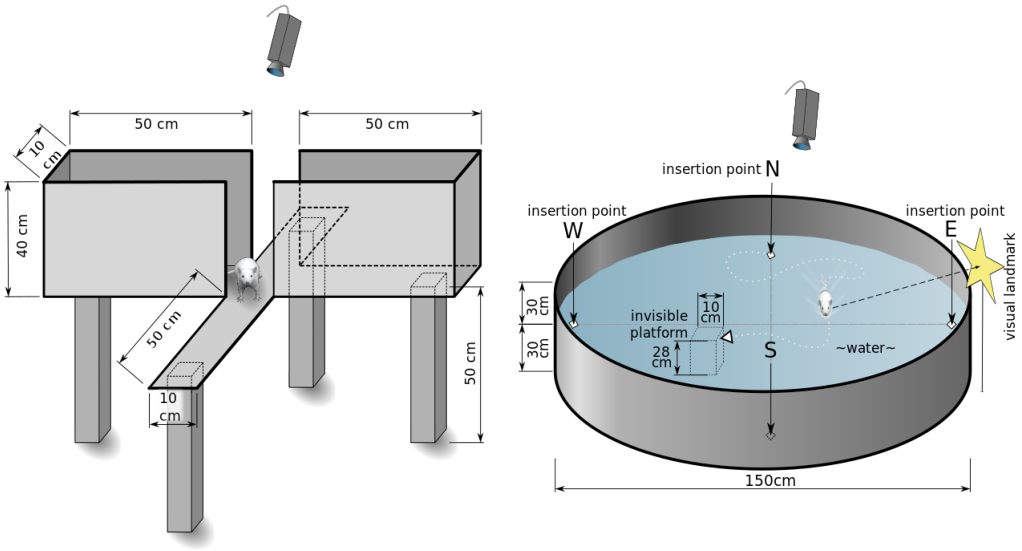
These comorbidities, together with the unpredictable seizures, may lead to social exclusion, isolation, restrictions or overprotection which become part of the condition [3].

1.2 Behavioral testing

Behavioral comorbidities can be tested by the methods of behavioral testing [10]. This thesis focuses on attempt to quantify the comorbidities based on the behavioral analysis, specifically movement, which is described in this section.

Study of biological processes, understanding of diseases and drug effects is frequently carried out on experimental animal models [10]. In this study we use an animal model of epilepsy. The comorbidities described in previous section are also common in animal models and are detectable by behavioral testing [10].

Behavior of an individual is shaped through connections between regions of the nervous system. Any change or pathology in central nervous system or any part of the body may cause changes in behavioral actions. These changes are then analyzed by so called behavioral tests [10]. Some of the common behavioral tests are elevated plus maze (in the figure 1.1), passive avoidance task, fear conditioning or Morris water maze (in the figure 1.1) [10]. These tests usually last tens of minutes per mouse and are suitable for studying e.g. learning and memory, anxiety, curiosity or motivation.



(a) Elevated plus maze [11]

(b) Morris water maze [12]

Figure 1.1: Behavioral test examples.

However, in this thesis, we take a different approach which we call "behavioral monitoring" in contrast to the traditional "behavioral testing". Instead of performing dedicated behavioral tests, we make use of video recordings obtained during long-term video-EEG monitoring of epileptic mice. The main purpose was to record the seizures. Most of the videos are, however, from interictal (i.e. between seizure) periods and may contain valuable data about the interictal behavior of the mouse which may reflect various psychiatric comorbidities [13]. Here we focus on a simple but very important behavioral parameter, which is locomotor activity, i.e. how much the mouse moves around the cage.

1.3 Motivation of long-term automatic monitoring

Behavioral testing is typically done by detailed observation of the animal in short (up to 30 minutes long) tests [10]. However, seizure rate fluctuates over days to weeks in patients as well as mice [14]. We suspect that also the comorbidities might fluctuate in such slow manner and therefore a single behavioral test might not be representative. Hence, we used a different approach: long-term behavioral monitoring. Less detailed, but carried out over several weeks, thus, capturing the possible long-term fluctuations of the behavior.

While the short testing may be done using manual labelling and the video recordings can be analysed visually by the investigator, manual analysis of the long-term recordings would be prohibitively time consuming. Therefore, large part of this thesis is devoted to the exploration of methods of automatic analysis of long-term videos.

We tested already existing method for video analysis, specifically pose-estimation, DeepLabCut (DLC) [15]. We also compared DLC with a simple method which we implemented, PixelCount (PIX), mainly based on the analysis of the difference of consecutive frames of the video. Step by step description of these analyses is provided in next chapters. We then compared the locomotor activity data extracted by each algorithm. The ultimate goal was to link the long-term variations in locomotor activity to the variations in the rate of seizures.

Chapter 2

Analyzed videos and hardware

In this chapter, there is a brief description of origin of the videos we were provided. Then there is short overview of the parts of computer used for the analyses in this thesis.

2.1 Provided videos

We analyzed videos of laboratory mice with artificially induced epilepsy [16]. The videos were recorded at the Department of Developmental Epileptology, Institute of Physiology, Czech Academy of Sciences. The mice were labeled jc20181211_1, jc20181218_1 and jc20181219_2 and in further analyses we will describe them without the underscore and numbers after that for simplification.

The mice in these videos were video-EEG monitored for several weeks continuously (except for accidental recording dropouts) in rectangular open plexiglass boxes (terrariums) of size 30 x 40 x 50 cm (length x width x height). Videos were recorded at 25 frames per second (FPS) by black and white industrial cameras. The recording was split into files containing 20 minutes each. Example frame of video is shown in figure 2.1.

Videos used in this study come from study of behavioral correlates of seizures and main aim of this study was to utilize already created videos. Because of that, the videos are video-EEG recordings. Video-EEG combines the recording of EEG with cable attached to the mice head and video, in this case video recording from above. Because of this, the cable is present at all times in the recordings and is inseparable part of the recording. This may cause disturbance in some analyses, but in the case of locomotor activity monitoring, we focus mainly on movement and when the cable is connected to the mouse, its movement corresponds to the mouse movement. Therefore, we get data about mouse movement not only from the mouse but also from the cable.

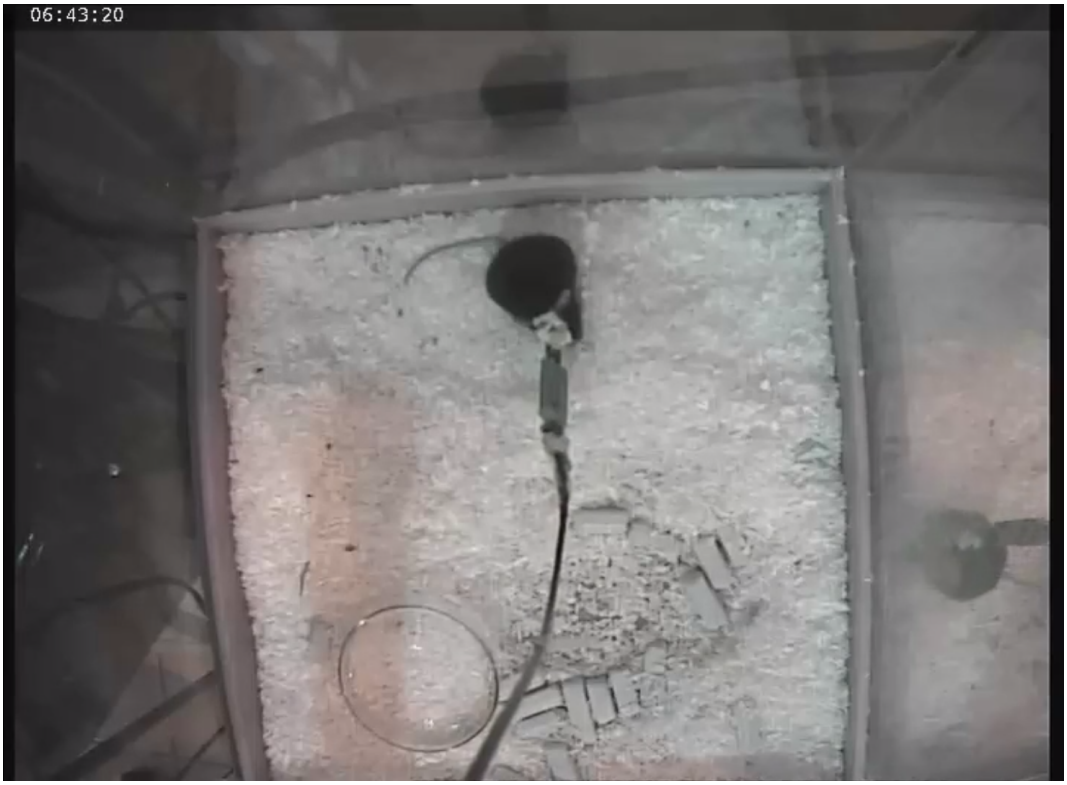


Figure 2.1: Example frame from an analysed video.

Figure 2.1 depicts terrarium of mouse, where we can see the video-EEG monitored mouse. There is also bedding material, food pellets and bowl with water present. The mouse itself is darker than the surroundings in the terrarium. The mouse has roughly same color as EEG cable. There are also apparent shadows, reflections and neighbouring terrarium.

2.2 Used hardware

All the preprocessing and analyses were carried out on laptop Lenovo IdeaPad Gaming 3 (15ACH6). Overview of specific important parts is given in table 2.1.

Computer part	Name	Detail
Processor	AMD Ryzen 5 5600H	12 CPUs 3.3GHz
Memory	RAM	16384 MB
GPU	NVIDIA GeForce RTX 3050 Laptop	11074 MB Total Memory

Table 2.1: Laptop description.

Operation system of the laptop was Windows 11 Pro 64-bit.

Chapter 3

Frame dropping and reencoding

A significant obstacle in the automated analysis of videos is the high processing time, especially when using state-of-the-art artificial neuronal network-based approaches such as the DeepLabCut (DLC). If we wanted to reduce the time while using the same algorithms, one of the possibilities is reducing the frame rate of videos, frame dropping. But this procedure itself takes some time. We thus investigated whether this procedure is worth it and whether it keeps the yield of analyses close to the original value.

3.1 Frame dropping

In signal processing, downsampling, also called compression or decimation, is a procedure of keeping every D-th sample, where D is called decimation factor. In our case, frame dropping is an analogous procedure, keeping every D-th frame and reducing the frame rate.

The original videos are filmed in 25 FPS. We decided to investigate the frame dropping factors 5 and 25, thus reducing the videos to 5 FPS and 1 FPS, respectively. Our assumption was that if we are looking for long term changes of movement, we do not need to look for instantaneous movements like trembling or scratching, but rather focus on longer-lasting movement in space (i.e. the locomotor activity).

Although frame dropping adds some time to preprocessing, we came across an error, which needed to be solved by re-encoding the videos. Frame dropping could be combined with this necessary re-encoding, thus saving some time and making the time of frame dropping itself negligible.

3.2 Re-encoding due to faulty videos

Some of the videos could not be handled by DLC directly in the format in which they were recorded, resulting in an error of wrong encoding. The videos loaded normally in video players and MATLAB so there was probably only problem with the compatibility with DLC.

At first, we thought it was a problem with AVI format itself due to the vague error message, so we re-encoded the videos to MP4 format. After further investigation, we realized that the error was due to dropped frames and AVI format would be probably usable in the end, but due to the fact that we began analyses in MP4 we continued with MP4 format. This procedure could be combined with frame dropping, as mentioned in previous section.

3.3 Method

For the frame dropping and re-encoding, we used FFmpeg. FFmpeg is multimedia framework supporting almost all formats used to processing videos in many ways[17].

For specific frame rates we executed command:

```
for %i in (.*avi) do ffmpeg -y -i "%i" -r N %~ni_decN.mp4
```

This command executed frame dropping for each AVI format video while also re-encoding and converting format. The resulting video is of N frames per second and in MP4 format.

for %i in (.*avi): This part initiates a loop that iterates over files matching the pattern .*avi in the current directory. $\%i$ is a placeholder for each file found.

do ffmpeg -y -i "$\%i$**" -r N** $\%\sim$**ni_decN.mp4**: This part of the loop executes the FFmpeg command for each file.

FFmpeg options:

-y: Overwrites output files without asking for confirmation.

-i "$\%i$**"**: Specifies the input file, where $\%i$ is the current file in the loop.

-r N: Sets the output frame rate to **N**.

$\%\sim$ **ni_decN.mp4**: Constructs the output file name. $\%\sim$ ni extracts the file name without the extension from the input file, and **_decN.mp4** is appended to it. The placeholder **N** should be replaced with the desired frame rate.

3.4 Comparison of frame dropping and re-encoding

In the table below, we present the approximate time taken by re-encoding and frame dropping using FFmpeg. We compared three frame rates - 25 FPS (original), 5 FPS and 1 FPS. Speed is argument returned by FFmpeg, corresponding to the length of the video divided by processing time of the video. Last column shows approximate real time taken by the procedure on a 20 minute video. Note that these numbers are illustrative and highly depend on the specifications of the computer and also other potential concurrent workload.

Frame rate [FPS]	**Speed [-]**	**Approx. time [s]**
25	22	70
5	60	20
1	120	10

Table 3.1: Frame dropping processing time.

Chapter 4

Analysis methods

In this chapter, we describe the two algorithms we used to analyze the locomotor activity of the mice. These algorithms are DeepLabCut (DLC) and Pixel Count (PIX). DLC is feature detector subset of DeeperCut, pose estimation algorithm [15]. PIX is simple Matlab script quantifying pixel change frame by frame, which is our own implementation.

DLC is an excellent tool for pose-estimation, which accurately marks pre-selected bodyparts. It can also be used for multi-animal projects. However, analyses with DLC are highly time consuming. In PIX we focused on simplicity, hopefully pushing the speed of analysis to limits. In the case of simple algorithms, there is always trade-off for accuracy, which we analyzed by comparison to already proven accurate DLC.

In the last section, we present overview of algorithms and procedures researched in this thesis. This is to provide some information about other already existing open source algorithms.

4.1 DeepLabCut

DLC is a deep convolutional network combining two key ingredients from algorithms for object recognition and semantic segmentation: pretrained residual neural networks (ResNets) and deconvolutional layers [15]. ResNets weights were trained on an object recognition benchmark called ImageNet [15] [18]. To fine-tune the network for a particular task, its weights are trained on labeled data [15].

While installation of DLC is described in detail at DLC github [19], we will provide brief overlook of steps and describe fixes for specific errors encountered. Note that from version 2.1 of DLC there is full front-end user experience provided in form of Graphical User Interface [19]. DLC is licensed under the GNU Lesser General Public License v3.0 [19].

4.1.1 Installation and environment creation

Firstly, for DLC installation, you need to have CONDA installed, since DLC works in its environment. CONDA is an open-source package and environment management system

used by multiple companies and provided by Anaconda [20]. It runs on Windows, macOS, and Linux. It is used to install, run, and update packages and their dependencies [20].

With Anaconda installed, next step is to download CONDA file from DLC github and creating the CONDA environment for DLC installation. This can be created easily in Anaconda Prompt with command:

```
conda env create -f DEEPLABCUT.yaml
```

This command needs to be used in folder where DEEPLABCUT.yaml is downloaded. After installing and setting up CONDA environment, DLC is prepared for project creation. Again, the process is described in detail in DLC manuals, we will just describe our specific steps.

4.1.2 DeepLabCut project

First step is to chose the dataset to fine-tune on - excellent results were achieved even on 100 frames [15]. We chose 10 videos as the training data. This number should prove sufficient, due to the fact that there was almost no change in the surroundings of the mouse and the mouse was seen from the same angle at all times.

The next step is to extract and manually label the frames - we set DLC to extract 10 frames from each video, adding up to 100 frames extracted in total. The DLC was set to the default settings, which means the extraction used K-means method. The frames were then manually labeled by the previously chosen body parts (shown in the table 4.1) and then the project was ready for model training. In our project, we chose these body parts:

Body
Nose
EarLeft
EarRight
Neck
SpineFront
ForelimbLeft
ForelimbRight
SpineBack
HindlimbLeft
HindlimbRight
TailRoot
TailMid
TailEnd
Cable
cable1
cable2
cable3

Table 4.1: DLC tracked body parts.

Note that there is the cable included due to the video-EEG recording. The cable is connected to head of the mouse and we chose to also track it. In this thesis, we focus on the locomotor activity, so it may seem unnecessary to track so many points, however, the detection of additional points does not add significant time to the analysis and the data about the body parts can be used for additional analyses beyond the scope of this thesis. Additionally, the locomotor activity is estimated as a distance between succeeding averages of tracked points, which means the more points tracked, the more accurate estimation we get. The distance is then converted from pixel units into metric units and multiplied by frame rate to achieve the speed in meters per second. In our study, we calculated the estimate of position as mean of forelimbs, hindlimbs and spine. This adds up to 6 points, which gives us reasonable assurance, that at all times at least one point will be present.

Figure 4.1: DLC extracted frame with manually labeled body parts.

After the frame labeling, the model was trained using default settings except maximum iteration changed to 200000 from default 100000. After the model training, DLC is ready for video analysis.

The trained model then goes through all the videos and produces .csv files with positions of all selected body parts. It also provides probability density which represents the 'evidence' that a body part is in a particular location [15].

DLC is able to create a labeled video, which allows to visually check the results. An example frame of labeled video with body parts from table 4.1 can be seen in the figure 4.2.

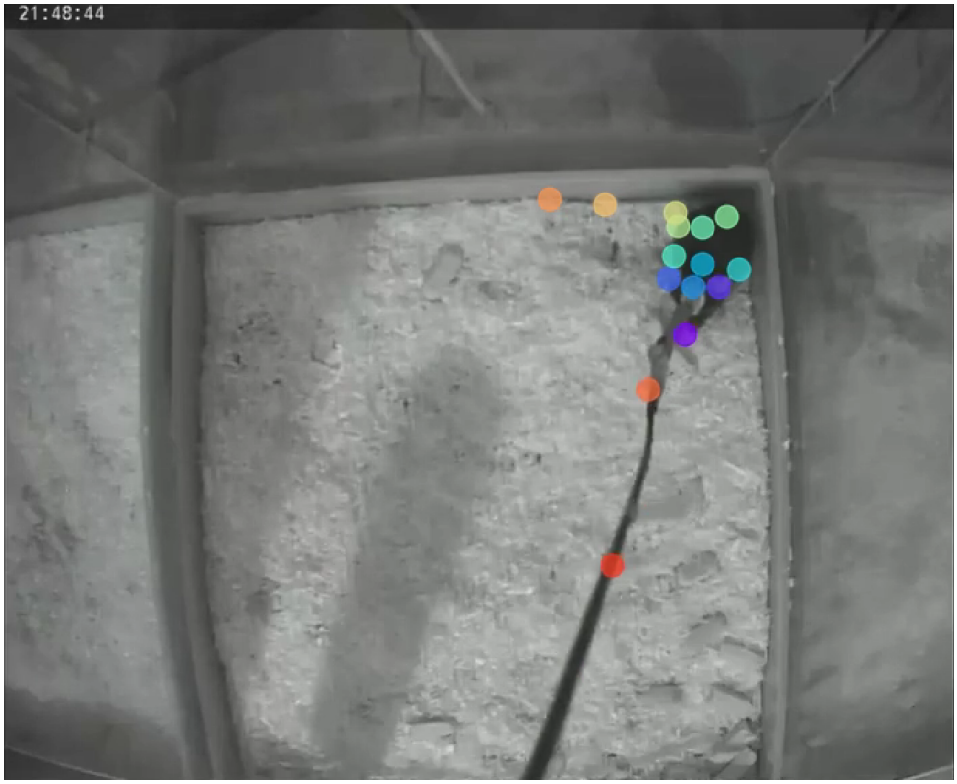


Figure 4.2: DLC automatically labeled frame.

4.2 PixelCount

The idea of this algorithm is to count the number of pixels changed between following frames. It is based on the assumption that number of changed pixels could substitute the tracking method, meaning the distance travelled as a metric is substituted with number of changed pixels frame to frame. If this assumption is correct, this method could prove much faster compared to mouse tracking methods.

Due to the simplicity of the method, there is noise contained caused mainly by background shadows. We investigated whether the method was yielding comparable results and this method could substitute the highly time consuming DLC analysis.

The output of this algorithm is a vector of numbers corresponding to the number of pixels changed between consecutive frames, which we use as a metric substituting the travelled distance. To find this metric, we first need to convert the frames to grayscale. This is due to the fact that the videos are saved in rgb - three separate channels. After that, we subtract frame_{i-1} from frame_i to obtain difference frame (procedure shown in the figure 4.3).

The difference frame is not the final step before counting and it needs to be processed. We tried to erode and dilate the grayscaled frames, but after some unsuccessful tries we replaced the erosion and dilatation with simple thresholding. We set the threshold to -20 experimentally (negative values of pixel change correspond to mouse appearing in new frame because the mouse is black - values near 0). After that, the thresholded frame pixels are counted and we obtain the final value.

Arguably, we could track both the negative and positive values - both where the mouse appeared and disappeared. This method could provide better results in cases of mouse standing up or falling on its back. Since we are interested mainly in the long term movement rather than smaller behavioral patterns like standing up, tracking only the part where the mouse appeared was deemed sufficient.

Note that there is always the cable movement included in the resulting values. This is not a problem, because the cable is tied to the mouse and its movement is related to mouse movement itself.

Figure 4.3 shows what the difference frame shows in case of a detectable movement. The white part in the difference frame would correspond to mouse "disappearance" area, while the black part corresponds to our "appearance" area, which we track. On the thresholded difference image, there can be seen something like the "contour of the mouse appearance".

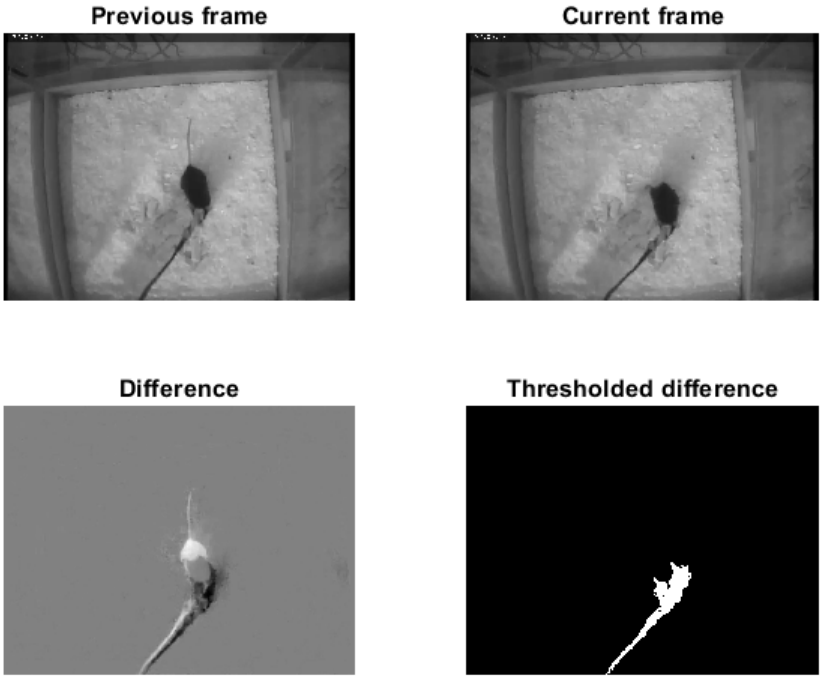


Figure 4.3: PIX example.

4.3 Different approaches to analysis

There have been other algorithms and methods developed focusing on behavioral testing in the past. These algorithms focus on pose estimation or analysing behavioral patterns. Some of the tracking methods are optical flow, Markov random field theory based monitoring and behavioral patterns clustering tool VAME. There are also other deep-learning tools such as SLEAP.

Optical flow is the distribution of apparent velocities of movement of brightness patterns in an image [21]. One of the most popular ways of analysing optical flow is Horn-Schunck method, minimising pixel-to-pixel variation among velocity vectors [22] [21]. Automated optical flow detection might prove useful in development of automated system for seizure recognition and characterisation [22].

Markov random field theory can be utilized for movement detection [23]. This method searches for optimal configuration of change detection map, using constraints between neighbouring pixels ("changed"/"unchanged" pixel will likely to be "changed"/"unchanged" too) [23]. Great robustness and accuracy of this method has been shown on monitoring arm of epilepsy patient [23].

Social LEAP (SLEAP) is a deep learning method based on earlier LEAP (LEAP estimates animal pose) algorithm [24]. It includes pose-tracking workflow, including interactive labeling, training, inference and proofreading [24]. SLEAP uses convolutional neural networks simpler than DLC. SLEAP may perform better than DLC in multi animal projects.

VAME - Variational Animal Motion Embedding is a tool used to cluster data obtained from pose-estimation tools [25]. It is used to find behavioral patterns and sort them after the tracking itself was concluded. The model uses recurrent neural networks [25].

We implemented one additional method, which tracked the mouse itself based on image thresholding - difference of color between mouse and its surroundings, but the method was struggling due to the presence of the cable. Because of that, we decided to abandon this method. We provide brief description of that method in the next section.

4.4 Mouse region with thresholding

In this algorithm, we also wanted to use the fact that the mice are black whereas the bedding is light. We focused on tracking the mouse and extract the locomotor activity by saving its positions. This procedure was done in several steps.

The image was first converted to grayscale image. This was done due to the videos being saved in three channels. Then we cut out the area outside of mouse terrarium and focused only on this part of the image.

After that, the image was thresholded (the threshold was found by analysing histograms of sample videos) so there would remain only a few objects, which should have corresponded to the cable and the mouse. In some frames it also included noise, corresponding mainly to shadows and food or bedding material.

We then selected the largest object region, corresponding to the mouse. But in large number of cases, the area of cable was larger than the area of mouse and after several unsuccessful tries with processing methods (e.g. erosion and dilation or selection of closest succeeding region) we decided to abandon the method.

Chapter 5

Relationship of locomotor activity and seizure rate

In this chapter we describe methods of analyzing relationship of epileptic seizures and locomotor activity and we will give explanation of why the metric could prove useful.

5.1 Sliding window analysis

This algorithm is a simple sliding window analysis, which processes the locomotor activity. In each window, the mean and standard deviation is computed. We tried different window lengths, ranging from 1 hour to 72 hours. The window lengths of integer multiples of 24 hours have the advantage of averaging out the circadian variations. We wanted to do so to study the long-term cycles - with frequencies of days to weeks. Additionally, longer windows than 24 hours flatten the data to such degree that they also provide no reporting value. Therefore, 24-hour windows with 18 hours overlap (i.e. 6 hour window shift) are used for the analysis of long-term fluctuations in locomotor activity.

This procedure outputs two signals, window mean signal and window standard deviation (STD) signal, which are timestamped to the end of the window. We extracted mean locomotor activity as a measure of mouse movement itself, and then standard deviation from which we calculated normalized standard deviation (STD divided by mean value) as a measures of the character of mouse movement. For example, if the mouse was slowly walking, the average could be the same as in jumping interrupted by sitting still. Such a difference in the character of the locomotor activity could be captured by the normalized STD.

To find some relation between seizures and locomotor activity, we need to prepare some metric to relate to. We did this by using the same sliding window on the timestamps of seizures converting them to signal which shows seizure count in the window - seizure frequency, also timestamped to the end of the window. Locomotor activity mean and normalised STD signals are then cross-correlated with the seizure count signal.

This method is advantageous in a sense that it could possibly be used as a seizure predictor or forecasting tool. E.g. one can imagine that an increasing or decreasing locomotor activity would indicate an approaching period of high seizure risk.

Main disadvantage of this type of analysis is that if longer windows are used, the values are flattened to such degree that data are unusable.

5.2 Time windows

In this method, the data are split into bins based on windows in day time, which correspond to day time, divided by hour. This means that data from 00:00-00:59 are assigned to first bin, 01:00-01:59 to second bin and so on.

Additionally, the data are split into bins of pre-seizure, post-seizure and non-seizure. These indicate presence of seizure in specific number of hours X. Pre-seizure data come from points where there will occur seizure in X or less hours. Post-seizure data come from points where there occurred seizure before X or less hours. Non-seizure data are the remaining data points, where no seizure occurred in preceding or upcoming X hours. Note that a point can appear in both pre-seizure and post-seizure categories, but a point appearing in a non-seizure category always belongs exclusively to it.

The split into bins of day time is advantageous due to the investigation of differences between seizure free parts and near seizure parts.

This method is useful for the exploration of the relationship between the locomotor activity and seizure rate but cannot be used for the prospective seizure forecasting due to its non-causal nature (the whole recording needs to be analyzed at a time).

5.3 Seizure detection and seizure forecasting

Due to the highly time consuming analysis of DLC, we were forced to use frame dropping. This significantly sped up the analysis, but ruled out the option to recognize some behavioral patterns - such as trembling. Because such behavioral patterns are the recognizing factor for seizure detection, we were forced to step down from the detection of single seizures to forecasting of groups of seizures [26].

Seizure forecasting is a procedure that quantifies the risk of seizure at any present or upcoming moment. Seizure forecasting can be characterised as a long-term seizure prediction. We do not want to predict seizures in upcoming minutes, but rather the risk of seizure group in upcoming hours or days. This can be illustrated on the similar example, weather forecasting, which informs us about the risk of thunderstorms, but does not predict individual lightning strikes.

To create a forecasting tool, we needed to find a relationship between the extracted locomotor activity data and the actual seizure risk (number of seizures). This was done in two steps on the averaged data, first was to analyse whether there is a linear relationship between the number of seizures and the extracted parameters in the same window. The second step was to find whether there was any relationship between the current window data and upcoming window seizure frequency.

We performed this analysis on mean values of locomotor activity and discrite time derivative (difference) of locomotor activity. This was done for both algorithms, for all mice. Due to the low number of mice and especially low number of seizure groups, this can not create forecasting tool, but rather provides a first step into locomotor activity based seizure forecasting.

Chapter 6

Results

6.1 Time taken by analysis on different frame rates

We analysed 20 minute videos of mouse labeled jc20181219_2 in three different frame rates with both algorithms for comparison. The frame rates were 25, 5 and 1 FPS. Rough average times achieved for frame rates were 15 minutes of analysis for 25 FPS video, spiking to 20 minutes in some cases, 3 minutes 10 seconds for 5 FPS and 38 seconds for 1 FPS.

Frame rate (FPS)	Average time (s)	Speed (length/time)
25	900	1.33
5	190	6.32
1	38	31.58

Table 6.1: Analyses processing time of 20 minute-long video files on different frame rates with DLC.

These numbers are estimates based on averages of 10 videos. The real average for all videos is highly dependent on workload throughout the analysis and on hardware of used computer. The time of analysis on the frame dropped videos decreases linearly with the decreasing frame rate.

In the table 6.2 we present processing time of PIX algorithm. In this analysis, the increase is even more significant.

Frame rate (FPS)	Processing time (s)	Speed (length/time)
25	205	5.85
5	36	33.33
1	5	240

Table 6.2: Analyses processing time of 20 minute-long video files on different frame rates with PIX.

6.2 Comparison of travelled distance with frame dropping

In this section we present the graphs of original and frame dropped videos for comparison. We also present close-ups to compare the instantaneous change although it is not so important for our analyses. These signals are included to show how the raw data looks and to illustrate the changes in mouse activity due to circadian rhythm (figures 6.1 and 6.2). It is also great for demonstration of differences between the algorithms.

There is an apparent increase in activity at night compared to the day (night = time when lights were switched off, 18:00-06:00). This correlates with the fact that mice are nocturnal animals and proves that the algorithms are tracking the quantity of movement correctly.

In the figure 6.1, we can see that there are peaks that extend out of the scope of the remaining values. These are outliers in some cases, corresponding to failure of tracking algorithm, although, they are present in such low volume, that they will have almost no effect in further analyses due to the averaging.

There is also a change in the amplitude of the overall signals when decreasing the frame rate. The amplitude decreases slightly for DLC in the figure 6.1 while it increases for PIX in the figure 6.2. This is caused by the calculation of the locomotor activity from output of DLC and PIX. DLC outputs the coordinates of the mice, which are averaged and distance is calculated between these points and converted to speed. Due to the triangle inequality, the distance at lower frame rate is always lower or equal to the distance obtained at the higher frame rate. On the other hand, PIX calculates the metric as number of changed pixels. On the lower frame rates, the difference of consecutive frames is usually bigger since the mouse travels longer distance from frame to frame. Thus, in this case, the amplitude increases since PIX does not normalize based on the frame rate. Note that the absolute values are of little interest for us, we are mostly interested in the fluctuations.

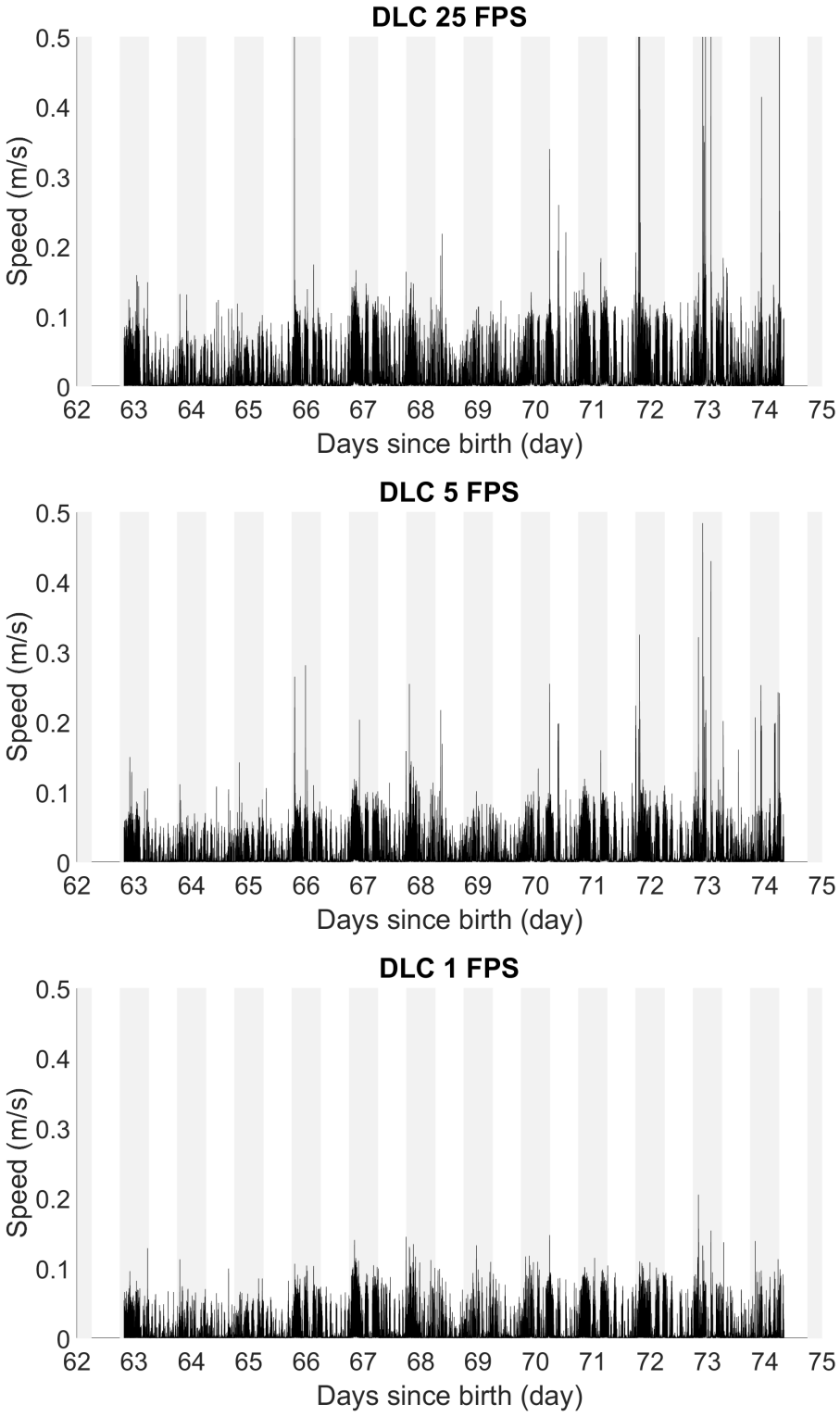


Figure 6.1: Raw DLC activity on different frame rates. Gray background indicates nighttime, note that there is increased activity. Truncated peak values at 25 FPS are reaching about 1.2 m/s. The amplitude is slightly decreasing with decreased frame rate.

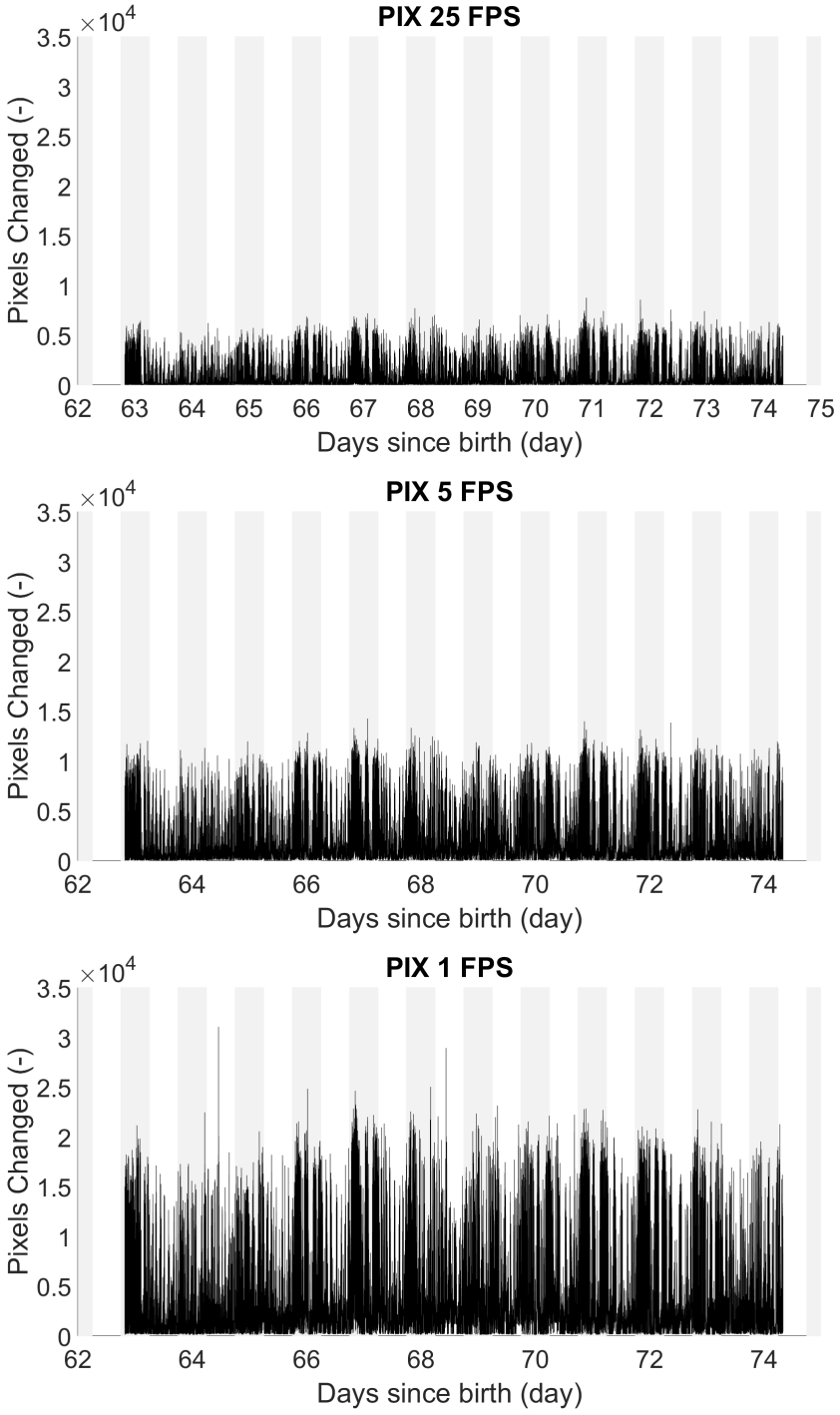


Figure 6.2: Raw PIX activity on different frame rates. Gray background indicates nighttime, note that there is increased activity. The amplitude is increasing with decreased frame rate.

To showcase the instantaneous changes of locomotor activity through one day, we present closeup on the first day of analysis. In the figures of closeups the increased daily activity at night can be seen in the figures 6.3 and 6.4.

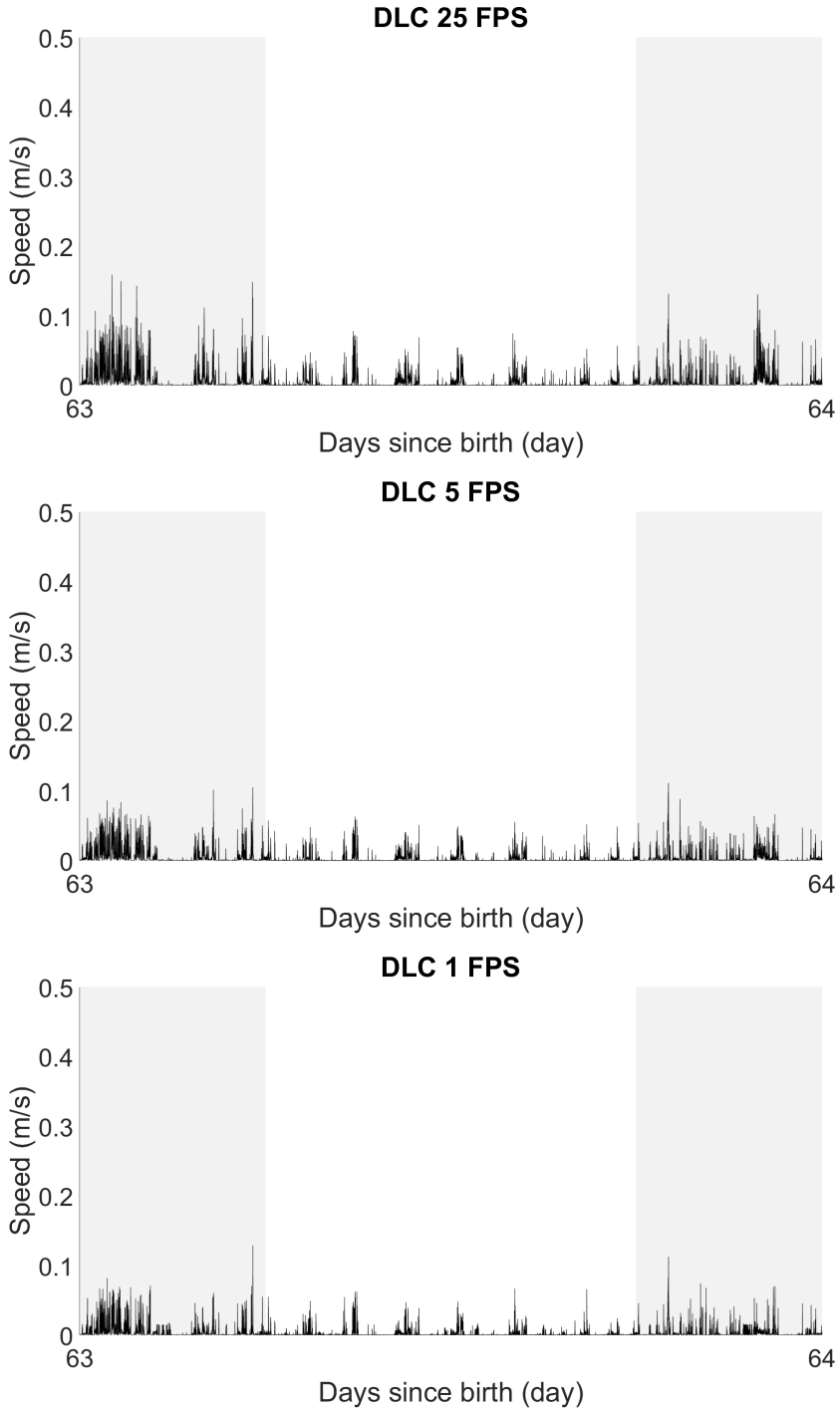


Figure 6.3: Raw DLC activity on different frame rates closeup. Gray background indicates nighttime. There is an apparent increase in activity at nighttime.

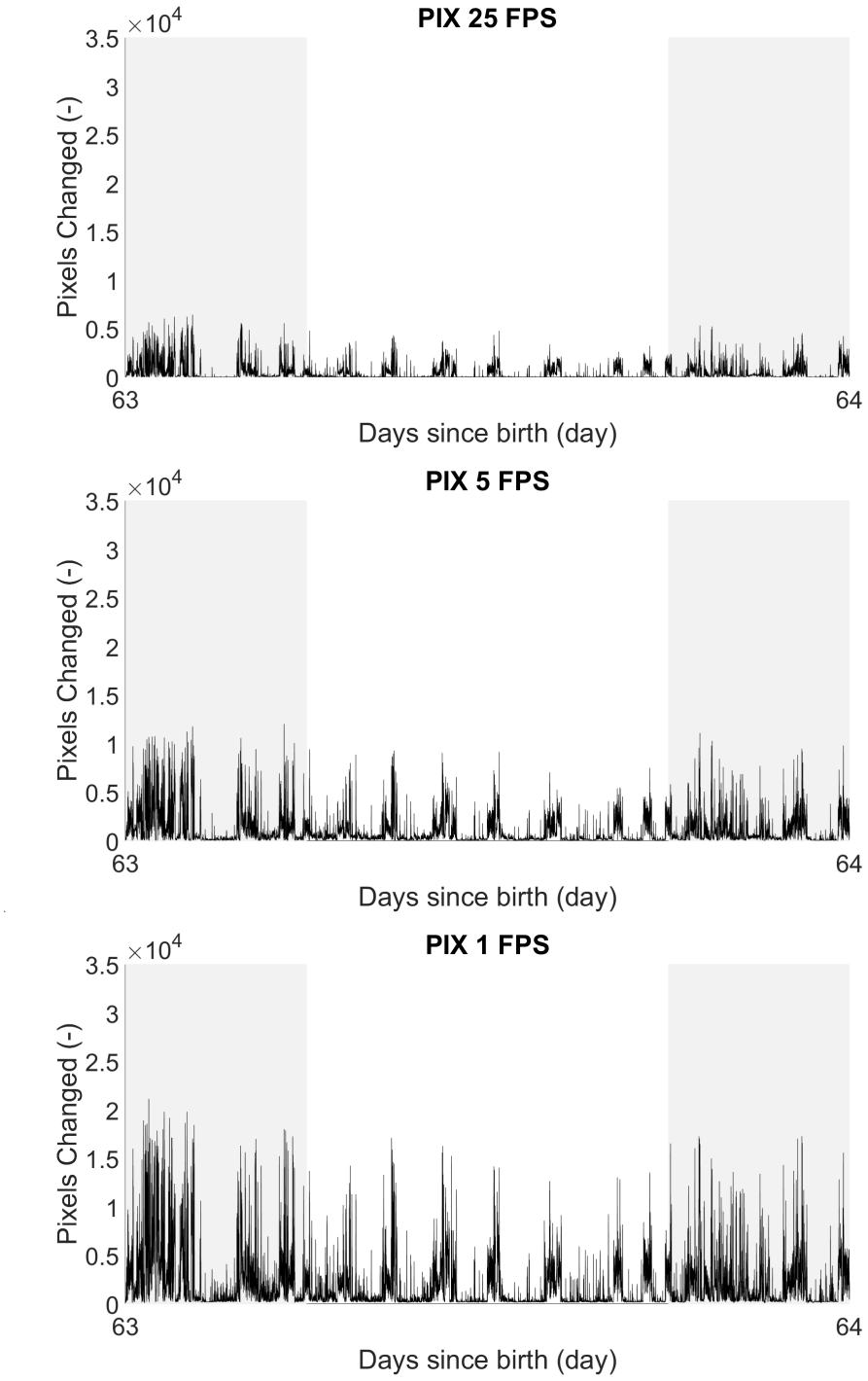


Figure 6.4: Raw PIX activity on different framerates closeup. Gray background indicates nighttime. There is an apparent increase in activity at nighttime.

In the previous pictures, there was a visible increase in the locomotor activity at night. However, we want to look past that and average out the circadian rhythm. Averaging with 24-hour window not only normalizes the circadian rhythm, but also provides information about daily locomotor activity, which we want to further investigate. Thus, for the purposes of our analyses, the figures 6.5 and 6.6 show signals averaged with 24-hour window.

It can be seen that both DLC and PIX have peaks around midnight of day 68 and 71, however, the peaks in DLC are more apparent and stand out among the surroundings. The 'shapes' of the signals also do not change with decreasing frame rate and remain almost the same.

We performed Pearson correlation on the signals with decreased frame rate. Values of correlation between different frame rates are presented in table 6.3.

Signals	Pearson correlation	P-value
DLC 25 x 5 FPS	0.99	<0.001
DLC 25 x 1 FPS	0.96	<0.001
PIX 25 x 5 FPS	0.99	<0.001
PIX 25 x 1 FPS	0.97	<0.001

Table 6.3: Correlations between averaged locomotor activity signals on different frame rates.

Based on the results of Pearson correlation, we decided to continue the further analyses on 5 FPS. It is the best possible trade-off between increasing the processing speed of further analyses and preservation of as much accuracy as possible.

Additionally, we performed Pearson correlation on these frame rates to have assurance, that DLC and PIX are not producing completely different data. In the table 6.4, we can see values of Pearson correlation, also performed on averaged signals. It can be seen that correlation increases with decreasing frame rate, this is likely due to the fact that PIX performs better on lower frame rates, since there is more apparent change between consecutive frames.

Signals	Pearson correlation	P-value
DLC x PIX 25 FPS	0.74	<0.001
DLC x PIX 5 FPS	0.78	<0.001
DLC x PIX 1 FPS	0.87	<0.001

Table 6.4: Correlations between averaged locomotor activity signals from different algorithms.

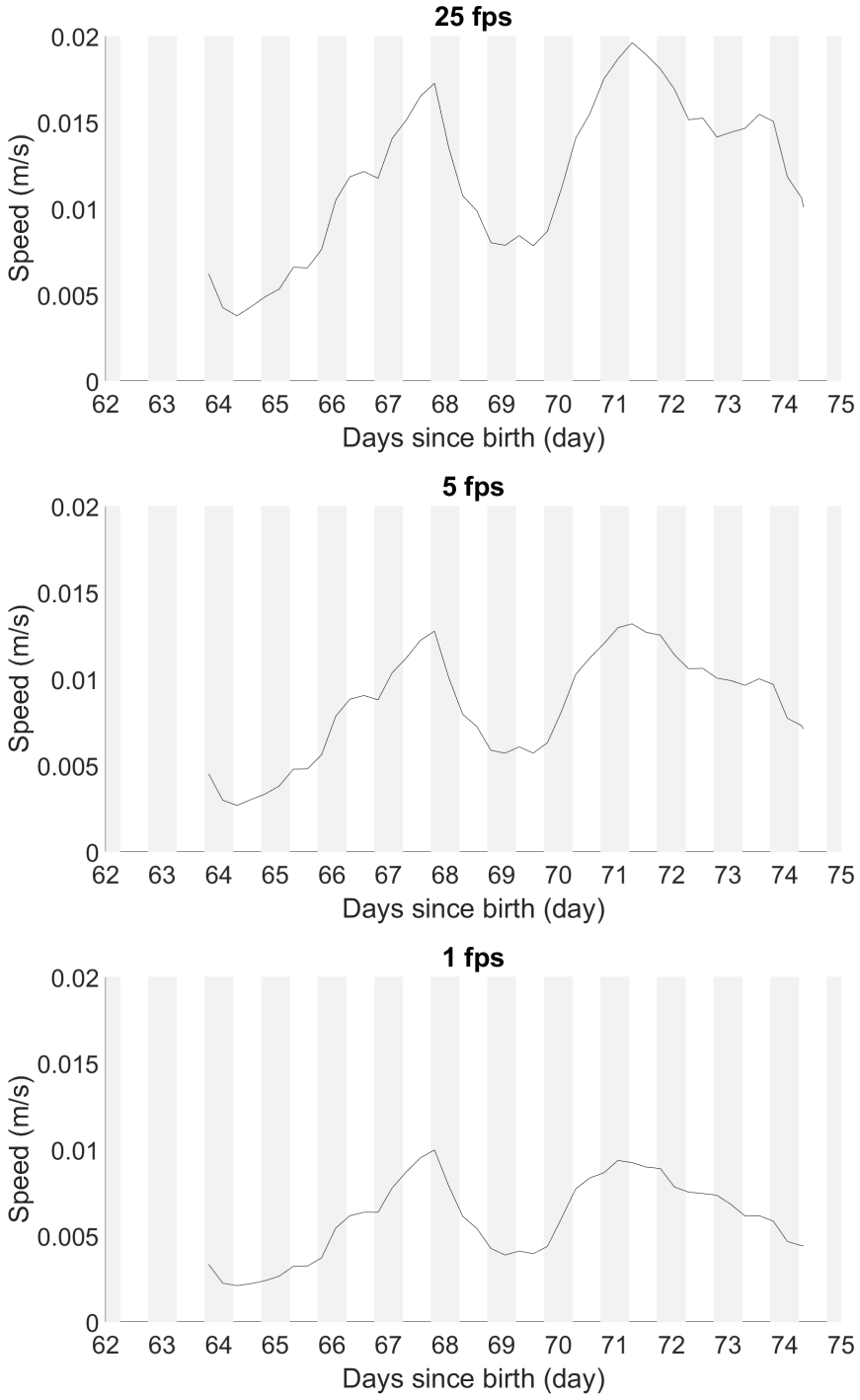


Figure 6.5: 24h window averaged DLC activity. Gray background indicates nighttime. Note that in this figure the values are not affected by circadian rhythm.

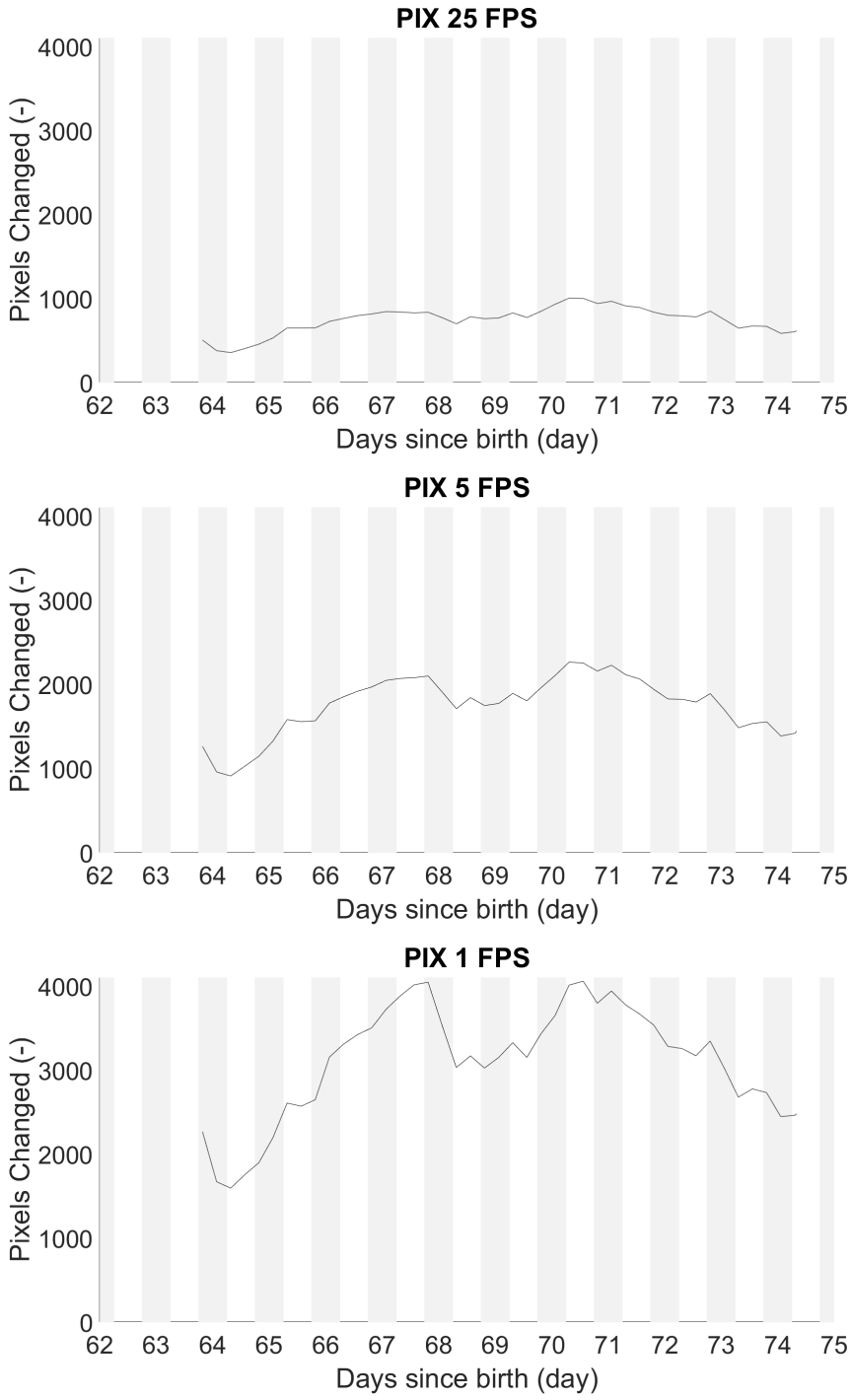


Figure 6.6: 24h window averaged PIX activity. Gray background indicates nighttime. Note that in this figure the values are not affected by circadian rhythm.

6.3 Fluctuations of locomotor activity

Here we present the results of analysing the signals of mouse activity with relationship to seizure presence and seizure count. The key part of these analyses was to prove whether there is some connection between long term change in locomotor activity and seizure of epileptic seizures.

Note that in the figures 6.7 and 6.12 there is barely any human eye noticeable difference around the seizures. On the other hand, in the remaining figures with averaged values there is human eye detectable long term change.

To rigorously confirm that there indeed is circadian rhythm detected, which needs to be normalised with specific length of sliding windows in analysis if we want to focus on long-term cycles, we calculated the periodogram and present the results in the figures 6.8 and 6.13. Periodogram of the signal is estimated by calculating the Fourier Transform (in the case of Matlab Fast Fourier Transform - FFT, function fft) of the signal, the absolute value of the FFT is then squared and divided by the number of samples of signal. We present these figures with x axis in days, to better visualise this idea. There is visible local maximum at 1 day in each mouse. This shows the occurrence of circadian rhythm.

Mean values of locomotor activity are shown in the figures 6.9 for DLC analysis and 6.14 for PIX analysis. We supposed that there would exist increase or decrease in mean values preceding the upcoming seizures. This increase is visible in the data from mouse jc20181219 and arguably also on jc20181218 data, while not even slightly in the mouse jc20181211.

In the figure 6.10 and 6.15 we present STD which is not normalized. This is provided to give some visual clue of how STD looks before normalization.

In the terms of normalised STD values, we can see apparent increase around seizures, again in mice jc20181218 and jc20181219, while increase in such manner is visible only around first group of seizures in mouse jc20181211. This is presented in the figures 6.11 and 6.16.

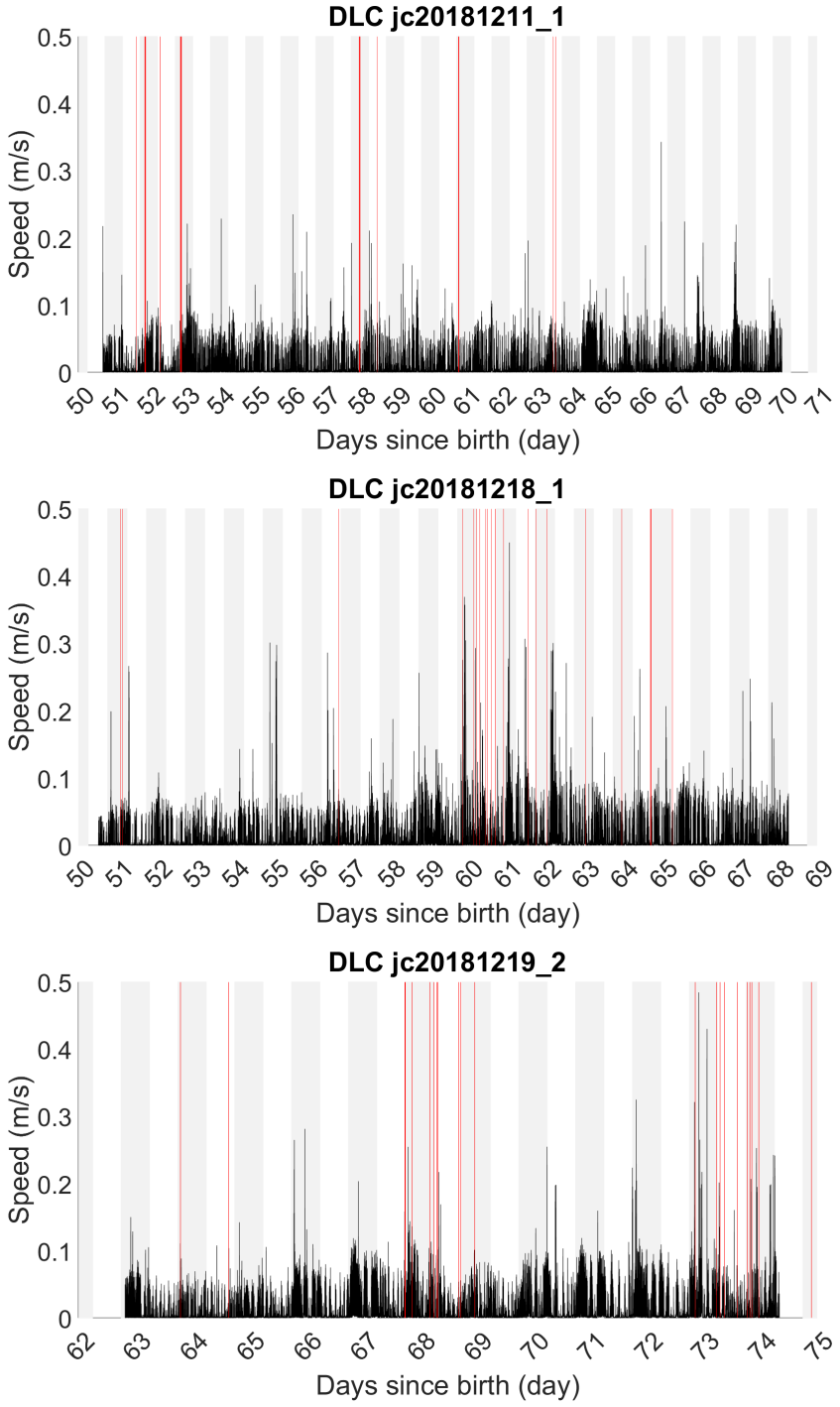


Figure 6.7: Raw DLC activity data of all mice with shown seizure clusters. Gray background indicates nighttime.

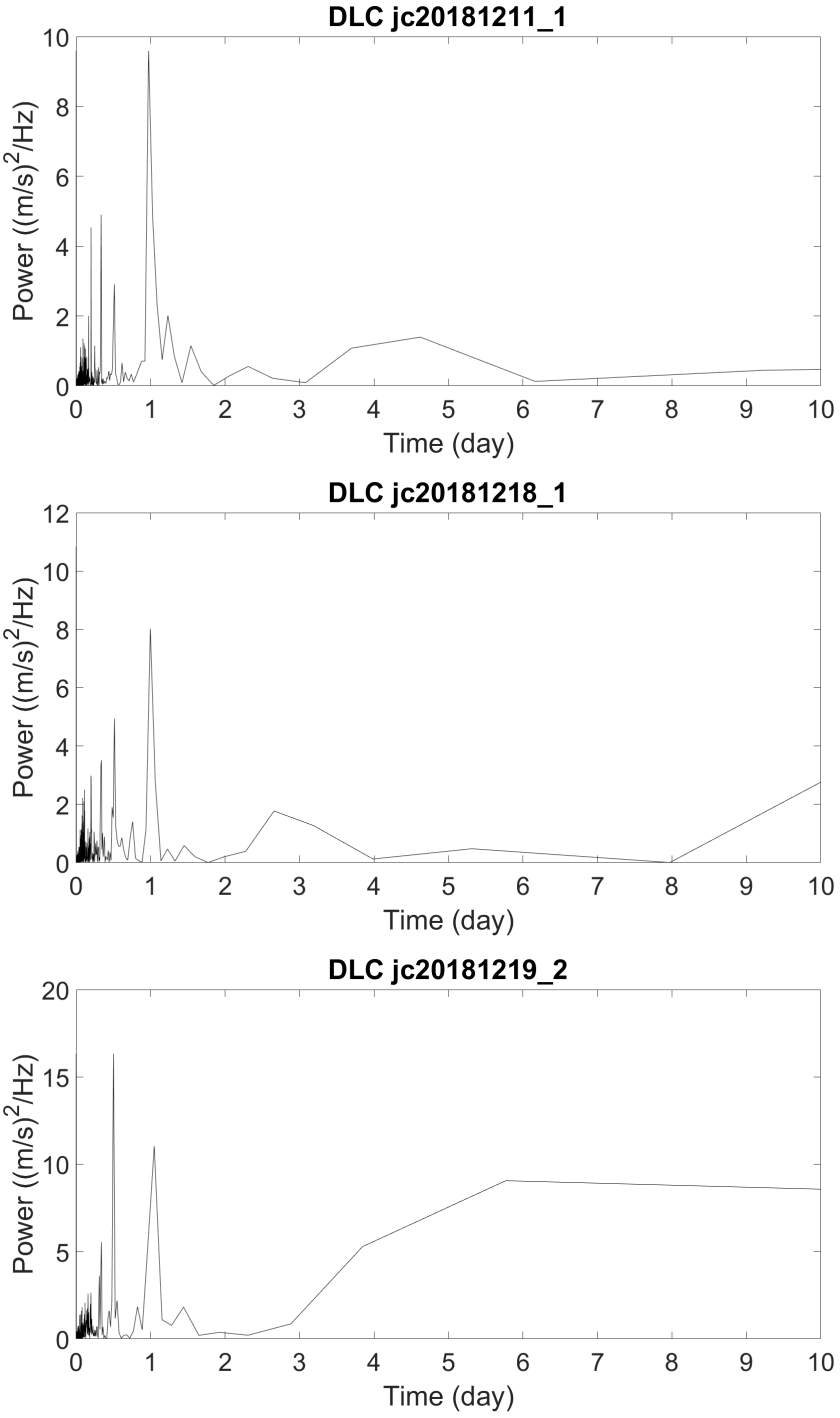


Figure 6.8: DLC periodograms of all mice. Note mainly the apparent peaks around periodicity of 1 day.

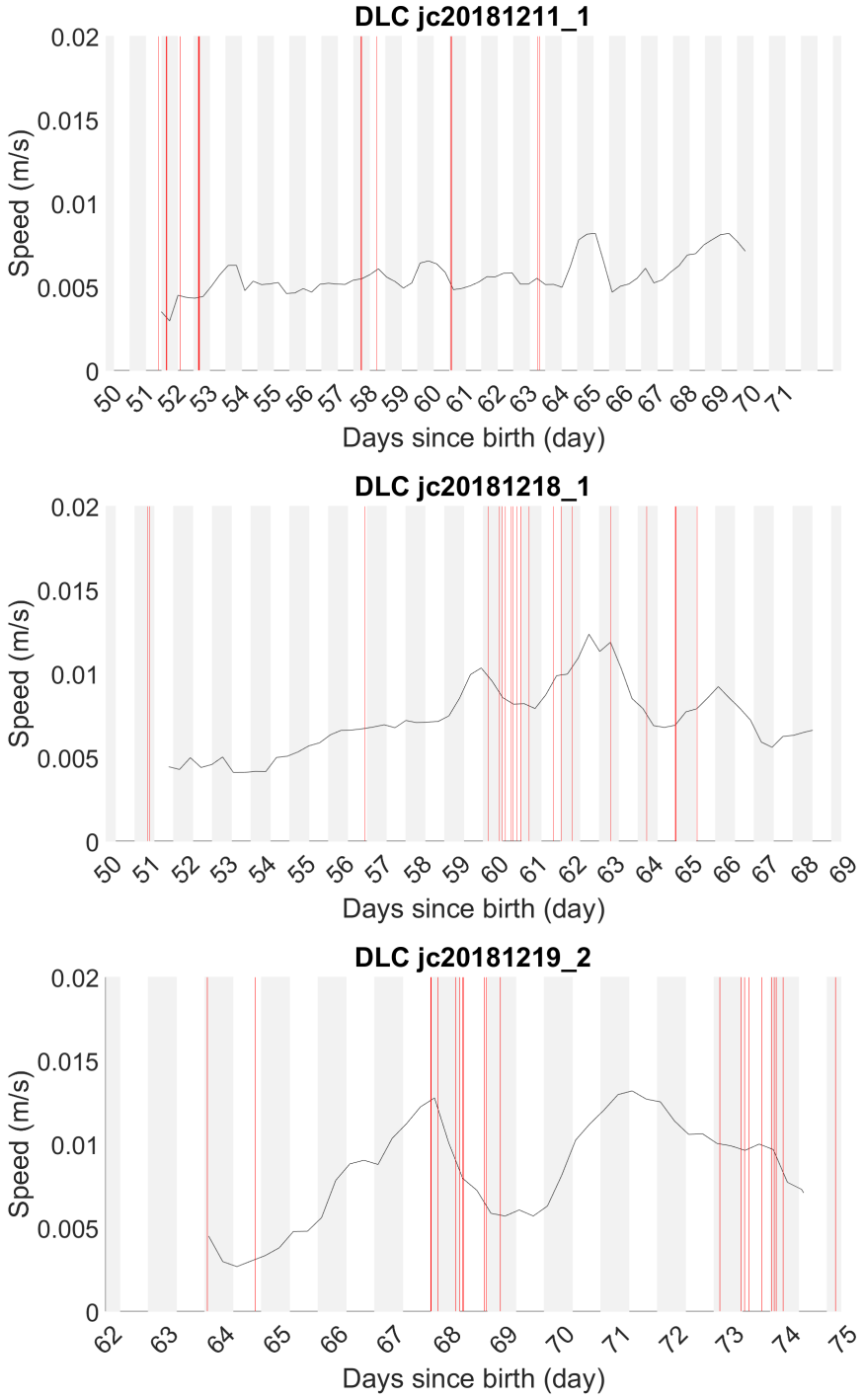


Figure 6.9: 24h window averaged DLC activity data of all mice with shown seizure clusters. Gray background indicates nighttime.

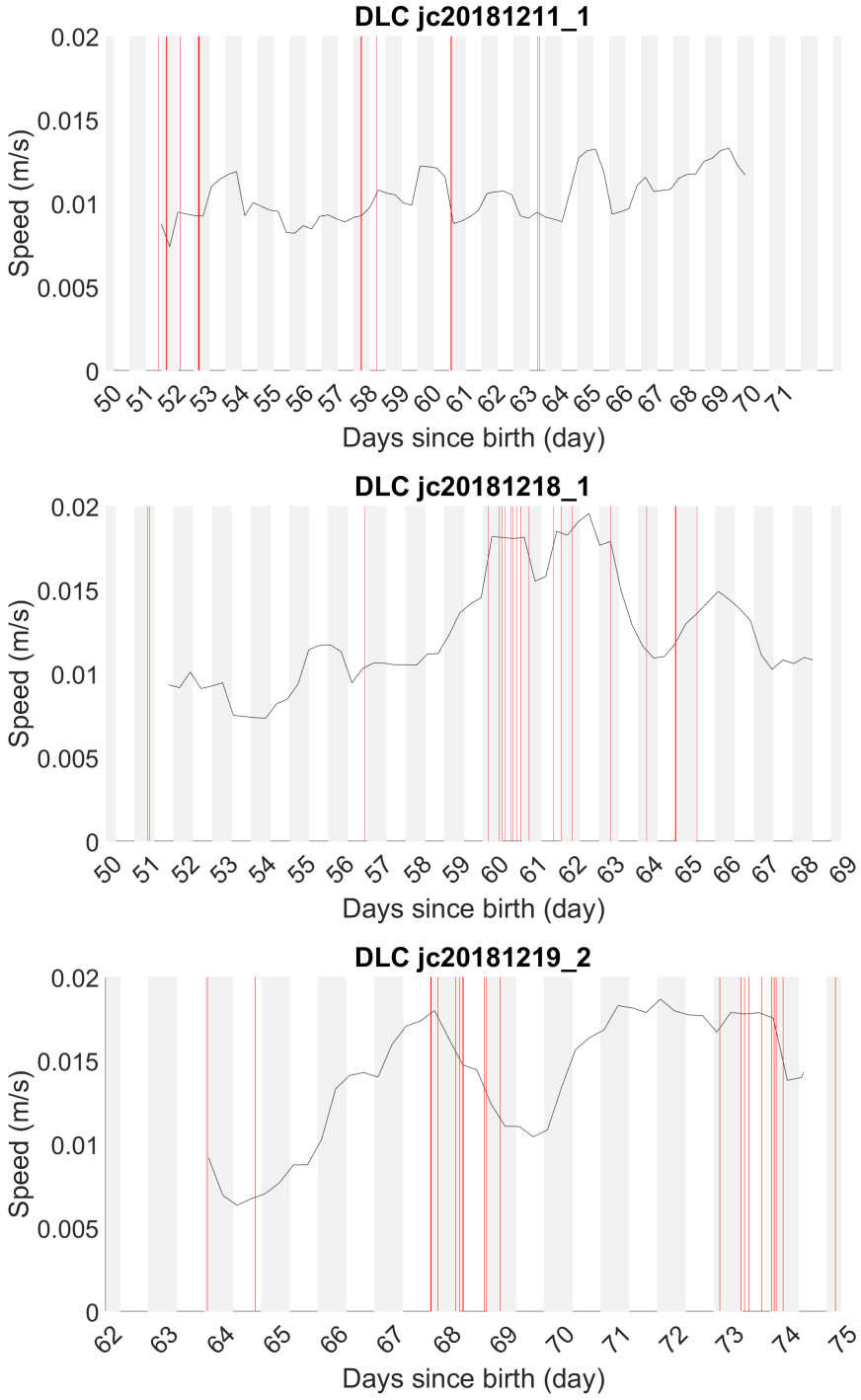


Figure 6.10: 24h STD of DLC activity data of all mice with shown seizure clusters. Gray background indicates nighttime.

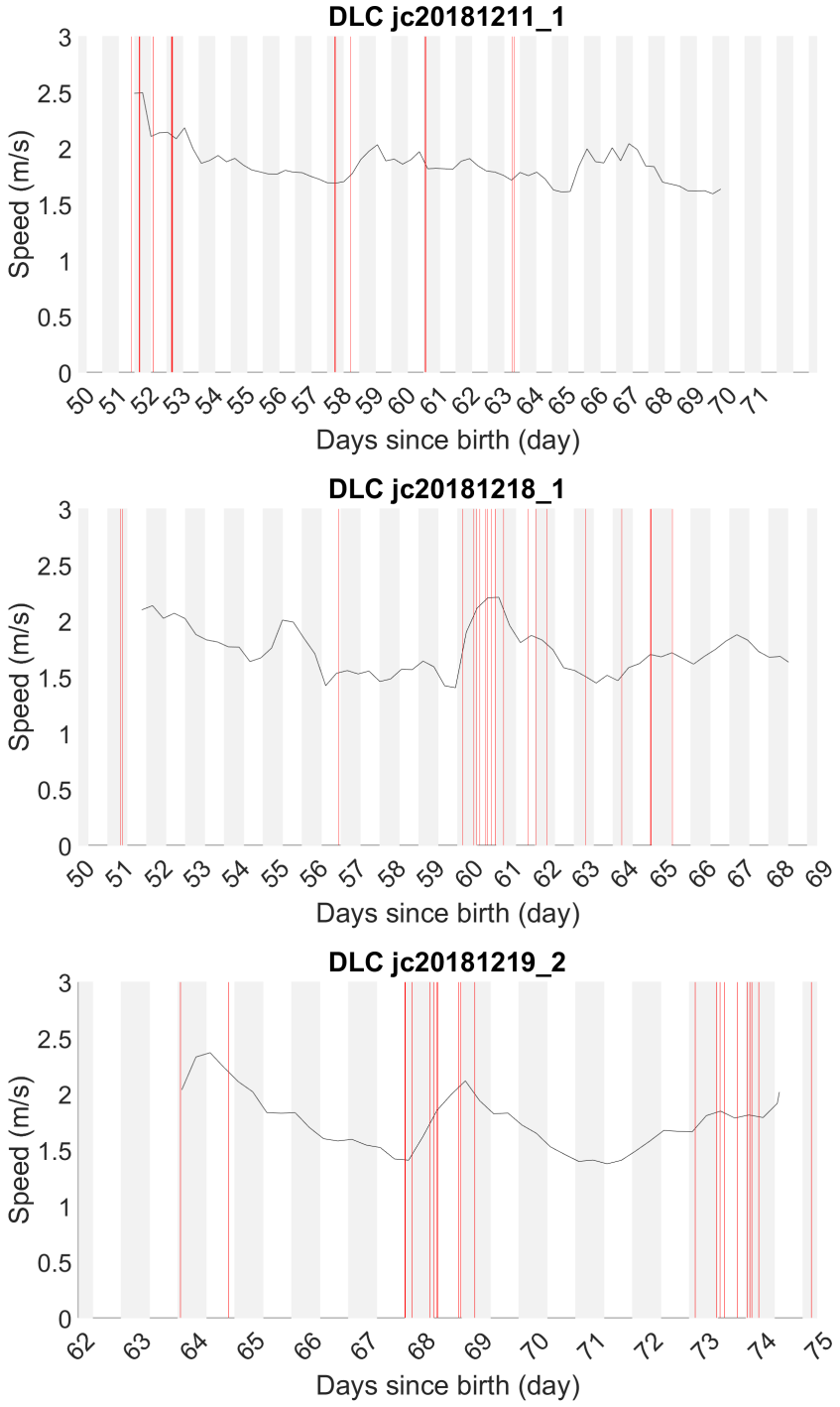


Figure 6.11: 24h normalized STD of DLC activity data of all mice with shown seizure clusters. Gray background indicates nighttime. There is visible increase around seizures in last two mice.

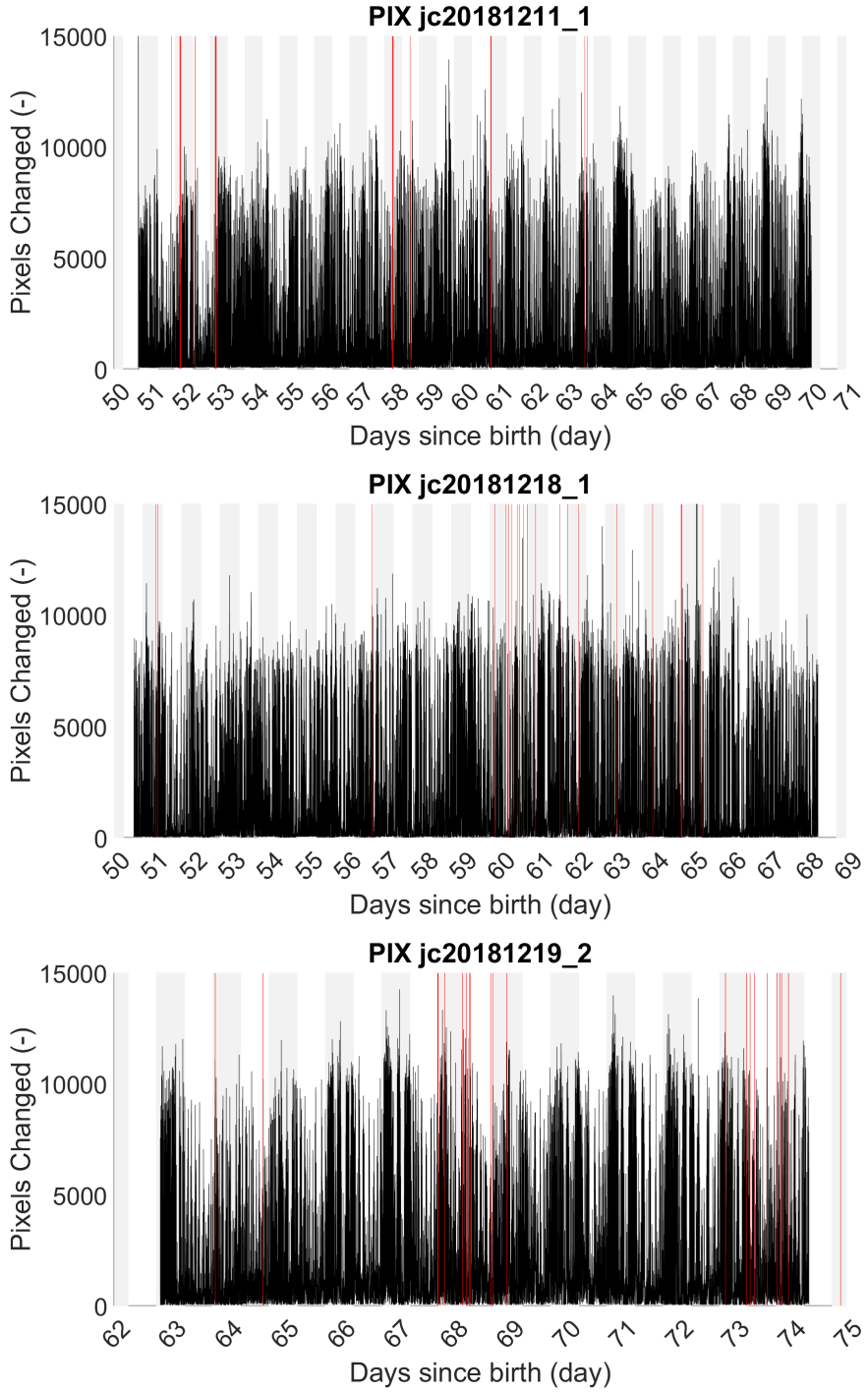


Figure 6.12: Raw PIX activity data of all mice with shown seizure clusters. Gray background indicates nighttime.

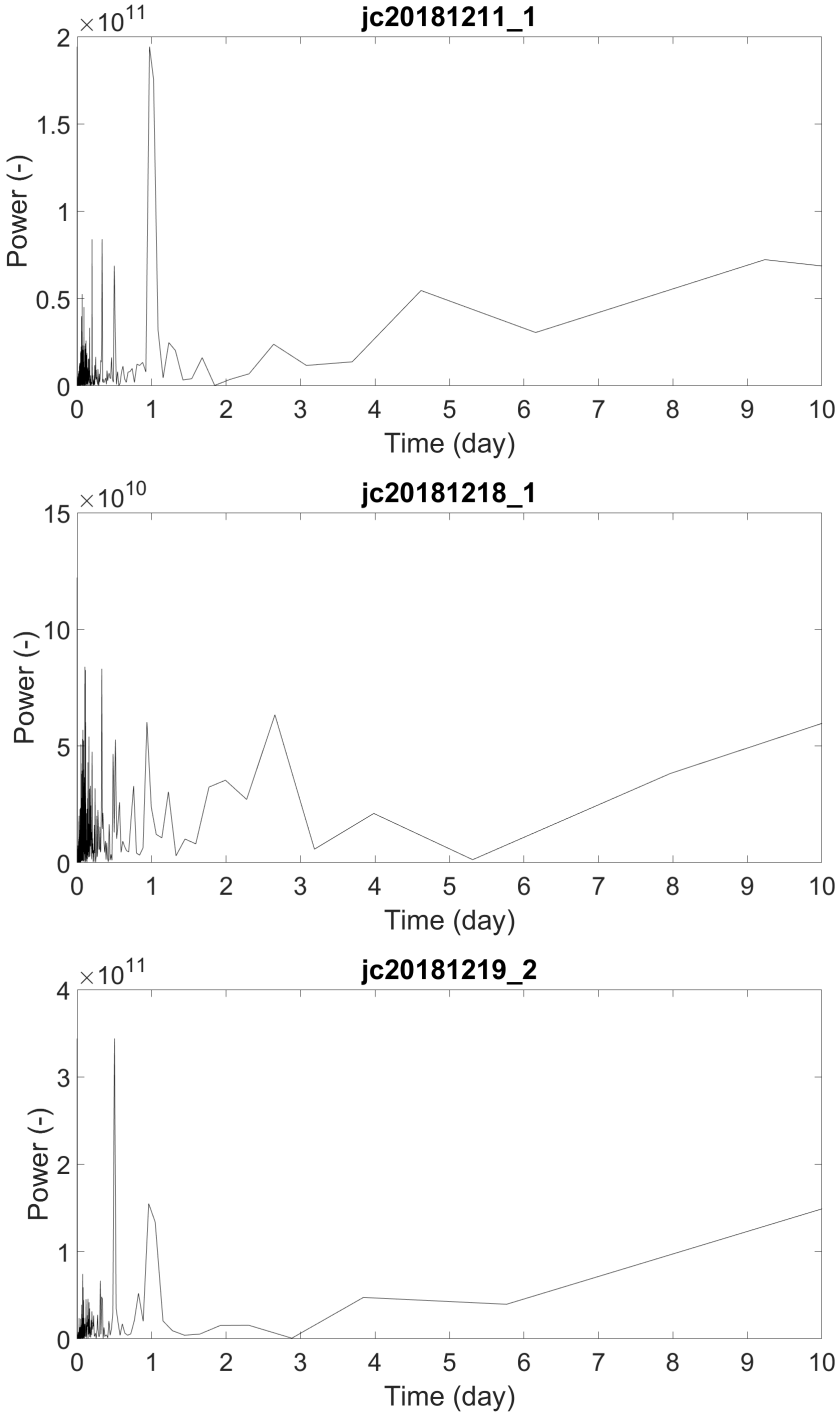


Figure 6.13: PIX periodograms of all mice. Note mainly the apparent peaks around periodicity of 1 day.

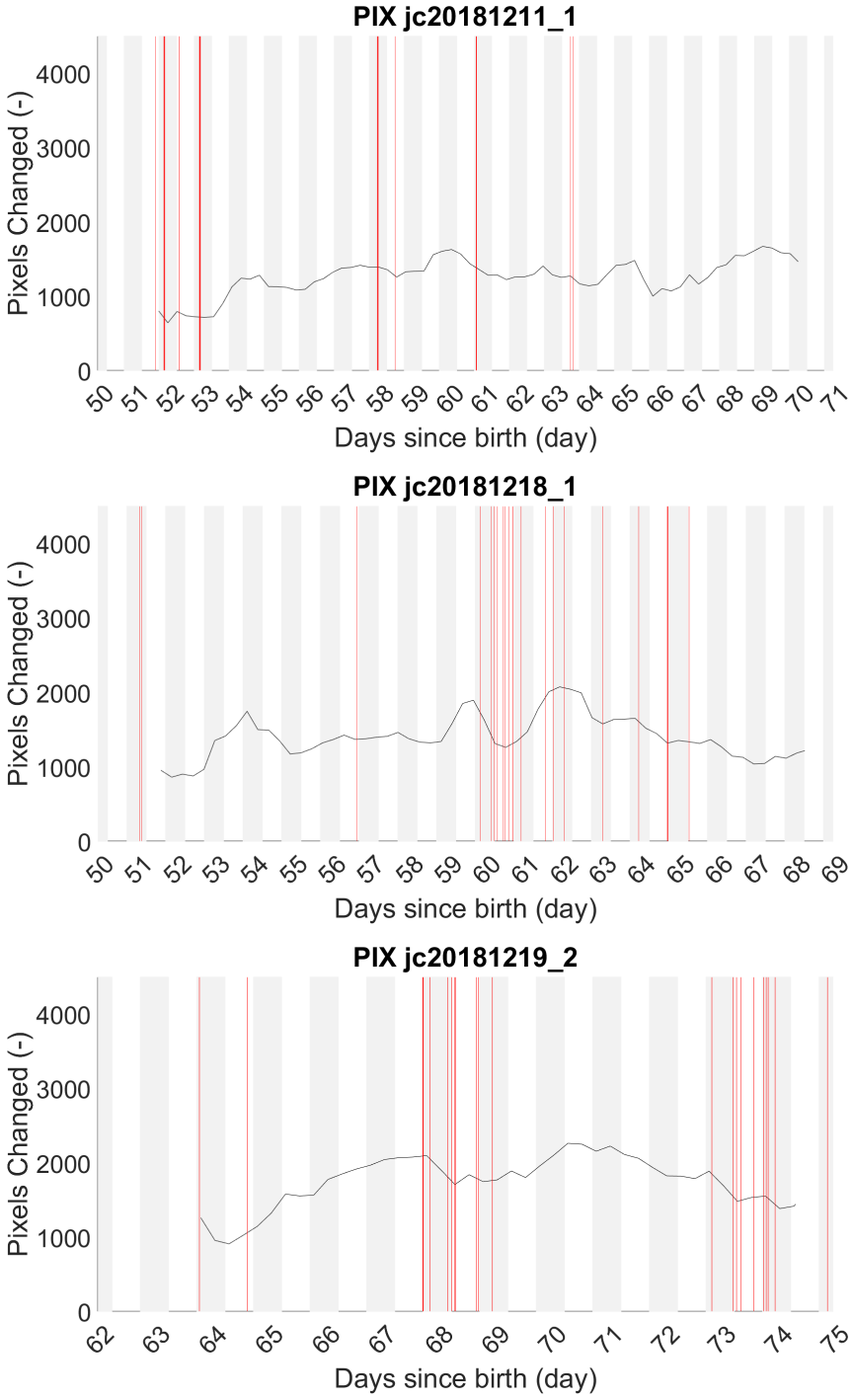


Figure 6.14: 24h averaged PIX activity data of all mice with shown seizure clusters. Gray background indicates nighttime.

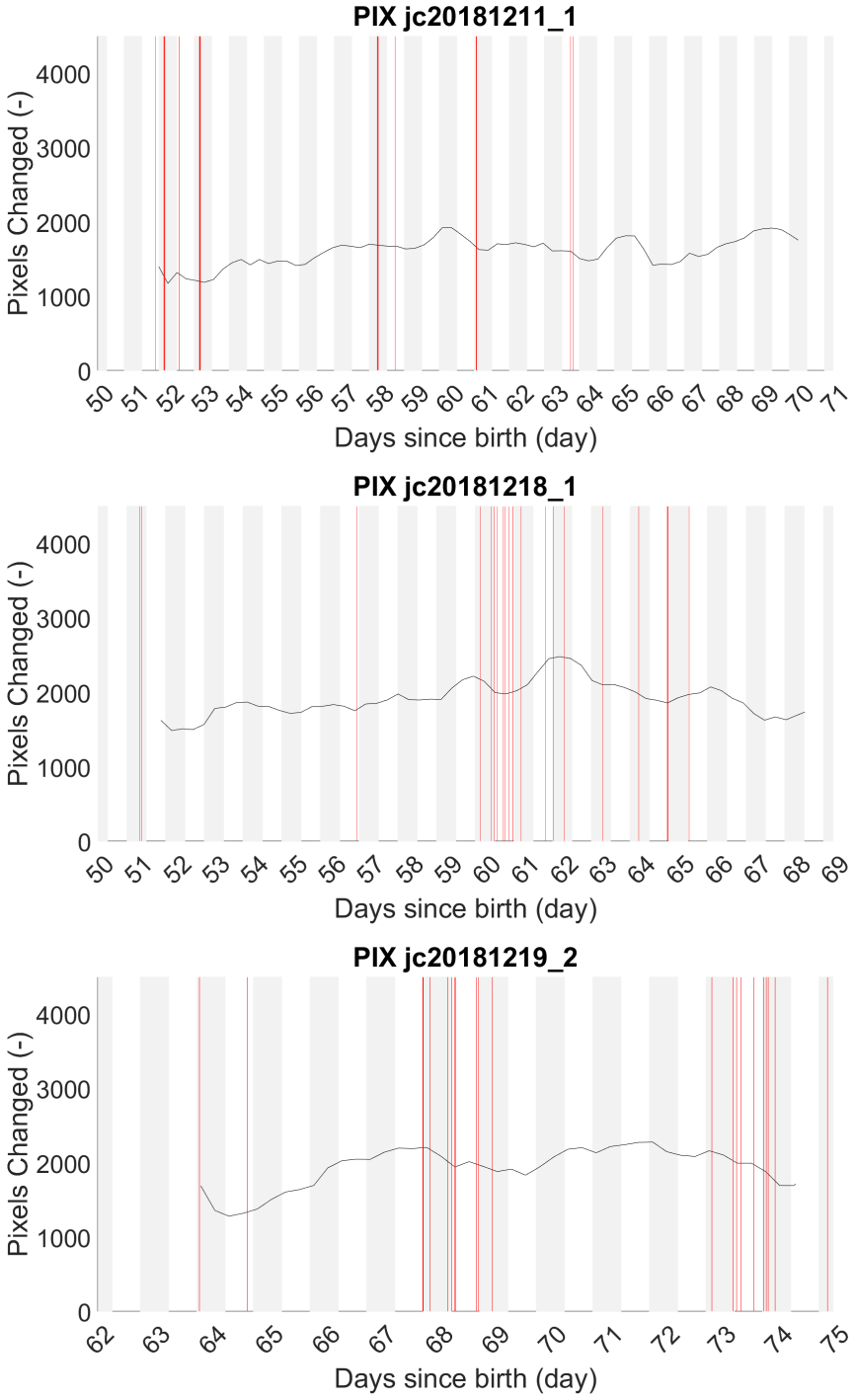


Figure 6.15: 24h STD of PIX activity data of all mice with shown seizure clusters. Gray background indicates nighttime.

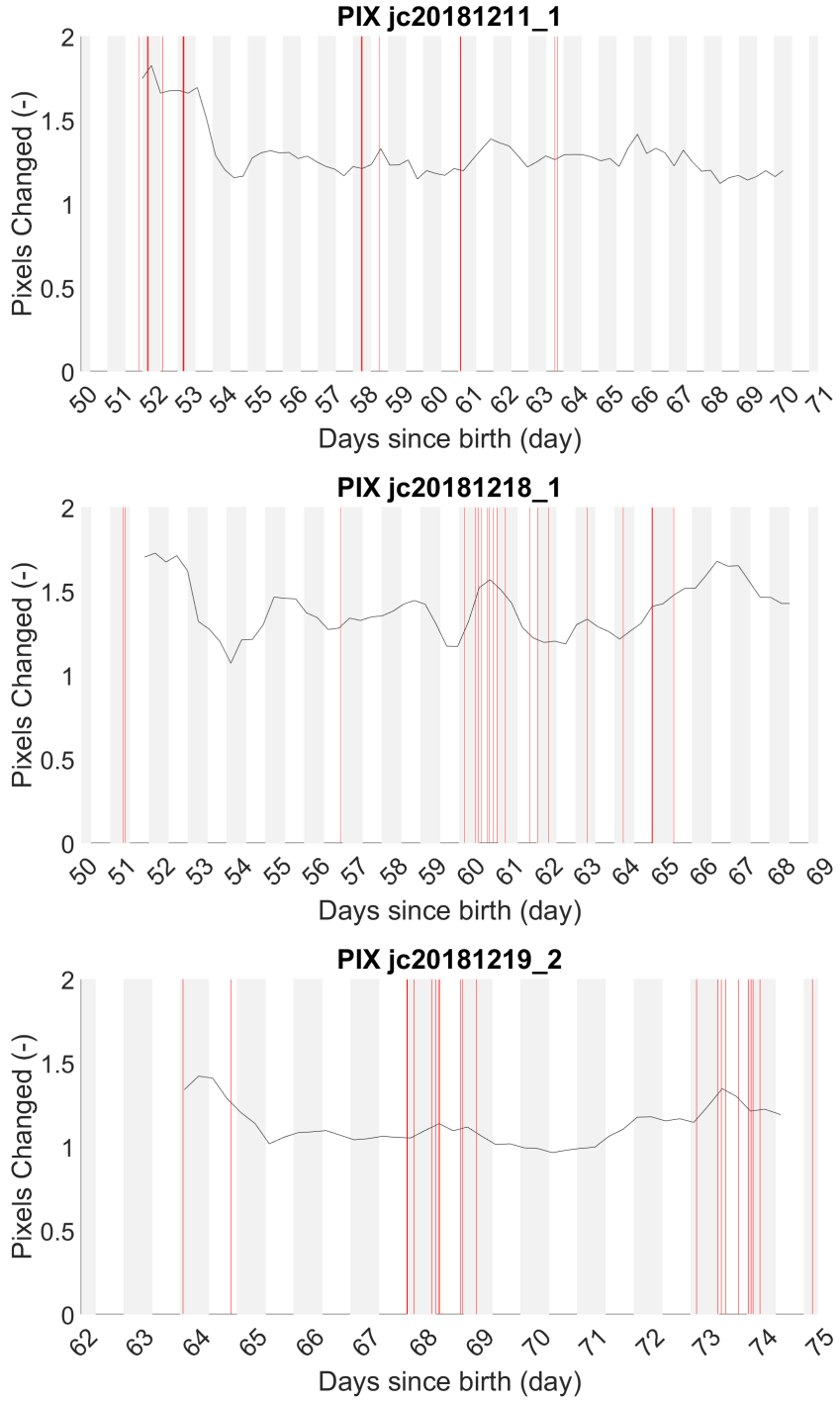


Figure 6.16: 24h normalized STD of PIX activity data of all mice with shown seizure clusters. Gray background indicates nighttime. There is visible increase around seizures in the last two mice.

6.4 Relationship between locomotor activity and seizure rate

In this section, we present the results of normalized cross-correlation between seizure rate signals with signals created by mean and normalized STD of locomotor activity in the time bins as described in previous chapter. Because of the overlap in sliding window analysis, the lag unit corresponds to 6 hours.

To support our hypothesis that the locomotor activity changes preceding seizures, we are looking for high values of positive or negative cross-correlation of mean values in positive lags. Additionally, STD should provide information mainly about seizures themselves. During the periods of high seizure rate, the motion is expected to be jerky which will result in a high STD. Thus, STD of locomotion activity could serve for a non-invasive estimation of the seizure rate.

In the figure 6.17 there is a cross-correlation from first mouse, jc20181211. The blue and red curves belong to the results obtained by DLC and PIX algorithm. Cross-correlations of mean values are in low values in positive lags. Values of STD are in high values around zero lag for both algorithms. Additionally, we can see that in the terms of difference between algorithms, there is only a slight difference in the negative lags of STD.

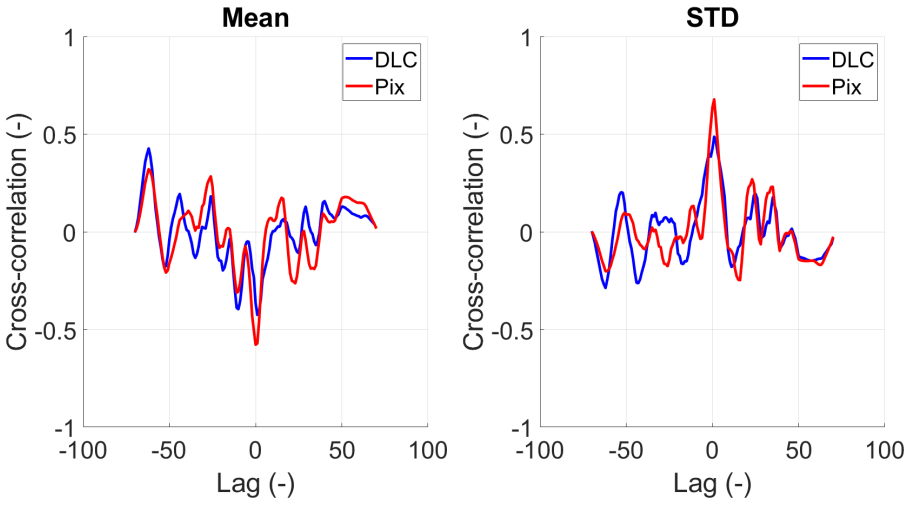


Figure 6.17: Cross-correlation results of jc201812111. Plot on the left shows cross-correlation between mean locomotor activity in sliding windows and seizure rate. Plot on the right shows cross-correlation between normalized STD of locomotor activity and seizure rate.

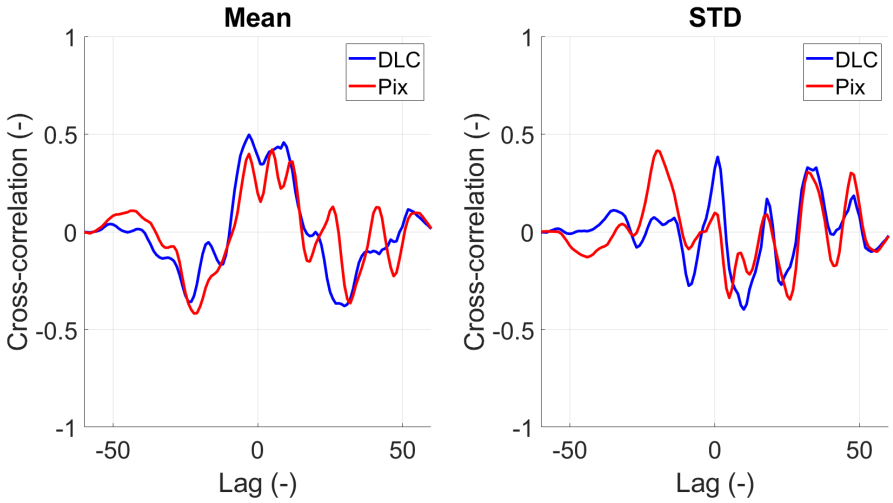


Figure 6.18: Cross-correlation results of jc201812118. Plot on the left shows cross-correlation between mean locomotor activity in sliding windows and seizure rate. Plot on the right shows cross-correlation between normalized STD of locomotor activity and seizure rate.

Figure 6.18 shows results from mouse jc20181218. In this case, the values reach peak in negative lag, although, the values around maximum extend to positive lag so the seizure preceding increase in locomotor activity is debatable. STD is again maximal around lag zero for DLC, on the contrary, PIX failed to detect this phenomen in this case.

The last mouse in this analysis was jc20181219, its cross-correlations are shown in the figure 6.19. In this case, we can see a periodicity, this was also visible in the figure 6.9. The first peak in mean values is in positive lag, which supports our the hypothesis that an increase in locomotor activity precedes an increase in seizure rate. STD values also show apparent increase around zero lag, although little shifted for different algorithms.

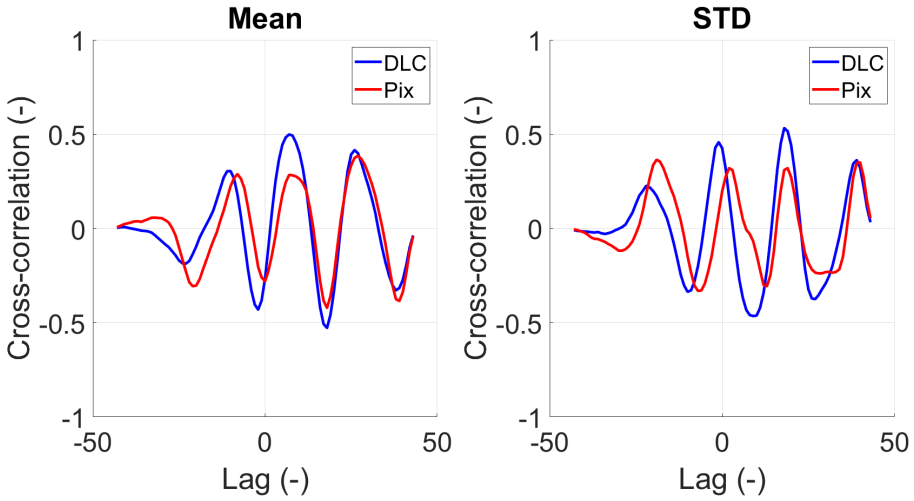
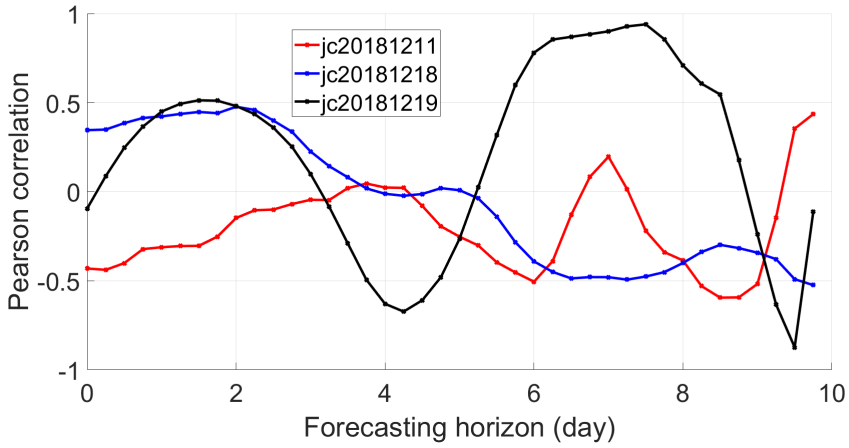


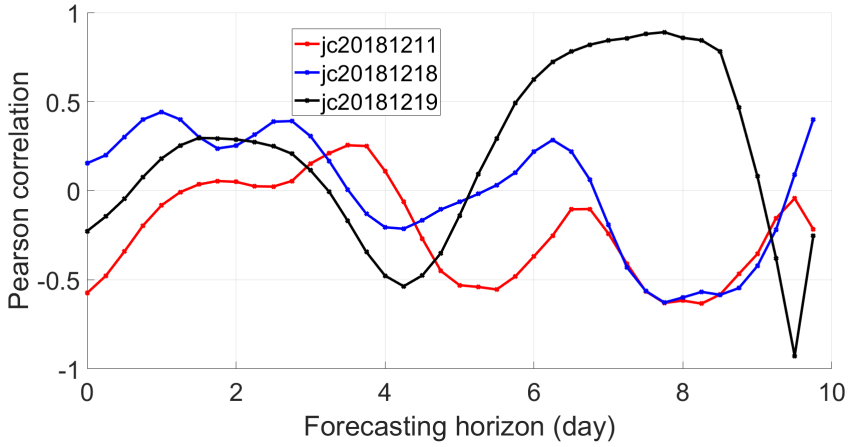
Figure 6.19: Cross-correlation of jc201812119. Plot on the left shows cross-correlation between mean locomotor activity in sliding windows and seizure rate. Plot on the right shows cross-correlation between normalized STD of locomotor activity and seizure rate.

6.5 Seizure forecasting

Due to the lack of data, we were not able to create a seizure forecasting tool, but we looked into the question of whether the locomotor activity could be of any help in seizure forecasting. In the figure 6.20, we present Pearson correlations between shifted seizure frequency and mean value of locomotor activity in 24 hour window. Then, in the figure 6.21, we present Pearson correlations between shifted seizure frequency and discrete time derivative (difference) of mean value of locomotor activity in 24 hour window. On the y-axis - the higher the absolute value of the curve, the stronger is the relationship between the locomotor activity (or its discrete time derivative) and the upcoming seizure rate. The high anti-correlation values show that locomotor activity decreases preceding seizures, high positive correlation shows increase in locomotor activity before seizures. The position on the x-axis tells us how long into the future the seizure rate is shifted.

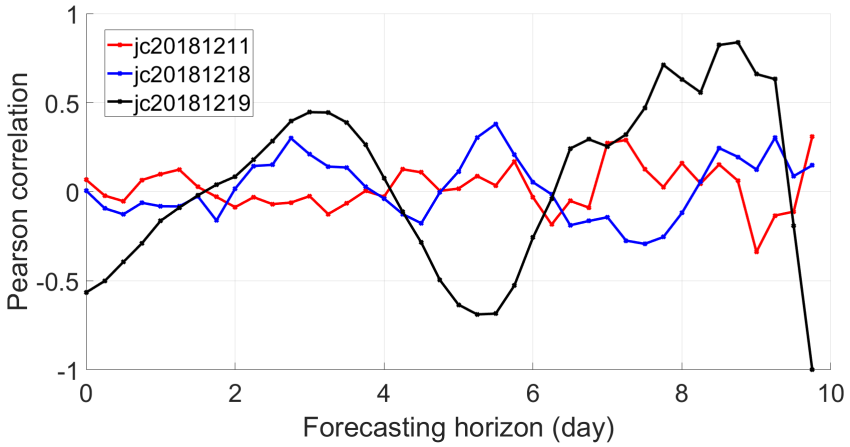


(a) DLC

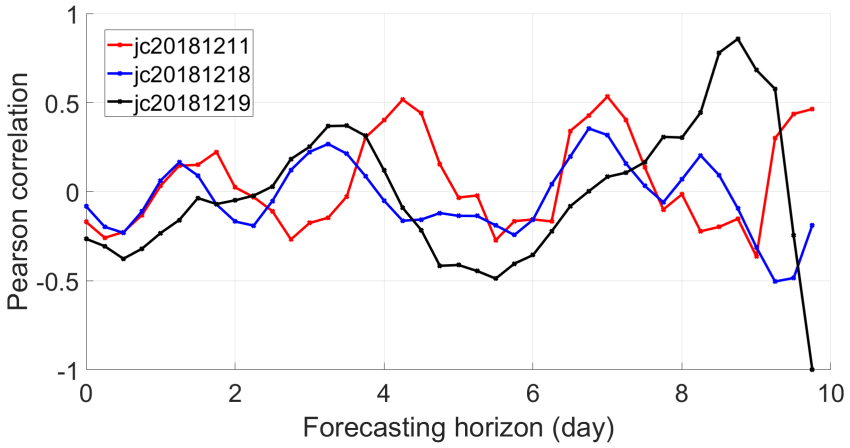


(a) PIX

Figure 6.20: Pearson correlation of mean locomotor activity with shifted seizure frequency. Shown for both algorithms and all mice combined.



(a) DLC



(a) PIX

Figure 6.21: Pearson correlation of difference of mean locomotor activity with shifted seizure frequency. Shown for both algorithms and all mice combined.

Values of correlation reach fairly high values around shift of 9 days in mouse jc20181219. This is caused mainly due to the fact that with this shift, it is only correlation of roughly half of the recording with one seizure group. We can see other peaks in positive correlation around shift of 2 to 4 days. Around shift 0 and shift of 4 to 5 days, there are minima, i.e. maximal anti-correlations. All these minima and maxima could provide information about seizure forecasting, whether proving that the locomotor activity increases before seizures in case of maxima, or decreases in case of minima. However, in this case, there would need to be longer recordings of mice with more seizure groups to investigate such phenomena.

6.6 Time of the day oriented analysis

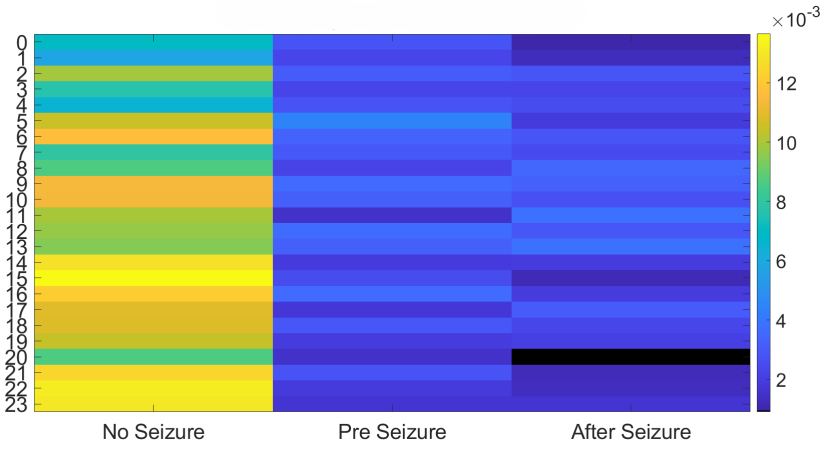
While using the sliding window as a seizure predictor or forecasting tool, there is a problem with the absolute values of data due to the skewness brought in by circadian rhythm. For this reason, we also decided to extract the average values for specific day times. This task is more on the illustrative side, although, with larger quantity of data, it could possibly be used to create normalization tool for seizure predictor or forecasting tool.

In the figures 6.22 and 6.23, there are three columns, which represent non-seizure, pre-seizure and post-seizure data. The rows correspond to daytime windows. Bins where there is data absent are marked with black color. In the figure 6.24 there is mean data for all mice presented.

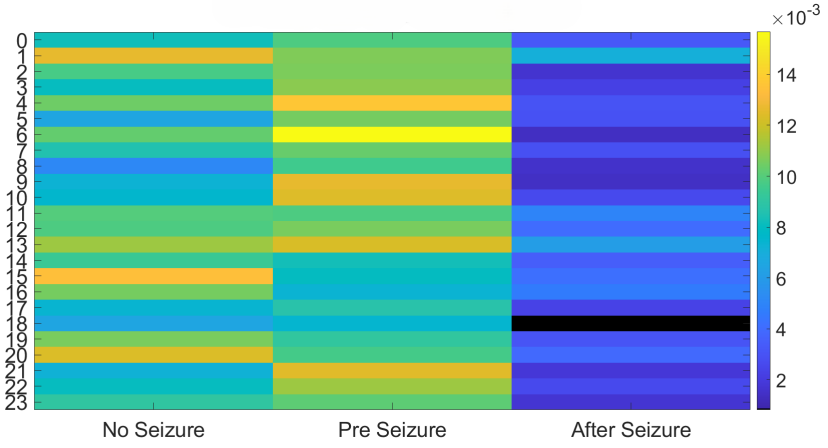
The results again vary between mice and similarly as in continuous analysis results, mouse jc20181211 provides no data from which any conclusions can be derived. On the other hand, remaining mice show that in the case of upcoming seizure, there can be a detectable increase of locomotor activity early in the morning (around 04:00 to 07:00) and at night (around 20:00 to 24:00).

Additionally, there is an apparent decrease in locomotor activity after seizures, which may point to postictal depression.

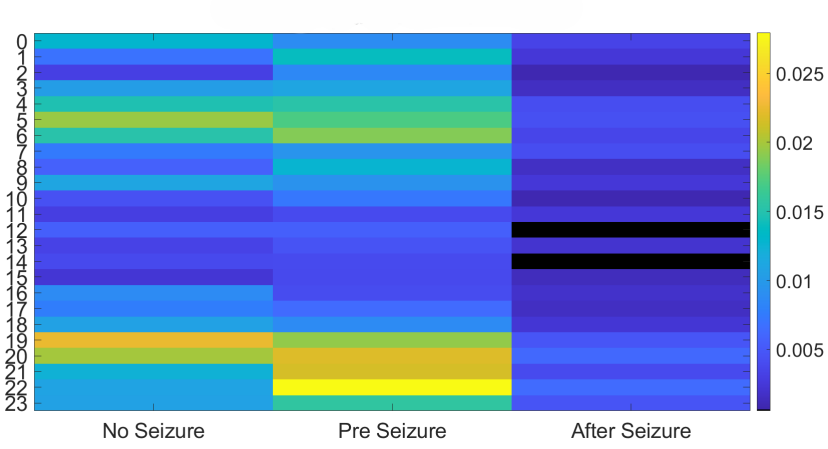
However, this analysis is confounded by high uncertainty due to the low number of data points (sometimes even zero) in certain bins.



(a) jc20181211

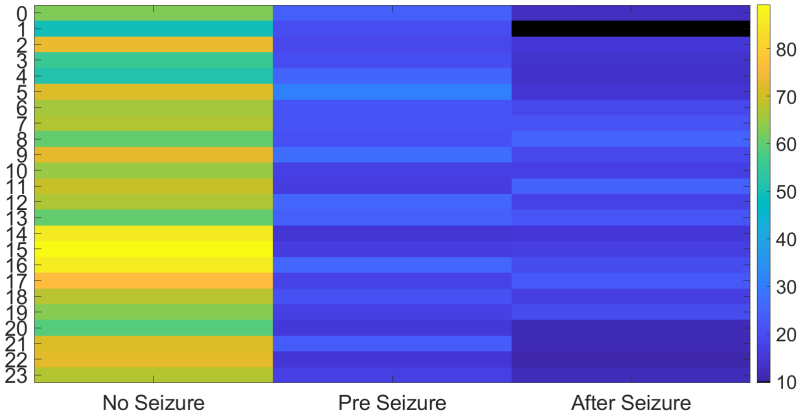


(b) jc20181218

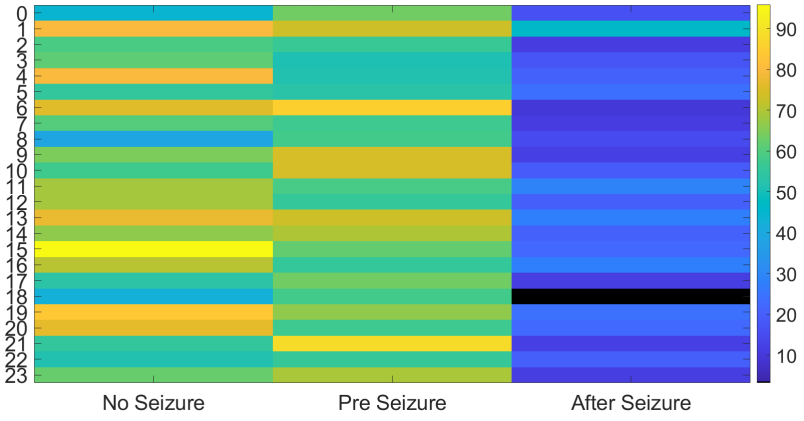


(c) jc20181219

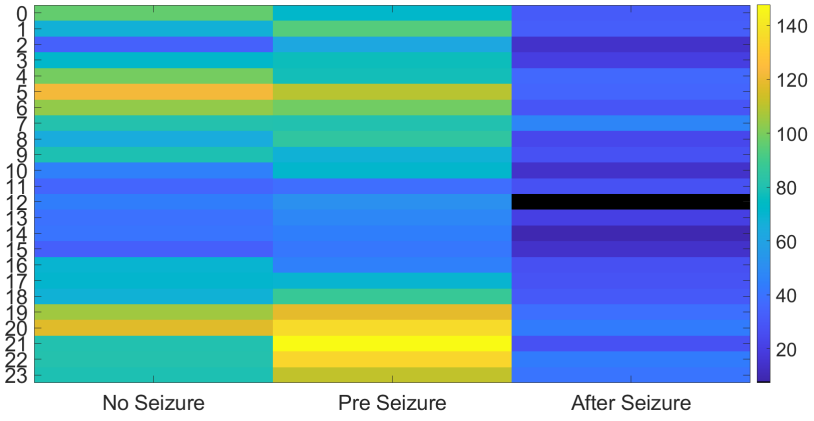
Figure 6.22: Hour by hour analysis based on DLC output. Black color visualizes zero data present in specific bin.



(a) jc20181211



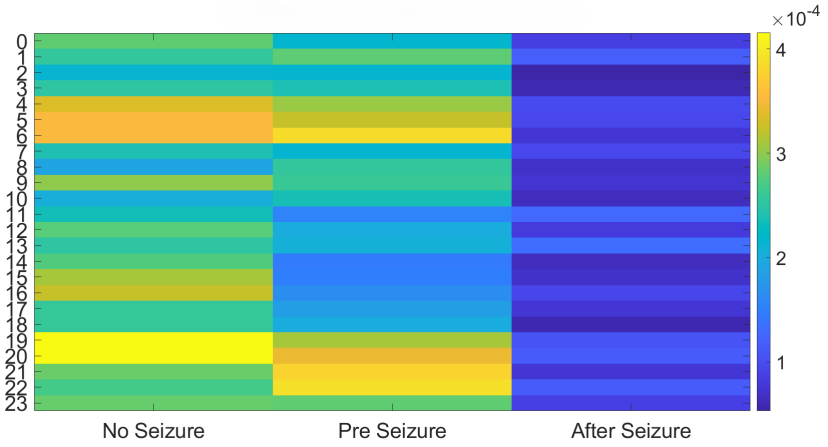
(b) jc20181218



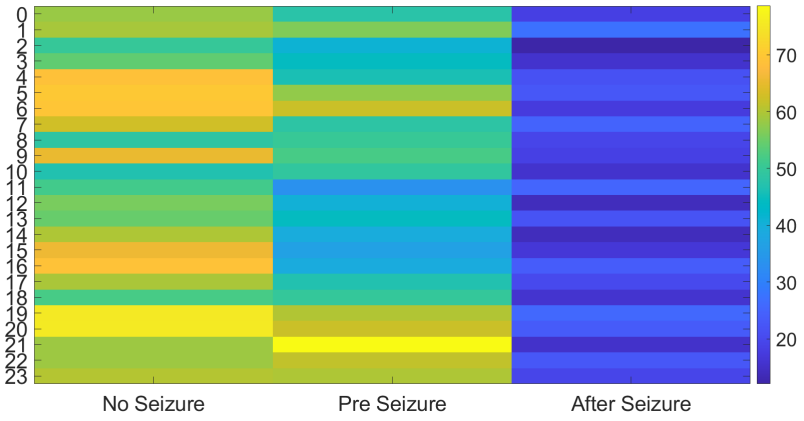
(c) jc20181219

Figure 6.23: Hour by hour analysis based on PIX output. Black color visualizes zero data present in specific bin.

In the figure 6.24 there are values which average all the mice together. The values in the figure are calculated as means of means. This figure serves as a summary of all the data.



(a) DLC data



(b) PIX data

Figure 6.24: Combined hour by hour analysis based on both algorithms.

The values from this averaged analysis provide information about overall increase before seizures in specific time windows. With larger scale data sets, this analysis could be used to normalize the values in the seizure forecasting tool to provide information about specific increase or decrease at certain time periods of day.

6.7 Circadian distributions of seizures

In the figure 6.25, there is a circadian distribution of seizures. The seizures generally do not follow uniform distribution. It can be seen that the most seizures occur at night (18:00 - 06:00).

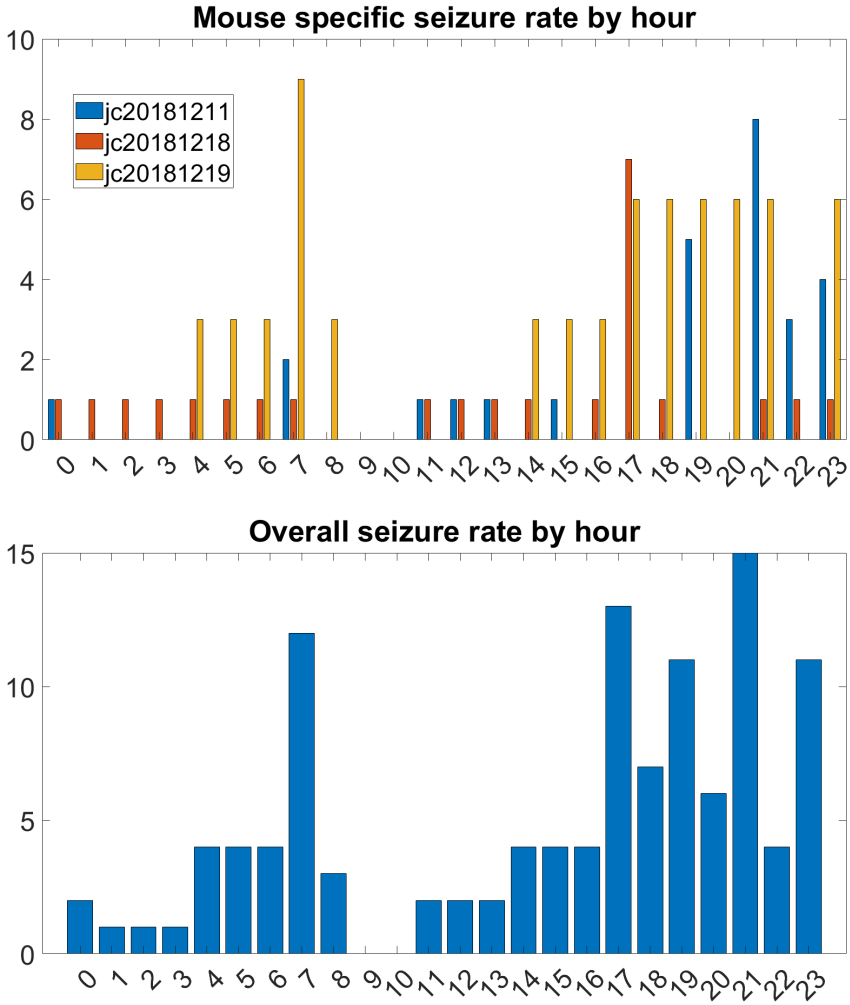


Figure 6.25: Circadian distributions of seizures.

Chapter 7

Discussion

It is known that the frequency of seizures fluctuates over days to weeks in patients as well as in animal models of epilepsy [14] [27]. In this study, we investigated whether also the long-term behavior fluctuates in such manner and whether this fluctuations are related. It is also known that epilepsy patients suffer from postictal symptoms, such as headaches, migraines, and psychoses [28], which may result in postictal depression. Ultimately, we investigated if any change of the locomotor activity precedes the periods of high seizure rate. Such knowledge can facilitate the development seizure forecasting techniques which have an extremely high potential of improving lives of people with epilepsy.

We examined the behavior based on locomotor activity, one of the most simple parameters to monitor. We hypothesized that the locomotor activity fluctuation could be related to seizure frequency. Our hypothesis was that the locomotor activity would increase or decrease preceding to seizures and would return to normal after seizures. Monitored increase in locomotor activity preceding seizures could then be used as a tool to determine seizure probability. This could help drug resistant epilepsy patients to overcome their fear and uncertainty from unpredictability of seizures [5].

The aim of our study was not to conduct any dedicated behavioral test. We did not monitor the mice doing any specific task or number of tasks. To conduct such study, the best tool would be to use phenotyping cages. These cages can monitor for example how much the mouse eats, drinks or runs on a wheel [29] [30]. However, these cages mostly do not allow EEG measuring and are typically costly. Videos used in this study come from study of behavioral correlates of seizures and main aim of this study was to utilize already created videos.

As a basis for monitoring of long-term locomotor activity we used two algorithms, DeepLabCut (DLC) and PixelCount (PIX). DLC is a tool for pose-estimation, which marks the pre-selected body parts [15] and PIX is Matlab script counting frame to frame difference while focusing on the areas marking the mouse "appearance".

DLC is accurate and saves the positions of pre-selected body parts. However, DLC is far slower than PIX, needs to be trained (fine-tuned) for specific task and may not work with change of surroundings (e.g. placement of items in terrarium). PIX is much faster algorithm and with a simple change of threshold it will work in any environment, but it does not provide values about actual locomotor activity, but some substituting metric. PIX is also prone to noise, mainly caused by shadows and requires contrasting colors of the animal and bedding material.

As a simple test of whether the algorithms tracked correctly, we looked at circadian rhythm. In both algorithms, there was apparent increased activity at night (when lights were turned off, from 18:00 to 06:00), which correlates with the fact that mice are nocturnal animals. There was also circadian rhythm detected by PSD.

The main problem of our study is the lack of data. Not only we had sets of videos of only three mice, the sets were also comprised from monitoring only over about two to three weeks. This is problematic due to the fact, that multi-day seizure fluctuations have reported periodicity of 7, 15, 20, and 30 days [14], because of that we have at maximum 2 periods of high seizure rate, in some cases not even one. Therefore, the cross-correlation deductions can not be backed up by large scale data. We are aware of that and provide the results and deductions as some guiding ideas, which could suit as starting point for future analysis on a larger data set.

Using state-of-the-art neural networks such as DLC for video analysis is highly computationally expansive. We have decided to speed up the process of analysis by reducing the frame rate of the videos, a procedure called frame dropping. We performed frame dropping on original videos, specifically from 25 FPS to 5 FPS and 1 FPS. This approach is feasible for the purposes of behavioral monitoring, since it analyses long-term locomotor activity, rather than instantaneous changes. On the other hand, this renders the data unusable for the purposes of behavioral testing, because some mouse movement patterns, such as scratching or grooming, are not reliably detectable on videos with lower frame rate. For the purposes of our analysis, we decided to use videos, which were reduced to 5 FPS. Videos with 5 FPS also keep far more data intact than 1 FPS videos. This process reduced the time of analyses roughly 5 times, which moved the combined processing time of all videos from several weeks to several days.

We performed analyses on three mice with two to three weeks of video recording, which could become foundation for further analysis. The focus of further study may be performing the analysis on more video recordings, mainly analysing longer recordings, which would include more grouped seizures and more periods of multi day seizure frequency fluctuations. Additionally, the analysis can be extended to long term behavioral testing - long term monitoring of behavioral parameters (e.g. grooming, scratching, drinking or eating). Mainly grooming is considered a parameter which correlates with wellbeing of mice and its relation preceding seizures could be examined. However, grooming and scratching analyses would need to be concluded on original frame rate, which substantially increases the analysis duration and may even require the use of a specialized computational facility.

There still remain many other behavioral patterns which could be analysed and linked to long-term frequency fluctuations other than locomotor activity. To continue with behavioral parameters, only DLC could be used, since it can monitor the movement of limbs and then an engine such as Variational Animal Motion Embedding (VAME) could be used to cluster such data into behavioral patterns. However, this monitoring and further processing would be time costly. To monitor simple behavior, such as drinking and eating - by placing the food and drink in specific areas and look for the percentage of time mouse spent there, videos could be reduced to lower frame rates. For this analysis, 1 FPS could be enough.

To summarize, we provided a foundation for long term behavioral monitoring. We presented one tool - PixelCount, which in terms of long-term locomotor activity can substitute complicated models like DLC. It is specifically tailored to this task, so it is not multi-purpose like DLC, but it proved reliable in this area. We also sped up the analysis by trying out frame dropping and found out that for the purpose of long-term behavioral monitoring, decreased frame rates are sufficient. We did not arrive to some general conclusion, due to the lack of data so our study rather provides basis for further analyses.

Chapter 8

Conclusion

We analyzed three mice with artificially induced epilepsy with two different algorithms. DeepLabCut (DLC) is a pose-estimation tool directly extracting the coordinates of pre-selected body parts from videos. PixelCount (PIX) is a custom-made Matlab script which substitutes the locomotor activity with number of changed pixels frame to frame.

Automated analysis was highly time consuming so we analyzed whether frame dropping can be used to speed up the analyses while preserving the accuracy of the results. We frame dropped the original videos from 25 FPS to 5 FPS and 1 FPS. With 5 FPS videos, we achieved Pearson correlation between sliding window extracted values from original and frame dropped videos of 0.99 ($p < 0.001$) with DLC data and 0.99 ($p < 0.001$) with data from PIX. With 1 FPS videos, we achieved Pearson correlation of 0.96 ($p < 0.001$) with DLC data and 0.97 ($p < 0.001$) with data from PIX. We chose to continue analysis with 5 FPS because of data preservation. Pearson correlation between DLC and PIX data on 5 FPS was 0.78 ($p < 0.001$).

We extracted the raw locomotor activity data, which was then median filtered with 10s window and filtered with 24 hour sliding window for the investigation of the long-term fluctuations. We performed cross-correlation on seizure frequency signals with mean and normalized standard deviation signals of locomotor activity, all three extracted with sliding window. Our hypothesis was that the long-term locomotor activity would change before seizures and that normalized standard deviation would change around seizures. In the end, the first hypothesis could not be verified due to insufficient data, but the normalized standard deviation increased around seizures.

In the last part, we extracted the mean values for specific daytime windows. Main purpose of including these tables was that it shows that with larger amount of data, the continuous monitoring could be normalized to possibly create the seizure forecasting tool.

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Appendix A

Used software

I declare that I list all the artificial intelligence tools that were used according to CTU FEE guidelines.

A.1 Spell check

To check correct spelling in the whole thesis, Overleaf LanguageTool was used [31].

A.2 Translation of abstract to Czech language

The abstract was translated from English into Czech using Microsoft Copilot (Microsoft Bing Chat AI) [32].

A.3 Simplification

In the section 3.3, the command and its explanation was simplified with the use of ChatGPT [33].