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Research Proposal:

Analysis calls of mouse pups as a means of predicting autistic behavior

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Abstract

Autism Spectrum Disorder (ASD) is a developmental disorder which is affected by environmental and genetic factors. Early diagnosis and identification of ASD is particularly important because it allows diagnosed children early intervention and improvement in their behavioral development. Observing abnormal behavior is the basis for a diagnosis currently, as such diagnosis is not objective and may take a long time.

Mice are good models for researching autism because they are highly social animals. The mice emit specialized sounds in the ultrasonic range (USV) to communicate in different social contexts. Using analysis of the USV in mice, allows to elucidate and deepen the understanding of the biological mechanisms that cause abnormal social development that are seen in ASD.

In this project, we aim at developing a machine-learning-based model that analyzes the USVs signals and identifies whether a mouse is healthy or suffers from ASD-like signs. For this purpose, we will use a previously developed segmentation and classification model to extract the relevant features for ASD identification.

Literature Review

ASD refers to a group of neurodevelopmental conditions that are characterized by a wide spectrum of symptoms, skills, and functional levels [1]. Impairments are identified in two domains: communication and social interaction and restricted, repetitive patterns of behavior [2]. ASD is a prevalent condition, with 1 in 68 children identified with symptoms, e.g., developmental delay, communication and social difficulty etc, that characterize the disease in the first two years of life [1].

Observing abnormal behavior is the basis for a diagnosis, but due to the heterogeneity of symptoms and their severity in ASD, the diagnosis in children may be in late ages [2] [3]. Despite the availability of reliable methods for diagnosis of ASD before the age of two in severe cases, the average age at which a child receives a diagnosis is currently at the age of 4-5. Early diagnosis of ASD is particularly important because it allows early intervention and treatment in a time when brain circuits are still plastic. Studies have found that interventions implemented before age 4 lead to improvements in daily living skills, social behavior, language and quality of life of children with ASD [1].

The importance of early intervention for children with ASD has resulted in attempts to quantify behaviors in infancy that may lead to early detection. Studies show that delayed production of canonical syllables in infants may be an early warning sign of ASD [4]. Another study has shown that siblings of children diagnosed with ASD, considered high risk for ASD, produced significantly fewer speech-like vocalizations, fewer consonant types and fewer canonical syllable shapes Compared to children from low-risk families [5]. These and other studies indicate that signs of ASD can be detected in babies even before the emergence of verbal speech.

One of the genes associated with the increased risk of autism is methylenetetrahydrofolate reductase (MTHFR) whose activity strongly affects the C1 metabolic pathway. In mice, MTHFR deficiency is associated with developmental delays and autistic symptoms [6].

Studies that use rodents, and especially mice, explore specialized sounds in the ultrasonic range called UltraSonic Vocalizations (USV) that are emitted in different social contexts [7]. Using the mouse model of autism helps to elucidate and deepen the understanding of the biological mechanisms that cause abnormal development that are seen in ASD, and develop treatment [6]. In mouse pups, production of USVs is affected by maternal isolation, cold, rotation and genotype and hence the standard test for vocalizations in mice is the ultrasonic distress call of pups separated from the mother or removed from the nest [8] [9]. These calls are used for investigate the number of calls and the type of syllables emitted by separated pups in mouse models of autism spectrum disorders [9].

Mice communicate both in the human-audible range with squeaks for long-distance warnings, and in the ultrasound range for short-distance communication, with calls in frequencies ranging from 25–120 kHz. Mouse USV frequencies are especially variable and complex. Mouse USVs consist of several different syllable types, whose temporal sequencing includes the utterance of repeated phrases [8]. The most common approach is to split the USV of the mice into 10 types of syllables which different from each other in their duration, in their main frequencies and in their spectrograms (e.g., flat, short, upward, downward, etc.) [9] [10].

In recent years, attempts have been made to classify syllables into fewer and more general categories as well as to classify using software, in order to improve the ability to detect alterations in model pups [11]. We will present some of the studies and approaches:

Table 1: USV classification tools comparison

	MUPET	**DeepSqeak**	**VocalMat**	**BGU Project**
Year	2017	2019	2021	2021
Main method	K-mean clustering	Faster RCNN	CNN	CNN
USVs database size	51,000~	56,000~	45,000~	~6000
Syllable categories number	60-200 Depend on user-defined	100 Blink to the smallest possible number of categories	11	others+10
Average accuracy result	85%	95%	86%	81%
Limitation	Clustering is applied on the spectral magnitude of the segmented syllables so that amplitude changes can impair classification to the different categories	It may be classified into a small number than wanted of categories	Shows lower accuracy for detecting USVs with multiple components	Small training dataset
Reference	[15]	[14]	[13]	[12]

The above studies aimed at classifying the USV calls into categories, whereas our main interest is in diagnosis, i.e., a tool that can classify the USV calls into “healthy” and “ASD-like”.

In a previous study conducted at Golan’s laboratory, three groups defined by genotype and maternal genotype as follows: Mthfr+/+ (wild‐type [Wt]) offspring from Wt mothers (Wt:Wt), Wt offspring from Mthfr+/− mothers (heterozygote [Het]) (Het:Wt) and Het offspring from Het mothers (Het:Het) were created. Analysis of the syllables that the mice from the three groups emitted, they found that pups with maternal Mthfr+/+ (Wt:Wt)

genotype had syllables with higher frequencies (start, end, and mean frequency), compared to pups with maternal Mthfr+/- (Het:Wt/Het:Het) genotype. In addion, offspring Mthfr+/- genotype (Het:Het) had lower mean and end frequencies, compared to pups with Mthfr+/+ (Wt:Wt/Het:Wt) genotype. In contrast, the start frequency and bandwidth were lower in the mice with maternal Mthfr+/- genotype and further reduced by offspring Mthfr+/- genotype, such that the Mthfr+/- pups from maternal Mthfr+/- (Het:Het) genotype showed the lowest start frequencies. In the males, the Mthfr+/- genotype at maternal and offspring lowered the frequencies of the start, end and mean frequency of the syllables. In both sexes, the bandwidth was lowered by maternal Mthfr+/- genotype and further lowered by offspring Mthfr+/- genotype. In summary, it was found that the start frequency, end frequency and bandwidth were the variables that were most significantly affected by the ASD factor in most clusters and bandwidth and duration showed the largest differences between ASD and control groups. From an analysis of the proportional production of the different syllables it was revealed that the male offspring of maternal Mthfr+/- genotype (Het:Wt and even more in the Het:Het pups), was a lower proportion of syllables with high frequency in syllables of both simple or complex structure and also maternal Mthfr+/- genotype was associated with a marked increase in the proportions of low frequency calls. Altogether, Mthfr genotype decreased the proportion of syllables at the range of 70,000kHz and above, and increased the use of syllables with a range around 50,000kHz. In addition, it was found that the syllables emitted by the experimental groups were shorter, simpler and with lower frequency syllables compared to the experimental groups [11].

Project Goals

Previous projects focused on developing models of segmentation and classification that have yielded good results. In this project, our goal is take the research one step forward, and use the models that were developed, to process entire USV signals, characterize them, extract features that may be relevant to autism diagnosis and develop a machine-learning-based model that can use the signals to identify whether a mouse is healthy or suffers from ASD-like features.

Hence the project goals can be divided into the following:

1. Feature extraction- extraction of the relevant features to distinguish between healthy and ASD-like mice. For this purpose, we will utilize the segmentation and

classification algorithms that were developed in the previous projects, based on which we will calculate various features, including, for instance, statistics of the voiced signals (e.g., average duration), frequency of voiced segments, distribution of type of calls, etc.

2. Classifier development- develop a classifier to distinguish between healthy and ASD-like mice. For this purpose, we will consider to expand the USV database by recording mouse pups in the first days of their lives.
3. Application- we will utilize the classifier to examine the effect of maternal and pup Mthfr genotype on pups communication (e.g., what is the risk of a pup having ASD-like USV, what parameters of communication are modified in the ASD-like pups, etc.).

Methods

Figure 1 presents a block diagram of the proposed scheme. The scheme consists of pre-processing, feature extraction and classification.

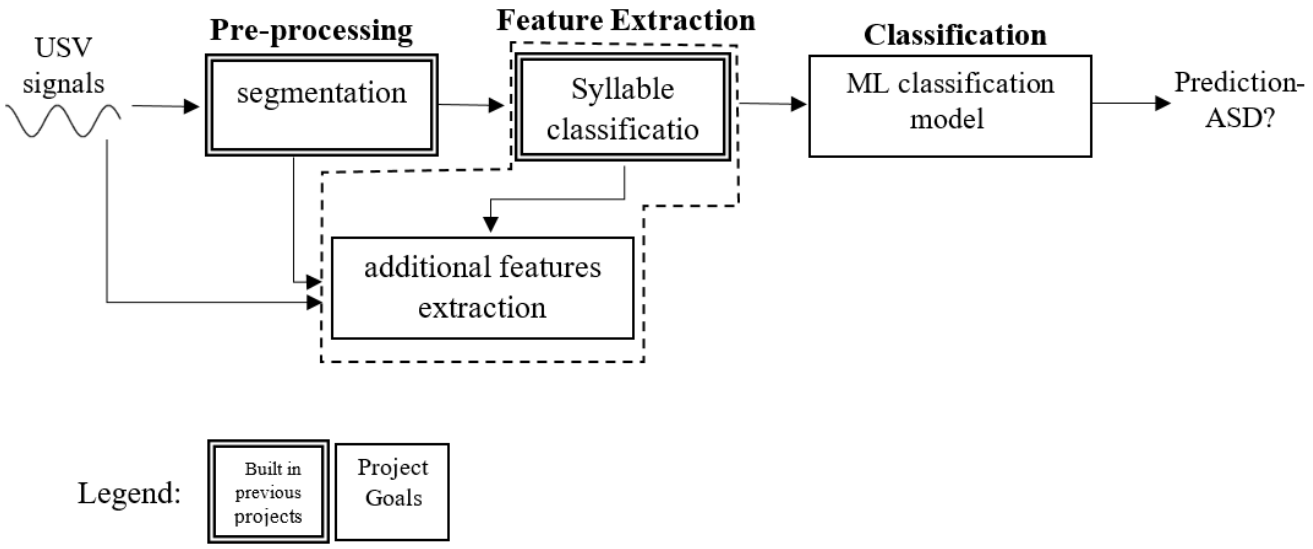

Figure 1: a general scheme of the proposed system

Pre-processing

The pre-processing stage includes a noise reduction filter and a segmentation algorithm which divides the entire USV signals into short segments which each includes one syllable and removes the silence segments.

Feature Extraction

During the feature extraction stage, diagnostic information is extracted from the USVs, including number of vocalic segments average, length of vocalic segments average, distribution of vocalic types, initial frequency average of vocalic segments , end frequency average of vocalic segments average, length of silent segments average during the speech signal, etc. We will then generate a matrix of features which will be used in the classification stage. The matrix of features describes the list of columns that contain variables to be processed, including all lines in the dataset. In the initial stage, we will base the finding of the features on a manual analysis of the signals and statistical differences that were found in Golan's laboratory. As a second step, we will use the outputs of the previous models and identify additional important features.

Classification

Classification is a systematic arrangement in groups or categories according to established criteria specifically. In this project, in addition to finding the features, we will focus on the development of a model that receives a matrix of features and can identify whether a mouse is healthy or suffers from ASD-like features.

The classification algorithm will be based on XGBoost. XGBoost is an algorithm that has recently been dominating in the world of applied machine learning for structured or tabular data. XGBoost is an implementation of gradient boosted decision trees designed for speed and performance [16].

Performance evaluation

In order to evaluate the performance of the classifier, we will divide our data into 'training', 'validation' and 'testing' sets. The training set will be used to train the models, the validation set will be validation and used for hyper-parameter selection, and the test set will be used for performance evaluation. The output of the classifier is a vector of scores that determine whether the mouse is healthy or suffers from ASD-like features.

Dataset

Available data: we will use a dataset that has been collected at Golan's laboratory in recent years. The dataset contains around 97,000 audio recordings of mice USVs (around

70,000 adults and 27,000 young), distributed between males, females, healthy, and mice with MTHFR gene deficiency (around 50%). In this project we will use recordings of young mice, as the goal is early detection of ASD.

Expansion of the USV data set: In these days we record additional set of data by recording isolation calls of postnatal day 4 and 6 pups. For that we use Avisoft-RECORDER, a versatile multichannel triggering harddisk recording system that designed for the special needs of bioacoustics. Having Up to 8 channels, each sampling at up to 1 MHz can be monitored simultaneously. Provides real-time spectrogram display, Waveform display and Energy display [17].

Project schedule

Table 2: project time plan

	Nov	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**
Literature survey										
Learning and controlling the models that built in previous projects										
Learning and organization the existing information										
Expand dataset										
Feature Extraction										
Developing a classification model										
Final report										

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