

Analyzing Long-Term Behavioral Patterns in *D. melanogaster* Larvae

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The mechanism by which recreational drugs are addictive is unclear. While a general understanding of addiction biology has been established, the molecular mechanism of addiction is not yet understood, limiting therapeutic development. Due to the complexity of the human brain, simpler models, such as the larvae of *D. melanogaster*, are optimal for investigating these mechanisms. *D. melanogaster* larvae are at the nexus of behavioral and single neuron research, allowing for simple yet effective research methodology. Paramount for applying this methodology is to be able to study both short and long-term behavioral effects; since *D. melanogaster* larvae limit experiments to 5 minutes by escaping experimental stages quickly, there is a need for a new method for long-term observation. Our laboratory previously created a modified 3D printer robot capable of returning escaping larvae to the stage, allowing this novel, 5-hour investigation into the long-term behavior of the larvae, including learning and development. A stimulus-free baseline intervention proved the efficacy of the long-term observational apparatus. Subsequent experiments with thermal gradient and nicotine stimuli showed significant changes in larval movement, as well as the long-term recovery from the stimulus. Overall, the results showcase the capacity of the automated experimental apparatus to make novel, long-term behavioral observations, presenting opportunities to better understand drug addiction and develop new therapies.