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Bioluminescence-based approach to monitor neural activity in freely moving *Drosophila* larvae

Marescotti M. 1,2, Lagogiannis K. 2, Davies R.W. 1,2, Webb B. 3, and Armstrong J.D. 1,2  

**Background:** Dissecting behavioral circuits requires us to examine activity in the brain as the animal processes sensory information and generates autonomous behavior. With a few exceptions most current methods require a constrained preparation, which does not translate well to application in freely moving animals. We developed a non-invasive bioluminescence-based approach to address this problem; targeting the expression of the calcium-reporter Aequorin (AEQ) to the *Drosophila* larval nervous system to measure neural activity. We report conditions that significantly increase the sensitivity of this assay, allowing us to measure activity in Kenyon cells (KCs) (100s) as well as in smaller populations of neurons, with clear signals obtained from less than 10 neurons in intact animals. Finally, we measure both spontaneous activity and evoked responses from KCs in freely-behaving larvae.

**Methods: Bioluminescence principle**

![Diagram](image)

**Results (I): Neuroluminescence from intact larvae**

![Diagram](image)

**Results (II): Neuroluminescence from freely-crawling larvae**

![Diagram](image)

**Conclusions and Ongoing work:**

We developed for the first time a method allowing real-time analysis of neuronal activity in intact larvae while they are freely moving. Our aims for the future are:

- Technical improvement of the system
- Analysis of smaller group of neurons, ideally single neurons
- Study the effect of more inputs on neural activity in the context of behaviors