Histamine-HisCl1 system for C elegans

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Abstract

We describe a chemical-genetic approach for inducible silencing of *Caenorhabditis elegans* neurons in intact animals, using the histamine-gated chloride channel HisCl1 from *Drosophila* and exogenous histamine. Administering histamine to freely moving *C. elegans* that express HisCl1 transgenes in neurons leads to rapid and potent inhibition of neural activity within minutes. *C. elegans* does not use histamine as an endogenous neurotransmitter, and exogenous histamine has little apparent effect on wild-type *C. elegans* behavior.

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Guidelines

Gloves are recommended while making or handling histamine-containing liquids. Avoid contact with eyes and skin.

Materials

Histamine dihydrochloride H-110 by Gold Biotechnology

HisCl1 plasmid and test strains Per Pokala et al 2014, request p by Contributed by users

Protocol

Histamine media

Step 1.

Put on gloves. Gloves are recommended while making or handling histamine-containing liquids. Avoid contact with eyes and skin.

Histamine media

Step 2.

Make 1M histamine (HA) stock - Dissolve 1.85g histamine dihydrochloride (Sigma-Aldrich H7250) per 10mL water. Sterile filter and store at -20˚C until use.

Histamine media

Step 3.

Make NGM-HA and NGM control agar.

In a 2L flask, add:

17g agar

2.5g peptone

3g NaCl

975mL water + stirbar

Autoclave (liquid mode, 121˚C for 1hr).

Also autoclave a empty 1L flask with a stirbar inside.

Histamine media

Step 4.

Place both flasks on heated stirplates at 60˚C.

Histamine media

Step 5.

When the 2L flask is cool enough to hold comfortably with a gloved hand (60˚C), add while stirring:

1mL cholesterol (5mg/mL in ethanol)

1mL 1M CaCl2

1mL 1M MgSO4

25mL 1M potassium phosphate pH 6.0

Histamine media

Step 6.

Using 50mL pipettes, sterilely transfer 500mL molten NGM agar to the 1L flask.

Histamine media

Step 7.

Add 5mL 1M HA stock to the 1L flask while stirring to make NGM-HA agar with 10mM histamine. The strength of neural inhibition can be titrated, with 1mM giving intermediate effects, and 10mM giving

maximal effects.

Histamine media

Step 8.

While the pH of standard NGM media does not change appreciably upon the addition of 10mM histamine dihydrochloride, it is strongly recommended that the pH of less strongly buffered solutions and media be checked and adjusted as needed (with control plates modified in tandem).

Histamine media

Step 9.

Pour control agar (from the 2L flask) and NGM-HA agar (from the 1L flask) into labeled petri dishes. 10mL for small (35mm), 25mL for large (100mm) dishes. NGM-HA plates stored at 4˚C retained their potency for at least 2 months.

Example experiment - anterior touch

Step 10.

The day before the experiment, pick 20-60 array-containing L4 animals.

Example experiment - anterior touch

Step 11.

If necessary, attach a hair to a P200 pipette tip:

Pluck or cut an eyelash or a hair from the back of your finger.

Place the tip of a P200 tip far above a flame until there is a bit of molten plastic.

Using forceps, quickly anchor one end of the hair into the molten plastic.

Wipe the hair with a Kimwipe soaked in ethanol. Use a second P200 tip as a sheath for storage.

Example experiment - anterior touch

Step 12.

Take histamine and control plates from 4˚C storage and store at room temperature overnight.

Example experiment - anterior touch

Step 13.

The day of the experiment, dry the plates without the lids for 1hr. Dry an additional food-free NGM plate.

Example experiment - anterior touch

Step 14.

Pick young adult worms to the food-free NGM plate. Let the animals crawl away from any carried-over food.

Example experiment - anterior touch

Step 15.

Gently pick 10 animals to each control plate, and 10 animals to each histamine plate. Histamine takes

effect within one or two minutes. After removal from histamine, animals can take one to two hours for full recovery.

Example experiment - anterior touch

Step 16.

Blind the plates: Remove any markings on the plate. Designate a one-character code for each strain and condition and write it on a thin piece of tape. Place the tape on the side of the plate, and cover the tape with another piece of tape. Shuffle the plates and leave undisturbed with the lid off for 30min.

Example experiment - anterior touch

Step 17.

After 30min, assay animals on each plate by gently stroking the area just posterior to the pharynx with the eyelash. Record the reversal length (number of body bends or head swings), and whether the animal performs an omega turn (sharp turn >135˚ or head touching the ventral surface). Animals that make a reversal less than a body bend are scored as 0.5. Also record if an animal pauses instead of performing a reversal; these animals are scored as having a reversal length of 0, but positive for a response.

Example experiment - anterior touch

Step 18.

After completion of assays, wipe the hair with an ethanol-soaked Kimwipe and re-sheath for use on another day.

Example experiment - anterior touch

Step 19.

Un-blind the plates by removing the covering tape.

Example experiment - anterior touch

Step 20.

Calculate the mean reversal length, fraction of animals that perform an omega turn, and fraction of animals that have any response, including a pause.