Tracking the Response of Dopaminergic Pathways in the Nucleus Accumbens

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Introduction

Quintessential bodily functions one performs throughout their lifetime lead to the constant inhibition of dopaminergic pathways through the nucleus accumbens. Located in the basal forebrain, there is a nucleus accumbens in each hemisphere of the brain. Only recently coming into importance when associated with the behavioral study of physical actions under the influence of drugs, the nucleus accumbens plays a vital role in mediating reward,[1] as dopamine signaling is vital in storing information in relation to environmental stimuli[5] for the body to associate certain experiences with the environment the experience was contracted in. As the reward circuit in the nucleus accumbens is not fully understood, new technologies such as fiber photometry have become capable of tracking the release of dopamine and how inhibition of dopaminergic pathways results in a specific bodily response in accordance to the surrounding environment[5].

Fiber photometry is a technique that has only recently been adopted in the area of behavioral studies. As the technology has become more accessible, there are new and important ways in which utilizing its functionality helps scientists observe minute tasks the brain performs which were previously unable to be seen with the help of electron microscopes. Fiber photometry is a method where optical fibers are implanted in the desired brain region. In these regions, calcium indicators deliver excitation light which then collects overall fluorescence tracked by calcium activity during the light excitation[3, 7]. Contrary to previous methods such as fluorescence micro endoscopy where the activity of individual neurons were captured within the field of view of the fiber optic, fiber photometry creates a larger model of the overall fluorescence of neurons expressing a genetically encoded calcium indicator, which in our case, was GCaMP, a calcium indicator created from a fusion of green fluorescent protein, calmodulin, and M13, which is a peptide sequence from myosin light chain kinase.[1, 5, 2]

The GCaMP protein has proven to be effective in the means of gathering information from masses of neurons, providing promising results from previous research. The first successful utilization of CaMP protein was in a study published by Newton *et al.* in 2002. Limitations of the technology of this time confined this research to study the broader area of neuroplasticity, researching cAMP response element-binding protein (CREB), a critical integrator of neuroplasticity, developing inducible transgenic lines of mice that express CREB, or a dominant-negative mutant of CREB in the forebrain region, particular in the nucleus accumbens, an area in the brain responsible for the mediation of reward observed through environmental stimuli. The study concluded that the nucleus accumbens CREB-dynorphin influence in their developed model suggest that the signaling cascade observed through the use of the cAMP protein demonstrate possible links to symptoms of depression. As this study was confined by the lack of advanced fiber photometrics, a thorough analysis of dopaminergic pathways in the nucleus accumbens was not performed. Dopaminergic pathways in the nucleus accumbens are the most integral function of the nucleus accumbens. Tracking the release of dopamine from dopaminergic innervations is ultimately what will help link symptoms of depression to the nucleus accumbens, which would occur by comparing the release of dopamine in accordance to an environmental stimulus, comparing the distance of the innervation to the extremity of the stimulus.

Furthering the research completed in relation to the linking of the nucleus accumbens to dopaminergic innervation is a study completed by Nicola *et al.* in 2014. For this study, a newly introduced neurophotometric apparatus was utilized. This apparatus allows for quicker brain imaging and recordings, collecting masses of neurons in targeted areas cancelling white noise while presenting the data collected in a graph format. In this study, the neurophotometric

apparatus was utilized to inactivate neurons in the dorsomedial striatum to further isolate the desired neurons. Neurons in two structures of the dorsomedial striatum send glutaminergic afferents to the nucleus accumbens are excited by reward-predictive cues from environmental stimuli. Reversible inactivation of either of the neuronal structures or of the ventral tegmental area reduces the magnitude of cue-invoked excitation in the nucleus accumbens, cancelling out white noise. Rats were trained to perform behavioral experiments in which two auditory cues were presented eliciting the response of a lever pull by the rats. Correspondence of the correct sound with the lever pull introduced a sucrose reward. The tones consisted of a siren tone (which cycled in frequency from 4 to 8 kHz over 400 ms) and an intermittent tone (6 kHz tone on for 40 ms, off for 50 ms).

Consistent with previous studies, it was found that bilateral infusions of either antagonist which inactivated either neuron in the two structures previously mentioned or the ventral tegmental area into the nucleus accumbens significant reduced the proportion of DS neurons (neurons that respond differentially to the direction of a stimulus), therefore cancelling excess information presenting solely the pathway of the dopaminergic innervations presented when the rat was given a sucrose reward after successfully completing the behavioral task, and when the rat was given no reward if the task was incorrectly completed[2, 5, 6].

Statement of Purpose:

There has been no known way to emulate the present data which shows the results of behavioral experiments through brain activity in the nucleus accumbens. The purpose of our study was to create and run a behavioral experiment and write a script in the language R in order to translate data from fiber photometry experiments into the program. Studying the release of

dopamine through dopaminergic innervations elicited from environmental stimuli will help scientists further understand the link between the functionality of the nucleus accumbens and underlying symptoms of depression and addiction. The acquisition board utilized operates at 1000Hz, but the photometry camera operates at 40Hz. The written program will help translate the compilations of photos taken by the photometry camera and have it presented in a form understandable to the program, therefore making it possible for us to view precisely what processes of understanding the rats tested were going through while undergoing the behavioral experiment, as this can all be tracked through the path of dopaminergic innervation.

Methods and Materials:

Fiber Optic Implant Process

Rats taken from Albert Einstein College of Medicine's secure breeding facility in which rats are grown. Each rat given tag in order to identify brain after perfusion process later to come. Implant apparatus hooks two ear bars under the jaw of the rat through the ear in order to stabilize head during surgery. Stereotaxic frame allows for precise measurement of skull in order to identify area of interest. Frame placed into position in accordance to both nucleus accumbens along with ventral tegmental area. Skull of rat is exposed, and based on measurements, drill is passed through skull to bypass bone in order to gain access to brain. The three implants are then measured lengthwise so as not to pass or come short of nucleus accumbens and ventral tegmental area. Measurement for implant placement varied for each rat, as skull structure differed. Throughout process of implantation, exposed skull area is covered with hydrogen peroxide and countered with halothane to prevent infection. Implants then left in the skull and surrounding skull-area covered with dental cement to prevent possible infection.

Behavioral Testing

Code written on MED-PC in order to create behavioral tasks to be completed by rats. Classes which were modified were taken from the MED-PC database, which include ON, OFF, #R, ADD, STOPABORT, and KILL. #R allowed to hose a variety of interactive behavioral tasks which were to be utilized by the behavioral chamber itself. Code was written to flash two separate lights (colored blue and yellow) along with one lever. Rats were trained to pull lever when blue light was turned on. If task was successfully completed, a sucrose reward was given. Fiber optics were then placed into the implants on the skull, and behavioral testing proceeded. Occurrence of both lights were randomized, for each trial, however the same response was expected (lever pull) and the same reward was given throughout all trials (sucrose)

Behavioral Study Data Collection

Data received throughout behavioral experiments stored on a computer. Data transferred to a separate location in order to unpack binary format. RStudio was utilized for the unpacking and analysis of data, and all commands and classes derived through the R database. The Str() command gives data's structure and class in a format readable to the computer. FPMPCdata[[(Rat name)]]$rat command shows a selected rat's name as given by us through the software before behavioral testing had begun for the first element of FPMPC Data. FPMPCdata[[2]]$frameIdx returns index of every time the fiber photometric camera took a frame as detected by the acquisition board. The acquisition board currently operates at 1000Hz, however the fiber photometric camera utilized operates at 40 Hz, so every 25 milliseconds a frame is captured and is saved into the FPframe (a class able to store quantifiable data). Acquisition port's ADC port was connected to the photometry system and an event was detected every time a frame was captured, which allows us to link the separate data streams (1000Hz and

40Hz) together. For every sample that was taken from the acquisition board, the voltage was recorded and saved at the connected ADC port. Along with this, every time the camera captured a frame, the ADC voltage dropped in order to accommodate the lower refresh rate. The script coded locates and separates the voltage drops, then saves them into a vector named frameIdx. In our program, frameIdx has n elements within it where n is equal to the number of rows in FPframe. Therefore, every element in frameIDX from 1 to n corresponds to a row of FPframe. This data is taken in separate sections. In this case, the sections were made so that the camera took its first image when the acquisition board took its 25^{th}. Essentially, frameIDX can be viewed as a function where for every x from 1 through n taken as an input, f(x) is a position in the acquisition board vector between 1 and 25n (the boundary for each section taken). F(x) was then used to select different events from acquisition board and was compared to the photometry data from the camera at row 'x' to understand what the rat was doing when an individual frame was taken. F(y) would essentially be used to find the corresponding row of TTLframe (row of 25 frames taken by photometry camera) to see if the rat's paw entered the receptacle after being presented with the correct light, and whether or not the rat pressed the lever, therefore successfully completing the behavioral task.

Results:

We first tested for calcium signaling in the left and right hemispheres of the nucleus accumbens during our behavioral experiment. Green and red represent left and right hemispheres respectively as collected by the acquisition board.

Dopaminergic Activity Eliciting Fluorescence Detected by Software:

Figure 1

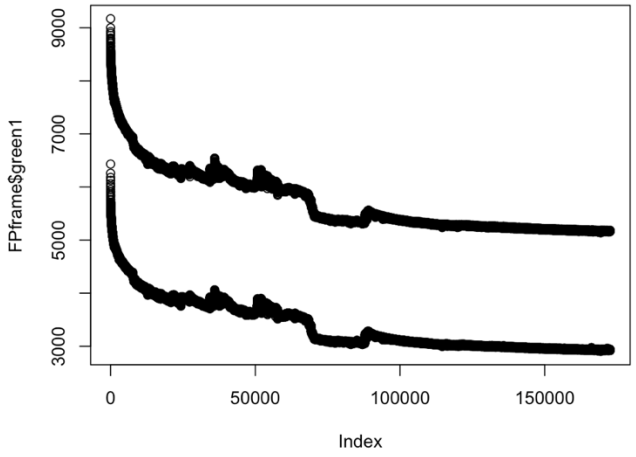

Figure 2

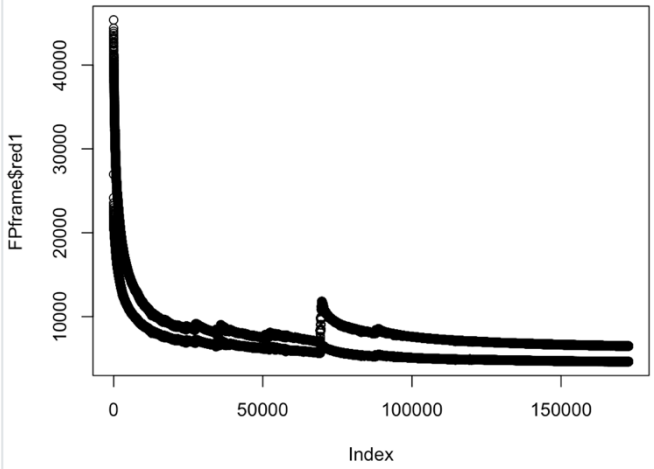

Fig. 1. Initially spikes are seen indicating dopaminergic activity – but not significant enough to conclude presence of calcium in nucleus accumbens.

Fig. 2. Steep decline for both fiber optic lines indicate that the fiber optics were loose at start of behavioral experiment. Spike in violet at approximately 60000 too large to indicate legitimate calcium detection, so z-scoring program was necessary to conclude finding.

Figure 3

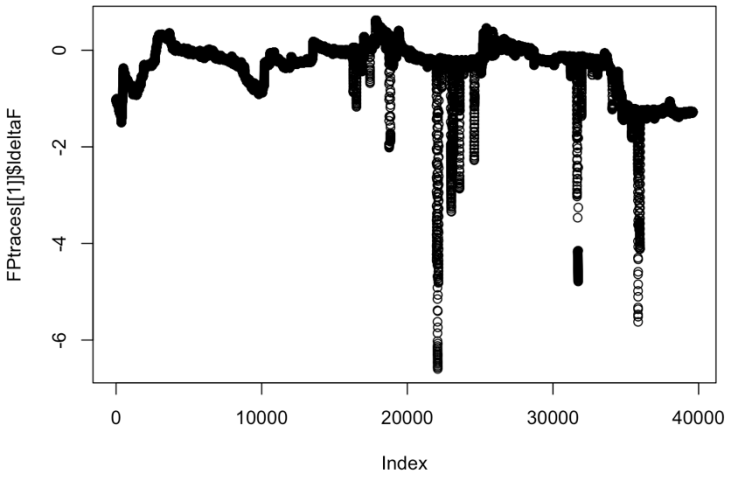

Fig. 3. Z-Scoring software written shows many outliers in the left hemisphere which were smoothed over with my program. Spikes above 0 indicate fluorescence of GCaMP implanted into nucleus accumbens indicating the presence of calcium – affirming our hypothesis.

Figure 4

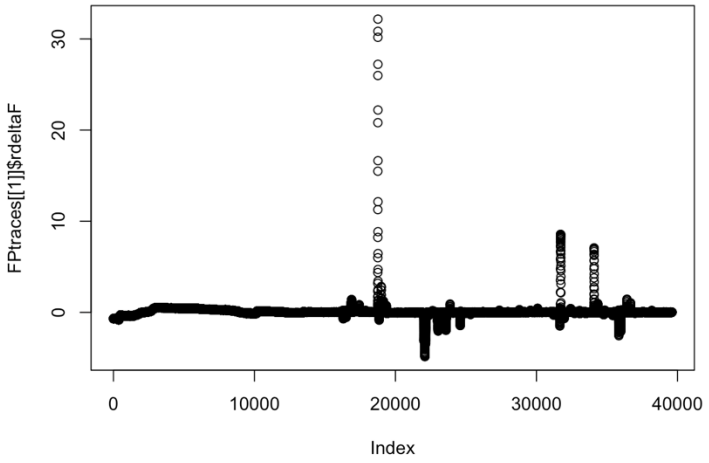

Fig. 4. Z-Scoring software collected data for right hemisphere of rat one's accumbens, presenting outliers obtained from auxiliary brain activity as well as spikes at approximately 19000 and 37000 indicating the presence of calcium in the accumbens.

Figure 5

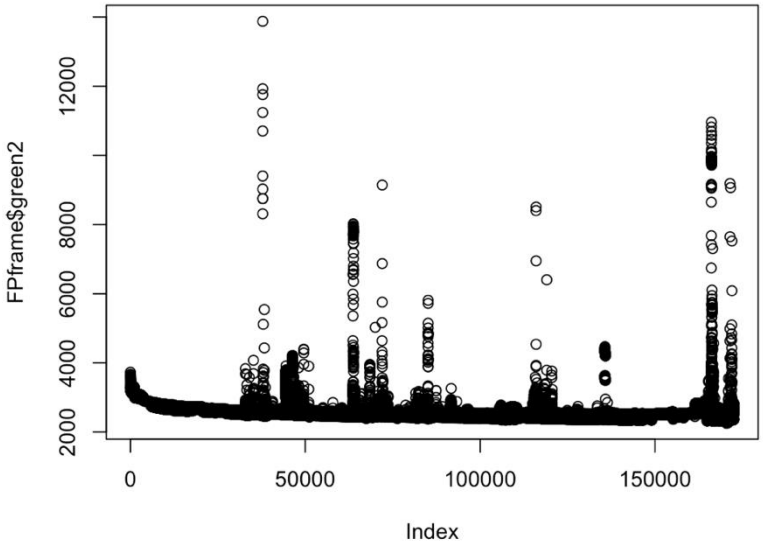

Figure 6

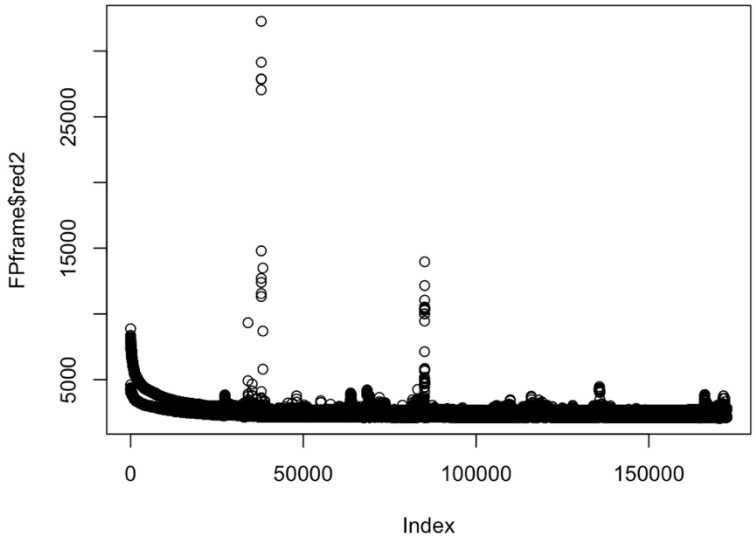

Fig. 5. Problem with acquisition board led excess shots to be taken by camera, thus outliers (opened points) were present and terminated from data through z-scoring program. Spikes present at hypothesized windows, thus showing the potentiality of calcium presence in the left hemisphere.

Fig. 6. Similar to Fig. 5., my program detected outliers and signaled them as open points, while still maintaining a solid frame of data for both the blue and the violet fiber optics. Promising spikes at approximately 70000 and 80000 were thought to have shown the presence of calcium, but z-scoring was required to cancel out white noise and to get more precise data points.

Figure 7

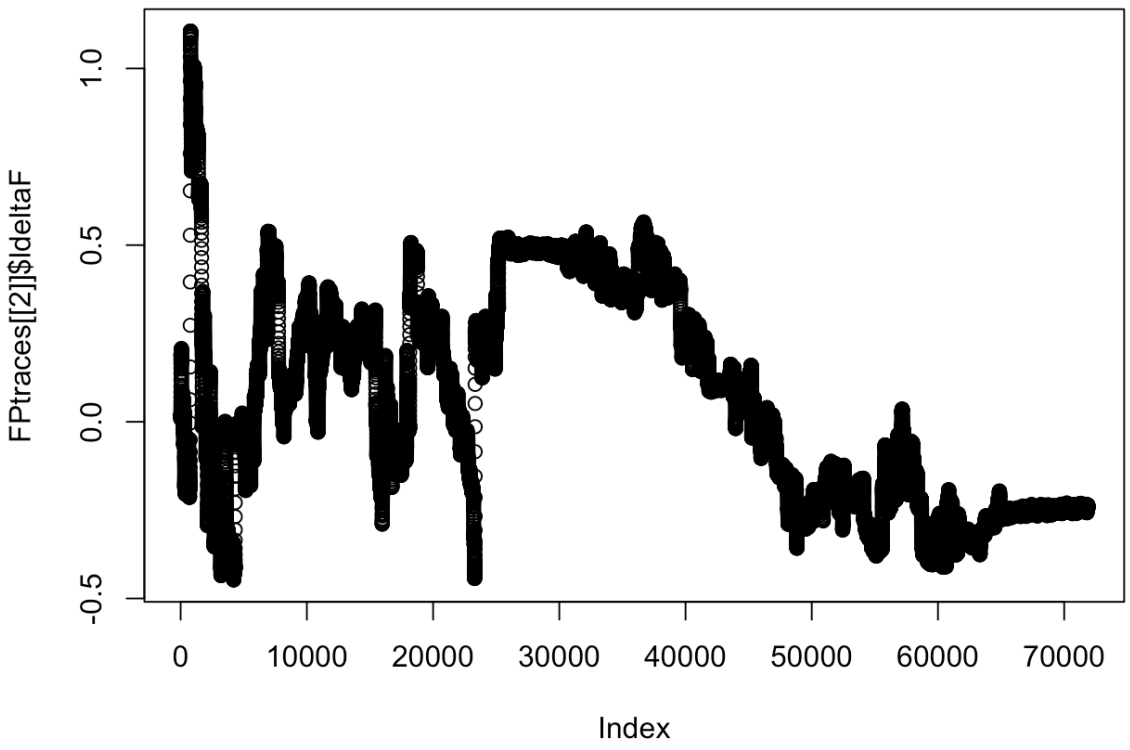

Fig. 7. shows no noticeable signs of calcium present in the nucleus accumbens. The spike at around zero was simply white noise that my program detected and kept as this was after the start of the session. All other points seem to be under 1.0 even after the z-scoring and smoothening by my program.

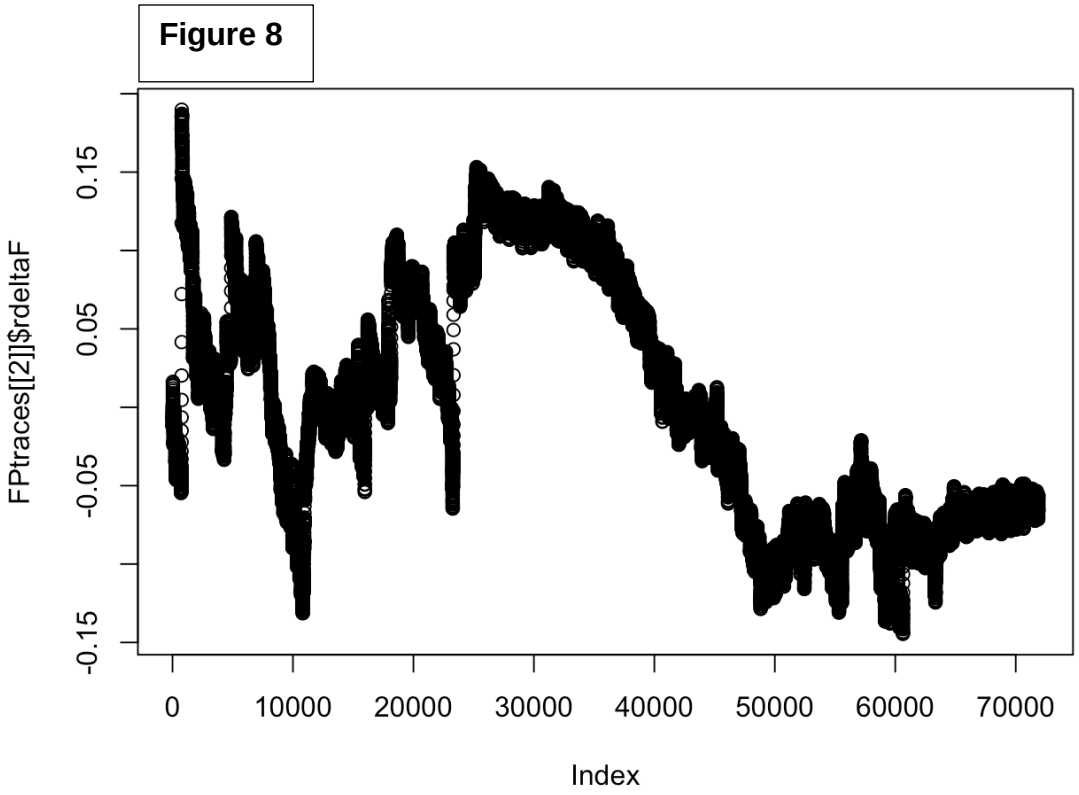

Fig. 8. More promising results were determined in the right hemisphere of the second rat tested, as a spike is seen at around 28000. This spike shows the presence of calcium in its left hemisphere, present during the reward stimulus.

After seeing these results, we were confident in our hypothesis. Initially when data was first collected, there was no obvious spike in dopaminergic activity in the nucleus accumbens when the rat was supposedly given a sucrose reward after completing a behavioral experiment. Obtaining data from both hemispheres allowed us to superimpose the two data sets, and our program smoothened out any remaining white noise present in the data from auxiliary brain activity. The final set of graphs deduced from both sessions from our control and test subjects showed spikes in brain activity specifically in the nucleus accumbens and the creation of

memory stores in the brain indicating that the drug had indeed rendered psychological functions useless during the testing. The creation of these memory stores in the Hippocampus of the brain further supports our hypothesis that there are calcium channels present in both hemispheres of the nucleus accumbens, and that targeting these areas to combat underlying symptoms of depression and anxiety will effectively eliminate psychological damage prone to rise from the repeated creation of the same memory store during the intake of a drug. Figure 2 showed that the fiber optic had become loose during the experiment and showed too large of a spike in calcium activity, so results were inconclusive for that model. Figure 1 showed more promising results, so z-scoring software was utilized to z-score data from both hemispheres of the accumbens, and the results were seen on Figures 3 and 4, both showed calcium spikes above 1 which confirms our hypothesis that the calcium cycle plays an important role during the intake of drugs and the presence of this cycle will elicit the impairment of bodily functions when intoxicated. To affirm results, the same test and data collection resulted in similar data. In Figure 5, the outliers were detected by my program and were signaled by open circles. Spikes, however, were presented at our hypothesized window of 70000 to 80000 in both Figure 4 and Figure 5. After running figures 4 and 5 through my z-scoring and smoothening program, we found in Figure 7 an unlikely spike at the beginning of the behavioral experiment which has been deemed to be white noise, while there is no significant spike in the left hemisphere of the second subject. When looking at Figure 8, however, it is seen that there were spikes present at around 28000, which was not within our hypothesized window, but the presence of calcium during the experiment is supported as the behavioral experiment had begun for around 10000 seconds, which is well within the point of impairment. All of this data supports our hypothesis that the calcium cycle plays a large role when the body is exposed to drugs and leads to eventual impairment. Targeting the calcium cycle

which elicits dopaminergic activity has proven to be a possible first step towards preventing physical or mental impairment bound to happen during the intake of a drug or over a longer period of time.

Discussion and Conclusion:

Fiber Photometry with neurophotometrics holds much potential which would be utilized in the clinical world in relation to detecting early stages of addiction and depression, suppressing the issues before they begin to negatively affect a patient's life.

The protein we used to locate calcium in the nucleus accumbens was GCaMP. Blue light through the fiber optic was calcium dependent, such as dLight and GCaMP, and violet light was non-calcium dependent. Through collecting data for the blue and violet fiber optics, we were able to find a connection between firing rates of dopamine through dopaminergic pathways in the nucleus accumbens and the reaction to a reward given after the rats were presented with a stimulus.

Currently, there is no known way to prevent the firing of dopamine in the nucleus accumbens when faced with a rewarding stimulus, such as doing a drug[5], but the presence of calcium in the nucleus accumbens makes it much simpler to understand the underlying issue of what leads to intoxication and eventual impairment as a result, which will aid scientists in locating target areas in the study of reward consumption and prevention of depression, anxiety, and other illnesses which are prone to rise through repeated intoxication.

Our data supports the hypothesis that there are calcium channels located in both hemispheres of the nucleus accumbens through which dopamine travels to elicit the experiences one may feel while intoxicated. We already knew that the nucleus accumbens was responsible

for the initial reaction during consumption of a drug, but with our data we now know the true origin of the initial spike in dopaminergic activity which ultimately renders the recipient of drug impaired. With this knowledge, we can work on ways to reverse the long-lasting effects drug intake can have on the recipient, such as depression or anxiety.

Our experiment has made it very clear as to where the origin of this initial spike is when a drug is taken. Now that the presence of calcium channels in the nucleus accumbens along with the path dopaminergic neurons take when presented with a rewarding stimulus is well-established, scientists may now have a better point to start at when targeting these areas to combat the long-term effects drugs can have on a person.

Our results answered an essential question but brings up many more with potentiality to be answered. Through the discovery that dopaminergic neurons are fired from the nucleus accumbens, the final destination of these neurons are unknown. Typically, neurons are designated to go to specific areas of the body based on their function, but dopaminergic neurons are well-known to affect the entire body, which is how someone is determined as impaired. However, now that the starting point of this initial firing is known, the path it takes to spread to the rest of the body rendering many bodily functions useless is still an open area for study.

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