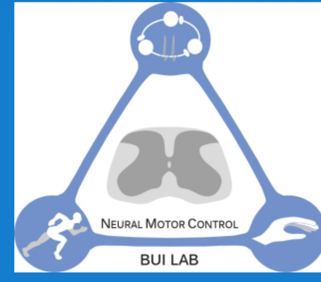
Study of Neural Activity in Zebrafish Larvae Using Calcium Imaging



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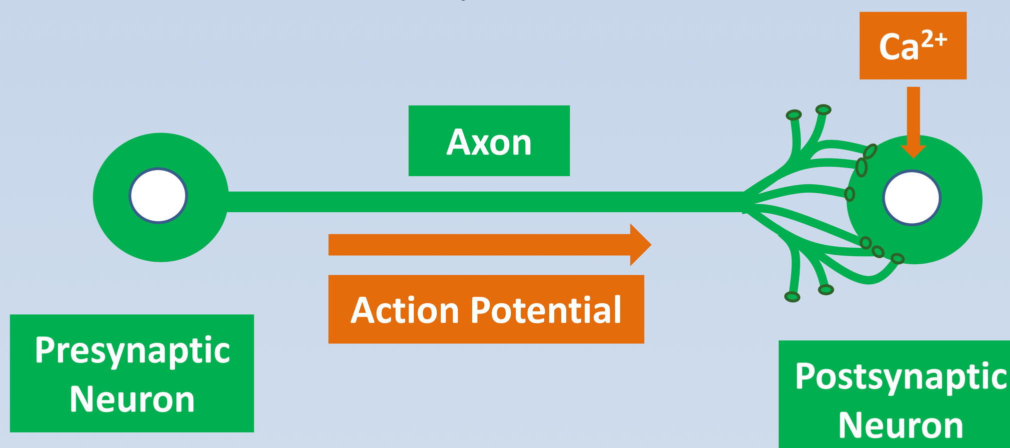
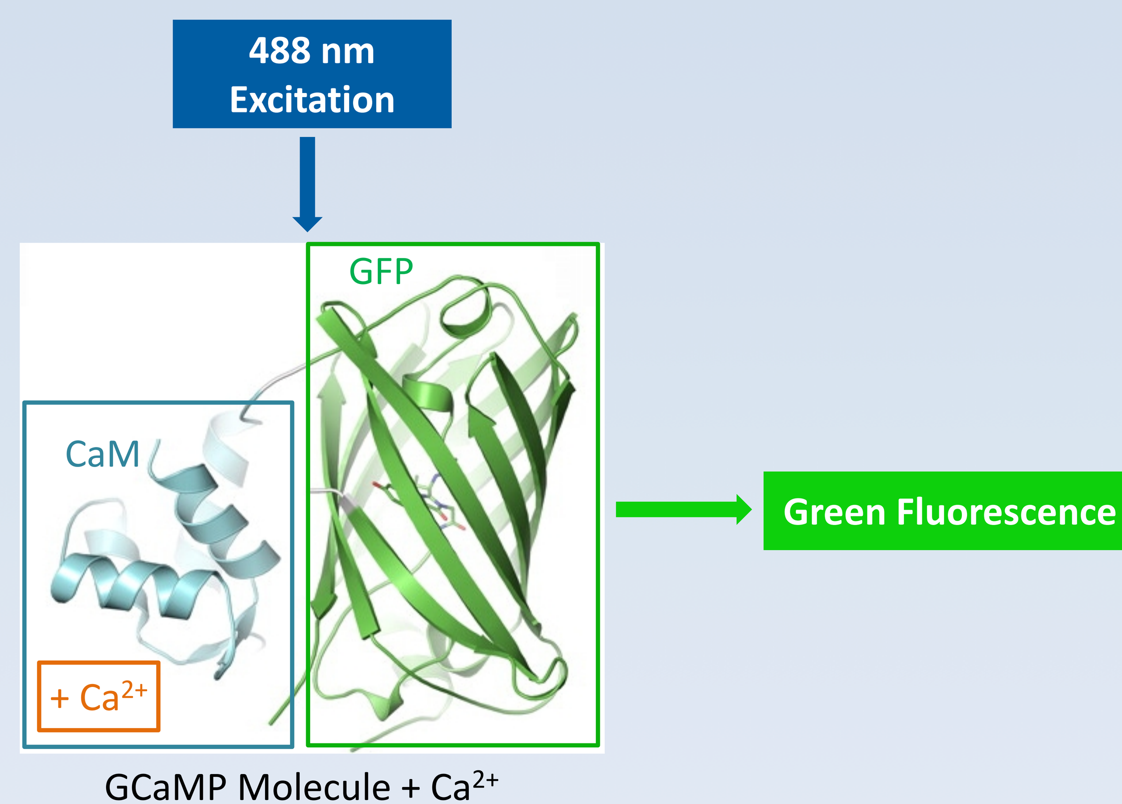
Introduction and Purpose

- Scientists are constantly trying to find new methods and techniques of visualizing the activity of neurons in organisms.
- When a neuron fires an action potential, calcium (Ca^{2+}) enters the intracellular space of the postsynaptic neuron and can be used as a visual marker of neural activity.



- The transparency of zebrafish larvae makes them a good model for studying neural activity.
- Genetically encoded calcium indicator (GCaMP6s) is a protein that consists of a modified GFP fused with calmodulin (CaM). When Ca^{2+} is bound to CaM, it allows the protein to be excited by 488 nm light (blue) and to emit fluorescent light (green).



- This study aimed to visualize the activity of neurons in zebrafish *Danio rerio* larvae *in vivo* after application of neurotransmitter agonist NMDA through calcium imaging.
- **Hypothesis**: Differences in neural activity during rest and during NMDA exposure could be resolved by calcium imaging using GCaMP6s.
- **Prediction**: Under GFP excitation, the fluorescent signals were predicted to be larger after NMDA exposure.

Methodology

1. Heterozygous *Tg(elav: h2b-GCaMP6s)* zebrafish were bred.
2. Homozygous *Tg(elav: h2b-GCaMP6s)* larvae offspring were anaesthetized and immobilized in 1.2% agarose gel.
3. Neural activity was recorded under light microscope and GFP excitation using CMOS camera (*Hamamatsu Orca Flash*).
4. Larvae were recorded before and after exposure to 150 µM NMDA.
5. Image capture analysis software (*HCImage*) was used to assess the fluorescent signals by comparing the intensity of the signal in the presence and absence of NMDA.

Results and Analysis

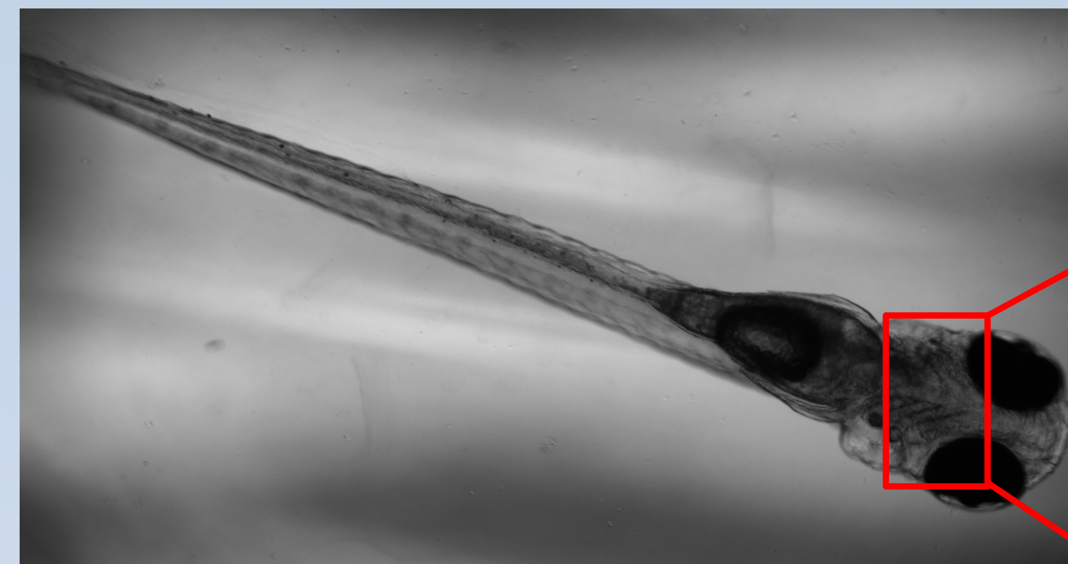
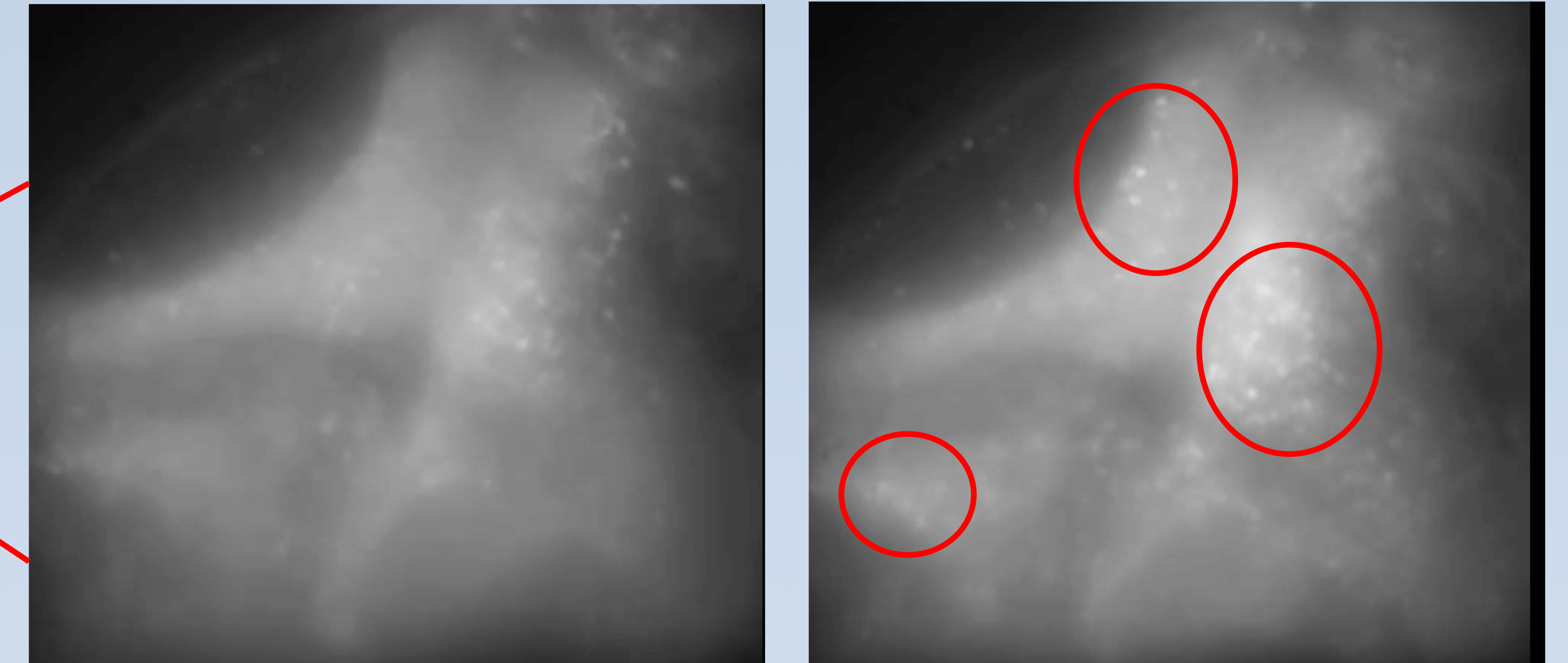


Figure 1. *Danio rerio* zebrafish under brightfield at 7 dpf (10X). Zebrafish larvae were immobilized using 1.2% agarose gel then placed under the light microscope to be observed using CMOS camera. Figure 1 shows the dorsal side of the zebrafish. This study focused on the neurons located in the brain region shown in the figure.



Figures 2A and 2B. Fluorescent neurons before and after NMDA exposure (40X). Zebrafish larvae were exposed to 150 µM NMDA at 7 dpf and observed under GFP fluorescence using CMOS camera. The GCaMP6s indicators in the organism emit fluorescence upon excitation. Regions of high neuron density and increased fluorescent light intensity are circled in figure 2B.

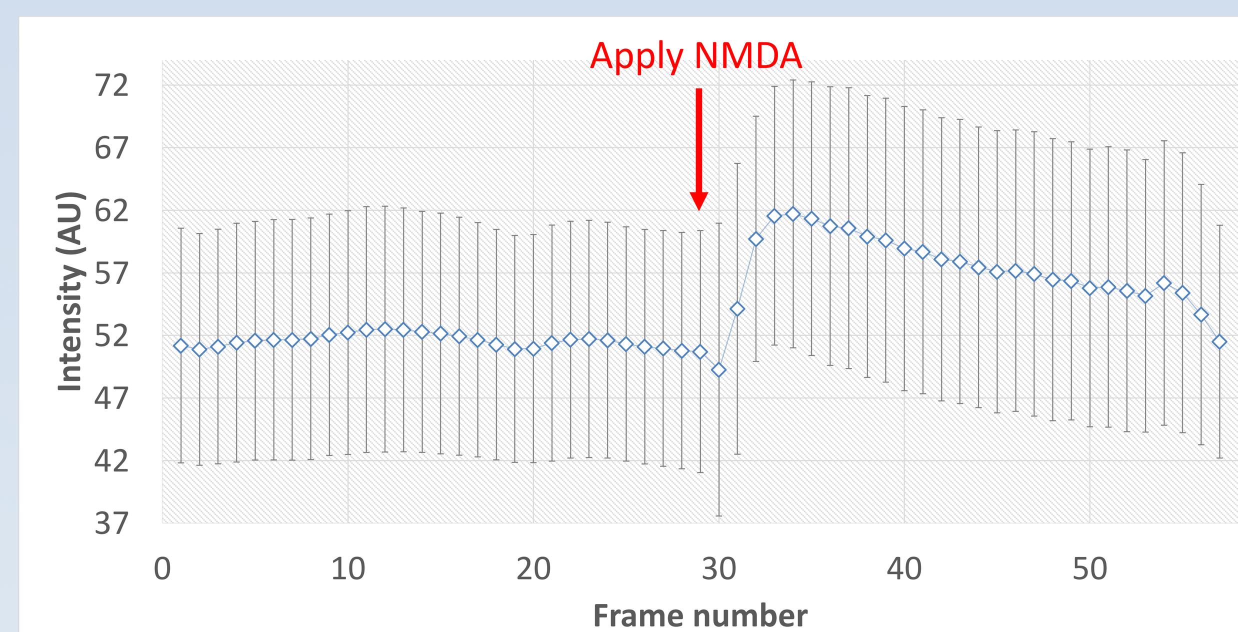


Figure 3. Average intensity (AU) of fluorescent signal as a function of the frame number. The average intensity of the signal from 20 neurons was measured over 57 frames (19 seconds) in ImageJ. A blank intensity was subtracted from the averages. NMDA was applied at approximately 10 seconds which corresponds to frame 29. A sudden rise in intensity can be seen at this point.

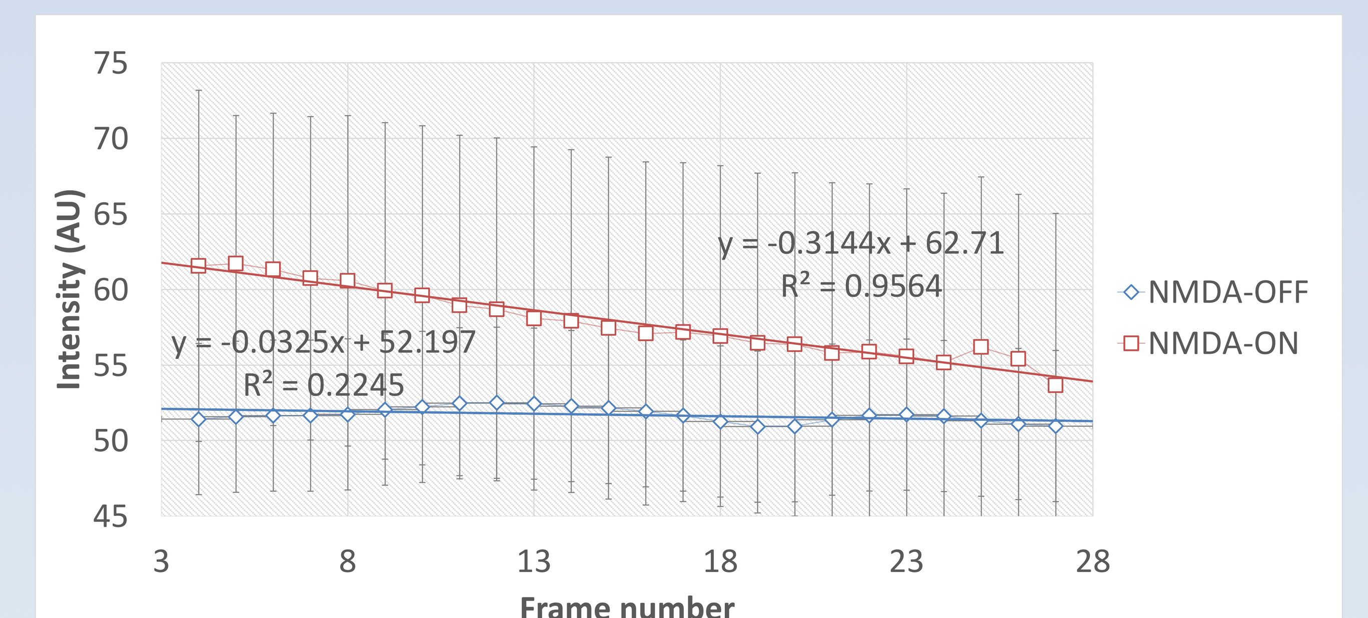


Figure 4. Intensity (AU) of fluorescent signal before and after exposure to NMDA as a function of the frame number. The data from figure 3 was split into two parts: intensity of fluorescence pre-NMDA application and intensity of fluorescence post-NMDA application. This data was overlapped to as a function of the frame number (pre- and post-NMDA) to obtain a clear visualization of the difference in intensities. Outliers were eliminated.

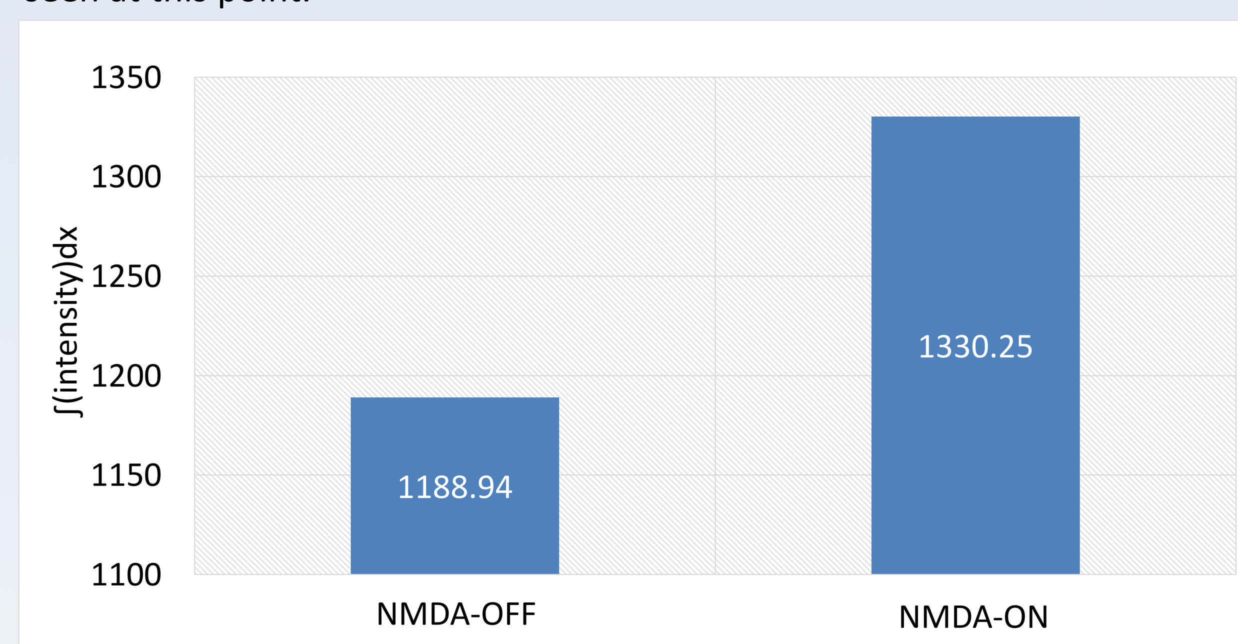


Figure 5. Integral of the intensity (AU) over 23 frames for NMDA-OFF and NMDA-ON. Integration of intensity over the number of frames for NMDA-ON and NMDA-OFF were both calculated and plotted as histograms using the regression lines in figure 4 during the period when the intensity was most linear, which corresponds to the [4; 27] frame interval pre- and post-NMDA.

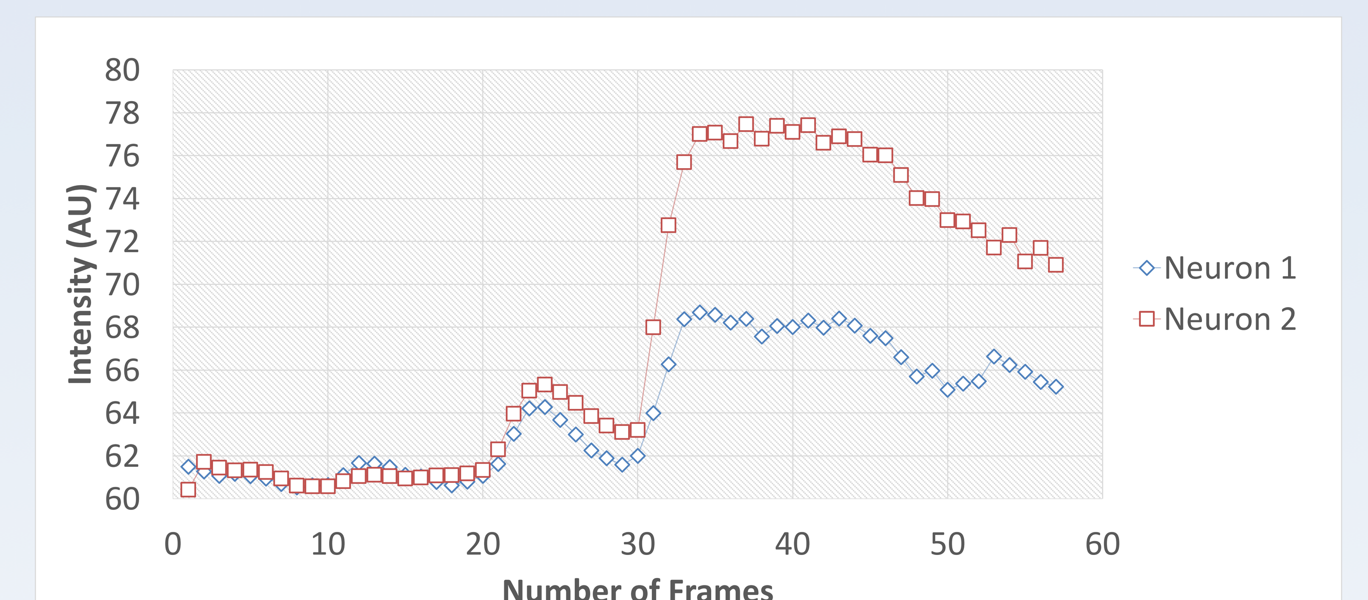


Figure 6. Individual intensity (AU) of fluorescent signal over the frame number for two neurons with different responses to NMDA. The intensities of a neuron with very small changes in response to NMDA (neuron 1) and a neuron with very large changes in response to NMDA (neuron 2) were shown in figure 6.

Conclusion

- As seen in figures 3, 4 and 5, it is clear that the average intensity of fluorescence increases in response to the application of NMDA. There is a strong response from the GCaMP6s indicators in the larvae. We can conclude that differences in neural activity during rest and during NMDA-induced excitation can in fact be resolved by calcium imaging using GCaMP6s. Our initial prediction can be validated, as the fluorescent signals were larger after exposure to NMDA.
- Better results and less error could have been obtained had the fish been properly immobilized before application of NMDA and had a higher concentration of NMDA been used.

Acknowledgements

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