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Protocol status: In development

We are still developing and optimizing this protocol

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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.

Keywords: ASAPCRN,
DREADD, Mouse

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 stolutions

> **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.