**Basic Protocol for Flavor-Nutrient Conditioning – Alhadeff Lab 3/5/20**

* C57Bl/6J mice (~8-10 weeks old)
* Flavor stimuli
  + Habituation: 0.05% sodium saccharin
  + Training – 0.05% sodium saccharin, .05% Kool Aid flavor
  + \*0.3% saccharine if sated
* 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
* PM training sessions will occur 3 hours after the end of each respective mouse’s AM session
* 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)

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

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

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

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

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

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) (60 mins for fat)

Day 11 – 30 min with both CS+ and CS- (no infusion ) (2 bottles alternate sides from the previous day) (60 mins for fat)

\**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.