Methods Rural-Like City Setup Outline

Animals and Housing

* Arrival and Habituation: Female mice (n=20) arrived at the facility on January 10th, 2024. DOB 11/28/23, strain C57BL/6J.
* N = 18; by the end of the experiment, one animal was sacrificed due to malocclusion (April 16), and another had dislodged the transponder from its neck, making it impossible to track.
* They were habituated to the colony room under red light and a reverse light cycle for a month.
* RFID Tagging: Mice were implanted with unique RFID transponder tags under isoflurane anesthesia in the neck region on January 31st, February 5th, and February 6th to enable individual identification and tracking. Weight of all animals was recorded.
* Stable 12-hour light/dark cycle.

Experimental Setup

* Introduction to Rural-like City Setup: On February 20th, the mice were introduced to a rural-like city environment designed to mimic natural conditions.
* Housing Configuration: The setup consisted of five large identically sized cages (Allentown, size: 10.5" x 19" x 6" h) connected to a central circular hub via tubes (length 12 inches-24 inches, diameter 1.5 inches).
  + Central Hub: The hub contained soil sourced from a rural environment, providing a naturalistic substrate.
  + Cages:
    - Nesting Materials and Water: Four out of the five cages contained nesting materials and water.
    - Food Locations: Two of the four cages also housed the permanent location of grain- and fat-based food.
    - Feeding Experimentation Device 3 (FED3) Cage: The fifth cage contained a FED3 device and acted as the reward competition paradigm. The device was equipped with an overhead camera to record animal behavior.

Antenna System:

* The total number of antennas installed throughout the city setup is 10.
* The four tubes connecting the four nesting cages to the central hub each had two RFID antennas installed to record animal activity. These antennas tracked when animals entered and exited each of the individual cages (Antennas #2-5, 7-10).
* The tube connected to the reward competition cage had only one antenna (#1), with the second antenna (#6) positioned beneath the FED3 device to record which animals were motivated to engage in the reward-based competition.
  + See the rural-like city setup below:

A diagram with colorful rectangles and numbers

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Bedding Change:

* First change on March 25th, to decrease stress related to scent marking territories only 75% of the bedding was replaced with new material.
* Second change on May 8th, handled the same as the first change.

Reward Competition Paradigm

* Introduction to Sugar Pellets: Feb. 26th mice were first introduced to sugar pellets by placing them in front of the FED3 device in the reward competition cage.
* Free Feeding Paradigm: Feb 27th, the FED3 device was set up in a free feeding program. In this setup, the device automatically dispensed a pellet, and once an animal retrieved the pellet from the central port, the next pellet was delivered after a short delay. Mice were allowed to participate in free feeding for four days, with each session lasting 1-2 hours.
* Fixed Ratio Paradigms:
  + Fixed Ratio (FR1): On March 4th, the FED3 program was switched to a Fixed Ratio 1 (FR1) paradigm, requiring the mice to correctly nose poke to receive a sugar pellet reward.
  + Fixed Ratio Reversal (FR1 Reversal): On April 10th, the program was changed to a Fixed Ratio Reversal, where mice had to correctly nose poke on the left side to receive a reward.
  + Fixed Ratio 5 (FR5): On May 16th, the program was further changed to Fixed Ratio 5 (FR5), where mice were required to nose poke correctly five times to receive one sugar pellet reward.
  + Total number of pellets dispensed, and total number of Left/Right pokes were recorded after each session.

Schedule: During the Fixed Ratio phases, the reward competition sessions were run for one hour each day, only on weekdays.

Behavioral Testing

* Individual Testing: On June 6th, each mouse was individually tested to assess whether they had learned the nose-poking behavior for rewards. Each animal was given 5 minutes in the reward competition cage with the FED3 device set to the Fixed Ratio 1 (FR1) paradigm. Weight of all animals was recorded.
* Grouping Based on Results:
  + Following the individual tests, the animals were categorized into three groups based on their behavior:

  1. Mice that successfully learned to nose poke for a reward.

  2. Mice that did not learn the association of nose poking for a reward.

  3. Mice that waited at the central port to receive a reward without performing a nose poke.

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End of Rural Experiment

* All 19 animals were sacrificed. Tumor was observed on one animal.
  + Based on the three groups listed above, the brains were assigned to either be perfused (n=9) or frozen (n=10, highlighted in red in the above list)

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Given to Lab PINAR | | | | | | |
|  | | **Perfused** | | **Tail Mark** | | **Weight** |
| Cage 1 | | 79FED08 | | 0 | | 24.7g |
| Cage 1 | | 70FEADE | | 1 | | 22g |
| Cage 1 | | 7A3FDE2 | | 2 | | 22.7g |
| Cage 1 | | 7A3931B | | 3 | | 28g |
| Cage 2 | | 7A2EFA1 | | 0 | | 24.7g |
| Cage 2 | | 7A3DC62 | | 1 | | 24.8g |
| Cage 2 | | 7A3C016 | | 2 | | 32.1g |
| Cage 2 | | 7A3A179 | | 3 | | 26.6g |
| Cage 2 | | 79FC3C6 | | 4 | | 30.8g |
| Frozen LAB LIU and MINGOTE | | | | |
| **Order Sacrificed** | **Frozen** | | **Weight** | |
| 8 | 79FEC60 | | 28.5g | |
| 10 | 7A02701 | | 25.4g | |
| 4 | 7A2E90C | | 25.4 or 25.7g | |
| 9 | 7A3C408 | | 24g | |
| 3 | 7A40088 | | 23.4g | |
| 6 | 79FEEA8 | | 31.8g | |
| 5 | 7A00464 | | 25.42g | |
| 1 | 79F8602 | | 23.6g | |
| 2 | 7A3DD05 Tumor | | 23.6g | |
| 7 | 7A3E5BC | | 25.7g | |
|  |  | |  | |

Data Analysis:

**Moves (average of moves in a week)– circadian rhythm:**

1. First week of data 🡪 March 2nd – March 9th (sat to sat; 1 week) – 12 days in the city
2. March 25th (Monday, cage change) 🡪 26 March - April 2nd (Tuesday to Tuesday)
3. One week before 2nd cage change 🡪 April 30th to May 7th
4. May 8th (Wednesday, 2nd cage change) 🡪 May 9th to May 16th
5. Last week in the city 🡪 May 29th to June 5th

Graph 1 - Average Moves in One Week

A graph of a number of mice

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Average Actogram (7 days, same as above) – each bar is 1hour, and an average of moves across the 20 animals (Torquet et al., 2018, supp 3A)

A graph showing a number of data

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11am

11pm

**Time spent in each cage:**

**Circular plots** – for each cage (orange, red, blue, yellow, green); each dot is a visit—example from Torquet 2018, supp 3B. For the weeks describe above

**A group of circles with numbers and numbers

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**A graph of events and events

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Histogram - Distribution of the time spent in each cage for each week described above (Torquet et al., 2018; figure 1 right)

**Social Interactions: nodes and link diagrams**

How much time do they spend together?

Do certain groups of animal’s nests together?

Do the “popular animals” are the ones that know how to nose poke for food?

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Description automatically generatedAnimals that know how to poke are:

A close-up of a black and white box

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Description automatically generatedA close-up of a diagram

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