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**Protocol status:** In development  
We are still developing and optimizing this protocol

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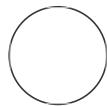
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## CNO Preparation and Consumption Monitoring

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### ABSTRACT

Chemogenetic models utilize the consumption of clozapine N-oxide (CNO) to activate Designer Receptors Activated Only by Designer Drugs (DREADDs) in order to modulate neuronal activity. This protocol describes the preparation of a CNO solution for consumption by mice, as well as the daily monitoring of water intake.

## Materials and Solutions

### 1 Materials:

- Clozapine N-oxide  Clozapine N-oxide Bioanalytical Systems Catalog #4936
- Sucrose  Sucrose Bioanalytical Systems Catalog #S9378
- Autoclaved water

### 2 Making CNO and vehicle water stock solutions


#### Note

- Store CNO and vehicle water in 4C fridge.
- Make fresh weekly.

- 2.1** CNO Water (2% sucrose, 200 mg/L CNO):  
20g sucrose and 300mg CNO in 1L autoclaved water
- Carefully weigh CNO to the nearest 0.05mg, then protect from light
  - Slowly add CNO in small amounts to autoclaved water while stirring, then add sucrose
  - Wrap bottle in foil to protect from light
- 2.2** Vehicle water (2% sucrose):  
20g sucrose in 1L autoclaved water

## Monitoring water intake

- 3** On day 0, discard the autoclaved water, and give 10mL of medicated water (e.g. CNO or vehicle water) in a water bottle per mouse. Record the time when mice were given medicated water.

 Mouse Water Bottle Bioanalytical Systems Catalog #CS-5010

Guardian Hamster Water Bottle (Amazon cat: B0713XMP98)

- 4** Monitor water intake at the same time every day of the experiment.

For each cage, perform the following substeps:

- 4.1** Remove from rack and check that there is sufficient food available on cage floor. Add more if needed.
- 4.2** Use a 10 or 25 mL serological pipette to measure how much water is still left in the bottle. Record this value.
- 4.3** For single-housed cages (e.g. mice with activity wheels), if water level is below 8.0 mL, use a clean pipette to add 5 mL more from the stock. If water level is below 4.0 mL, add 10 mL more. Record how much water is added.
- 4.4** For group-housed cages, top off with enough water to ensure at least 5mL per mouse plus overage (e.g. 15mL for two mice, 35mL for 5 mice).
- 4.5** Return cage to rack and repeat for remaining cages.
- 5** Enter recorded water levels into an Excel spreadsheet to track water intake per mouse over time.