AMPLITUDE AND FREQUENCY FEATURE EXTRACTION OF NEURAL ACTIVITY IN MOUSE VENTROLATERAL STRIATUM UNDER DIFFERENT MOTIVATIONAL STATES USING FIBER PHOTOMETRIC SYSTEM

S. Imai [(1)], *Y. Mitsukura* [(2)], *K. Yoshida* [(3)], *I. Tsutsui-Kimura* [(3)], *N. Takata* [(3)] *and K. F. Tanaka* [(3)]

[(1)] Graduate School of Integrated Design Engineering, Keio University, Yokohama, Kanagawa, Japan
[(2)] Faculty of Science and Technology, Keio University, Yokohama, Kanagawa, Japan
[(3)] Department of Neuropsychiatry, Keio University School of Medicine, Shinjuku, Tokyo, Japan
{s.imai, mitsukura, k.yoshida81310261, ikimura, takata.norio, kftanaka}@keio.jp

***Abstract*—In this paper, we focused on the motivation of mice and aimed to extract neural activity features of D2 medium spiny neurons (D2-MSNs) in the ventrolateral striatum of mice under different motivational states. Motivated behavior is defined as the activation of goal-directed behavior, and this enables actions such as ingestion, sleeping and reproduction, which are essential for living. In human society, motivation allows us to participate in society, improving our quality of life. Loss of motivation has been causing problems such as withdrawal from society. If the symptom is heavy, it can even threaten our lives, and treatment is necessary. The mechanisms which lead to loss of motivation are yet to be understood, and an effective treatment does not exist. To solve these problems, we decided to find features in the neural activity which affect motivation, because understanding the mechanism may contribute to the establishment of treatment. In the experiment, the neural activity was recorded using gene-encoded ratio metric calcium ion (Ca^{2+}) indicator and by constructing a fiber photometric system, which enabled recording of neural activity at specific brain region for specific type of neurons. While the recording took place, mice performed food-incentive lever-pressing tasks, which were used to define motivational states. The experimental results show that both the amplitude and frequency components of Ca^{2+} fluctuation have features which are good predictors of motivational states.**

I. INTRODUCTION

The number of people not currently being employed, educated or trained in developed countries is increasing lately. These people are called "NEETs", an abbreviation for 'not in education, employment or training'. In America, 16.9% of young people aged between 16 and 29 are said to be NEETs [1]. Some people even choose to withdraw from social life, seeking extreme degrees of isolation and confinement. This phenomenon does not only apply to America, but also to other countries around the world. In Japan, this issue is called 'hikikomori', where 541,000 people choose to confine themselves in their rooms. These social issues can be traced back to loss of motivation. With severe symptoms, people are deprived of living ordinary lives, and their lives may be threatened. Therefore, treatment is necessary. Psychotherapy is often used as treatment. It is a method for individuals to change their negative thinking patterns and behaviors by identifying unhealthy patterns of thinking the individual tends to fall into. However, this treatment is not always effective since it focuses on just the mental aspect of the symptom, and not the fundamental mechanisms in the brain. Other approaches include pharmacologic approach, which intends to potentiate dopamine release in the central nervous system. Dopamine is a neurotransmitter that is reported to regulate motivation, and increasing dopamine release in the central nervous system by using dopaminergic drugs was able to enhance motivation [2]. However, how dopamine regulates motivation is yet to be known, and this treatment is not effective in all patients. To establish a fundamental treatment to this symptom, it is necessary to understand the mechanisms which cause loss of motivation.

Some researchers sought to understand the neural mechanisms which lead to motivational loss using mice, which were known to have similar brain structures to humans. Preceding research focused on D2 medium spiny neurons (D2-MSNs) in the ventrolateral striatum, and demonstrated that these neurons in this specific brain region control motivated behavior [3]. The experiments consisted of recording mouse behavior in food-incentive lever-pressing tasks while gradually destroying D2-MSNs with diphtheria toxin, and as a result, the correlation between dysfunction and reduced motivational behavior could be recognized. However, this method was not successful in measuring the real-time neural activity taking place during motivational behavior, since the neurons were being destroyed. Recording the real-time neural activity is important, because we can uncover what pattern of neural activity is responsible for controlling motivated behavior. If this pattern in neural activity could be understood, we may be able to efficiently test whether a certain drug is effective in enhancing motivation, or may be able to stimulate neural activity towards that pattern.

In this research, we therefore observed the real-time neural activity of D2-MSNs in freely moving mice performing the lever-pressing tasks. The neural activity was recorded as calcium ion (Ca^{2+}) fluctuations, using fiber photometric system. This system delivered light to the target neurons through an optical cannula implanted in a mouse's brain, and measured intensity of light emitted by fluorescent proteins, which were dependent on Ca^{2+} concentration. Ca^{2+} generate versatile intercellular signals that control key functions in all types of neurons, and they are important indicators of neural activity. The advantage of the fiber photometric system is that it can record neural activity of specific neurons, which in our case are D2-MSNs. Using the above techniques, we analyzed neural

activity in the ventrolateral striatum during motivational behavior.

II. Experiment

A. Fiber Photometric System

We used fiber photometric system to observe neuronal Ca^{2+} fluctuations. Recording of neural activity has been carried out in many ways including the electrophysiological methods, which measure direct electrical signals through electrodes. However, these methods cannot measure neural activity of specific neurons, and thus fiber photometric system was used. Ca^{2+} plays a vital role in generating versatile intracellular signals, and controlling diverse variety of cellular processes including gene transcription, muscle contraction and cell proliferation. In the neurons, high Ca^{2+} concentration is associated with high neural activity. When electrical or chemical signal reaches a cell, the cell membrane potential changes, which opens Ca^{2+} channel, allowing the ions to enter the cell and increase ion concentration.

The system was designed by Olympus Engineering Co. Ltd. Light with wavelength of 435 nm was emitted from LED and was delivered to the target neurons through optical fiber cannula implanted into the mouse's brain. All mice used in the experiment were transgenic mice expressing Yellow Cameleon-Nano50 (YC-Nano50) in D2-MSNs. YC-Nano50 is an ultrasensitive calcium indicator, composed of yellow fluorescent protein (YFP) and cyan fluorescent protein (CFP) [4]. Neuronal calcium fluctuations cause structural changes in these proteins and CFP and YFP emit fluorescent signals accordingly, which travel back through the optical fiber. Then, they are separated by dichroic mirrors and are detected by photomultiplier tubes (PMTs). Finally, YC ratio (a ratio of yellow to cyan fluorescence intensity) is calculated, which is a measure of Ca^{2+} concentration in cells. The recording was carried out with sampling frequency of 1000 Hz. Frequency of neuronal activity in mice brain are reported to be as high as 200 Hz, and 1000 Hz is considered an adequate sampling frequency to detect features in the activity [5]. The research group which developed YC-Nano50 also used this sampling frequency to record Ca^{2+} fluctuation.

B. Fixed Ratio Operant Task

We tested the mice with lever-pressing operant tasks to define motivational states in free-moving mice (Figure 1). The trainings and tests were carried out in an operant chamber. Inside the chamber, an electrically controlled lever was present, which triggered delivery of food reward. The task consisted of trials, which started with presentation of the lever (Figure 2). In each trial, a mouse had to press the lever 10 times to earn one food pellet. Once the food pellet was delivered, the lever retracted and the mouse had 30 seconds to consume the pellet and prepare for the next trial. The session lasted for 60 minutes or until the mouse received 100 food rewards. Start and end of a trial, as well as the times of lever press were recorded automatically with the sampling frequency of 1000 Hz.

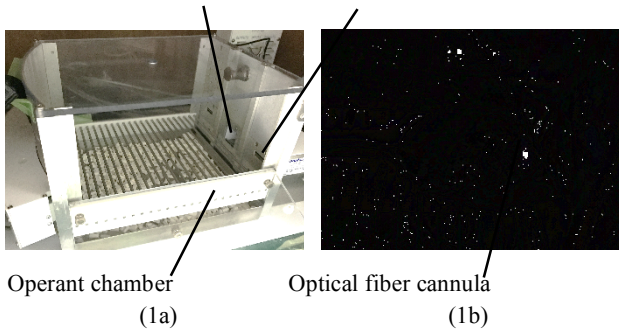

Figure 1. Experimental landscape. Operant chamber (1a) and mouse under Ca^{2+} fluctuation recording (1b). Optical fiber cannula is implanted in mouse's brain, and light is being delivered to the target neurons D2-MSNs.

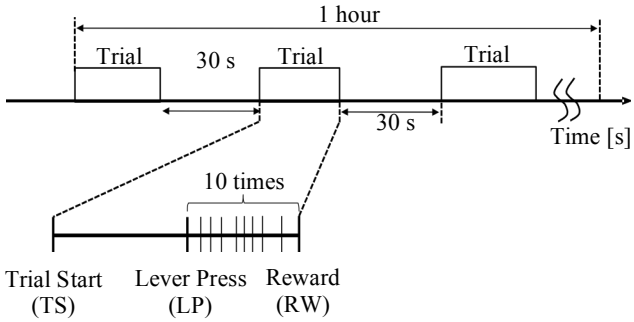

Figure 2. Operant task procedure. Task consists of trials, which ends once lever is pressed 10 times.

A mouse was trained to press the lever by giving it one food reward after each lever press. Once it was able to obtain 50 rewards within 60 minutes, the training progressed to the next phase in which two lever presses were required to earn one food reward. Once this phase was accomplished, the number of lever presses required moved on to 3, and then to 10. The training ended once all these tasks were accomplished.

The task to measure motivational states and Ca^{2+} fluctuation was carried out with 7 transgenic D2-YC mice, 22 times in total. All mice had finished training and had received the surgery to implant an optical fiber cannula into the ventrolateral striatum for fiber photometric recording.

III. Methods

In the experiment, Ca^{2+} fluctuation measured as YC ratio in ventrolateral striatum was recorded while mice performed lever-pressing tasks, and time-series data on Ca^{2+} fluctuation and the lever pressing times were obtained. These two time-series data were used to extract neural activity features under

Table 1. Correlation coefficients between amplitude features and TS-LP, LP-RW time lengths

	TS-LP	LP-RW	Mean TS-LP	Mean LP-RW	Gradient TS-LP	Gradient LP-RW	Value TS	Value LP	Peak value at LP-RW
TS-LP		0.157	-0.071	-0.048	-0.057	-0.013	0.009	-0.041	-0.097
LP-RW	0.157		-0.129	-0.079	-0.045	0.230	-0.088	-0.142	0.082

Table 2. Significant differences of seven amplitude features between two motivational states

Definition	TS-LP			LP-RW		
Motivation	*High*	*Low*	*Significant difference*	*High*	*Low*	*Significant difference*
Mean TS-LP	0.2929	0.115	Yes	0.4353	0.1163	Yes
Mean LP-RW	0.259	0.1192	Yes	0.1704	0.0831	No
Gradient TS-LP	1.42E-04	-1.08E-05	Yes	7.71E-05	6.02E-05	No
Gradient LP-RW	-7.40E-05	-1.23E-04	Yes	-2.86E-04	-1.89E-05	Yes
Value TS	0.2728	0.3367	No	0.394	-0.013	Yes
Value LP	0.3264	0.2469	No	0.4372	0.1081	Yes
Peak value LP-RW	2.5997	1.93	Yes	2.1808	2.6822	Yes

different motivational states. We intended to analyze Ca^{2+} fluctuation in terms of both amplitude and frequency.

A. Preprocessing

As a preprocessing, we adopt the following steps.

Step 1 : Smooth the Ca^{2+} fluctuation signal by using 100-point moving-average method.

Step 2 : Detrend by using cubic spline interpolation to remove the effect of fluorescence photobleaching from the signal.

Step 3: Apply band-pass Butterworth filter. For the amplitude analysis, band width was set to 0.01-1 Hz, and for the frequency analysis, to 1-200 Hz.

Step 4: Calculate z-score so that amplitudes of signals with different scales could be compared.

Z-score is defined as follows:

$$\mathrm{z} = \frac{x - \mu}{\sigma} \qquad (1)$$

Where x is the time-series data, μ is the mean and σ is the standard deviation.

B. Definition of Motivational States

From the time-series data of lever-pressing, motivational states were defined. We hypothesized that there were two types of motivations; to start goal-directed behavior and to continue such a behavior. We used the below parameters to define motivational states, which were used in the preceding research.

- Time length from trial start (TS) to the first lever press (LP), expressed as TS-LP, to define motivational states to start goal-directed behavior
- Time length from the first lever press (LP) to when mouse finishes the tenth lever press and earns reward (RW), expressed as LP-RW, to define motivational states to continue goal-directed behavior

Short and long time lengths can be interpreted as high and low motivational states respectively.

C. Amplitude Analysis

We first focused on slow Ca^{2+} fluctuations because these slow fluctuations in amplitudes may have correlations with motivational states.

We extracted 7 features from Ca^{2+} fluctuations. That is to say, for each trial composing the task, we calculated the mean amplitude for TS-LP and LP-RW, amplitudes at TS and LP, the gradient of Ca^{2+} fluctuation for TS-LP and LP-RW, and the peak value of Ca^{2+} fluctuation after the mouse earned reward. We applied correlation analysis to each feature. If correlation coefficients of those features and either TS-LP or LP-RW recorded high values, we may say those features correlate to motivational states.

Correlation coefficient of two variables is defined as follows:

$$\rho(A,B) = \frac{1}{N-1}\sum_{i=1}^{N}\left(\frac{A_i - \mu_A}{\sigma_A}\right)\left(\frac{B_i - \mu_B}{\sigma_B}\right) \qquad (2)$$

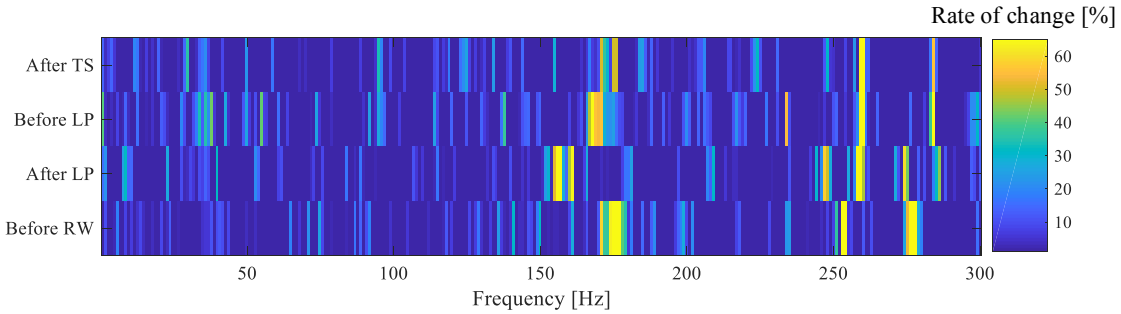

Figure 3. Color map shows rate of change in frequency components for two motivational states defined by TS-LP time lengths. The colors show the rate of change at one second period after trial start (After TS), one second period before first lever press (Before LP), one second period after first lever press (After LP), and one second period before end of trial (Before RW).

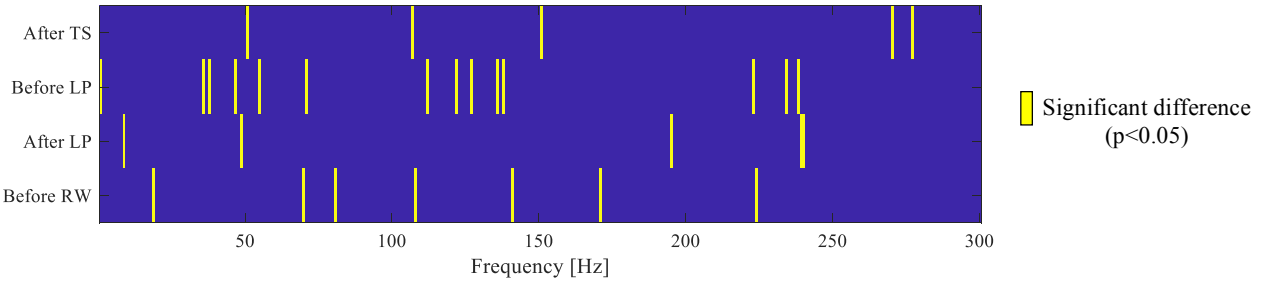

Figure 4. Color map shows the frequencies and the time periods at which significant differences ($p<0.05$) are recorded, for two motivational states defined by TS-LP time lengths. The yellow color represents frequency components with significant differences at one second period after trial start (After TS), one second period before first lever press (Before LP), one second period after first lever press (After LP), and one second period before end of trial (Before RW).

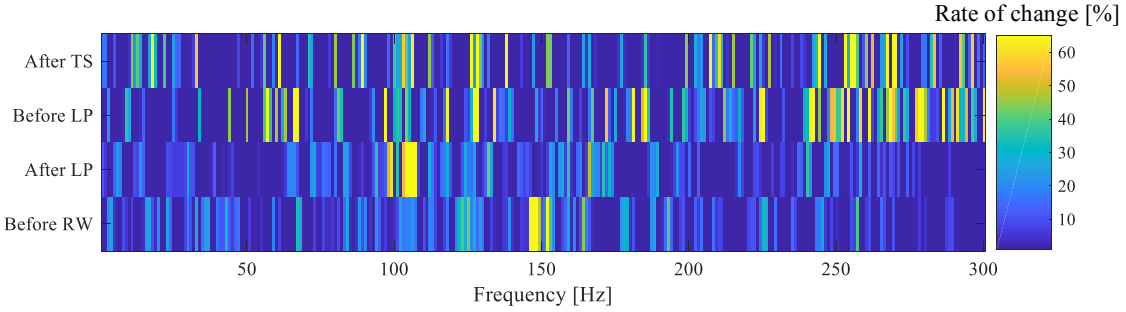

Figure 5. Color map shows rate of change in frequency components for two motivational states defined by LP-RW time lengths. The colors show the rate of change at one second period after trial start (After TS), one second period before first lever press (Before LP), one second period after first lever press (After LP), and one second period before end of trial (Before RW).

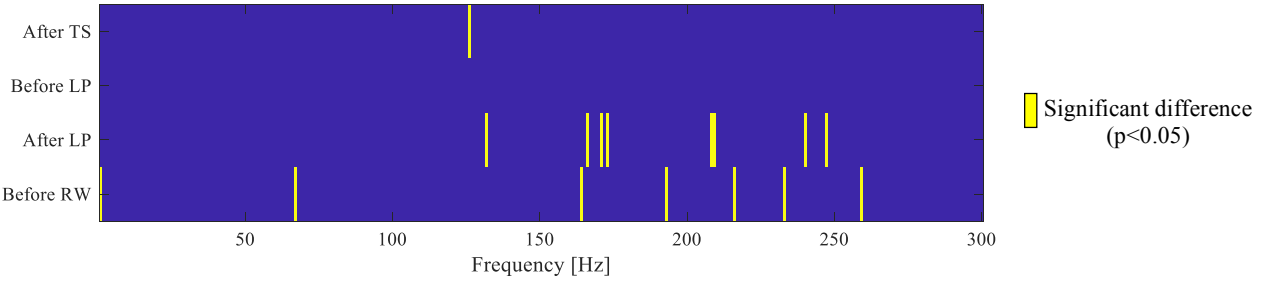

Figure 6. Color map shows the frequencies and the time periods at which significant differences ($p<0.05$) are recorded, for two motivational states defined by LP-RW time lengths. The yellow color represents frequency components with significant differences at one second period after trial start (After TS), one second period before first lever press (Before LP), one second period after first lever press (After LP), and one second period before end of trial (Before RW).

Where A and B are two variables, μ_A and μ_B are the means, and σ_A, σ_B are the standard deviations of A and B . N is the number of data-points used.

Considering that correlation analysis cannot necessarily justify that differences in features are not coincidental, we also carried out significance tests of these features under two motivational states. The two motivational states were defined by sorting TS-LP and LP-RW time lengths. The top 10% of trials with short time lengths were labeled as motivated states, and the bottom 10% were labeled as less motivated states.

D. Frequency Analysis

We also focused on the fact that frequency-controlled Ca^{2+} oscillation has a role in transmitting biological information [6]. The study of amplitudes alone cannot extract these features, so we carried out frequency analysis with Fast Fourier Transform (FFT). Two motivational states were defined by sorting TS-LP and LP-RW time lengths. The top 10% of trials with short time lengths were selected as motivated states, and the bottom 10% were selected as less motivated states. For both the TS-LP and LP-RW defined motivational states, FFT was performed on data just after TS, just before and after LP, and just before RW. Data length was set to 1000, and 1000-point Hamming window was used. This window size was used, because we first intended to find dominant features around each time events (TS, LP, RW), and considered that looking at the frequency components on a large time scale of seconds was necessary. Fourier spectra ranged 1-300 Hz were extracted and transformed to power spectra.

IV. Results and Discussions

Table 1 shows the correlation coefficients of features obtained from Ca^{2+} fluctuations, TS-LP and LP-RW. Considering that coefficients -1 and 1 suggest strong negative and strong positive relationship, it can be said that all of the coefficients are very small, and the features do not seem to have correlations with motivational states. The highest correlation coefficient 0.23 was recorded between LP-RW and gradient LP-RW. It can be said that higher motivational state to continue behavior had the largest correlation with smaller rate of decrease in Ca^{2+} concentration.

Table 2 shows the values features take under two defined motivational states, and whether there were any significant differences. With TS-LP definition of motivational states, significant differences were present for the mean amplitude and the gradient of Ca^{2+} fluctuation of TS-LP and LP-RW, and the peak value after the mouse earned reward. With LP-RW definition of the motivational state, significant differences were present for the mean amplitude of TS-LP, the gradient of LP-RW, the amplitudes at TS and LP, and the peak value after the mouse earned reward. For both definitions of motivational states, higher amplitudes of Ca^{2+} fluctuation could be associated with higher motivational states. For the motivation to start behavior, the rate of increase in Ca^{2+} concentration between start of the trial to the first lever press affected motivational state. For the motivation to continue behavior, the rate of decrease between first lever press to the 10^{th} lever press was responsible.

Figures 3-6 show the results of frequency analysis. Figure 3 and 4 show rates of spectra changes in frequency components and the frequencies at which significant differences were confirmed between two motivational states. The states were defined by TS-LP time lengths, meaning motivation to start a goal-directed behavior was considered. Figure 5 and 6 are color maps showing the results obtained in the same way, except that motivational states were defined by LP-RW time lengths, meaning motivation to continue goal-directed behavior was considered.

Figure 3 shows high rate of change in frequency components exceeding 60 % were recorded at high frequencies over 150 Hz. The largest changes were likely to be concentrated in the bands of 150-175 Hz and 250-300 Hz. The frequency components showing significant differences between the two states were distributed over wide range of frequencies. We can also notice that there were more significant differences between frequency components of two motivational states just before the first lever press compared to just after the trial start.

For the motivational states defined by LP-RW, the highest rate of change was likely to be concentrated in the frequency bands of 100-110 Hz for one second after LP, and 140-150 Hz for one second before RW. For TS-LP, patterns were not recognized, suggesting it was likely that the frequency components affecting motivation to continue behavior appeared after the lever was pressed. This is also supported by the fact that there was only one frequency component which showed significant difference in the trial start to the first lever press period, compared to 10 components in the period of the first lever press to the end of the trial.

As suggested above, we were able to extract features related to amplitude and frequency of Ca^{2+} fluctuations in D2-MSNs of the ventrolateral striatum under different motivational states.

V. Conclusions

The purpose of this study was to extract neural activity features of D2-MSNs in the ventrolateral striatum of mice under different motivational states. In order to achieve this objective, Ca^{2+} concentration of the target neurons was recorded using fiber photometric system while mice performed lever pressing tasks, and the results were analyzed in terms of both amplitude and frequency.

Our contributions from the experimental results are as follows:

- It was confirmed that higher amplitudes of Ca^{2+} fluctuation in D2-MSNs were associated with higher motivational states, both for the motivation to start behavior and to continue behavior.

- It was confirmed that the rate of increase and decrease in Ca^{2+} concentration seemed to affect the motivation to start behavior, and the motivation to continue behavior.

- It was confirmed that high frequency components of Ca^{2+} fluctuation over 150 Hz were likely to have effects on the motivation to start behavior.

ACKNOWLEDGMENT

This research was supported by MEXT KAKENHI (Scientific Research (S)) Grant Number YYK7S01.

REFERENCES

[1] J. Bynner, and S. Parsons, "Social Exclusion and the Transition from School to Work: The Case of Young People Not in Education, Employment, or Training (NEET)," Journal of Vocational Behavior, vol. 60, no. 2, pp. 289-309, 2002.

[2] T. T. –J. Chong, and M. Husain, "Chapter 17 – The role of dopamine in the pathophysiology and treatment of apathy," Progress in Brain Research, vol. 229, pp. 389-426, 2016.

[3] Iku Tsutsui-Kimura, et al., "Dysfunction of ventrolateral striatal dopamine receptor type 2-expressing medium spiny neurons impairs instrumental motivation," Nature Communications, vol. 8, 2017.

[4] K. Horikawa, et al., "Spontaneous Network Activity Visualized by Ultrasensitive Ca2+ Indicators, Yellow Cameleon-Nano," Nature Methods, vol. 7, no. 9, pp. 729-732, 2010.

[5] D. L. Buhl, et al., "Selective Impairment of Hippocampal Gamma Oscillations in Connexin-36 Knock-Out Mouse In Vivo", The Journal of Neuroscience, vol. 23, no. 3, pp. 1013-1018, 2003.

[6] E. Smedler, and P. Uhlén, "Frequency decoding of calcium oscillations," Biochimica et Biophysica Acta (BBA) – General Subjects, vol. 1840, no. 3, pp. 964-969, 2014.