Towards a Computer Vision System for Monitoring and Stimulation of Rodents

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***Abstract*—The Behavioral Neurophysiology research area investigates the electrophysiological correlates of behaviors, normally using animals such as rodents as subjects. Examples of studies in the field include the investigation of neural processing dysfunctions and synaptic plasticity in animal models of autism and changes in synaptic plasticity in animal models of epilepsy. This area is in constant need of new equipment to aid research. With this goal, this work aims to develop a system capable of receiving video information in real-time using computer vision algorithms to define the positioning of the animal, plus the processing of ultrasound audio and brain electrophysiology signals. These data are to be represented in a user-friendly way and, from these data, we also aim to generate brain stimuli depending on the type of test being performed.**

***Index Terms*—Behavioral Neurophysiology, Behavioral Box, Computer Vision, Animals Monitoring**

I. Introduction

Studies in the area of Neurophysiology are extremely important for a better understanding of the processes that occur in the brain, for example as a result of diseases or external stimuli. This research topic is constantly growing and needs new methods and techniques [1]. Most of the studies carried out use laboratory animals with some biological and behavioral similarities to humans. With the use of animals, human society accepts, through its ethics committees, the performance of more invasive tests that would be unfeasible to be performed with human beings. In this direction, laboratory rats and mice are considered ideal animal models for research [2]. Different tests are designed with them to investigate different aspects of behavior, including anxiety, cognition, spatial memory, and learning [3]. Currently, one way to obtain information about what happens in the brains of animals is through electrode implants that provide electrophysiology data from different parts of the organ. Data can be synchronized with video and audio to study the behavior of different models of behavioral problems, such as autism or epilepsy. Usually, studies use a device called a Behavioral Box, which basically consists of a complex environment, where data from tasks performed are collected by researchers.

In this context, in the present work, we propose to create a tool that will help the research carried out in rats or mice through the development of a software/hardware architecture that is capable of receiving information from the electrophysiology of the animal's brain, from video and audio (ultrasound) in real-time. Basically, we use computer vision techniques to find the animal's position and definition of the studied area. Furthermore, our proposal provides a means of presenting the data to the user in a friendly manner and enabling the generation of reports, as well as allowing the researcher to choose a part of the electrophysiology reading to be used as a stimulus depending on the position of the animal. Finally, our proposal should provide users with an offline video upload tool where they can carry out all their analysis in a friendly environment, without the need for programming or other more specific skills.

II. Electrophysiology in Rodents

Electrophysiology consists of the study of the electrical properties of cells and tissues. It involves measurements of electrical potential differences at a variety of scales from simple ionic channel proteins to complete organs. For the acquisition of electrophysiological signals, the animals must be submitted to surgical implantation of electrodes. The electrodes are designed, built, and implanted in areas of interest in the brain, in this case, the areas were the prefrontal cortex and hippocampus, but the decision is up to the researcher as to the type of study being carried out [4]. The implanted leads are connected to an acquisition system which is then connected to the computer. There are several techniques for analyzing the behavior of experimental laboratory animals. One of them is the open field test, as it is the application test of our proposal.

A. *Open-field maze test*

An open-field maze consists of an area surrounded by a wall high enough to prevent the subject from escaping. Typical maze shapes are circular or square with a large enough area, defined based on the size of the tested subject, to provoke a feeling of openness in the center of the maze [5]. Figure 1 shows a video frame recorded in an open field test carried out in a behavioral study laboratory. In the figure, it is possible to better understand the environment for performing the open field test.

A number of variables can be studied in the open-field maze with most parameters involving different types of motor activity. Ambulation (walking) is the most commonly studied

Fig. 1. Open field test environment.

behavior, but others, such as latency or elevation, can also be measured. Most of the time, rodent behavior is analyzed in an empty maze. But, the addition of objects, one or many to the floor of the maze, adds the ability to see how the subject interacts with new additional stimuli. The relevant parameters when objects are presented are normally the number of approaches to an object or, in some cases, a preference or aversion for one object over another.

III. Computer Vision Software Architecture

In the context of software development, several solutions can be defined to meet requirements, but analysis must be carried out to define the most suitable for a given application development context [6]. The software architecture of a program or computer system is the structure or structures of the system, which comprise software elements, the externally visible properties of those elements, and the relationships between them [7]. In our work, we are typically developing Computer Vision software, which is an area of Computer Science that studies and seeks to develop theories and methods aimed at the automatic extraction of useful 3D information contained in images that are captured by devices such as a video camera and scanner [8]. The organization of a computer vision system depends on the application and its implementation also depends on whether its functionality is prespecified or if there is some part of learning during operation. Despite this, there are some typical functions that are found in general computer vision systems [9]:

- Image acquisition: a digital image is produced by one or several sensors. Depending on the type of sensor, the result can vary between a two-dimensional image, a three-dimensional scene, or even a sequence of images. Pixel values generally indicate the intensity of light in one or several color bands.
- Pre-processing: before a computer vision method is applied to an image to extract information, it is usually necessary to process the image to ensure that it satisfies the conditions of the method. Examples include remapping, noise reduction, and contrast enhancement.
- Feature extraction: mathematical features of the image at various levels of complexity are extracted. Basic examples include edge, corner, or point detection.
- Detection and segmentation: at some point in the process a decision is made about the relevance of image regions for further processing. Examples include selecting specific regions of interest and targeting one or more regions that contain an object of interest.
- High-level processing: at this point, the input is usually a small dataset. The subsequent process includes verifying the satisfaction of the data, estimating parameters about the image, and classifying the detected objects into different categories.

IV. Related Work

We looked at the literature for existing software architectures used in mouse and rat behavioral research. Among the various works in this line, we find three specifically that deal with similar problems. The first two focus on aspects of computer vision for the quantitative determination of behavior tests [3], while the last one shows an electrophysiological recording software in rodents [10].

The MouBeAT is an abbreviation for *Mouse Behavioral Analysis Toolbox*. It is open-source software designed to analyze different behavioral tests on rodents semi-automatically. These tests include Open Field (OF), Elevated Plus Maze (EPM), Y-Maze (YM), and Morris Water Maze (MWM). MouBeAT showed a high correlation with manual assessment in all parameters analyzed for all behavioral tests, reinforcing its value as an analysis tool. This tool is available for free online [3]. It works offline with a previously recorded video, and uses the ImageJ tool, a Java-based image processing program, to work with configurations and choosing the behavioral analysis test.

X-PloRat is software that allows recording the location, displacement, and other properties of animal behavior in confined spaces. X-PloRat offers a variety of reports and can help in training students in behavior observation as well as validating other types of records [11]. This software allows the user to customize their study area and specify the keyboard keys that will be used to represent a specific behavior. The software then assists in recording information on the animal's position in the cage while the user manually informs what type of behavior is happening at the moment. This information can later be used for reporting.

Invasive Neurophysiology-Brainstorm (IN-Brainstorm) is another open source software that integrates various aspects of data analysis for most modalities and signal types of basic electrophysiology: from single cells to arrays of distributed channels, from events from peak to local field potentials, from ongoing recordings to event-related responses, and from in-vitro preparations to free-behavior models. The end result is a unique and expansive software toolkit that bridges the gap between recording scales and data modalities, records invasive neurophysiology with structural anatomy data, and thus provides a unifying analytical environment for the neurophysiology research community [10]. IN-Brainstorm's capabilities provide comprehensive solutions for data import and analysis, including peak classification, extraction of local field potentials, and correlations between these measurements across multiple channels. The creators claim that because it is an intuitive graphical user interface, no programming skills are required to access and use the advanced methods available.

The differential of our proposal in relation to works in the literature [3], [10], [11] is that we use both types of recording on video and electrophysiology, simultaneously, for data analysis and generation of reports, as well as providing functionality that allows brain stimulation according to the event chosen by the user.

V. Implementations

Currently, studies with rodents carried out in the Behavioral Studies Laboratory of UFRN use different software tools to obtain the necessary information for several types of research. The audio data of the ultrasonic microphones are saved with the help of Matlab, while the video capture is done separately and later processed by a first version of the software that we have developed previously at the beginning of this work, and the electrophysiology data are saved and analyzed with the OpenEphys software. This division of information in different places brings challenges in terms of data synchronization and makes it difficult to perform some tests. An example of a test would be the stimulus to the animal's brain given a certain event, which may depend on the position of the animal or the sound emitted by it.

Given the above circumstances, this work aims to solve these problems by including in a single software architecture the acquisition and processing of video, audio, and electrophysiology data, as well as in real-time allowing the choice of events for the brain stimulus.

A. System Recquirements

The system architecture proposed in this work has the following requirements for its correct functioning:

- **Visualization:** The user must be able to visualize in real-time the video, audio, and electrophysiology information on the screen.
- **Calibration:** With the image paused, the user should be able to choose the study area (cage), the animal's shape, and areas of interest.
- **Detection:** After calibration, the video should show the position of the animal and save the information whenever the animal passes through the areas of interest.
- **Brain Stimulation:** The user must be able to choose between one of the following events:
  - Go through an area of interest;
  - Output a value in sound decibels;
  - Click on a defined key on the keyboard.

  And from that event to be able to choose a stimulus to be executed in the animal's brain;
- **Report:** At the end of the test, the data must be saved on the machine to generate reports.

B. Hardware

To host the system, we basically use a Workstation computer with 2TB HDD, 500GB SSD, 16GB RAM, and NI-6251 PCI-e Input/Output card. The software has been modeled with UML. The computer receives information from cameras, microphones, and electrophysiological recordings. We used two video cameras, one being a Logitech C920 Webcam that captures video at up to 30 frames per second with a resolution of up to 1080p and another Logitech Brio 4k capable of capturing videos at up to 60 frames per second with a resolution of up to 4k. The hardware also has 4 ANL-940-1 ultrasonic microphones from Med Associates. This kind of microphone and ultrasonic amplifier accurately measure the amplitude and frequency of ultrasonic vocalizations emitted by laboratory rodents. This device is suitable for measuring motivational behavior in studies of drug addiction, mother-infant separation, isolation stress, social interactions, sexual encounters, and many other paradigms. The microphone should be centered over the desired location and pointing towards the subject of interest. The analog output can be connected to third-party data acquisition systems, and in the case of this work, the output is connected to the National Instruments NI-6251 PCI-e I/O board.

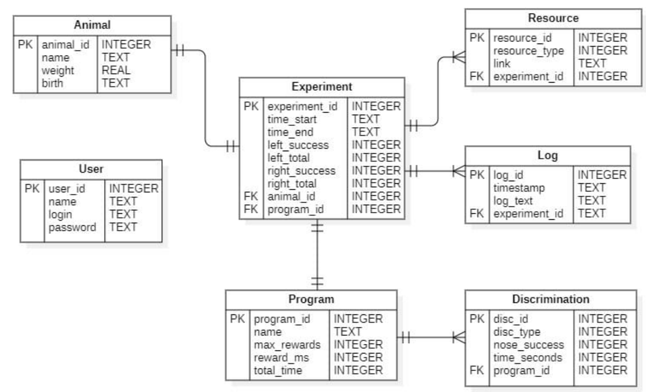

Fig. 2. Diagram showing the entity-relationship model developed for the proposed system.

The set of electrodes surgically implanted in the animal's brain is connected to a plate that collects the information, called an electrophysiological recording plate. The board used for this work is the 16-channel Intan RHD-2000. Each channel is connected to an electrode that records information from a part of the brain.

C. Software

The development of the software architecture, with the conceptual modeling, is being done using UML. This specification language is usual for developing new systems that make use of object-oriented programming, in its various phases, such as requirements analysis, systems analysis, and design. The UML language was conceived in order to establish a single standard to be used to specify the characteristics of computer systems designed to meet the needs of users of these systems [12]. A first diagram of the entity-relationship model of the proposed system is shown in Figure 2.

We also use the COMEDI package, which is a collection of drivers for a variety of common data acquisition plug-in boards. Drivers are implemented as a core Linux kernel module, providing common functionality and low-level individual driver modules. This is used to communicate with the National Instruments I/O board for real-time data acquisition.

The Computer Vision algorithms are implemented in Python, with the OpenCV library (Open Source Computer Vision Library). OpenCV provides a common infrastructure for computer vision applications and for accelerating the use of machine perception in commercial products. This library will be used to acquire the position of the animal in the cage.

We also use Qt, which is a free and open-source widget toolkit for creating graphical user interfaces as well as cross-platform applications that run on various software and hardware platforms such as Linux, Windows, macOS, Android, or embedded systems. Qt is used to develop graphical user interfaces (GUI) and cross-platform applications that run on all major desktop platforms and most mobile or embedded platforms. PyQt is a Python binding of Qt, implemented as a Python plugin. PyQT is being used to develop the graphical interfaces for this work.

VI. Preliminary Experiments and Partial Results

Given the need to carry out immediate research at the behavioral study laboratory, we decided to start with a prototype for detecting the position of the animal, initially working offline. Using the OpenCV library (python), we made an application that allows the user to choose the study area (cage), choose an area of interest, and perform the animal calibration. The software then processes the video frame by frame and outputs two files: a processed video and a table with frame-by-frame information on the position of the animal, information if the position is in the area of interest, and the number of times in which the animal passed through the area.

Figure 1, above, has shown a frame of an open field test video performed in the behavioral study laboratory. Figure 3 shows a further frame of the same video after performing the processing in the software. It is possible to see that the position of the animal is being successfully detected. Further, each time the animal passes through the area of interest (green square), a green light in the upper left corner is turned on in the video, for viewing purposes.

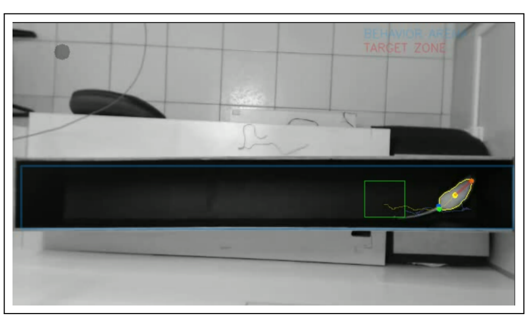

Fig. 3. Open field test processed video frame.

The current system has limitations because it requires a test to have been performed and recorded in advance, and we are only performing the detection of the animal, by now. The electrophysiology information and ultrasonic microphones are currently captured with Matlab and synchronized with the processed video after passing through the software so that the search can be carried out with the data found.

VII. Conclusion

Our ongoing work goal is to create a tool capable of assisting research in the area of behavioral studies with rodents carried out at the Brain Institute of UFRN (Natal, Brazil). It can be assumed that the objective will be achieved with the final architecture design and development of the software that performs the acquisition of the multimodal data necessary for the study (video, audio, and electrophysiology signals). Our main goal is to develop a friendly visualization tool for these data, in a synchronized way, which allows the users to capture any kind of stimuli generated inside the animal brains by the electrodes given a chosen event, plus other information such as the animal's pose and, eventually, its motion behavior.

The proposed system development follows the software architecture design methodologies, combining our knowledge in computer vision, mainly image processing techniques, electrophysiology, and graphical interfaces, that will be applied during the next steps. This work will allow the tests carried out in the Behavioral Study Laboratory to become more automatic, facilitating the generation of reports and contributing to new discoveries in research in the area.

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