Freely-Moving Two-Armed Bandit – Sabatini Lab
consolidated protocol from Lynne Chantranupong and Michael Wallace

Habituation phase – 1 week prior to behavioral arena exposure

- Singly house mice, water deprived to 80% original body weight (BW)
- Handle mice for ~5 minutes/day
- Administer water by syringe during handling
- Place 1-1.5 mL of water in home cage to maintain water-restricted BW

Pre-training/Daily setup – Configuring Arduino and data directory

1. **Configure Arduino**
   a. Open Arduino IDE
   b. Open *MultiNoseportController*
   c. Compile and load onto Arduino board

2. **Configure MATLAB**
   a. Add *Mouse-Bandit-Behavior-markov folder to path*
   b. Run *NewGUI* in command window

3. **Configure NewGUI – flush tubing**
   a. Select *Connect to Arduino*
   b. Number of iterations = 100
   c. Left/Right duration = 450 ms
   d. Select *Flush Left* until all air bubbles are removed
   e. Select *Flush Right* until all air bubbles are removed
   f. Wipe away remaining water in ports

4. **Running an experiment – choosing data directory**
   a. Define mouse name
   b. Select: *Choose Directory to Save In* (select desired directory location)
   c. Type in desired parameters in GUI
   d. Select: *Run Experiment*

5. **Stopping a session**
   a. Select: *Stop Experiment*
   b. Clean box

Pre-training – behavioral arena setup/calibration

- Flush tubing with clean water by increasing solenoid valve opening time to 500 ms, 100 iterations on GUI.
- Change solenoid valve opening time to 50 ms, 1 iteration on GUI. Confirm that water delivery occurs in the form of a droplet.
- Calibrate ports (weekly) by:
   1. Set solenoid opening time to 50 ms, 100 iterations
   2. Aim for 300 uL of water to be dispensed during this time
   3. Adjust solenoid opening time accordingly until both ports release 300 uL of water.

Day 1/Stage 1: Mice learn that water is available from side port lick spouts

Bait center port and side ports with approximately 10 uL of water.
Weigh mouse then place in behavioral arena.

Set GUI parameters to:
      Center poke trigger = 0
      Center poke reward window = 0
      Left/Right reward probability = 1/1
      Min/Max block range: 1000

Learning criterion: train mice until they have reached a total of 150-200 rewarded trials then, proceed to next training stage.

Stage 2: Introduce trial structure – center to side nose poke for reward delivery

Bait lick spouts with 10 uL of water
Weigh mouse then place in behavioral arena
Session time = 40 min

Set GUI parameters to:
      Center poke trigger: 15 s
      Center poke reward window: 1
      Left/Right reward probability: 1/1
      Min/Max block range: 1000

Learning criterion: >100 correct trials/session; proceed to next training stage.

Stage 3: **Learning block structure – rewarded trials switch sides with each block transition**

Bait center and rewarded ports with 10 uL of water.
Weigh mouse and place in behavioral arena.

Set GUI parameters to:
      Center poke trigger: 15 s
      Center poke reward window: 1
      Left/Right reward probability: 1/0*
         *Counterbalance rewarded side every session to minimize development of bias
      Min/Max block range: 30

Learning criterion: ~70% accuracy across 6 blocks; proceed to next training stage

Stage 4: Introduction of physical barrier between reward ports and initiation port

Note: A well-trained, experienced mouse can perform a single trial within 300-400 ms which is too fast to resolve a photometry signal for a single trial. Therefore, physical barriers are introduced to increase trial time.

Barriers reach a maximum length of 6 cm. These are introduced in a gradual, stepwise manner so that animals can adjust their movements to accommodate the barriers. Barriers begin at 1.5 cm in length then, increase to 3 cm then, finally to 6 cm.

Install appropriate barriers.
Bait center and rewarded ports with 10 uL of water.
    Note: Once mice achieve criterion on shortest barrier, stop baiting center port.

Set GUI parameters to:
    Center poke trigger: 15 s
    Center poke reward window: 1
    Left/Right reward probability: 1/0*
        *Counterbalance rewarded side every session
    Min/Max block range: 30

Learning criterion: ~70% accuracy across 6 blocks then, can increase barrier length and/or move on to the final training stage.

Trouble shooting tips: If the mouse is struggling with the introduction of the barriers, change block range to 20 until mice perform well, bait rewarded port to help and place tape over unrewarded port if necessary. Then, increase block range to 30.

Stage 5: Final block structure for well-trained mice

Install longest barrier.
Bait rewarded ports with 10 uL of water.
Weigh mouse and place in behavioral arena.

Set GUI parameters to:
    Center poke trigger: 5 s
    Center poke reward window: 1
    Left/Right reward probability: 0.9/0.1
    Min/Max block range: 30