

Two-Bottle Choice (2BC) Behavior SOP

Overview

We use 2BC in conjunction with STAR to assess the impact of repeated binge drinking phenotypes. This procedure reveals wide individual differences in alcohol self-administration which can be quantified using STAR but is an auxiliary component to the STAR framework and can be altered or removed entirely depending on experimental question. Our protocol was based on previous work throughout the alcohol, and uses an experimental timeline based on the [drinking in the dark procedure](#), but water is always concurrently available similar to [limited access 2BC procedures](#).

Step-by-Step Guide

1. Set up a cage for each animal who will be running this part of the experiment
 - **Optional:** a control cage was also run to estimate the normal change in bottle weight due to dripping, evaporation, and other processes which might cause the bottles to change in weight over the course of a few hours
2. Place animals in individual cage to acclimate to environment while bottles are being prepared
 - Animals in this phase will have 2BC access for 2 hours, 4 hours, (checking in 2 hours into experiment) or be in abstinence (4 days of 2-hour access, 1 day of 4-hour access, 2 days of abstinence; repeat cycle twice for a total of two weeks)
 - If animal is in abstinence for the day, leave them in their homecage
3. Fill up bottles ~80% with solution and tightly secure the stopper
 - **Important:** Turn tube ~45° downwards and using gloved hand, tap on ball bearing in spout – solution should come out, releasing bubbles into the tube and leaving solution on glove, showing it's flowing. Should not drip freely at all
4. Weigh each pair of tubes and write down the weights before gently placing them on the cages
5. After 2 hours, remove and weigh all tubes. Replace any back on cage if animal is running for 4 hours that day. After 4 hours, remove and reweigh those tubes as well.
 - Once animal is done with 2 hour access, weigh the animal and then give them a normal water bottle for the cage while the others finish up the 4 hour access period. All animals will be fed at the end of the day once returned to homecage
 - The volume of each solution consumed is inferred from the change in weight of the bottles between the beginning and end of the session
6. **CRITICAL:** When converting from weight to volume $1g \neq 1mL$ because ethanol's specific gravity is lower than water (0.79 g/ml at room temperature). Thus the conversion will depend on ethanol concentration.
 - Conversion from weight to volume for a v/v ethanol in water solution is calculated as $[mL = g \times 0.79 \frac{EtOH(v)}{total(v)} + \frac{H2O(v)}{total(v)}]$
 - Example: 1g of a 15% v/v ethanol solution has a volume of 0.9685mL. $[0.9685 = 1 \times 0.79 \times 0.15 + 0.85]$
7. Ethanol density must also be account for when [calculating grams of ethanol from volume consumed](#).
 - Conversion from volume of solution to grams of ethanol consumed is calculated as $[g = mL \times 0.79 \times \%v/v]$

- Example: 1mL of a 15% ethanol v/v solution contains 0.1185g of ethanol. [$0.1185 = 0.79 \times 0.15$]
8. Empty all tubes at end of day and triple rinse them and the rubber stoppers with water. Make sure tubes are labeled with the solution they contained so that cross contamination is eliminated as much as possible