Online Decoding System with Calcium Image From Mice Primary Motor Cortex

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***Abstract*—With the development of calcium imaging, neuroscientists have been able to study neural activity with a higher spatial resolution. However, the real-time processing of calcium imaging is still a big challenge for future experiments and applications. Most neuroscientists have to process their imaging data offline due to the time-consuming of most existing calcium imaging analysis methods. We proposed a novel online neural signal processing framework for calcium imaging and established an Optical Brain-Computer Interface System (OBCIs) for decoding neural signals in real-time. We tested and evaluated this system by classifying the calcium signals obtained from the primary motor cortex of mice when the mice were performing a lever-pressing task. The performance of our online system could achieve above 80% in the average decoding accuracy. Our preliminary results show that the online neural processing framework could be applied to future closed-loop OBCIs studies.**

I. Introduction

Brain-computer interface (BCI) aims to achieve direct information communication between the brain and external devices. It takes the physiological signals of the brain as the initial signal, extracts the useful information of the intention, identifies and controls the peripheral devices, and realizes the direct interaction between the brain and the external physical environment [1]. Establishing a BCI system that can achieve neural decoding in real-time is an important exploration for biomedical engineering and neuroscience, which can help us better understand the cognitive model of the brain and the mechanism of its processing information [2].

One of the most widely used BCI technologies is the electrophysiological recording which collects signals of neuron clusters through patch-clamp, neuropixels probe, and so on [3]. However, limited to the scale of the neural collecting, we can`t get hundreds or thousands of neuron changes at the same time in this way. Moreover, the collection has spatial sparseness and cannot record a specific cell or spatial organization information. With the development of functional optical imaging, these limitations are hopeful to be resolved. Calcium imaging technology is one of the popular functional optical imaging methods. Its main principle is to combine exogenous fluorescence signal with the physiological phenomenon and reflect the concentration of free calcium ions in cells through the change of fluorescent dye signal to represent the functional state of cells [4]. The method allows simultaneous observation of multiple functionally or location-related brain cells [5]. It is widely used to monitor the changes of calcium ions in related neuron groups in real-time, thus determining their functional activity. The technology allows scientists to witness the transmission of neural signals in time and space in a neural network [6].

Two-photon microscopy imaging technology is a usual calcium imaging method combining confocal laser scanning microscope and two-photon excitation technology, which has been used in many studies. Zong et al. had developed a fast high-resolution, miniaturized two-photon microscope [7]. However, due to the low imaging speed, image distortion and thefixed head of living animals, more and more researchers prefer single-photon microscopy. As the device of single-photon microscopy can be fixed on the head of animals, the animals move body freely and real-time dynamic imaging through wide-field microscopy can be captured. Barbera et al. proposed that neural clusters in the dorsal striatum encode locomotion-relevant information by analyzing the data collected by single-photon microscopy [8].

The primary motor cortex is an important region that controls body movement and converts the action instructions from the brain into neuron signals to encode movement [9]. Komiyama et al. researched the calcium imaging in layer 2/3 and found that correlated activity in specific ensembles of functionally related neurons is a signature of learning-related circuit plasticity [10]. Capturing and analysing calcium imaging data from the primary motor cortex of mice will help us to realize neural decoding [11].

In this paper, we establish a real-time Optical Brain-Computer Interface System (OBCIs) and present a novel online neural signal decoding framework. We trained three mice to perform a lever-press task and collected calcium imaging data from layer 2/3 motor cortex by single-photon microscopy. The support vector machine (SVM) was used as online decoder and our OBCIs could achieve a good performancewith average decoding accuracy exceeds 80 percent. The implementation of the optical brain-computer interface system based on single-photon calcium imaging provides a basis for further closed-loop OBCIs.

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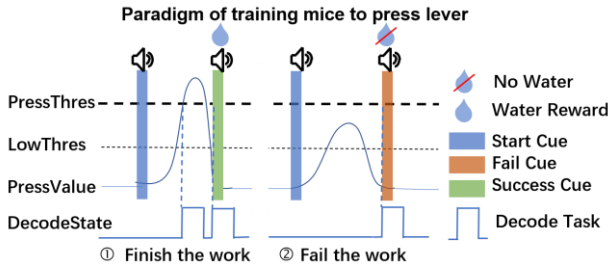

Figure 1. A experiment paradigm of mice for real-time decoding task.

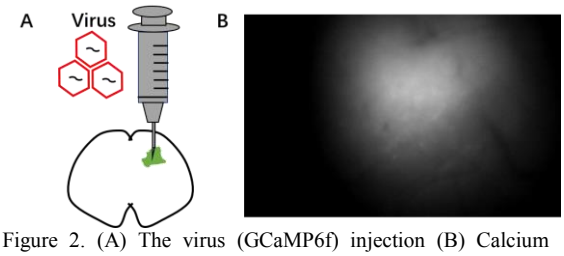

Figure 2. (A) The virus (GCaMP6f) injection (B) Calcium imaging from mice primary motor cortex

II. Materials and Methods

A. Behavioral Task and Surgery

We choose the three C57BL/6 wild-type mice weight 25g as our experiment subject. These mice were trained to press a lever for getting water rewards(Figure 1). Each trial begins with the start sound stimulus (Start Cue). Mice need to reach out their forelimbs and press the pressure sensor bar to meet the following criteria: (1) pressure exceeds the high-threshold we set; (2) pressure decline to the low threshold after reaching the high-threshold; (3) the change of pressure should be completed in schedule time we set. After the mice finish the task successfully in this trial, system offers sugar-water reward and sound stimulus (Success Cue). In other cases (timeout or pressure which is below the threshold), system will consider as a failure and send another sound stimulus (Fail Cue). Next trial need to wait for the refractory period time after the failure trial. In each session ,we trained for 30 minutes (consisted of about 200 trials). By sufficient training (8-10 sessions), the average successful rate of these mice reached 78.80%. After the behavioral training, we operated on the mice to prepare them for subsequent image acquisition.

All surgical and experimental procedures conformed to the Guide for The Care and Use of Laboratory Animals (China Ministry of Health) and were approved by the Animal Care Committee of Zhejiang University, China. Mice were anesthetized with 35ml Avertin (1.25% solution, 0.015ml/g, i.p.) and we drilled a 1.5mm-diameter hole in the skull to provide the desired viral injection location. Next, 100nl virus (pAAV2/9-hSyn-GCaMP6f pMT311) was injectedinto the desired location through micro-infusion (Figure 2A). And then, we laid a pre-sanitized 1.5 mm-diameter glass coverslip (Thickness: 0.13mm) over the skull hole and glued it with Krazy glue. After recovery for 2 weeks,mice were installed baseplate of Miniscope for following calcium imaging acquisition. Figure 2B is an example calcium image from the primary motor cortex.

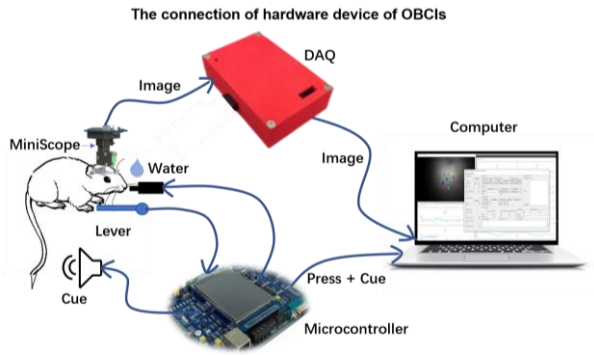

Figure 3. The diagram of connection of hardware device in the OBCIs system.

B. System Design

To analysis the calcium image from mice, we need to get the synchronous behavior data. The specific connection of the hardware device is shown in Figure 3. The system transmitted the acquired images back to the computer through the DAQ and acquired press change by the pressure sensor (Lever). We utilize serial communication to control the microcontroller to send the sound stimulation and feed water operation for training mice to complete the behavioral task.

We used the UCLA MINISCOPE as our calcium imaging device and wrote the experimental software. It implements most functions: image acquisition, microscope parameters adjustment, ROI selecting, fluorescence tracing. Our OBCIs can acquire calcium data at 10-30 frames per second. In addition, Our OBCIs can make training datasets of neuron signals, training the SVM model, and real-time decoding neural signals. During the online experiment, calcium imaging and behavioral event signals obtained from mice are stored simultaneously, which could be used for further offline analysis. All codes of the experimental system were implemented in C++.

C. Online Decoding Method

The workflow of our OBCIs system is shown in Figure 4A. The first step is to select the neuron for decoding. Before the decoding experiment, we need to preprocess the calcium signals from mice. After getting the calcium imaging, motion correction and image enhancement are performed to get a better view of neurons, and then we can manually select the ROI. The selection criteria for neurons were high ratio-to - noise and the shape of neurons is doughnut-shaped or macaron-shaped. Figure 5A shows the image after enhancement and Figure 5B shows the neurons selected in our experiment. The more neurons used as the decoding input, the more movement-related information can be extracted. But we still need to consider the number of neurons since the excessive number will cause a burden on the real-time performance of system decoding. Through offline experiments, we found that ten neurons as the decoding input could reach better results with less input.

Feature extraction is a critical part that will affect the following decoding results. Now the popular methods applied to offline process are the CNMF method and MIN1PIPE method [13 14]. But these methods cost so much time which dissatisfies with our system requirement of real-time processing. Here we chose the traditional method to calculate

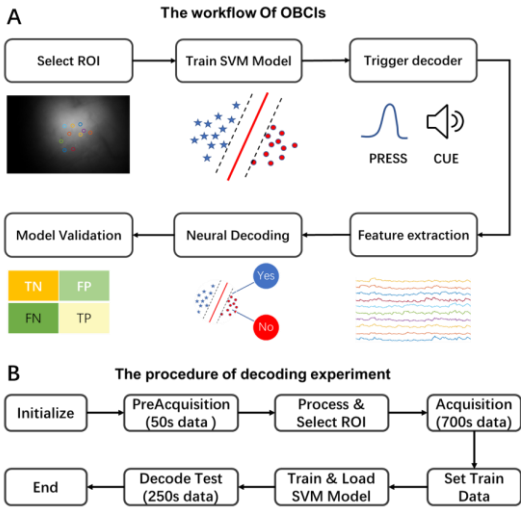

Figure 4. (A) The workflow of OBCIs in decoding experiment. (B) The experiment procedure of mice decoding including selecting ROIs, training model and decoding neural signals.

the values of the region of interest. By comparing the values of ROIs calculated by the maximum, mean, and Gaussian fitting, we found that there is no significant difference in decoding and chose the average value of ROI as the neuron characteristic. The changes of ROIs and press of mice are shown in Figure 5C.

Lee et al. had applied the linear discriminant analysis (LDA) to the real-time neural decoding problem [15]. However, LDA default assumes per class meets a normal distribution and is limited to the linearly separable problem. The Support Vector Machine (SVM) is the most widely used classification method. Though Lee et al. also applied it to the neural signal decoding framework, it is still a proposed framework tested by offline data and has not been widely applied to online experiments [16]. Here we applied the SVM method as our decoder by modifying its execution steps. To get rid of the linear constraint, we apply the Radial Basis Function (RBF) kernel as our SVM model, set the degree to 3. After the experimental test, the optimal parameters are finally selected. Using the time delay of neural information can often improve the decoding effect of neurons. Our decoder input at a time is a hundred features (ten neurons from ten frames of calcium image). We classified data as true when the pressure exceeded the threshold, and we identify the mice as performing the lever-pressing; Otherwise, the mice were classified as false and did not press thelever.

Here we use two conditions as the decoder triggers: sound stimulation of success or failure and pressure exceeding the high-threshold. When the decoding condition is triggered, the system will uses the neuron information to decode the behavior state. The details of decoding experiment was displayed in Figure 4B. The calcium imaging time in mice collected in each experiment was about 15min because of the bleaching property of single-photon. Due to the limited imaging time, we divided the training and decoding according to the experimental time. Firstly, calcium imaging data of mice were collected for 50 seconds as the preprocessing data of the decoding task to select the location of neurons. We artificially select neurons according to the criterion of high correlation

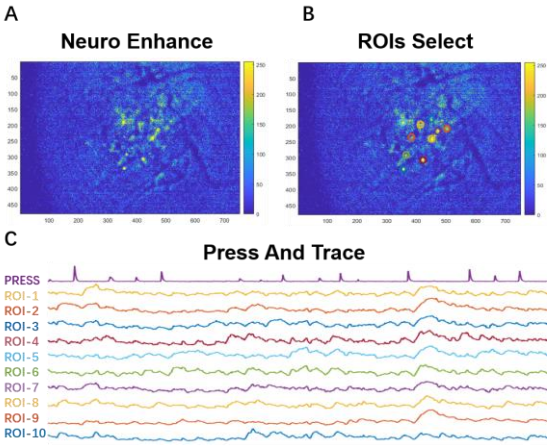

Figure 5. Select ROIs by processing data from preacquisition. (A) show the image after movement correction and ΔF/F process. (B) show the ROI we select for real-time decoding. (C) are press value and the trace (mean value of ROIs) as input of SVM decoder.

between neurons and behavior, as a mature automatic neuron recognition algorithm has not been introduced into the system yet. After 10 neurons were selected, 700 seconds of experiment data were collected as training data. While collecting data, our software synchronizes mice calcium imaging and behavioral information to generate online training data sets for the predictive model. We put the training data into the SVM and get the predicted model. After the model was loaded, we conducted the same experiment for 250 seconds to test and recorded the online prediction system. At the same time, the system also saves the data for subsequent offline decoding analysis.

III. Result

Three mice were trained for ourexperiment We collected calcium imaging signals from the M1 of these mice (4 sessions from each of them on different days) and decoded behavior state through our OBCIs system.

The specific decoding results of the three mice are shown in Table I. We modified the SVM classification method for online classification decoding. the results from the offline process of neuron decoding are the same. The average accuracy of these online decoding is 81.04% and the highest

TABLE I. Decoding Accuracy In Different Sessions

Mice	Decoding Accuracy of Mice			
	Session1	*Session2*	*Session3*	*Session4*
H12	80.77%	90.91%	88.14%	82.22%
H18	79.41%	87.50%	83.02%	80.00%
V14	71.64%	72.92%	86.81%	77.14%

We apply the RBF kernel as our SVM model. According to the classification standard, OBCIs automatically set training data. We go on with the decoding experiment and test the decoding result.

TABLE II. Decoding Time Cost In Different Sessions

Time Cost (ms)	Time of Decoder costing in different sessions			
	Session1	*Session2*	*Session3*	*Session4*
H12	2.576	2.272	2.864	2.590
H18	2.484	2.354	1.528	1.360
V14	1.312	2.076	2.414	1.934

The time of decoder we calculated was the average value in each session (collected in different days).

can reach 90%. The decoding results from H18 is the best but the result from V14 is relatively poor. The possible reasons are the poor quality of calcium imaging and unobvious firing of neurons. Also, V14`s success rate of paradigm is lower than others and the increase of negative samples in the experimental data will lead to the imbalance of positive and negative samples, which will also affect the effectiveness of the SVM decoder.

Session 1- 4 was tested and recorded in chronological order. Decodeing result in the first session is always not satisfactory. The decoding effect improved with the increase of training days and reached its best in Session 2/3. During these days, the mice were trained daily to complete the task. As the mice learning experimental paradigm, the neurons in the primary motor cortex always strengthen the mechanisms that encode movement. When the mice performed the task, the firing of neurons in the motor cortex was even more pronounced, which makes a great contribution to our online decoding. This will make contributions to the balance of positive and negative samples and improved decoder results.

In Session 4, we found that the online decode accuracy of mice slightly may reduce. We think this is a usual phenomenon. Long-term single-photon irradiation has a photobleaching effect on the fluorescent protein. With the increase of time, the imaging quality of calcium imaging begins to decline may also be an important reason.

From Table II, we could see that the time cost is so short (average is 2.417 ms) and all the cost time in sessions was less than 3ms. We collected calcium images at a rate of 20 frames per second whose time interval is much greater than the time decoding cost. It can be used to decode online neural signals, which provides the possibility for the closed-loop animal experiments of single-photon calcium.

In addition, the poor decoding effect is also greatly related to the selection of neurons and the extraction of neural signals. Due to popular algorithms for automatic neuron recognition can`t apply to real-time processing, we select neurons artificially. There may be some errors in the neurons location and neurons unrelated to behavior may also be selected. We selected the 10 pixels area to calculate the mean value as the calcium signal emitted by neurons while neurons actually vary in shape and size. Applying mature signal extraction methods, such as neuronal background modeling, can obtain more pure neuronal signals and improve the decoding effect of neurons. So the next step for us is to improve the signal extraction method in the system.

IV. Conclusion

In this paper, we establish a real-time Optical Brain-Computer Interface System that can achieve neural decoding from calcium imaging and present a novel online neural signal decoding framework. OBCIs include many functions: image acquisition, neuron manual selection, feature extraction, SVM model training, and signal decoding. Through some experiments, we proved the feasibility of the real-time decoding framework and achieved high decoding accuracy. It provides the possibility for the subsequent closed-loop experiments of the optical brain-computer interface. It can be said that calcium signal has great potential in future neural decoding.

For future improvements, we`d like to use methods such as Kalman filtering to realize the prediction of the pressure value for subsequent experimental studies. Also, the neuron selection, signal extraction, and decode method can be our target to improve. A more efficient feature extraction method for neuron signal and choosing a better classifier such as the neural network can be applied to improve decoding accuracy.

In this paper, we give some analyses of the decoding results. However, all the results are based on data from only three mice, which is very preliminary. Further research requires more experimental data.

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