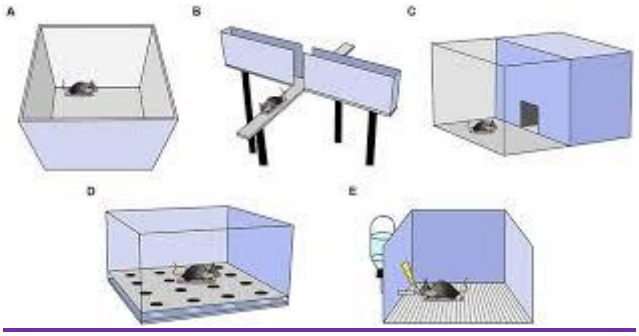

Open Field Test (OFT) – Assessing General Locomotion and Anxiety

Objective:

To evaluate exploratory behavior, locomotion, and anxiety-related responses in rodents.

Equipment:

- Open-field arena (standard size: 40 cm × 40 cm × 40 cm)
- **EthoVision** tracking software
- Overhead camera

Procedure:

1. Allow the subject to acclimate to the testing room for at least 30 minutes before testing.
2. Place the rodent in the center of the open field.
3. Record movement for **10–15 minutes** using EthoVision.
4. Analyze total distance traveled, time spent in the center vs. periphery, and movement patterns.

Expected Results:

- **High center time & increased locomotion** → Reduced anxiety, increased exploration.
- **Low center time & decreased movement** → Higher anxiety-like behavior.

Elevated Plus Maze (EPM) – Assessing Anxiety

Objective:

To measure anxiety-related behavior in rodents based on their willingness to explore open vs. enclosed arms.

Equipment:

- Elevated plus maze (two open and two closed arms, elevated 50 cm above ground)
- Overhead camera and **EthoVision** software

Procedure:

1. Place the rodent in the center of the maze facing an open arm.
2. Allow free exploration for **5 minutes** while recording behavior.
3. Measure time spent in open vs. closed arms and total entries into each.

Expected Results:

- **More time in open arms** → Reduced anxiety.
- **More time in closed arms** → Higher anxiety levels.

Rotarod Test – Assessing Motor Coordination and Balance

Objective:

To evaluate motor coordination, balance, and fatigue resistance.

Equipment:

- **Rotarod apparatus** with adjustable speed
- Timer

Procedure:

1. Train the subject for **two days** with increasing speed (from 5 to 40 rpm).
2. For testing, place the rodent on the rotating rod at a starting speed of 5 rpm.
3. Gradually increase the speed until the subject falls off.
4. Record the latency to fall over **three trials per day**.

Expected Results:

- **Longer latency to fall** → Better motor coordination.
- **Shorter latency** → Motor impairment.

Morris Water Maze (MWM) – Assessing Spatial Learning and Memory

Objective:

To measure learning and memory based on the ability to locate a hidden platform using spatial cues.

Equipment:

- Circular water tank (diameter: 120 cm)
- Hidden platform (submerged ~1 cm below water surface)
- **EthoVision** tracking system

Procedure:

1. Train the rodent for **4–5 days** with four trials per day.
2. Place the subject in the water at different quadrants and allow **60 seconds** to find the platform.
3. If the subject fails, guide it to the platform and let it rest for **15 seconds**.
4. On the **probe trial (Day 5 or 6)**, remove the platform and measure time spent in the target quadrant.

Expected Results:

- **Shorter escape latency** → Efficient learning.
- **More time in the target quadrant** → Good memory retention.

CatWalk Gait Analysis – Assessing Locomotor Function

Objective:

To analyze gait patterns, weight distribution, and limb coordination.

Equipment:

- **CatWalk XT system** (automated gait analysis system)

Procedure:

1. Train the rodent to walk across the CatWalk platform.
2. Record **three successful runs** per subject.
3. Analyze parameters like step cycle, stride length, swing speed, and paw intensity.

Expected Results:

- **Regular stride and symmetrical weight distribution** → Normal motor function.
- **Irregular gait patterns or asymmetry** → Neuromotor deficits.

Social Interaction Test – Assessing Social Behavior

Objective:

To evaluate social motivation and interaction in rodents.

Equipment:

- Three-chamber apparatus
- Social stimulus (age- and sex-matched rodent)
- **EthoVision** tracking software

Procedure:

1. Place the test subject in the middle chamber and allow habituation.
2. Introduce a novel conspecific into one of the side chambers.
3. Allow exploration for **10 minutes** while tracking time spent in each chamber.

Expected Results:

- **More time spent near the conspecific** → High social motivation.
- **Avoidance of the conspecific** → Social withdrawal or deficits.

Fear Conditioning Protocol – Assessing Associative Learning and Memory

Objective:

To evaluate associative learning and memory by measuring an animal's ability to link a neutral stimulus (tone) with an aversive stimulus (mild foot shock).

Equipment:

- Fear conditioning chamber (equipped with metal grid floor for foot shocks)
- Speaker for auditory cue (tone)
- Overhead camera and **EthoVision** tracking software
- Software for automated freezing detection

1. Habituation (Day 1 - Optional)

- Place the rodent in the conditioning chamber for **5 minutes** with no stimuli.
- This step helps reduce baseline anxiety unrelated to fear conditioning.

2. Conditioning Phase (Day 2 - Training Day)

Procedure:

1. Place the rodent in the conditioning chamber and allow it to explore for **2 minutes** (baseline recording).
2. Play an auditory tone (e.g., 80 dB, 5 kHz) for **30 seconds**.
3. In the last **2 seconds of the tone**, deliver a **mild foot shock (0.4–0.7 mA, 2 seconds)**.
4. Repeat this **tone-shock pairing 2–5 times**, with **30–60 seconds inter-trial intervals** (ITI).
5. Leave the rodent in the chamber for **1–2 minutes** before returning it to its home cage.

3. Contextual Fear Test (Day 3 - 24 Hours After Training)

Objective:

To test memory for the training context.

Procedure:

1. Return the rodent to the **same chamber** without any auditory cue or foot shock.
2. Record behavior for **5 minutes** and measure **freezing behavior** (complete immobility except for respiration).

Expected Results:

- **Increased freezing behavior** → Indicates strong memory of the fear-inducing environment.
- **Reduced freezing** → Impaired contextual fear memory.

4. Cued Fear Test (Day 4 - Tone Recall Test in a Novel Context)

Objective:

To assess memory of the auditory cue independent of the original environment.

Procedure:

1. Place the rodent in a **new chamber** (alter context by changing floor texture, wall color, or adding a new scent like vanilla).
2. Allow the rodent to explore for **3 minutes** (baseline recording).
3. Play the **same auditory tone** used in training (without a shock) for **3 minutes**.
4. Measure **freezing behavior** during the tone presentation.

Expected Results:

- **High freezing during tone presentation** → Strong memory of the tone-shock association.
- **Low freezing** → Impaired associative fear memory.

Data Analysis

- **Primary Measure**: Percentage of time spent freezing during each phase (baseline, post-shock, contextual recall, cued recall).
- **Software**: EthoVision or automated freezing detection software (e.g., FreezeFrame, ANY-maze).

Light-Dark Box Protocol – Assessing Anxiety and Exploration

Objective:

To measure anxiety-related behavior in rodents by assessing their preference for dark vs. light environments. This test relies on rodents' natural tendency to prefer dark, enclosed spaces, and it is commonly used to evaluate anxiety-like responses.

Equipment:

- **Light-Dark Box**: A divided arena with a dark chamber and a light chamber, usually made of opaque material with one side illuminated (e.g., 300 lux).
- **EthoVision** tracking software
- Overhead camera

1. Habituation (Day 1 - Optional)

- Place the rodent in the **center** of the light chamber to allow acclimatization for **5 minutes** without any stimuli.
- This step helps reduce baseline anxiety and prepares the animal for testing.

2. Testing Phase (Day 2 - Anxiety Test)

Procedure:

1. Start the test by placing the rodent in the **light side** of the box.
2. Allow the rodent to freely explore the two chambers for **10–15 minutes** while the light stays on in the light chamber.
3. Track the following behaviors:
    - **Time spent in the light chamber** (less anxious behavior).
    - **Time spent in the dark chamber** (anxious behavior).
    - **Entries into both chambers**.

Expected Results:

- **More time spent in the dark chamber** and fewer entries into the light chamber → Increased anxiety.
- **More time spent in the light chamber** → Reduced anxiety and increased exploration.

3. Data Analysis

- **Primary Measures**: Time spent in each chamber, number of entries into each chamber, and total distance traveled.
- **Software**: EthoVision can be used to analyze the amount of time spent in each compartment and to track locomotor activity.

Barnes Maze Protocol – Assessing Spatial Learning and Memory

Objective:

To assess spatial learning and memory by measuring an animal's ability to locate a hidden escape hole based on spatial cues.

Equipment:

- **Barnes Maze apparatus**: A circular platform (90 cm diameter) with 12 equally spaced holes.
- **Escape box**: A small, darkened box placed under one of the holes.
- **EthoVision** tracking software
- Overhead camera

1. Habituation (Day 1)

Procedure:

1. Place the rodent on the center of the Barnes maze with **no escape box** for **2–3 minutes** to reduce initial anxiety.
2. After a brief habituation, place the **escape box** in one of the holes and allow the rodent to explore the maze for **5 minutes**.
3. Do not provide any negative stimuli. Simply guide the rodent to the escape hole if it cannot find it within the time limit.

2. Acquisition Phase (Days 2-4)

Procedure:

1. Place the rodent in the **center of the maze** with the **escape box** placed under one of the holes.
2. Allow the rodent to explore for a **maximum of 3 minutes** or until it enters the escape box.
3. If the rodent fails to find the escape hole within the allotted time, guide it to the escape hole to ensure it learns the location.
4. Repeat the trial for **4–6 consecutive days**, and record the following metrics:
    - **Latency to find the escape hole** (time to reach the hole).
    - **Total distance traveled**.
    - **Number of entries into incorrect holes** (indicating learning efficiency).

3. Probe Test (Day 5 or Last Day of Acquisition)

Objective:

To assess memory retention of the escape location after several days of training.

Procedure:

1. On the final day (or **Day 5**), remove the escape box from the maze and place the rodent in the center.
2. Allow the rodent to explore the maze for **5 minutes**.
3. Measure the **time spent in the target quadrant** (where the escape hole was located).
4. Track **number of entries into the target hole** and general exploration patterns.

Expected Results:

- **Shorter latency to find the escape hole** and fewer entries into incorrect holes → Better learning and memory.
- **Increased time in the target quadrant** during the probe test → Good memory retention of the escape location.

4. Data Analysis

- **Primary Measures**: Latency to find the escape hole, distance traveled, number of incorrect entries, and time spent in the target quadrant.
- **Software**: EthoVision can automatically track the rodent's path, latency, and entries into each hole.