

Hydroxyurea ablation of mushroom bodies
Per A. Rodan, 2002

de Belle JS, Heisenberg M. (1994) Associative odor learning in Drosophila abolished by chemical ablation of mushroom bodies. Science. 263:692-695.

“In *Drosophila*, MB Kenyon cells are derived from four neuroblasts (MBNbs) that divide continuously from embryogenesis until the end of metamorphosis. MBNbs and one lateral neuroblast (Lnb) are the only proliferating cells from 0 through 8 to 12 hours after larval hatching (ALH). We fed hydroxyurea (HU) to newly hatched wild-type *D. melanogaster* larvae. This treatment should kill MBNbs and delete all MB Kenyon cell lineages with the exception of the 40 to 300 cells per hemisphere that arise during the course of embryonic development.”

1. Collect eggs on apple juice plates at 25°C in 1-hour intervals. Then, incubate the plates at 25°C and 70% relative humidity for 23.5hr each (this time was determined empirically to result in the most efficient MB ablation).
2. Collect the newly hatched first instar larvae from each time interval and transfer them to a microcentrifuge tube containing a paste of heat-killed yeast with (treatment) or without (control) 50µg/ml hydroxyurea (Sigma).
3. Allow larvae to feed for 4hr at 25°C.
4. Wash larvae and transfer them to regular food bottles that have some yeast paste on the food.
5. Incubate the larvae in their new bottles at 25°C.
6. Collect adults, 2-4 d old, and proceed with behavioral testing.

To visualize the degree of mushroom body ablation

- a. Collect eggs in Step 1 from a cross between your flies of interest (virgin females) and UAS-lacZ (males).
- b. After completing your behavioral tests in Step 6, proceed with β-galactosidase staining as described in that protocol.

Used in:

Rodan, A.R., Kiger, J.A. Jr., and Heberlein, U. (2002) Functional Dissection of Neuroanatomical Loci Regulating Ethanol Sensitivity in Drosophila. The Journal of Neuroscience 22:9490-9501.