

Progressive ratio motivational task using mROBuckets

Carroll Lab - 2017

Food Deprivation Protocol

Materials:

- 1- Holding cage
- 1- Rodent food
- 1- Toploading balance scale

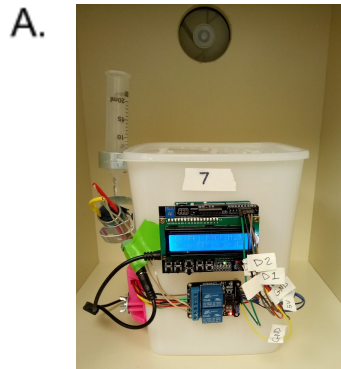
Overview: Approximately two weeks prior to behavioral testing, all mice are single housed with food bins removed. Food deprivation (FD) is a two-week process to achieve a target body weight between 80-85% of free feeding weight. Mice are fed 3.0 grams of food daily for the first 6 days and weighed daily starting on day 5. On days 7-14, mice are fed 0.2 grams less than the previous day until 85% target weight is achieved. Maintain target weight beginning at the start of the third week. Once behavioral testing has begun, feed mice one hour after testing. This ensures that mice are not rewarded with food based on testing performance, but rather on a time dependent schedule. Food weight may vary while aiming to maintain target weight, but once a mouse has reached the Progressive Ratio task, food weight should only vary by 0.1 grams as necessary.

Feeding Schedule:

1. Record mouse weight on day 1 to determine their free-feeding weight
2. Remove food hopper from mouse cage and feed mice 3.0 g of rodent food on days 1-6, once per day
3. On day 5, begin weighing the mice using a toploading balance scale
4. On day 7, reduce the food amount by 0.2 g and feed the mice 2.8 g
5. Feed mice 0.2 g less each day, until all mice reach 85% target weight of their free-feeding weight
6. Begin testing mice after 85% target body weight is achieved - this generally takes 10-15 days
7. Feed mice daily, adjusting food weight accordingly by 0.1 g increase or decrease in food weight to maintain the target body weight for each mouse
8. Once behavioral testing has begun, feed mice one hour after daily task completion. For example, if mice are in experiment from 2:00pm - 3:00pm, feed at 4:00pm.
9. Monitor the health and weight of each mouse daily
10. Once mouse has completed all behavioral tasks, place mouse back on free-feeding diet
11. Continue monitoring mouse weight until their weight returns back to 100% of free-feeding weight

Operant Testing Protocol

Materials:



- 1- Modified ROBucket mouse operant chamber (Devarakonda et al., 2016), equipped with two photobeam sensors positioned on either side of the following three nose-poke wells: (1.) active well, (2.) inactive well, and (3.) liquid reinforcement dispensing “reward” well.
- 1- Bottle 70% EtOH.
- 1- Bottle of DI H₂O.
- 1- Container of liquid reinforcements (20% sucrose water).

FR1 Operant Training: In order to train mice to nose-poke for a sucrose reward, mice are trained on a fixed-ratio-1 reinforcement schedule, where a single well press elicits the delivery of a food reward. There is a 1 second time-out after each active well press.

1. Bring mice into the experimental room, and allow them to habituate 1 hour prior to operant training - remove standard cage water bottles (if applicable) prior to habituation
2. Only one well in the operant box is designated as “active.” Counterbalance the active well between mice
3. Place each mouse into the operant chamber, and begin the operant training session.
4. Leave the animal in the operant chamber until either 60 minutes has passed, or the animal has received 50 reinforcements
5. After criterion is met, return animal to home cage and clean the chamber with 70% EtOH
6. At the end of each testing day flush the solenoid valve with 70% EtOH and DI H₂O twice
7. Repeat steps 2-5 for each animal daily until FR1 acquisition criteria are met.
8. Acquisition criteria for the FR1 schedule is met when:
 - a. Mice exhibit discrimination criteria of $\geq 3:1$ for the active versus inactive well for 3 consecutive days.
 - b. Mice receive ≥ 20 reinforcements for 3 consecutive days.
9. After criterion has been met for an individual mouse, move them onto the next phase
10. If mice do not achieve acquisition criteria after 17 days of FR1, exclude them from FR1 analysis and further testing

FR5 Operant Training: In order to prepare mice for a large number of pokes require to elicit reward, mice are trained on a fixed-ratio-5 reinforcement schedule, where five active well pokes elicit the delivery of a food reward. There is a 1 second time-out after each active well press.

1. Habituate mice in the experimental room for 1 hour prior to operant training - remove water bottles prior to habituation if applicable
2. Place each mouse into the operant chamber and begin the operant training session
3. Leave the animal in the operant chamber until either 60 minutes has passed, or the animal has received 50 reinforcements
4. After criterion is met, clean the chamber with 70% EtOH
5. At the end of each testing day flush the solenoid valve with 70% EtOH and DI H₂O twice
6. Repeat steps 2-5 for each animal daily for 3 consecutive days
7. After 3 days, move mice onto the progressive ratio schedule

Progressive Ratio Reinforcement Schedule: In order to test motivation to receive a reward, mice are tested on a progressive ratio schedule of reinforcement, where the number of well presses required to elicit reinforcement is calculated using the equation: Reinforcements = $\lceil 5e^{(N \cdot 0.2)} \rceil - 5$, where N is equal to the number of food reinforcements already earned plus 1. The number of rewards earned on the final day of stabilization is defined as the “breakpoint”.

1. Bring mice into the experimental room, and allow them to habituate 1 hour prior to operant training - remove water bottles prior to habituation
2. Place each mouse into the operant chamber, and begin the operant training session
3. Remove the mouse from the operant chamber after 60 minutes has passed, or after 10 minutes has elapsed without the mouse obtaining an active well poke
4. After each testing session, clean the chamber with 70% EtOH
5. At the end of each testing day flush the solenoid valve with 70% EtOH and DI H₂O twice
6. Repeat steps 2-4 for each animal daily until performance is stabilized
7. Performance is considered stabilized when the number of rewards earned in a 60 minute session deviates by $\leq 10\%$ for 3 consecutive days, or if the number of rewards earned is within 1 reward from the previous day for 3 consecutive days

References:

Devarakonda, K., Nguyen, K. P. & Kravitz, A. V. ROBucket: A low cost operant chamber based on the Arduino microcontroller. 1–7 (2016)