

**Dopamine feeding and locomotor assays**  
**Per C. Singh, 1999**

**Drug preparation:**

Ingredients:     5% sucrose  
                  2% yeast  
                  water

Drug A:           10 mg/ml 3-iodo-tyrosine (3IY)

Drug B:           1 mg/ml L-3,4-dihydroxyphenylalanine (L-DOPA)

1. Heat the water to boiling in the microwave.
2. Add the sucrose, yeast, and drug of choice.
3. Mix on a vortex or rotator until all ingredients are solubilized. (This can take awhile.)
4. Food can be made fresh, or kept at 4°C overnight for the second day of feeding.
5. Feeding can be done on either small or large vials.
  - a. For small vials, insert a 3 cm x 7 cm 3MM paper tightly fit along the inside wall of the vial, a Whatman circle on the bottom, and 1-2 ml of food solution per vial.
  - b. For large vials, insert a 7 cm x 8 cm 3MM paper tightly fit along the inside wall of the vial, a 24 mm. GF/A filter on the bottom, and 2 ml of food solution per vial.

**Feeding the flies:**

1. Allow newly eclosed flies to feed for 24 hours at 25°C and 90% relative humidity (RH).
  - a. For the open field assay, put 4 flies/vial.
  - b. For locomotor tests in tubes, put 25 flies/vial.
  - c. For inebriometer runs, put 100 flies/vial.
2. After the first 24 hours (for open field assay only), cut off the flies' wings, in order to avoid flight during the ethanol exposures.
3. Return the flies to their feeding vials and allow them to feed for another 24 hours.

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## ASSAYS

### **Open field assay**

1. Allow the flies to clean their feet by transferring them to a fresh vial that has 3MM paper and 0.5 ml water in it. Let them clean for 20 minutes.
2. Put the flies in your open field box with constant flow of air. [6 x 6 x 1.5 cm acrylic exposure chamber.] Let them rest for 1 minute.
3. Start videotape.
4. Film the flies for 5 minutes with constant air flow only.
5. Apply ethanol vapors to your experimental flies [EtOH/air: 50/15 at 3 psi] and air only to your control flies [air only: 50 at 3 psi] for 25 minutes.
6. Analyze locomotion using DIAS software.

### **Locomotion assay in tubes (to determine baseline locomotion)**

(See tracking assay protocol for more details on videotaping with the booze-o-mat.)

1. Allow the flies to clean their feet by transferring them to a fresh vial that has 3MM paper and 0.5 ml water in it. Let them clean for 20 minutes.
2. Place flies in booze-tubes then quickly load them into the booze-o-mat.
3. Apply only air: 50 at 6 psi.
4. Analysis: Quantify basal locomotion for each individual fly using DIAS software.

### **Inebriometer assay**

(See inebriometer protocol for details.)

1. Allow the flies to clean their feet by transferring them to a fresh large vial that has 3MM paper and 1 ml water in it. Let them clean for 20 minutes.
2. Run flies as normal.

Used in:

***Bainton RJ, Tsai LT, Singh CM, Moore MS, Neckameyer WS, Heberlein U (2000)  
Dopamine modulates acute responses to cocaine, nicotine and ethanol in  
Drosophila . Current Biology 10:187-94.***