

Dopamine Egg-Laying Assay

In addition to dopamine's effects on locomotion, it has also been shown to affect egg-laying behavior in adult hermaphrodites (Schafer and Kenyon, *Nature*, 1995), as exogenous dopamine exposure significantly inhibits the number of eggs laid. In our lab, we use exogenous dopamine-inhibited egg laying as a simple assay to uncover the mechanisms of 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 **5 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.
5. Pick **5 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 5 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 count the number of eggs on the plate. *You may remove the adult worms at this stage to allow for better visibility or later scoring.* Divide the number of eggs by the number of worms and record the results for Replicate #1.
10. Repeat for the remaining replicates.
11. Calculate the **mean and standard deviation** for the 3 replicates, and compare different strains or conditions with a **Student's T-test**.