2. Experimental Protocols

2.3. BAT Taste-Induced Odor Preference Ortho Enriched Condition

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22007 IACUC Protocol

**Water Restriction**

Rats will be placed on a water restriction regime while undergoing behavior procedures (both during training and testing phases), to ensure they are motivated to drink during the experiment. In addition to the fluid they receive during daily experimental sessions (between 5 and 8 ml), rats will also receive access to an additional 15 ml of water per day in their home cage.

Note, this restriction will never amount to an entire day without water. This restriction is absolutely standard in behavioral neuroscience studies, daily evaluation and recording of weight will allow monitoring of health; in the unlikely event of weight loss that is larger than 15% normal body weight (as determined by Charles River, the breeder), that particular rat will be removed from the protocol.

**Preference Test**

This paradigm allows us to determine our animals’ preferences to different tastes and concentration ranges, which helps us in accessing the palatability relationship between tastants.

Length of training/ testing: Approximately 7 days.

Animals will be adapted to a variant of the “Davis Rig,” which allows an examination of taste preferences for a small set of odors in a single session. The rig is nominally a 1-lick spout chamber, but the spout is behind a sliding panel in the chamber. A computer- controlled conveyor belt allows any one of 12 actual lick spouts to be positioned behind the panel, which then opens for a period of 10 sec at a time (one “trial”). In adaptation sessions, the animal will learn to wait for the panel to slide out of the way, and then to drink from the proffered spout. Once the animal has learned to approach and lick water, sessions will ensue in which each trial is a randomly selected 1 of 2 odors is offered. An infrared detector, very similar to the beams that signal approach to the doors of many stores, will allow us to know exactly when the animal’s tongue extends to reach the lick spout. At the end of a 60- 90 min session, the animal will have consumed between 5 and 10 ml of fluid, and the resultant data- number of licks for each fluid- will provide us with more information as to the animal’s particular odor preferences.

**Taste-potentiated odor association (TPOA) Orthonasal Olfaction**

This test investigates how taste and odors interact with one another, which is important when looking at the mechanisms behind taste preferences.

Length of training/ testing: Approximately 8 days.

1) in training sessions, tastes or water will be presented accompanied by the smell of either Citral or Methyl Valerate diluted to 0.01% in water, a specialized lick spout will be used that emerges from a variant of the “Davis Rig” in a pseudorandom order and 2) a second testing session, in which water is available in the odorized lick spouts, will evaluate any learned aversions for the odor itself.

In a subset of these animals for 3 days prior to the BAT preference test the animal will receive 0.01% of Ethyl Butyrate and Carvone in their home cage water (50mL of solution per 24 hours).

Handling and care

Per protocol animals must be in vivarium for 1 week prior to any experimental procedures/surgeries.

These can be done days in a row, or with a couple days in between.

Animals should be handled for ~10 min each unless otherwise stated 3-4 times a week.

At least once a week (during cage changes if in early stages of handling) rats should be weighed and logged on the cage card.

If you are not logging your handling sessions by weighing them, you should be writing them in your notebook (good to do both)

After surgery or when on water dep animals must be weighed daily

* For the first couple days once they arrive don’t handle them
* Handling day 1: first day place them in room they will be used in for 30 min with cage top off, but metal grate on to acclimate to sounds and smells.
* Handling day 2: After adjusting to the room with the lid off you can take off the cage and wait in the room with them. Go on phone, clean up around, do work on laptop etc.
* Handling day 3: Next day can start putting your hand in the cage, near the edge, just letting them get used to you. I like to stroke my thumb along my fingers to make a sound. I always make that sound when I enter the cage and before I pick them up from then on to help them know it’s me and what’s happening/cue them.
* If they are receptive to your hand (sniffing, letting you touch them in the home cage) you can start petting them and interacting with them the same day. If not, just leave your hand in there as a benign object that doesn't cause startle/is aversive.
* Handling day 4: Next day repeat the slow hand introduction, interacting with the hand in the home cage. Skittish rats may just be allowed out onto the cart, more ‘sociable rats’ can be pet more and even start to be picked up onto the lap, picked up out of the cage. But they should be given plenty of freedom to return to the cage, leave your lap, or avoid your hand. Important that the rats learn that you and your hand are a positive or neutral stimulus and NOT an aversive one.
* Handling day 5: All rats should be picked up and be spending time on your lap (may be less time for certain rats, but they should still be getting used to the lap, give more skittish ones the option to escape onto the cart)
* Handling day 6+: Final stage of handling! Introduce your hand into the cage to let them know you are there, then pick up and weigh, and pour out into your lap and let them roam/practice injection pinches/practice head touching/give them pets!

I usually start picking up rats with the scoop method then transition into under the shoulders. NEVER pick up by the tail. You may HOLD the tail while you scoop when a rat is hard to corral, but do not pick them up solely by their tail.

Always clean everything with 70% EtOH between animals. Try to do this with some time to air out the ethanol smell before the next animal.

Be very careful to look for startle responses like freezing (Whiskers stop moving when they’re startled)

Never interrupt grooming (it’s a coping mechanism for them)

**Introducing touch**

* Touch rats in ways they touch each other.
* Right below the shoulder blades is a good spot to touch.
* Really only use fingers because hands are too big.
* Faster rat like strokes of touching is better.

Be careful of sudden transitions - move slowly when handling them.

You can manipulate them by using your hand to get them comfortable with your hands and maneuvering them. You can make them turn right by using your hand *etc.* use it as a platform they have to touch to get down from or up to some place.

There is a certain amount of pressure you can figure out to put on them to make them immobile without startling them so experiment with how much pressure that is.

Start touching them in ways you might use to pick them up.

Mess with the parts of the body that you want to touch and pick up from

Get them used to the sounds of gloves, any clicking, hand motions that loom, and changes of light in front of them.

Use short pick-ups with the immediate option of them being able to escape/get down.

Include chew block, enviro-pak, and cardboard tube in the cage

Prep

Order animal 1 week before (**7-9 weeks old**)

Handle

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| 0  Rats Arrive | 1  Handling | 2  Handling | 3  Handling | 4  Handling  **pre-exposure** | 5  **pre-exposure** |
| 6  **pre-exposure** | 7  **pre-exposure** | 8  BAT hab 1  **pre-exposure** | 9  BAT hab 2  **pre-exposure**  ***Start water dep*** | 10  BAT hab 3 | 11  BAT hab 4 |
| 12  BAT hab 5 | 13  Pre-preference test  60 trials | 14  Conditioning  30 trials | 15  Conditioning  30 trials | 16  Conditioning  30 trials | 17  Conditioning  30 trials |
| 18  Conditioning  30 trials | 19  Conditioning  30 trials | 20  Post- preference Test  60 trials | 21  Post-preference Test  60 trials |  |  |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Animal** | **Enriched (E) or Unenriched (U)** | **Enriched Odor** | **Paired Odor** | **AB or BA** |
| TG43 | E | MV/EB | 2-hexanone | AB |
| TG44 | E | MV/EB | Butyl acetate | AB |
| TG45 | E | MV/EB | 2-hexanone | BA |
| TG46 | E | MV/EB | Butyl acetate | BA |

**Day 1 – Cond**

-TG43 – 2 bottles of 2-hexanone with sucrose

Bottle 1 Bottle 2

|  |  |  |  |
| --- | --- | --- | --- |
| PRE: |  | POST: |  |

-TG44 – 2 bottles of 2-hexanone without sucrose

Bottle 1 Bottle 2

|  |  |  |  |
| --- | --- | --- | --- |
| PRE: |  | POST: |  |

-TG45 – 2 bottles of BA with sucrose

Bottle 1 Bottle 2

|  |  |  |  |
| --- | --- | --- | --- |
| PRE: |  | POST: |  |

-TG46 – 2 bottles of BA without sucrose

Bottle 1 Bottle 2

|  |  |  |  |
| --- | --- | --- | --- |
| PRE: |  | POST: |  |

**Day 2 – Cond**

-TG43 – 2 bottles of BA without sucrose

Bottle 1 Bottle 2

|  |  |  |  |
| --- | --- | --- | --- |
| PRE: |  | POST: |  |

-TG44 – 2 bottles of BA with sucrose

Bottle 1 Bottle 2

|  |  |  |  |
| --- | --- | --- | --- |
| PRE: |  | POST: |  |

-TG45 – 2 bottles of 2H without sucrose

Bottle 1 Bottle 2

|  |  |  |  |
| --- | --- | --- | --- |
| PRE: |  | POST: |  |

-TG46 – 2 bottles of 2H with sucrose

Bottle 1 Bottle 2

|  |  |  |  |
| --- | --- | --- | --- |
| PRE: |  | POST: |  |

**Day 3 – Cond**

-TG43 – 2 bottles of 2-hexanone with sucrose

Bottle 1 Bottle 2

|  |  |  |  |
| --- | --- | --- | --- |
| PRE: |  | POST: |  |

-TG44 – 2 bottles of 2-hexanone without sucrose

Bottle 1 Bottle 2

|  |  |  |  |
| --- | --- | --- | --- |
| PRE: |  | POST: |  |

-TG45 – 2 bottles of BA with sucrose

Bottle 1 Bottle 2

|  |  |  |  |
| --- | --- | --- | --- |
| PRE: |  | POST: |  |

-TG46 – 2 bottles of BA without sucrose

Bottle 1 Bottle 2

|  |  |  |  |
| --- | --- | --- | --- |
| PRE: |  | POST: |  |

**Day 4 – Cond**

-TG43 – 2 bottles of BA without sucrose

Bottle 1 Bottle 2

|  |  |  |  |
| --- | --- | --- | --- |
| PRE: |  | POST: |  |

-TG44 – 2 bottles of BA with sucrose

Bottle 1 Bottle 2

|  |  |  |  |
| --- | --- | --- | --- |
| PRE: |  | POST: |  |

-TG45 – 2 bottles of 2H without sucrose

Bottle 1 Bottle 2

|  |  |  |  |
| --- | --- | --- | --- |
| PRE: |  | POST: |  |

-TG46 – 2 bottles of 2H with sucrose

Bottle 1 Bottle 2

|  |  |  |  |
| --- | --- | --- | --- |
| PRE: |  | POST: |  |

**Day 5 – Cond**

-TG43 – 2 bottles of 2-hexanone with sucrose

Bottle 1 Bottle 2

|  |  |  |  |
| --- | --- | --- | --- |
| PRE: |  | POST: |  |

-TG44 – 2 bottles of 2-hexanone without sucrose

Bottle 1 Bottle 2

|  |  |  |  |
| --- | --- | --- | --- |
| PRE: |  | POST: |  |

-TG45 – 2 bottles of BA with sucrose

Bottle 1 Bottle 2

|  |  |  |  |
| --- | --- | --- | --- |
| PRE: |  | POST: |  |

-TG46 – 2 bottles of BA without sucrose

Bottle 1 Bottle 2

|  |  |  |  |
| --- | --- | --- | --- |
| PRE: |  | POST: |  |

**Day 6 – Cond**

-TG43 – 2 bottles of BA without sucrose

Bottle 1 Bottle 2

|  |  |  |  |
| --- | --- | --- | --- |
| PRE: |  | POST: |  |

-TG44 – 2 bottles of BA with sucrose

Bottle 1 Bottle 2

|  |  |  |  |
| --- | --- | --- | --- |
| PRE: |  | POST: |  |

-TG45 – 2 bottles of 2H without sucrose

Bottle 1 Bottle 2

|  |  |  |  |
| --- | --- | --- | --- |
| PRE: |  | POST: |  |

-TG46 – 2 bottles of 2H with sucrose

Bottle 1 Bottle 2

|  |  |  |  |
| --- | --- | --- | --- |
| PRE: |  | POST: |  |

Odds with sucrose

Evens without sucrose

Pre Exposure Begins - Day 12

Make fresh daily.

Use graduated cylinder to measure 100mL of DI/miliQ water. Add to amber 100mL bottle. In fume hood, select odorant. Use p10 to carefully pipette 10ul of odorant into 100mL bottle (pipette up and down to mix). Top 100mL bottle and shake. Label with animal and odor. Remove *ad lib* water bottle. Place lick spout with ball into amber bottle and into animal cage at same time daily (within the same hour timepoint). Measure how much water is consumed by animal.

Pre exposure odors: Methyl valerate and Ethyl butyrate.

Switch which odor is pre-exposed after 2-3 days (e.g. if started with MV, switch to EB)

* Add infused water (0.01% concentration of each odor) into its home cage.
* 50ml of Ethyl Butyrate in 100mL in conical tube with spout, remove *ad lib* water.
* Unenriched also get conical tube and spout.
* Remains until water dep starts end of BAT hab 2.

Habituation (hab) in BAT - Days 15-19

Weigh animal daily

Clean rig thoroughly with 70% EtOH, with 10min to air out in between animals

Fresh lick spouts used for each animal

MiliQ water fresh on hab1

**Hab 1**

Rig habituation,

no shutter or tastants. 30min

**Fan off**

**Hab 2**

Rig habituation,

no shutter or tastants. 30min

**Fan off**

Water restriction starts

**Hab 3**

Licking Habituation-

shutter open with 1 water bottle (stink\_hab3), 30 min

**Fan off**

9am 10-15ml water

**Hab 4**

2 bottles water - 30 trials, 15 each bottle (stink\_hab4)

**Fan off**

9am 10-15ml water

**Hab 5**

2 bottles water - 30 trials, 15 each bottle (stink\_hab5)

**Fan off**

9am 10-15ml water

Make Tastants

For Preference tests (pre and post):

**2μL 2-Hexanone in 20mL distilled water use to dip kimwipe/paper towel and put it on the spout. Same with Butyl Acetate. Liquid odor CANNOT be on the tip of the spout where the animal licks.**

Bottles:

1. 20mL MiliQ water
2. 20mL MiliQ water
3. 20mL 0.01% Citral – **0.01% 2-Hexanone (On Spout) and 20mL distilled water**
4. 20mL 0.01% Citral - **0.01% 2-Hexanone (On Spout) and 20mL distilled water**
5. 20mL 0.01% Methyl Valerate - **0.01% Butyl Acetate (On Spout) and 20mL distilled water**
6. 20mL 0.01% Methyl Valerate - **0.01% Butyl Acetate (On Spout) and 20mL distilled water**

→ 60 presentations of 6 bottles (10 presentations each; hab4/5 = 2 bottles presented 15 times)

Number of presentations: 60

Licktime: 5s

IPI: 30s

Maxwaittime: 60s

Session time limit: 70min

Store in glass bottles

\*\* FOR CONDITIONING DAYS, 2 BOTTLE PROTOCOL DESCRIBED BELOW\*\*

Paired odor: In 50mL miliQ, + 3.423g sucrose, same as pre-preference test on spout.

Unpaired odor: same as pre-preference test on spout.

1. Make sure to clean the cage bottom and top with ethanol in between animals and let it air out for ~10 minutes.
2. When handling odors switch gloves in between putting odors on spout and handling the animals.
3. Never have two animals in the room at the same time or an animal in the room when preparing the odors

Con 1/3/5 (**paired odor**), bottles:

1. 0.2M sucrose + paired odor on spout
2. 0.2M sucrose+ paired odor on spout

Con 2/4/6 (**unpaired odor**), bottles:

1. 0.01% on Spout unpaired odor and 20mL distilled water
2. 0.01% on Spout unpaired odor and 20mL distilled water

→ 30 presentations of 2 bottles (15 presentations each)

Number of presentations: 30

Licktime: 5s

IPI: 30s

Maxwaittime: 60s

Session time limit: 100min

Pre-preference test - Day 20

6 bottles: 2 bottles water, 2 bottles Citral, 2 bottles Methyl Valerate - 60 trials, 10 each bottle (stink\_prepref\_test)

**Fan off**

9am 10-15ml water

Conditioning (con) - Days 21-26 (AB)

**Con 1**

2 bottles **paired odor** - 30 trials, 15 each bottle (stink\_con\_paired1)

**Fan off**

9am 10-15ml water

**Con 2**

2 bottles **unpaired odor** - 30 trials, 15 each bottle (stink\_con\_un1)

**Fan off**

9am 10-15ml water

**Con 3**

2 bottles **paired odor** - 30 trials, 15 each bottle (stink\_con\_paired2)

**Fan off**

9am 10-15ml water

**Con 4**

2 bottles **unpaired odor** -30 trials, 15 each bottle (stink\_con\_un2)

**Fan off**

9am 10-15ml water

**Con 5**

2 bottles **paired odor** - 30 trials, 15 each bottle (stink\_con\_paired3)

**Fan off**

9am 10-15ml water

**Con 6**

2 bottles **unpaired odor** - 30 trials, 15 each bottle (stink\_con\_un3)

**Fan off**

9am 10-15ml water

Post-preference tests - Day 27-28

6 bottles: 2 bottles water, 2 bottles 2-Hexanone, 2 bottles Ethyl Butyrate - 60 trials, 10 each bottle (stink\_prepref\_test1)

**Fan off**

9am 10-15ml water

6 bottles: 2 bottles water, 2 bottles 2-Hexanone, 2 bottles Ethyl Butyrate - 60 trials, 10 each bottle (stink\_prepref\_test2)

**Fan off**

9am 10-15ml water