

Protocol for voluntary tunnel handling of mice

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1. General idea

To reduce stress we use tunnels to handle laboratory mice. In the following, we describe how to systematically habituate mice to this procedure within two weeks.

2. References

Gouveia and Hurst 2013 (<https://doi.org/10.1371/journal.pone.0066401>)

Gouveia and Hurst 2017 (<https://doi.org/10.1038/srep44999>)

Hurst and West 2010 (<https://doi.org/10.1038/NMETH.1500>)

and own experiences

3. Introduction

The protocol described here is a modified form of handling methods described in the papers mentioned above. The main changes are that

- mice voluntarily enter the tunnel; they will only be guided with the hand into the tunnel if they do not enter voluntarily
- mice voluntarily leave the tunnel, i.e. they are not tipped out, instead one end of the tunnel is closed with a hand and the other end is slightly tilted downwards

Note that this protocol was developed to match our husbandry and experimental conditions. Thus, we use comparatively long tunnels, which are also provided in the home cages as enrichment. During the handling training the mice are transferred from their home cage to a separate cage and back to the home cage. Our protocol is only meant as an illustration of the procedure. It can be adjusted to other needs, e.g., by transferring the mice directly back to the home cage.

4. Material and Equipment

- Home cage including equipment, lid, and filter top
- Tunnel for handling (e.g., a transparent Perspex tube, 4 cm in diameter, 17.5 cm in length)
- Scale, weighing dish, cover for the weighing dish

5. Procedure

First Weighing

- Directly after the arrival, i.e., mice are still in the transport box
- Including health check and fixation

Preparation

- Prepare new home cage and put it nearby

Implementation

- Place hand for 30 seconds in the transport box
- Fill weighing dish with soiled bedding from the transport box
- Because the mice are unfamiliar with the tunnel yet, mice are picked up by their tails
 - Take hold of the base of the tail
 - Place hand or arm below the mouse to relief weight
 - If a mouse is difficult to take hold of, place one hand in front of the mouse to slow it down and grip the base of the tail
 - Very young mice might be picked up directly by hand
- Place mice in weighing dish and note its weight
 - A cover might be necessary to prevent mice from climbing out of the weighing dish
- Place the mouse on the lid of the home cage for fixation and health check, and directly transfer mice to the new home cage

Phase 1

Duration: one week (five workdays), can be prolonged as needed

Frequency: once a day

Characteristics of handling: separate cage with soiled bedding and house from the home cage

Characteristics of cleaning: home cage bedding in weighing dish

Phase 2

Duration: one week (five workdays), can be prolonged as needed

Frequency: once a day

Characteristics of handling: separate cage with soiled bedding from the home cage

Characteristics of cleaning: home cage bedding in weighing dish

Phase 3/End phase

Duration: after the end of phase 2 ongoing

Frequency: as needed

Characteristics of handling: separate cage with soiled bedding from the home cage

Characteristics of cleaning: weighing dish without home cage bedding

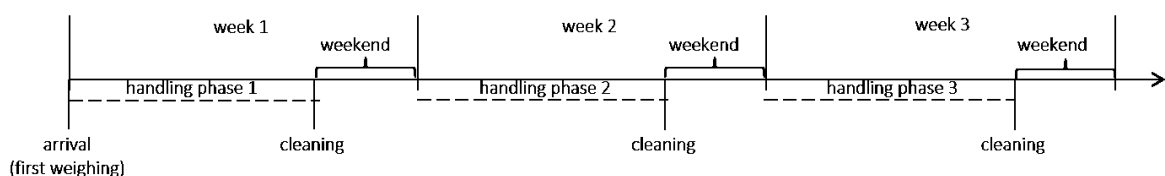


Fig. 1: Example of a time line for training the voluntary tunnel handling. In this example, the mice arrive at a Monday, and cleaning procedures take place once per week on Fridays. This procedure can be adjusted according to the situation.

Handling

- Once per day between 8 and 10 AM

Preparation

- Put separate cage nearby

Implementation

- Place a hand for 30 seconds in the home cage
- Prepare the separate cage:
 - Phase 1: take soiled bedding (ca. 1-2 hand full) from the home cage and scatter it in the separate cage, transfer the house from the home cage to the separate cage
 - Phase 2 and Phase 3: take bedding (ca. 1-2 hand full) from the home cage and scatter it in the separate cage
- Habituation to the tunnel
 - According to our experience, mice habituate faster to tunnel handling if the tunnel is permanently located inside the home cage
 - If the tunnel is not part of the home cage, we suggest to run some bedding material through the tunnel to make the tunnel smell familiar
- Place the tunnel in front of a mouse; if it does not voluntarily enter the tunnel, gently guide the mouse with the hand into the tunnel
- Lift the tunnel for 30 seconds
 - Phase 1 and 2: during lifting the ends of the tunnel are not closed; optionally the opening behind the mouse can be closed to prevent the animal from going backwards
 - Phase 3: both tunnel ends are closed with the hands during lifting
- Place the mouse into the separate cage
 - To allow the mice to leave the tunnel voluntarily, close one end of the tunnel with a hand and tilt the other downwards (maximum 45 °)
- After handling all mice, put the house back into the home cage
- Directly place all mice back into the home cage using the tunnel; this time, the tunnel is not lifted for 30 s

Cleaning

- The health check can be conducted during the handling session

Preparation

- Fill the new home cage with bedding material
- Place a new house, nesting material and additional equipment in the new cage
- Put a new lid nearby

Implementation

- Place the hand for 30 seconds in the home cage
- Scatter a hand full of soiled bedding material from the old home cage in the new one
- Prepare the weighing dish:
 - Phase 1 and Phase 2: place a hand full of bedding from the old home cage in the weighing dish
 - Phase 3: no bedding in weighing dish
- Pick up the mice from home cage using a tunnel

- Place tunnel in front of a mouse; if it does not voluntarily enter it, gently guide the mice into the tunnel
- Place mouse in weighing dish and note its weight
 - To allow the mouse to voluntarily leave the tunnel, close one end of the tunnel with a hand and tilt the other downwards (maximum 45 °)
 - To allow the mouse to re-enter the tunnel, place tunnel in front of the weighing dish. If the mouse does not voluntarily re-enter the tunnel, it can be guided with a hand.
- Let the mouse re-enter the tunnel and lift it for 30 seconds
 - Phase 1 and Phase 2: during the lifting the ends of the tunnel are not closed; optionally the opening behind the mouse can be closed to prevent the animal from going backwards
 - Phase 3: both tunnel ends are closed with the hands during the lifting
- When the mouse is in the tunnel, the health check can be conducted
- Place the mouse into the new home cage