Seizure Cage Detection

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***Abstract*—The piezoresistive sensor is a device capable of detecting movements by means of a change in electrical resistivity when pressure is applied. Combining these sensors in different positions, underneath an animal cage can provide a method for detecting movements of an animal under study. Thus, the goal of this project was to potentially detect seizures patterns and classify them in an animal subjected to seizure induced therapie. The results were promissing but they show that there is still some work to be done.**

***Index Terms*—seizure,cage,detection,piezoresistive,sensors, frequency,positioning,classification,k-Nearest-Neighbours**

1. Monograph

I. Introduction

EPILEPSY is one of the most common neurological problems afflicting humans, and although it has a large environmental component, genetics is thought to be important in the pathogenesis. The identification of candidate genes in mouse epilepsy models also facilitates the discovery of human disease genes[1]. Many features of seizures and the means by which they are induced are conserved in mammals, indicating common neural substrates or pathways[1]. This process requires the animals to be subjected to seizure induced therapies, which must be monitored. Behavioral monitoring can enable the detection of explicit spontaneous seizures, however it is not uncommon that seizures are readily provoked by routine handling. It is important to point out that it takes some experience to distinguish an unusual behavior to be an epileptic seizure from a movement disorder or a nonparoxysmal motor abnormality[1]. Considering that monitoring mice in their cages can be tedious task , a video camera may provide a convenient way to detect aberrant behavior. However, the current video camera technology available for these kind of studies does not provide enough frames per second to detect some faster movements. Therefore, it would be of high interest to provide a more reliable alternative, to detect those characteristic movements.

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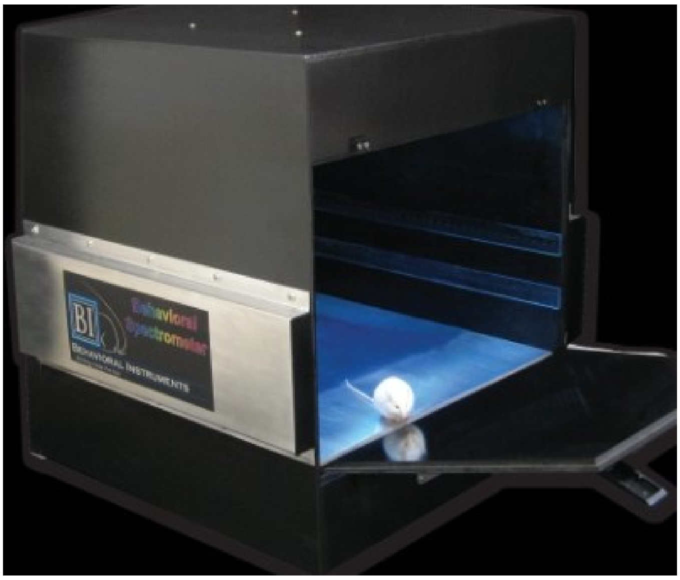
II. State of the art

ONE of the latest developments in this area is the Behavioral Spectrometer. This approach was developed by Brodkin, et al. [2]. The aim of the study was to create an automated system capable of providing detailed description of mice behaviour. The system consists on three types of sensors: one camera from which position and posture are extracted; accelerometers stuck to the floor are used to capture vibrations; and infrared beams, used to detect when the animal rears. The team uses a clustering based algorithm which contains 23 different patterns and comparises them with the recorded signal. They obtained results which were highly correlated with the classification made by humans (r = 0.97).

Another interesting study was published by Vasickova et al. [3] This article reported the design of a system for detection of epileptic seizures in a sleeping person. This system is one of the possible next steps of this work. It was designed to be applied in bed and it uses accelerometers. By using a windowed Fourier transform, it is possible to find out the frequencies that represent a seizure.

Figure 1: Behavioral Spectrometer



Borujeny et. al [4] proposed a detection of epileptic seizures based on wireless sensor network. The main difference to the suggested approach was the site where the sensors were. Since the sensors were wireless, they were attached directly in the patient, which allowed a continuous monitoring. Another obvious advantage was the increase of the recorded signal's quality as electrodes tend to pick up many motion artifacts due to the patients motion. To analyse the recorded signal they used two different approaches: one based on artificial neural network, and another based on K-Nearest-Neighbor.

III. Seizures in mice

A. Characterization

A protocol that aimed to induce seizures in mice was developed. Each step consisted of an injection of a cocktail of substances, each one with it's own role. To this investigation, the injection of pilocarpine was the essential one since it is the substance responsible of inducing seizures. Posteriorly, a seizure can be classified with the Racine scale. As the main purpose of this project is to detect seizure in mice, it is necessary to set well what is a seizure event and what variations can be found on those events. The classifications of seizure events are made using an adaptation of the Racine's stage. This scale has 5 levels, each of which is well defined by certain characteristics.

TABLE I: Racine scale adapted to mice

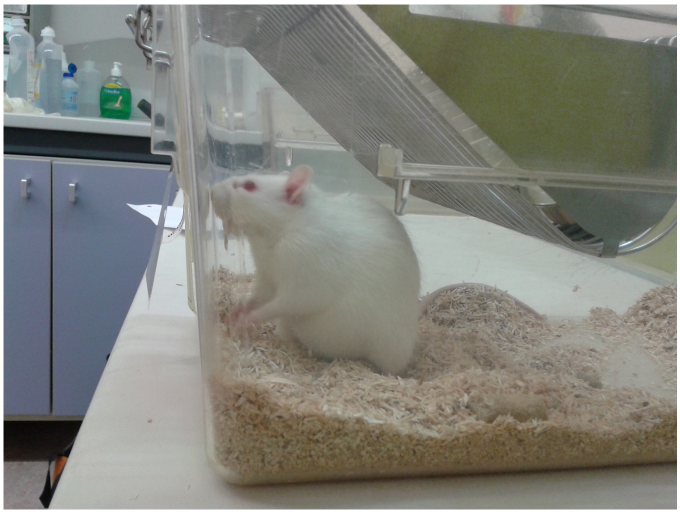
Level	Behavioral signs
Racine 1	Mouth and facial movements
Racine 2	Head nodding and stiff tail
Racine 3	Foreclimb clonus and chewing
Racine 4	Seizures characterized by rearing and tonic immobility
Racine 5	Seizures characterized by rearing and falling

Although the different levels of the Racine Scale describe very specific events, since the moment of the administration of the neurotoxin until the seizures can be observed, it is important to notice that the mouse changes its behavior, remaining quieter in its cage, avoiding water and food and laying in one of the corners of the cage. This seizures detection circuit was devoloped in order to be used for two different studies. The aim of the first study was to characterize the synaptic plasticity phenomena in hippocampus region by inducing seizures in the mice and the role of the vasoactive intestinal peptide (VIP) in this type of phenomena. The aim of the second study was to observe the modifications in VIP in its receptor, VPAC1 in the temporal lobe epilepsy.

B. Piezoresistive sensors

Piezoresistivity is a common sensing principle for micromachined sensors. First discovered by Lord Kelvin in 1856[5], the piezoresistive effect is a widely used sensor principle. Simply put, an electrical resistor may change its resistance when it experiences a strain and deformation. This effect provides an easy and direct energy/signal transduction mechanism between the mechanical and the electrical domains. By strict definition, piezoresistors refer to resistors whose resistivity changes with applied strain. The resistivity of semiconductor silicon changes as a function of strain. Resistance changes are often read using the *Wheatstone bridge* circuit configuration. A basic *Wheatstone bridge* consists of four resistors connected in a loop. An input voltage is applied across two junctions

Figure 2: Mouse with Level 4 seizure in Racine's scale

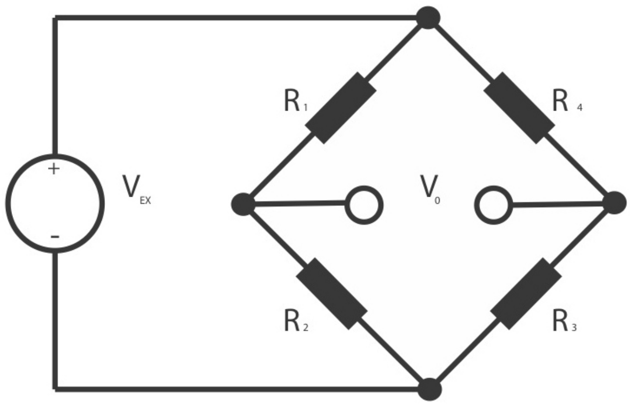


that are separated by two resistors. The voltage drop across the other two junctions forms the output. One or more resistors in the loop may be sensing resistors, whose resistances change with the intended variables[5].

The output voltage is related to the input voltage according to the following relationship:

$$V_{out} = \left(\frac{R_1}{R_2 + R_1} - \frac{R_4}{R_3 + R_4} \right) V_{in} \tag{1}$$

Figure 3: Wheatstone bridge scheme

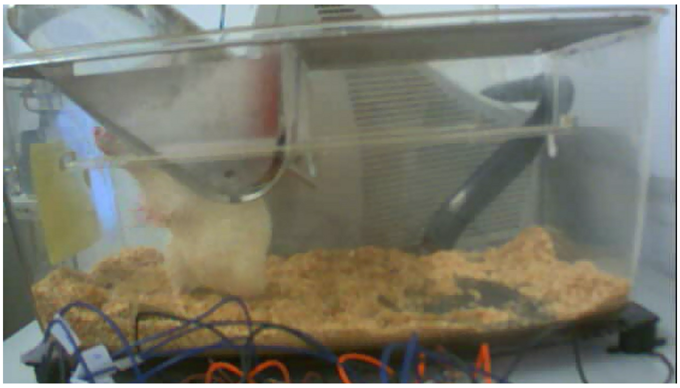


C. Piezoresistive sensors vs Camera monitoring

C.1 Advantages

The current approach of determining the hours of ocurred seizures is made by analysing the video recorded during the course of the study. This method, however, is characterized by some problems. The first is the obvious tedious task that is to watch a video of extended duration, in order to classify the rodent behavior. The second is more deterrent, and is the fact that the camera has a poor time resolution, and some higher frequency, with less amplitude movements might be undetected due to the inability of the camera to discern them. Using piezoresistive sensors, one can, in theory, precisively identify the time intervals in which there were seizures. by classifiying the signal in terms of frequencies. Also, because the sensors are very sensitive to higher frequency variations, the finer high frequency movements are detected providing a good solution to the second problem.

Figure 4: Video frame of the actual seizure detection method at Instituto de Medicina Molecular, Lisboa



In the figure 4 it is possible to notice the poor video resolution, where one can't notice if the mouse is only moving around or having a real seizure.

C.2 Disadvantages

The disadvantage of this method resides in the fact that a very precise training sample is required for the classifier to produce good results. Also, the limited amount of weight a piezoresistive sensor can handle before breaking might be a limitation for some applications.

D. Other applications

In an attempt to model human pathological anxiety in rodents, a wide range of behavioral testing paradigms have been developed[6]. Such is the case of the Elevated Plus Maze (EPM), in which a rodent is placed in an elevated maze with four arms, two of which are closed. Rodents also have an innate fear of heights, so elevating the EPM off the floor adds to their anxiety level[7]. Also, they prefer closed arms (shaded) and tend to avoid open arms. Therefore, EPM assesses anxiety by comparing the time spent in enclosed arms vs. open arms[7].This is an example where the piezoresistive sensors can be used to monitor the rodent behavior in the EPM.

Another application would be to apply the piezoresistive sensors to monitor rodent movement in the Open Field Test (OFT). In the OFT the rodent is placed in a square or circular arena, whose floor is divided into three different virtual areas. The degree of activity in the open field is evaluated by the distance travelled and amount of time that the animal moves into these three virtual areas[8]. This test evaluates the conflict between exploration of a novel environment and a rat's inherent aversion to open spaces. As for testing the spatial memory in rodents, the Radial Arm Maze (RAM) can be employed. It uses a central plataform with arms radiating outwards, at the end of which there is a food reward, which food-deprived subjects must locate[9]. The animal must be able to remember the baited arms location as well as which arm it has already entered (working memory), but it also must know to avoid non-baited arms across trials (reference memory). The monitoring, of course, can also be implemented with the piezoresistive sensors.

These are all examples on which the piezoresistive sensors can be applied, however the possibilities are endless. Almost everything that requires the monitoring of animal behavior, or even perhaps the analysis of the movement of a human patient in a bed for classification purposes, might be feasible with the method presented.

E. Classification

Regarding the classification of seizures, the objective was to recognize a certain pattern among the frequencies present in the signals. Specifically, it is expected that the signals of a seizure moment are characterized by higher frequency components in relation to the rest of the signals.

In order to access the frequency components along time, it was employed an STFT to the signals. This process yields the frequency components of the signal, broken down into pieces of predefined length. The classifier used to recognize the frequencies patterns was the K-nearest neighbor.

E.1 K-nearest-neighbor

The K-nearest-neighbor algorithm uses a database in which data points are separated into different separate classes (training set), and uses the similarity (by means of distance) between new points and those from the classes to predict to which class they belong (classification)[10].

The reason it is called the K-nearest-neighbor is because the procedure it follows to classify the new sample, is the following: a k positive integer number is specified; the k data points closest to the sample to classify (with the lowest distance) are selected; the class of those data points from the database is accessed, and the class with the most points from the k selected is the class attributed to the new sample[10].

2. Report

IV. Seizure cage detection procedure

A. Data acquisition

The data aquisition protocol includes many different components: the piezoresistive sensors and their coupling to the cage, the low-frequency circuit that captures the changes in the mouse location in the cage and the calibration of this circuit, the high frequency circuit that detects the higher frequecies that are normally assotiated with seizures, the Arduino-Matlab interface and the digital processing.

A.1 Sensors and mechanic coupling to the cage

In order to acquire the vibrations correspondent to seizure events in mice, a FC22 compression cell was used (figure 5), featuring a small size (about 34 mm of diameter), fast response time and preamplification, having a span between 0.5V and 4.5V at its output. The interface between the load cell and the cage is done using an acrylic plaque. To avoid tangential movements to take place, a material (e.g. rubber) between the plaque and the load cell could be used, but there was no possibility to achieve that goal.

The damping effect of all those mechanical interactions is bypassed with a higher amplification in the circuit that captures the frequency changes and with the use of the acrylic plaque. The effects of vibrations at the outside environment (i.e. the movements of the table or support where the cage is laying) are negligible, due to the magnitude of the mouse's movements.

Figure 5: FC22 Compression Load Cell

In order to get proper signals to study the seizure events' frequency and the positioning of the mouse inside the cage there are two parallel circuits between the output of the FC22 sensors and the Arduino analog inputs, with the data being acquired with a sampling frequency of 50 Hz. The circuits are depicted in figures 6, 8 and 7.

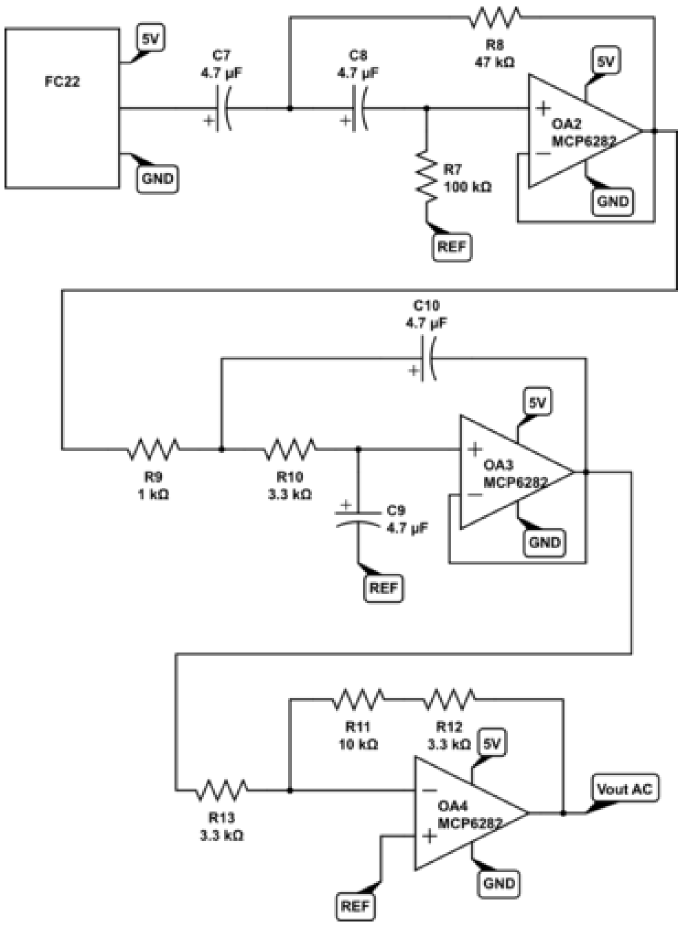
A.2 AC signal circuit

As depicted in the figure 6, the signal after the FC22 output goes through a second order bandpass filter (with Sallen-Key montage), between 0.48 Hz and 18.64 Hz (aprox.), removing the DC components of the signal, and recording only the fast variation on it. The corner frequency is given by:

$$\omega_0 = 2\pi f_0 = \frac{1}{\sqrt{R_1 R_2 C_1 C_2}} \tag{2}$$

This signal is referenced to 2.5V and amplified about 4 times, by the ratio between resistors R_{11}+R_{12} and R_{13}, in order to discretize small changes in the signal and to take advantage of the 5V span for the analog input of the Arduino. In order to get a bigger choice span for the sampling frequency, and also to remove frequencies that don't give any information about the seizure events in mice (such as the 50 Hz illumination or other parasite frequencies) a high cutoff frequency as low as about 18 Hz was chosen to the low pass analog filter.

Figure 6: Frequency circuit scheme



It is also presented here the small block that allows to reference the AC signal circuit in order to profit from the whole 5V window from Arduino's analog input.

A.3 Positioning circuit

As one can see in the figure 8, the signal is carried out through a lowpass filter (again in a Sallen-Key montage), with a cutoff frequency approximately equal to 0.48 Hz. Once applied, the filter will remove the AC part of the signal. The signal is referenced to 2.5V, and the operational amplifier is feed by the ground (0V) and by the 5V from Arduino.

A.4 Calibration

While the AC signal circuit gets the AC signal, that is more important to analyse in terms of frequencies present, the Positioning circuit has an important role in displaying

Figure 7: Buffer circuit scheme

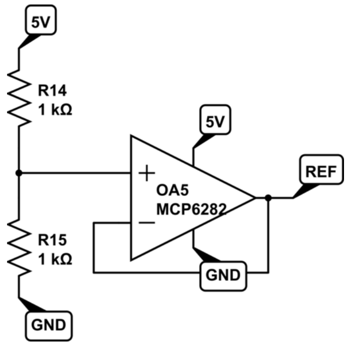
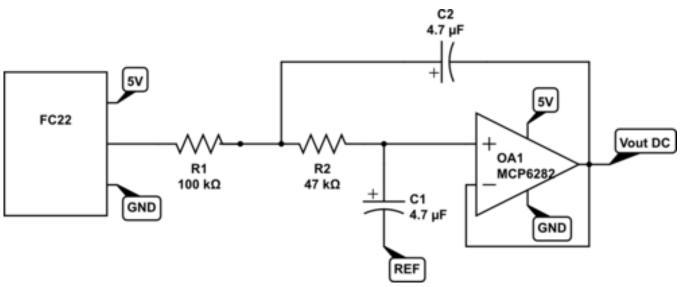


Figure 8: Positioning circuit scheme



variations in the mass distribution in relation to the cages centroid. The whole process has two primary objectives: the first one is to make sure that all the four sensors retrieve the same voltage when they sense whether the same pressure on them, or the same torque (intrinsic to a same distance over the cage); the second one is to ensure that in equilibrium, with the cage and the mouse on its centroid, the voltage given by the sensors is equal between them.

Using the method of Least Squares Fitting, it is possible to easily find the four y=ax+b specific equations (one for each sensor), making the whole system coherent to different applied forces. The matrix form for the LSF method is given by:

$$b = \left[F^T F\right]^{-1} F^T Y \tag{3}$$

where

$$b = \begin{bmatrix} a_0 & b_0 \\ a_1 & b_1 \\ a_2 & b_2 \\ a_3 & b_3 \end{bmatrix} \quad F = \begin{bmatrix} x_0^0 & x_0^1 \\ x_1^0 & x_1^1 \\ x_2^0 & x_2^1 \\ x_3^0 & x_3^1 \end{bmatrix} \quad Y = \begin{bmatrix} y_0 \\ y_1 \\ y_2 \\ y_3 \end{bmatrix}$$

where the x_n are the known masses for the n measurements taken.

The process takes the following steps:

1. Connect the Arduino to the computer and use the *escrevercalibracao.ino* script, to acquire the values of calibration process;
2. Registration of the sensors' positions on the acrylic plaque, and determination of its centroid;
3. Set the cage over it, in a stable position, equidistant of the four sensors, where it's centroid is the same of the acrylic plaque. The cage must be filled with food, water and sawdust;
4. Take the first measurement, with only the cage on top of the acrylic with an empty beaker on it, corresponding to a mass of 0g;
5. Then take as much registrations as possible, with known masses given by the quantity of water poured inside the beaker, taking into account the relation of 1 ml = 1 g of water;
6. Use the *lercalibracao.ino* script in order to obtain the a and b parameters for the LSF method;
7. Proceed with the disconnection of the Arduino from the computer, and by the time of the acquisition, the Arduino will automatically send calibrated values to Matlab, using the a and b parameters for each sensor previously saved in Arduinos EEPROM memory.

A.5 k-NN applied

In order to classify the signal obtained, the k-NN algorithm was employed. The arrays of frequency components for each time of the classified signals, accessed with the STFT as described above, were used as training set. The parameters used for the STFT were: a window of 1 second and 50% overlap, using 1024 points for the FFT. When a new signal is to be classified, the STFT is employed, and the distance between each array of frequency components of the training set and the new signal is calculated. The class of the k most similar arrays (the k used was 3) and the new array is accessed and the most representative class, is the class of this array. However, this method was experimentally determined as non-accurate: there was a great variation for each segment of signal. Therefore, after the classification, a window of pre-determined length l (l=10 was employed), was used to determine the number of positive classified points within that window, and if this number exceeded a certain threshold t (t=5 was employed) that segment would be classified as positive. This window slides through the whole signal, and at the end the signal blocks are classified. This method yielded satisfactory results.

V. Results

THE following image shows the signal at the output of AC signal circuit and the Positioning circuit measured with the oscilloscope. The yellow line is the output of the Positioning circuit, and the blue one is the output of the AC signal circuit.

The figures 10 and 11 show the Matlab output after the signal aquisition with Arduino.

The following figures show the classification process of two signals. As the process of acquiring data was very hard to achieve, there were two ways of creating and validating the k-NN classifier. First, half of the acquired data was used to train the model, and the other half to test it, so the test data and the training data didnt match, so that the result has a higher reliability. Then all the signal

Figure 9: Example of the electronic's output from Seizure Cage Detector

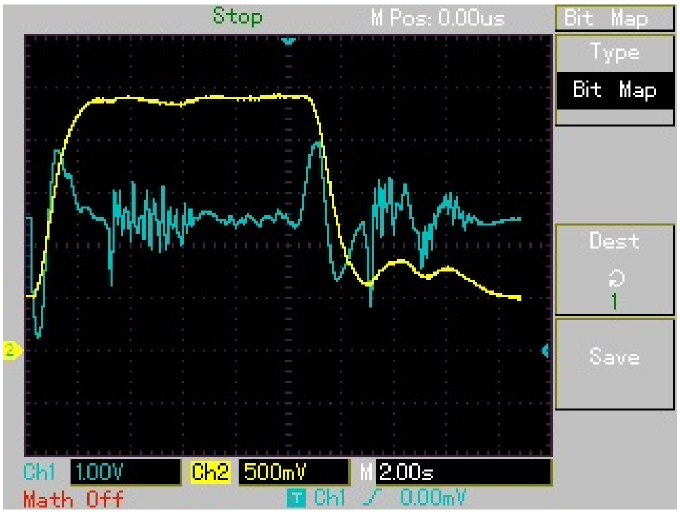
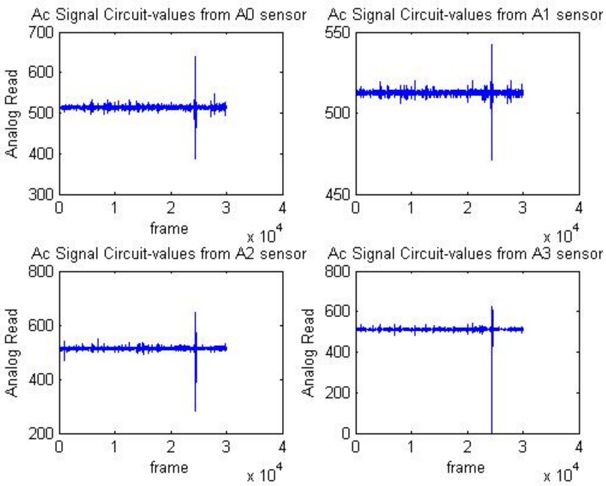


Figure 10: AC signal circuit Matlab output



available was used to train the model, and the classification of seizure and non-seizure events was performed over the same entire signal used before. Those results can be observed in figures 12, 13, 14 and 15 and as one can see, the rate of succesfull classifications is quite high. It is important to notice that in the above mentioned figures, a seizure event takes the value of 1 in the classifier, and a non-seizure event takes the value 0.

A. Validation

Regarding validation of sensors, it was used a device with a frequency of vibration of 10Hz. The device used is not perfect, meaning, when plotted, it was possible to observe the first harmonic (20Hz) besides the fundamental frequency. Although the detection of the first harmonic, one can conclude that the sensors can detect coherently and with precision the device's frequency (figure 16).

Figure 11: Positioning circuit Matlab output

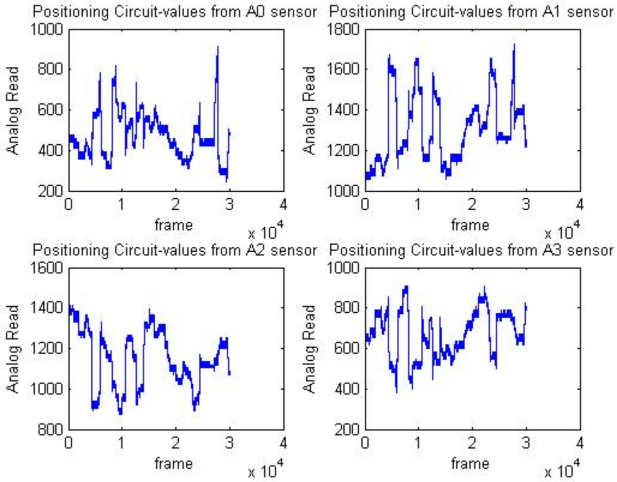
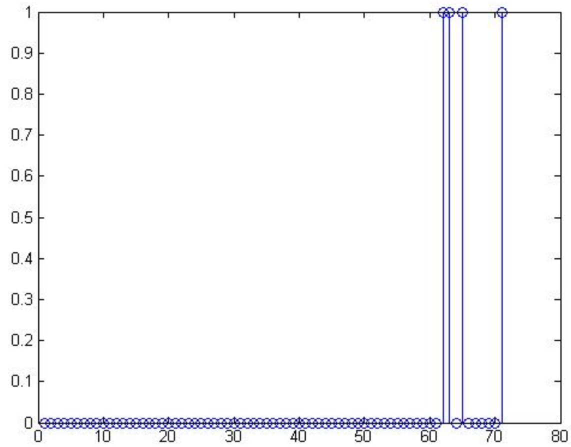


Figure 12: Classification of a non-seizure event with half of training set



VI. Discussion

A. Data to construct the training model

REGARDING the data used to build the training model, a group of data collected from the sensors representing time periods where no seizures were observed and a group representing time periods where the mouse had seizures (these classification were permformed during the experiences). The aim when the no seizure group of data was incorpored in the training was to include as many as possible different rat states meaning: sleeping periods and periods of less/more movement in order to create a train model which included a complete characterization in frequency domain of the mouse's behaviour. It was not possible to collect data from sleeping situations, besides that, it can be considered that the data group for non-seizure had too many periods of excessive mouse's

Figure 13: Classification of a non-seizure event with full training set

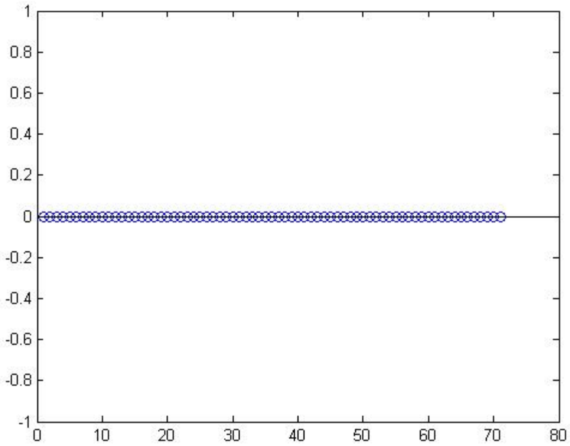
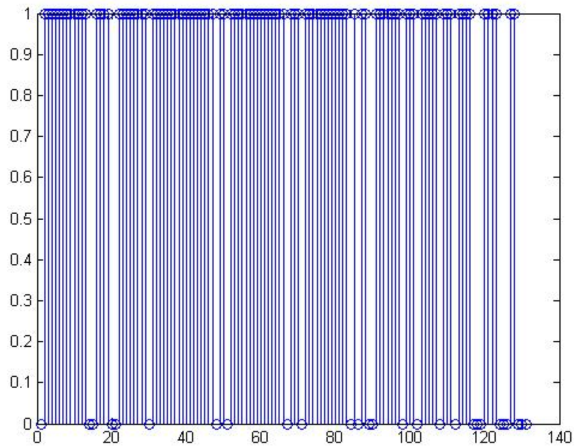


Figure 14: Classification of a seizure event with half of training set



movement. This is a point to improve in future approaches. The data group related with seizure periods was collected in one recording session, and covers almost all Racine scale (it was possible to record levels 1, 3, 4 and 5, according to human classification). So, it was possible to achieve a good description about the frequencies related to seizure periods (but not good enough to classify the seizure states according to the racine scale). Nevertheless, more recording sessions of both periods would increase the available data to build a better training model.

B. Other aspects of the training model

It is possible to test the quality of training model by calculating two parameters. One of them is a parameter defined by the fraction of misclassifications from the predictions of the model; another is a parameter which is

Figure 15: Classification of a seizure event with full training set

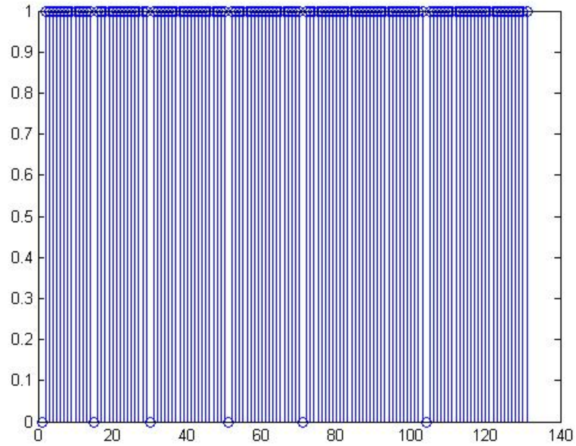
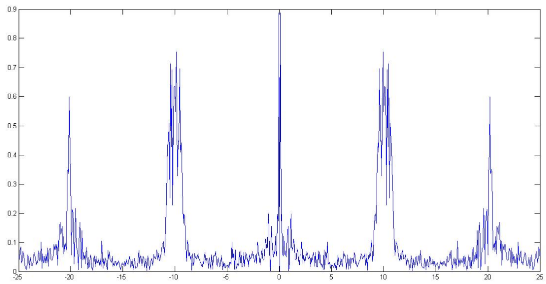


Figure 16: Functionality test of the seizure cage detector



an indication of the percentage of misclassification of new data. Matlab gives the chance to modify some parameters of the K-NN algorithm. One with a great interest is the distance metric used. There are several choices and by default Matlab uses the *euclidian* one. It was observed that the distance that minimized the quality parameters was the *euclidian* one. This distance is basically the *euclidian* one divided by the standard deviation.

Another parameter which is possible to modify is the number of neighbours used in the model to define if a certain point is or is not a seizure, according to the distance to that number of neighbours. To construct this model, it was chosen that 3 would be a good number to use, because it is an odd number, given no raise to draws. Obviously, a large number of neighbours wouldnt permit a good classification.

Another parameter of interest is the prior probability. A prior probability for a mouse to have a seizure of 0.05 was chosen, and not to have a seizure of 0.95. This choice is based in a simple fact: the probability of a mouse to develop a seizure is much small than the probability of not developing it. Of course, to define with a high accuracy these two values, it would be necessary to have more available data to define a distribution of probabilities.

To classify a given temporal interval as being a seizure event, it was defined that the number of consecutive points classified as a seizure had to be superior to a given threshold. It was defined that at least, the algorithm had to classify 5 windows out of 10 as a seizure to interpret that temporal interval as a seizure. It is possible to modify the parameters mentioned above, creating new combinations of them and consequently to achieve other results which can be or not be better. The values chosen for each of the parameters are, obviously, very important and so, in the future approaches, it would be of the great interest to pick them with more accuracy.

C. Error sources related with instrumentation and montage

One of the main problems related with the used instrumentation is the low resolution from Arduino, when converting voltage to bits. To convert 5V, Arduino uses 1024 bits which corresponds to 4.9 mV per bit. So, a large number of bits would be a great improvement to data quality. This problem originated a lot of noise in the frequency padrons of the signal since it introduced parasitic frequencies across the whole range of frequencies. Even great amplification of the AC signal could not solve this problem.

The circuit components are another possible error source, mainly because its manufacturing dispersion, which have to be carefully handled when analysing the exact cut-off frequency of a filter, for example. If the manufacturing dispersion of some component of a certain filter is high, it could introduce an uncertainty related to the exact cut-off frequency of filter, and so, could lead to a bad data interpretation. The main purpose of using a Sallen-Key montage to build all the filters was to increase the attenuation above the defined cut-off frequency. The aim of the project was to properly identify when or not a mouse was having a seizure. As explained, that achievement was made by analysing the AC part of the signal detected. However, it is possible to analyse the DC component in order to obtain other information such as: the position of the mouse and consequently determine the position of the center of mass. For example, it is possible to define the moments when the rat is closer to one of the sensors. It is also possible to set the times when the rat is removed from the cage or when water or food is poured in the cage. The DC component could also be used to differenciate the different seizure stages.

VII. Conclusion

DESPITE having some minor error in the classification, considering that the main purpose of this project was to develop a system to detect seizures in an animal, it can be concluded that it was a success to an acceptable extent. Regarding the signal acquisition, the circuit had a high sensibility to fine movements, allowing for an accurate representation of the activity of the animal inside the cage. The second objective however, which consisted of comparing the piezoresistive sensors with other methods to determine which yielded the best approach, wasnt feasible due to time and financial restraints.

The main difficulties discovered during the development of the system were the following:

1. The variability in the values of the resistors and the capacitors, which resulted in different cutoff frequencies for the filters employed
2. The Arduino introduced high irregularities in the frequency components due to its low voltage resolution;
3. The lack of a long enough dataset to be used as a training set was a major limitation in the performance of the classifier;
4. The poor human classification accuracy in time, also represents a major factor of uncertainty for the training of the classifier;
5. The impossibility to test the system regularly, being restricted to the dates when the experiments would be performed, also made the readily system adjustments impossible.

VIII. Future work

FOR some future improvements, the logical following approach would be to extend the classification to the different ranks of the Racine scale. This would require a sufficiently large amount of data an accurate rank and time accuracy by the medical experts. Also, the system could be readily applied to the other applications described above such as the EPM, the OFT or the RAM. Another use using the same principle, and perhaps which would be of high interest would be to use more resistant sensors and apply the system to a human patient bed to access several behavioral or pathological characteristic.

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