

### **TP3: Model of anxiety and evaluation of the anxiolytic effect of ethanol.**

-Anxiety is defined as “a state of intense apprehension, uncertainty, and fear resulting from the anticipation of a threatening event or situation” (American Heritage Medical, 2007).

Animal models of anxiety are based on the assumption that anxiety in animals is comparable to anxiety in humans. Being anxious is an adaptive response to an unfamiliar environment, particularly when confronted with danger or threat. However, pathological variants of anxiety can seriously hamper the daily life of those affected. To study the neurobiological mechanisms of normal anxiety as well as its pathological variations, animal models are essential tools.

**What are the characteristics of an ideal animal model to study anxiety and anxiolytic agents?** First, it should display reduced anxiety when treated with anxiolytics (predictive validity). Second, the behavioral response of an animal model to a threatening stimulus must be comparable to the response known for humans (face validity). And third, the mechanisms underlying anxiety as well as the psychological causes must be the same (constructive validity). Possessing these three characteristics simultaneously is difficult for any animal model.

Given that physiological and behavioral responses to aversive (threatening) stimuli are similar in humans and animals, it can be assumed that animal models may serve at least two distinct purposes: (1) to screen for potential anxiolytic and antidepressant effects of new drugs and (2) to study the specific pathogenetic aspects of the cardinal symptoms of anxiety disorders.

**-Experimental models of anxiety:** there are several models of anxiety, particularly in rodents. They mainly consist of exposing animals to stressful psychological conditions (e.g., separation of animals in isolated cages or continuous exposure to light, noise, etc.) or physiological conditions (e.g. induction of chronic pain) for periods of time. variables.

**-Assessment of anxiety behavior:** Often, by evaluating alterations in the animal's exploration behavior. Example: rodents (mice, rats, etc.), under normal conditions prefer narrow and closed places and avoid light and open spaces. Several tests based on this principle exist to assess the anxious behavior of rodents. In the test of light dark box: the animal is placed on a corner of the illuminated compartment and its movement is recorded for 5 min to evaluate the time spent in the two compartments. The longer the animal is spending time in the dark compartment the more it is expressing an anxiety like behavior and the longer the animal is spending time in the light compartment the more it is expressing an anxiolytic effect.

**Marble burying test:** An assay to describe anxiety or obsessive-compulsive disorder (OCD) behavior. It is based on the observation that rats and mice bury harmful or harmless objects in their litter box. Thus, the more the animals are anxious the more they will bury marbles and vis versa.

To carry out this test, the rodents are placed for thirty minutes in a standard cage filled with 5 cm thick wooden litter with a known number of regularly spaced marble balls. After thirty minutes, the quantity of buried balls is measured. A marble is considered buried if 2/3 of its surface is covered with litter. In the study, there should be at least one group receiving the vehicle and another group receiving the test substance.

Protocol: 10 healthy adult male NMRI mice are separated into 2 cages to form 2 groups (n=5). One of the groups is treated orally with 30% ethanol (0,5ml). The other is treated with distilled water. The behavioral assessment of anxiety of the animals in the 2 groups is carried out using the Marble burying test as indicated above.

-At the end of the experimental session for each animal the number of the buried marbles is registered.

### **Questions**

- Use excel to calculate the mean and the standard deviation of the number of buried marbles for each group. Run a statistical analysis by t-student test.
- Present the obtained results on a histogram.
- Interpret and discuss the obtained results.