

# Bioassay of Anxiolytic Activity



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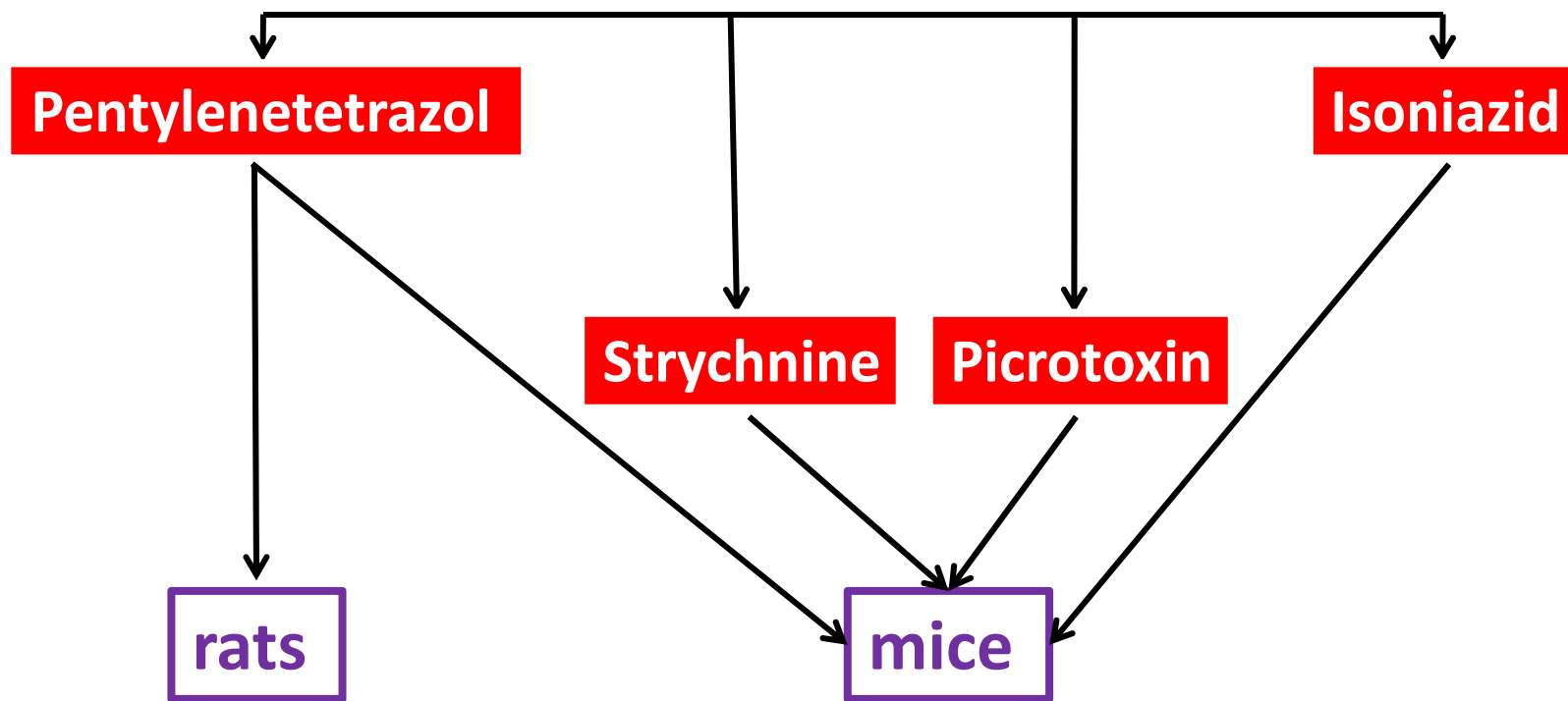
# General Considerations

- **Anxiolytics** or minor tranquilizers are drugs used for the **treatment** of anxiety.
- They act on the **limbic system** at appropriate doses.
- **Pathological anxiety** is manifested as
  1. **somatic** disorders
  2. **emotional** discomfort that **interfere** with normal life

# General Considerations

- The **complex** characterization of anxiety necessitates performing **several** tests.
- For *in vivo* studies use a **battery** (several) of
  1. **anticonvulsive** test
  2. **anti-aggressive** test
  3. **behavioral** test

# 1. Anticonvulsant Activity Tests



## 2. Anti-aggressive Tests

- Tests that measure the effect of anxiolytics on experimentally induced disturbed behavior.
- These tests evaluate the **animal interaction** (i.e. 2 animals are present at the same time)
- A compound exerting anxiolytic activity reduces experimentally induced aggressive behavior.

## 2. Anti-aggressive Tests

1. **Foot shock**-induced Aggression
2. **Isolation**-induced Aggression

## 2. Anti-aggressive Tests

### a. Foot shock-induced Aggression

#### Principle

animal interaction → aggression responses

Anxiolytics → ↓ aggression responses

## 2. Anti-aggressive Tests

### a. Foot shock-induced Aggression

#### Procedure

two male mice



box with grid floor (steel rods)



3min intermitted electrical current  
→ grid floor



Fight response



*Foot shock-induced aggression apparatus*

aggressometer

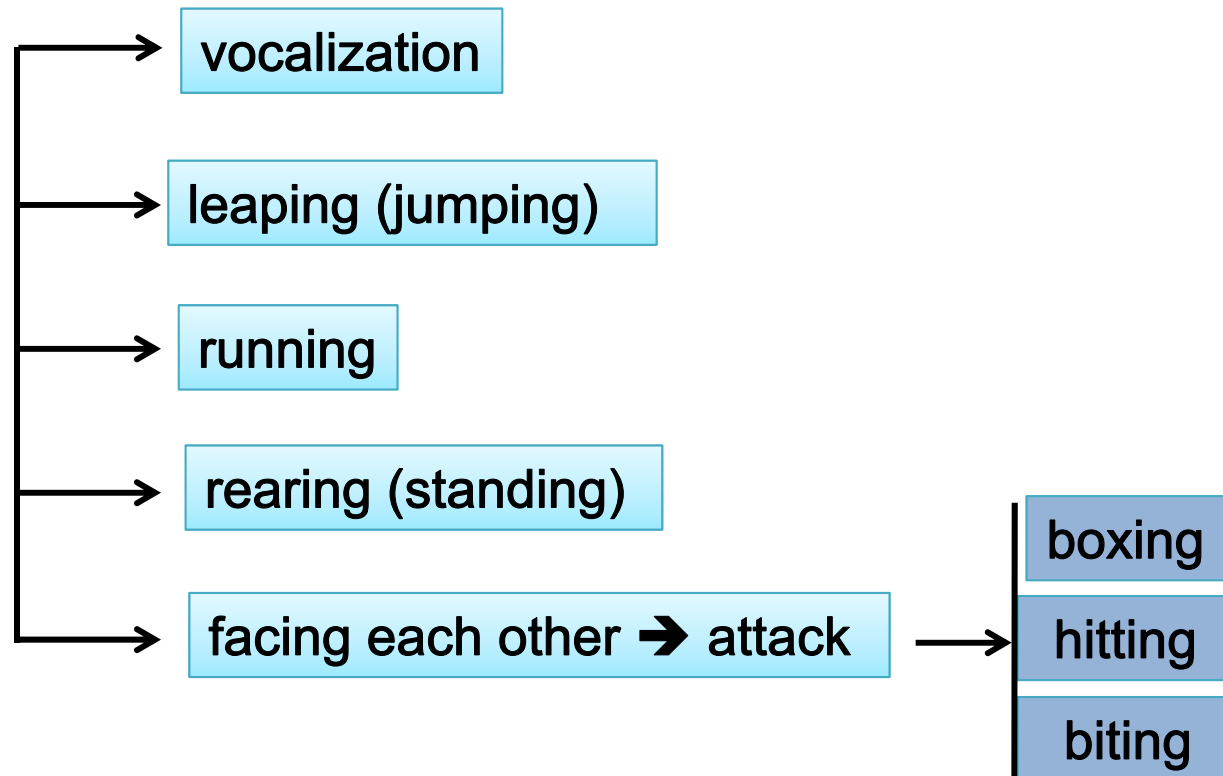


## 2. Anti-aggressive Tests

### a. Foot shock-induced Aggression

#### Evaluation

#### Fight response



## 2. Anti-aggressive Tests

### a. Foot shock-induced Aggression

#### Evaluation

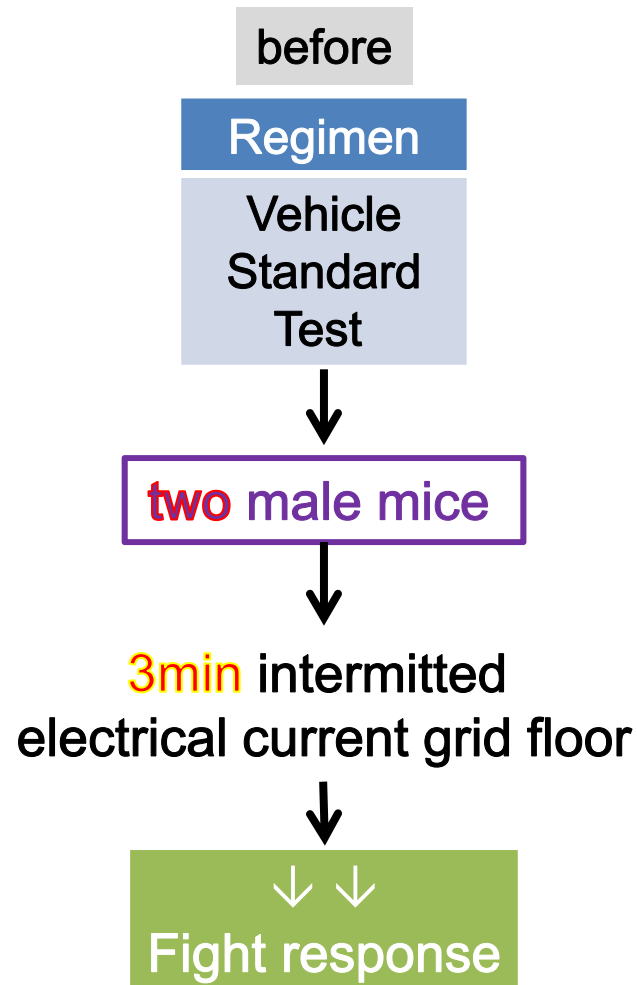
For each  
animal **pair**

Fight response  
(within **3min**)

1. **total number** of fights
2. **percent inhibition** of aggression from control (model)

## 2. Anti-aggressive Tests

### a. Foot shock-induced Aggression



## 2. Anti-aggressive Tests

### a. Foot shock-induced Aggression

**Anxiolytic activity**



**number of animals with fighting response in 3min**

**>>>> percent inhibition of aggression from control (model)**

## 2. Anti-aggressive Tests

### b- Isolation-induced Aggression

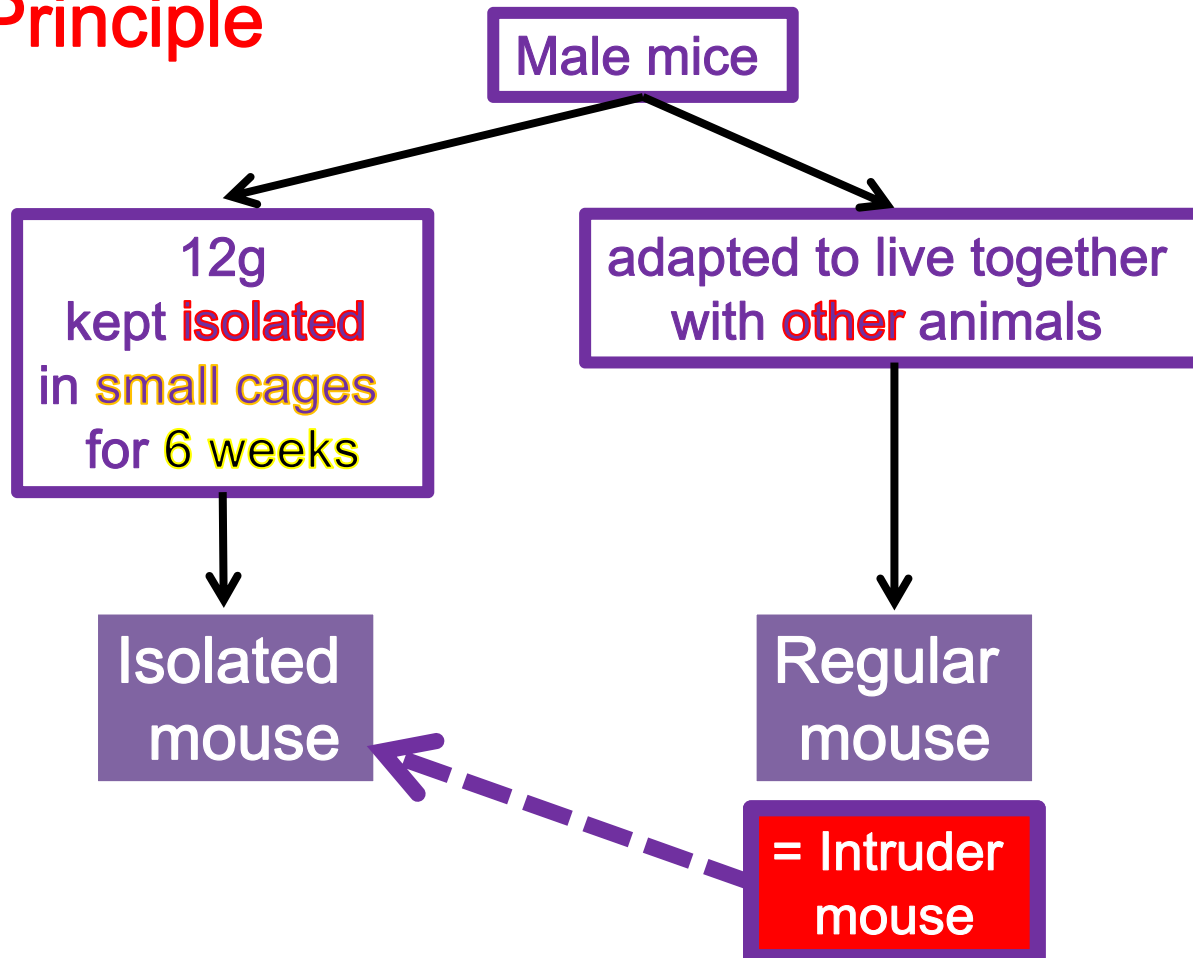
#### Principle

- This test evaluates **experimentally-induced** aggressive behavior.
- Male mice, submitted to **prolonged isolation**, develop **aggressive behavior** against animals of the **same sex** .
- Anxiolytics decrease aggression responses.

## 2. Anti-aggressive Tests

### b- Isolation-induced Aggression

#### Principle



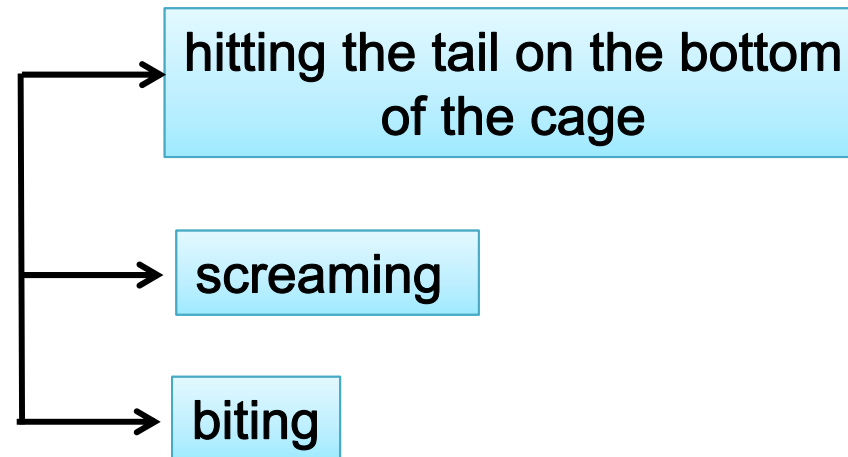
*apparatus*

## 2. Anti-aggressive Tests

### b- Isolation-induced Aggression

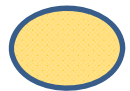
#### Evaluation

Aggressive behavior  
of isolated mouse



N.B.

**REACTION TIME** until the **first attack** <<<  
10s  
+  
relatively **constant** for each mouse



# 2. Anti-aggressive Tests

## b- Isolation-induced Aggression

### Procedure

Aggressive behavior

is evaluated for

Isolated mouse

when placing an

= Intruder mouse

in its cage

Aggressive behavior

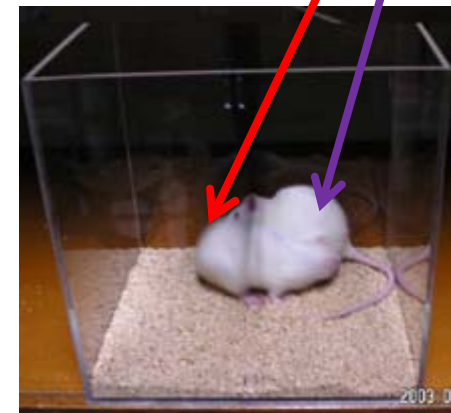
is evaluated

before

Regimen

Vehicle  
Standard  
Test

after





## 2. Anti-aggressive Tests

### b- Isolation-induced Aggression

**Aggressive response**

number of animals with  
fighting behavior << 10s

**Anxiolytic activity**

>>>>  
number of animals with  
complete suppression of the  
fighting behavior within 10 s

## 3. Evaluation of Behavior

- a. **Light-dark Model**
- b. **Elevated Plus Maze Test**
- c. **Open Field Locomotor Activity**
- d. **Rotarod Test**

## 3. Evaluation of Behavior

### a. Light-dark model

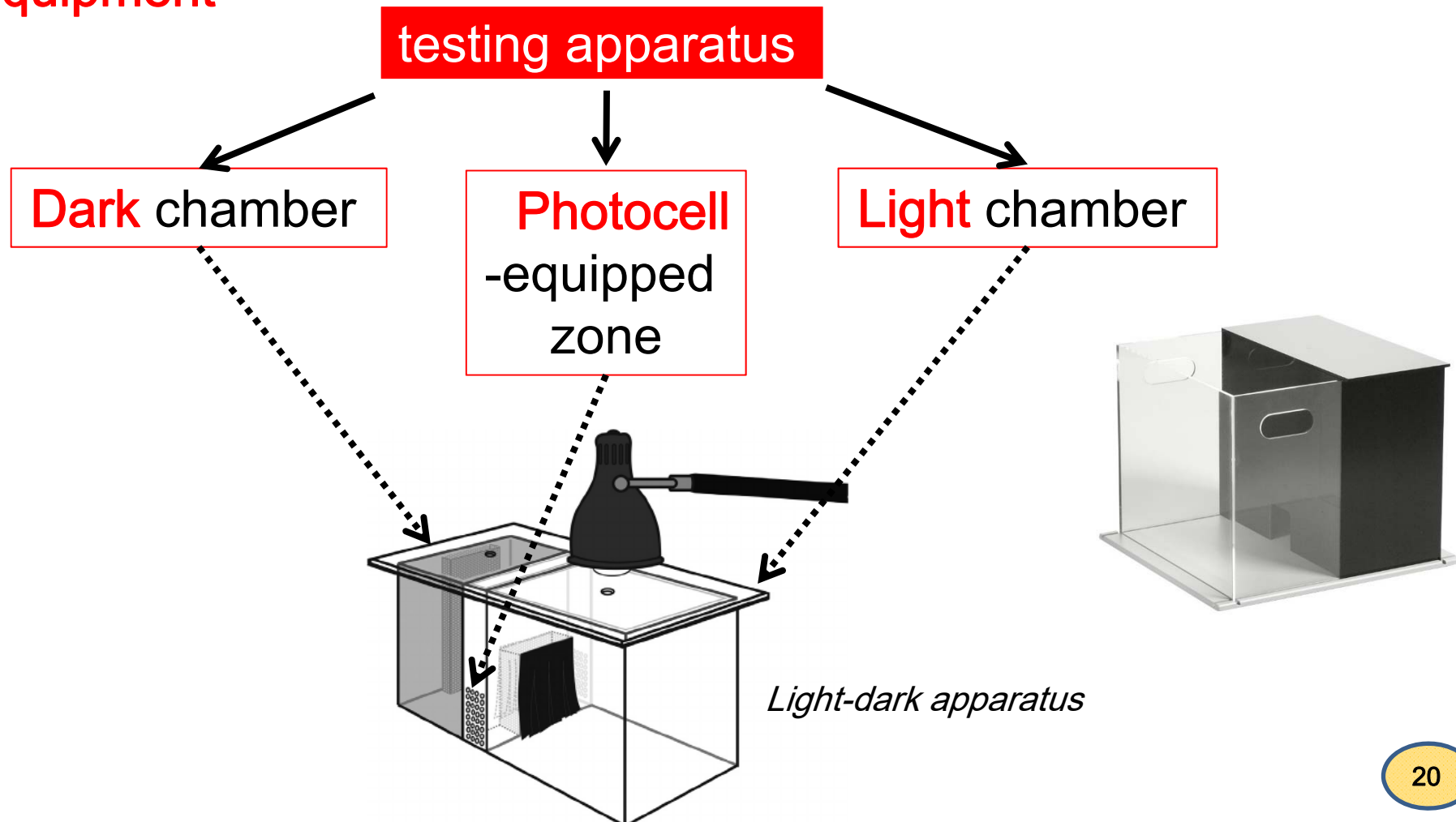
#### Principle:

- **Mice** and **rats** tend to EXPLORE a NOVEL (new) ENVIRONMENT
- They **avoid** from a **brightly-lit open field**
- Anxiolytics **increase crossings** to the brightly-lit open field.

# 3. Evaluation of Behavior

## a. Light-dark model

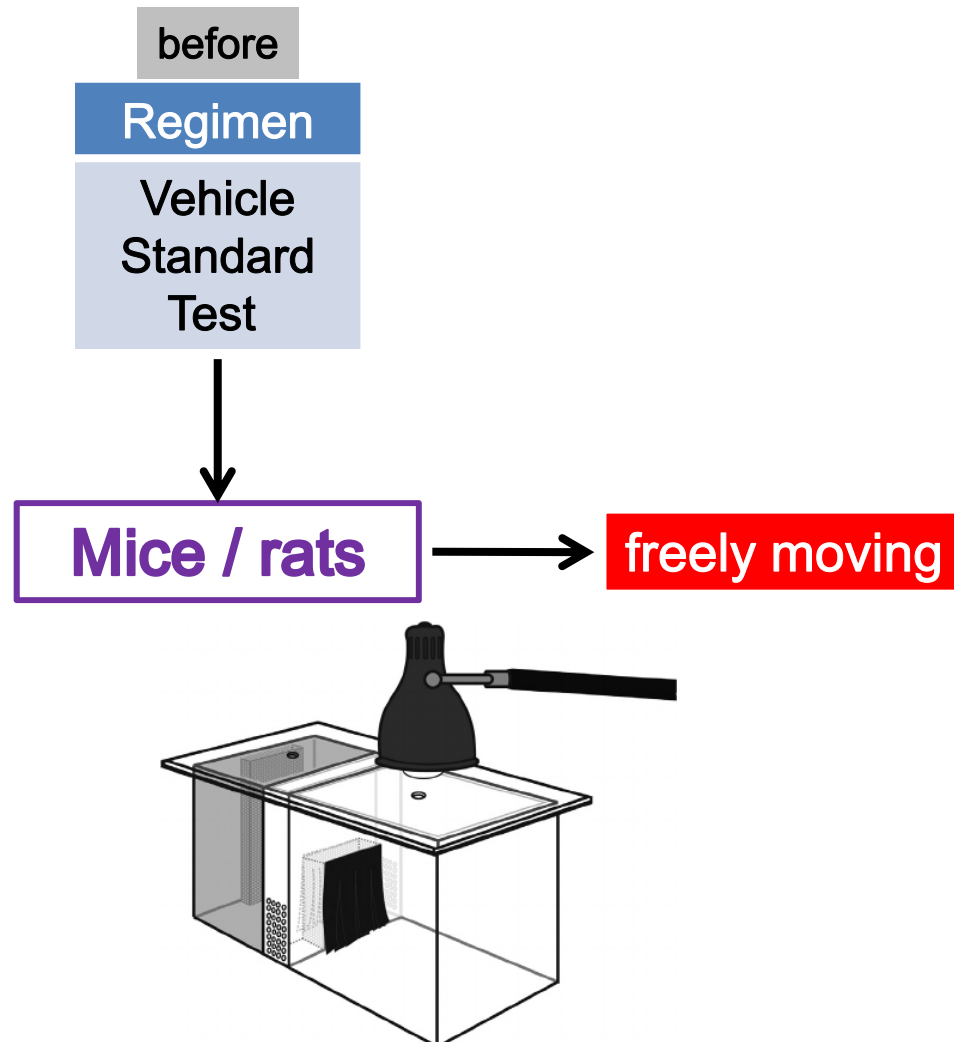
### Equipment



# 3. Evaluation of Behavior

## a. Light-dark model

### Procedure



# 3. Evaluation of Behavior

## a. Light-dark model

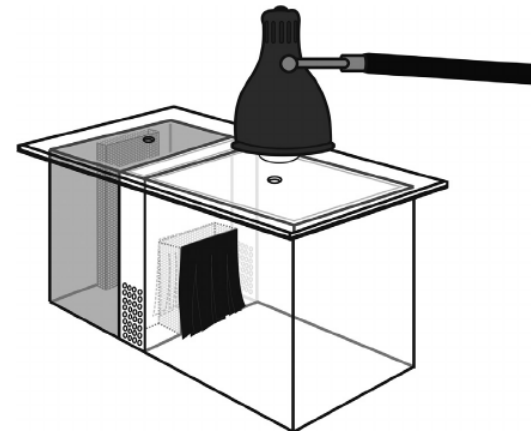
### Evaluation

Normal mice / rats

freely move across the  
photocell-equipped zone

<<<<<

number of animals with  
crossing to the light-chamber



# 3. Evaluation of Behavior

## a. Light-dark model

Evaluation

anxiolytics → more locomotion → more crossings



↑ numbers of crossings  
which is counted by the photocell

**Anxiolytic activity**

>>>>>

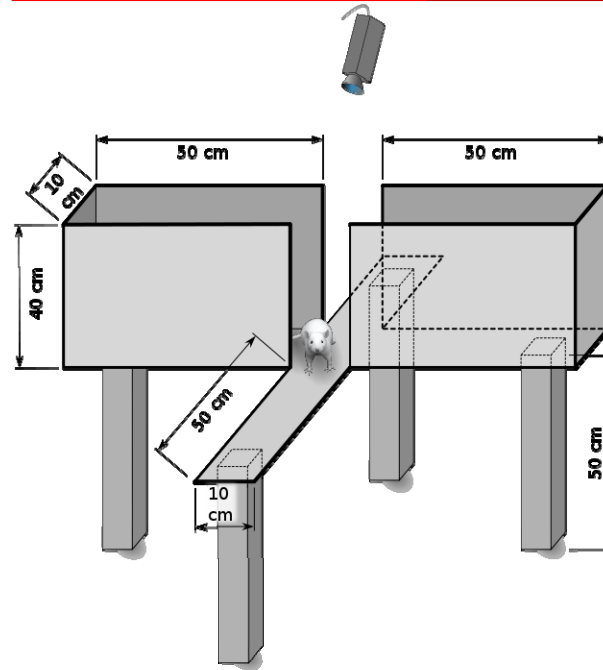
number of animals with  
crossing to the light-chamber

# 3. Evaluation of Behavior

## b. Elevated Plus Maze Test

### Principle:

- Anxiolytic compounds, by decreasing anxiety, increase the OPEN ARM EXPLORATION time.



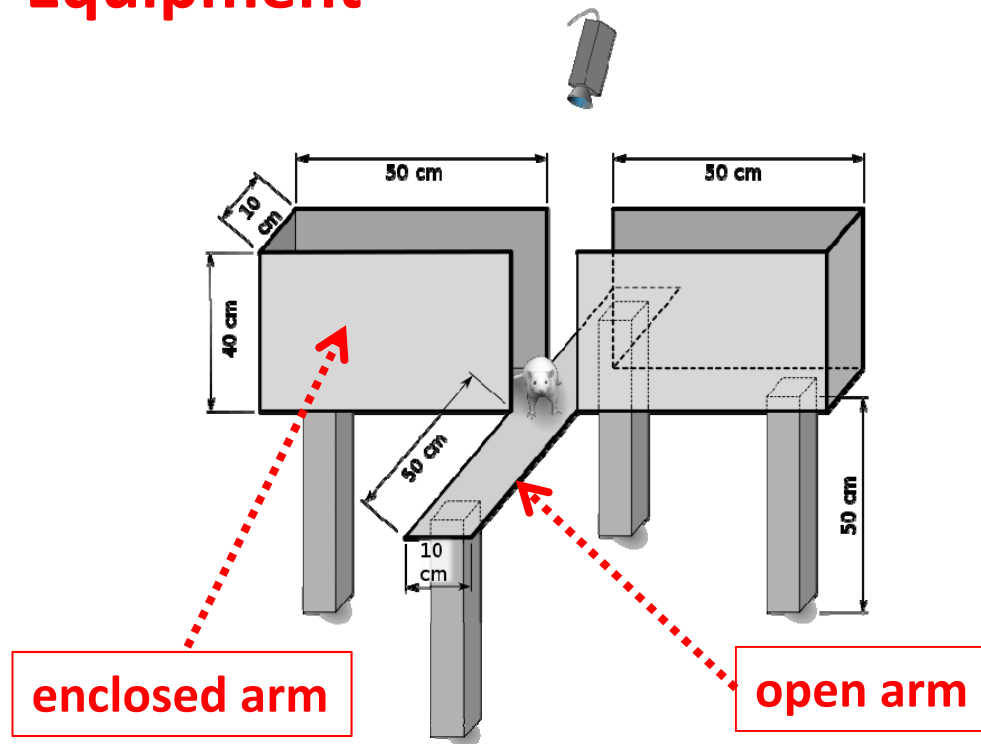
*Elevated plus maze test apparatus*



# 3. Evaluation of Behavior

## b. Elevated Plus Maze Test

### Equipment

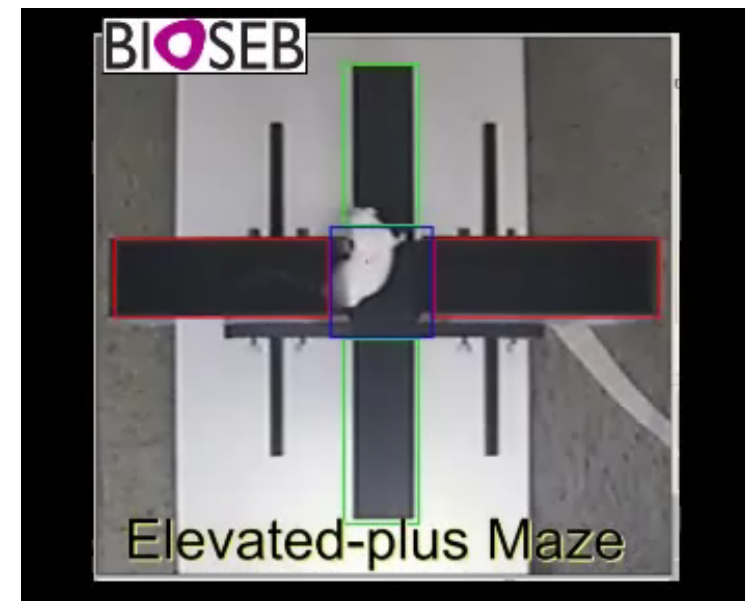


*Elevated plus maze test apparatus*

### Elevated plus maze

is elevated to a height of **50 cm**

with 4 **opposite** arms to each other



## 3. Evaluation of Behavior

### b. Elevated Plus Maze Test

#### Procedure

Rats

are **housed in pairs**  
10 days prior to testing

**handled** by the investigator on  
**alternate** days to reduce stress

# 3. Evaluation of Behavior

## b. Elevated Plus Maze Test

### Procedure

in a sound attenuated room

Rats

placed in the **center** of the maze

facing one of the **enclosed** arms

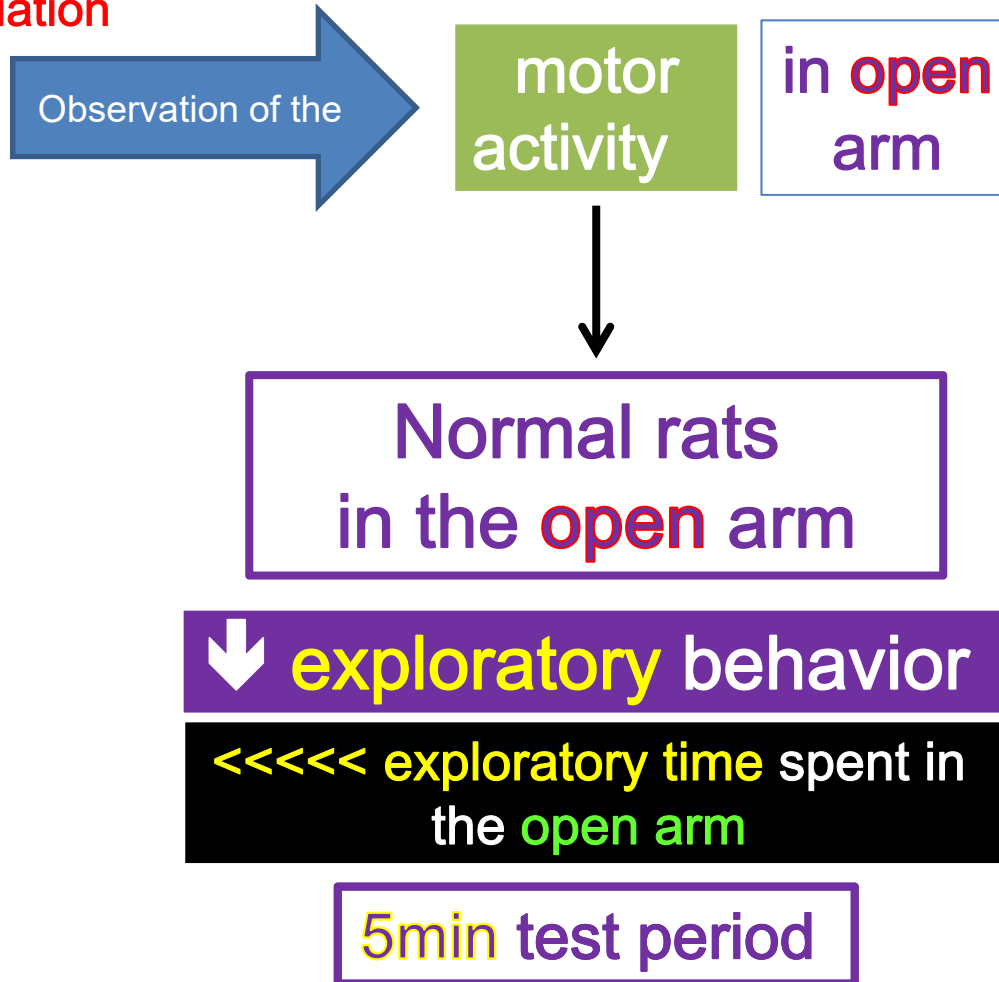
animal behavior  
within 5min remote TV camera

observations made from  
an adjacent room

# 3. Evaluation of Behavior

## b. Elevated Plus Maze Test

Evaluation



## 3. Evaluation of Behavior

### c. Open Field Locomotor Activity

#### Principle:

- In **rodents**, **open field activity** tests are used to
  1. measure **locomotor activity**
  2. determine **motor deficits**
  3. determine **anxiety**
  4. determine effects of **anxiolytic drugs**
  5. test **hippocampal damage** (stroke)
  6. test **basal ganglia damage** (Parkinson)

# 3. Evaluation of Behavior

## c. Open Field Locomotor Activity

### Procedure

Rodents

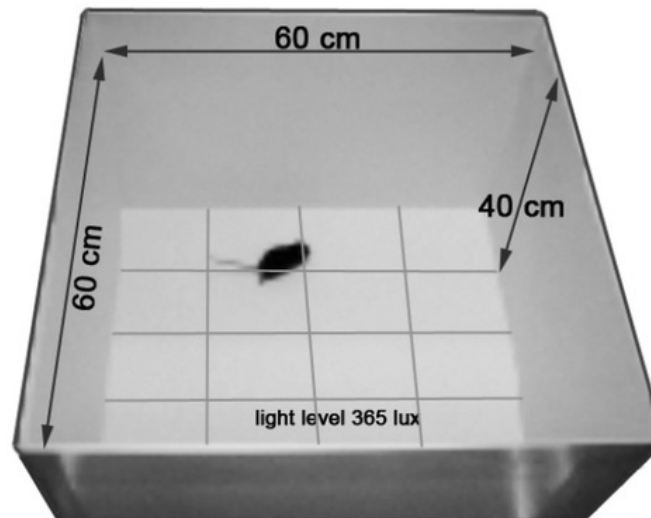
1

Locomotor activity

2

Pattern of exploration

in open field  
within 5min



*Open field apparatus*

# 3. Evaluation of Behavior

## c. Open Field Locomotor Activity

Evaluation

Observation

in open field within 5min

Normal rodents

1- Locomotor activity  
(Distance traveled within 5min)

2- Pattern of  
exploration  
within 5min

Center versus periphery

Rearing (stand) total number

Grooming (clean) total number

# 3. Evaluation of Behavior

## c. Open Field Locomotor Activity

Evaluation

anxiolytics

↑ Locomotion Activity  
↑ Center occupation

**Anxiolytic activity**

>>>>> total distance traveled in 5min  
>>>>>locomotion

>>> rearing, grooming & center  
occupation  
[in 5min test period]



## 3. Evaluation of Behavior

### d. Rotarod Test

#### Principle:

In **rodents**, this is the most widely used test to assess

1. sensorimotor **coordination**
2. **motor** function
3. experimental **basal ganglia** damage
4. experimental **cerebellum** damage

# 3. Evaluation of Behavior

## d. Rotarod Test

Equipment

**Rotarod**

a horizontally oriented rotating cylinder

**Rodents**

naturally

try to stay on  
the rotating cylinder

till they fall



*Rotarod apparatus*

## 3. Evaluation of Behavior

### d. Rotarod Test

#### Procedure

Rodents

placed on a rotating Rotarod

till they fall  
(Fall latency)

# 3. Evaluation of Behavior

## d. Rotarod Test

Evaluation

↓ Fall latency

A. damage

- 1- basal ganglia
- 2- cerebellum

B. ↓ motor function

- 1- muscle coordination
- 2- muscle relaxation

C. anxiolytic activity

(dt muscle relaxation together

with taming/calming effect)