**Protocol for Flavor-Nutrient Conditioning Extinction Experiment– Alhadeff Lab 10/17/22**

* C57Bl/6J mice (~8-10 weeks old)
* Flavor stimuli
  + Habituation: 0.05% sodium saccharin
  + Training – 0.05% sodium saccharin, .05% Kool Aid flavor
* IG infusions: 16.67% glucose and 6.667% Intralipid (if we do straight infusions, we will infuse at least 0.6ml over 10 min, which is the volume that is self-ingested during the first 15 min of FNC – Zukerman et al., 2014)
* mouse licking triggers .6 ml infusion over 5 mins
* PM training sessions will occur 3 hours after the end of each respective mouse’s AM session
* Both control and stim mice should be included in one run of this experiment. The controls should be run together and should always be run before the stim mice. For the controls, there is a protocol in MED-PC that allows you to set the thresholds for the *time* when the bottles should be taken away based. These thresholds have been calculated based on previous data in hungry mice and represent the average time it takes a stim mouse for a given session to reach the average number of licks a control mouse will reach for the same session. After the controls are run, the experimenter should record the number of licks and compute the average number of licks for that session. This average will be the threshold for the number of licks the stim mice are allowed. There is a separate protocol in MED-PC that allows one to set a threshold on the number of licks. NOTE: since we do not have retractable lickometers, these protocols simply tell the experimenter when to take the bottles away. As a result, somebody should remain in the room for the duration of the experiment to take the bottles away at the appropriate time for each mouse. Special care should be taken to ensure no solution is left behind after bottle removal
* Rations are to be given ~1h after the last session on each day
* For all 2 bottle sessions, the bottle sides will be alternated between sessions
* Be sure that the bottles are always flush with the holders but even more so for all 2 bottle sessions! Both bottles should be equally accessible to the mice

Habituation 1 (water dep)

Day 1 – 30 min session with unflavored saccharin (1 bottle center)

Day 2 - 30 min session with unflavored saccharin (1 bottle center)

Habituation 2 (food dep)

Day 3 – 30 min session with unflavored saccharin and IG water (1 bottle center)

Day 4 – 30 min session with unflavored saccharin and IG water (1 bottle center)

Training (food dep)

Day 6 AM – 30 min with CS- and IG water (1 bottle center) – Control Mice (Time Threshold)

Day 6 AM – 30 min with CS- and IG water (1 bottle center) – Stim Mice (Lick Threshold)

Day 6 PM – 30 min with CS+ and IG glucose OR fat (1 bottle center) – Control Mice (Time Threshold)

Day 6 PM – 30 min with CS+ and IG glucose OR fat (1 bottle center) – Stim Mice (Lick Threshold)

Day 7 AM – 30 min with CS+ and IG glucose OR fat (1 bottle center) – Control Mice (Time Threshold)

Day 7 AM – 30 min with CS+ and IG glucose OR fat (1 bottle center) – Stim Mice (Lick Threshold)

Day 7 PM – 30 min with CS- and IG water (1 bottle center) – Control Mice (Time Threshold)

Day 7 PM – 30 min with CS- and IG water (1 bottle center) – Stim Mice (Lick Threshold)

Day 8 AM – 30 min with CS- and IG water (1 bottle center) – Control Mice (Time Threshold)

Day 8 AM – 30 min with CS- and IG water (1 bottle center) – Stim Mice (Lick Threshold)

Day 8 PM– 30 min with CS+ and IG glucose OR fat (1 bottle center) – Control Mice (Time Threshold)

Day 8 PM– 30 min with CS+ and IG glucose OR fat (1 bottle center) – Stim Mice (Lick Threshold)

Habituation 3 (food dep)

Day 9 AM– 30 min session with unflavored saccharin (2 bottles, left bottle has .025%, the right bottle has .05%)

Day 9 PM– 30 min session with unflavored saccharin (2 bottles, left bottle has .05%, the right bottle has .025%)

*\*look at data and potentially add a second day of habituation (identical to Day 9) if necessary.*

Testing (food dep)

Day 10 – 30 min with both CS+ and CS- (no infusion) (2 bottles)

Day 11 – 30 min with both CS+ and CS- (no infusion ) (2 bottles alternate sides from the previous day)

Day 12 – 30 min with both CS+ and CS- (no infusion ) (2 bottles alternate sides from the previous day)

Day 13 – 30 min with both CS+ and CS- (no infusion ) (2 bottles alternate sides from the previous day)

Day 14 – 30 min with both CS+ and CS- (no infusion ) (2 bottles alternate sides from the previous day)

Day 15 – 30 min with both CS+ and CS- (no infusion ) (2 bottles alternate sides from the previous day)

\**NOTE*: To rule out the development of a side preference, during habituation/testing, the side each mouse’s CS+ appears on for the first day of testing will be counterbalanced such that half of the mice with Grape as their CS+ will see Grape on the right for the first day of testing while the other half will see Grape on the left that day. Similarly, half of the mice with Cherry as their CS+ will see Cherry on the right for the first day of testing and the rest will see Cherry on the left that day.