Dopamine Paralysis Assay

Several important studies have demonstrated dopamine's role in modulating locomotion in *C. elegans*. Mutants in dopamine-related signaling genes, including biosynthetic enzymes and dopamine receptors, display defective 'basal slowing' - a behavior that slows locomotion when a worm encounters food (Sawin, Ranganathan, and Horvitz, *Neuron*, 2000). In normal worms, food sensing by the CEP neurons stimulates dopamine release, which acts through dopamine receptors to inhibit locomotion by the motor neurons (Chase et al., *Nat Neurosci*, 2004). This intrinsic behavior is also manifested when worms are exposed to exogenous dopamine in their environment (Schafer and Kenyon, *Nature*, 1995), as exogenous dopamine exposure displays a dose-sensitive response that ends in complete paralysis of worms (Chase et al., *Nat Neurosci*, 2004). In our lab, we use exogenous dopamine-mediated paralysis in a simple assay to identify the genes, cells, and circuits that are required for dopamine signaling.

Procedure

1. On the day before you plan to conduct the dopamine paralysis assay, pick **30 - 40 L4-stage worms** to a new seeded plate for each of the strains or conditions you will test.
2. Pour **30 mM dopamine plates** (see Plate Pouring) or ensure that there are enough plates stored in the cold room. Plates should be used within 10 days of pouring, and should not exhibit black discoloration.
3. Allow the worms to grow at **20 deg C for 18 - 24 hours**.
4. You will conduct **3 separate trials** of ~10 worms for each strain or condition you plan to test. Gather the appropriate number of 30 mM dopamine plates and ensure that they are equilibrated to room temperature (if stored in the cold room).
5. Pick **10 worms** to the center of a blank dopamine plate using as little bacteria as possible. Any big globs should be scraped off afterwards.
6. Once all of the worms are transferred, start a timer for **20 min** and put the plate aside (**Replicate #1**).
7. After 5 minutes, pick 10 new worms to a blank dopamine plate as before, and start another timer for 20 minutes (**Replicate #2**).
8. Repeat the process after another 5 minutes (**Replicate #3**).

9. When Replicate #1 has incubated for 20 min, put the plate on the microscope stage and let it sit undisturbed for **30 seconds**.
10. Count the number of worms that are moving during a **15 second period**. Movement is counted as a **spontaneous body bend**, which is defined as the movement of a point in the animal immediately posterior to the pharynx through a minimum or maximum amplitude (Chase et al. 2004).
11. Record the results for Replicate #1.
12. Wait until Replicate #2 has incubated for 20 min, put the plate on the microscope stage and let it sit undisturbed for 30 seconds.
13. Count the number of worms that are moving during a 15 second period. Record the results for Replicate #2.
14. Repeat the scoring and recording for Replicate #3.
15. Conduct at least **3 trials** for each strain or condition you are testing.
16. Calculate the **mean and standard deviation** for the 3 replicates, and compare different strains or conditions with a **Student's T-test**.