BEHAVIOURAL TESTS PERFORMED AT THE LABORATORY OF NEUROBIOLOGY OF CIPF

We perform the following types of studies:

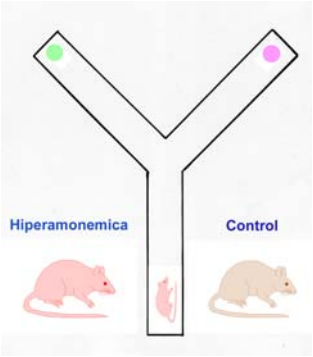
1. **Analysis of cognitive and motor functions in rats**
2. **Study of the alterations of cognitive and motor functions in pathological situations and of the mechanisms responsible for the alterations.**
3. **Evaluation of therapeutic treatments to restore the cognitive and/or motor functions.**
4. **Analysis of the possible secondary effects of drugs on cognitive and motor function.**
5. **Analysis of circadian rhythms of activity and of their alterations in pathological situations or by the use of drugs**
6. **Analysis of the electroencephalogram (EEG) and of their alterations in pathological situations or by the use of drugs**

We perform a wide range of behavioural studies in rats to analyze different aspects of cognitive and motor functions.

A. Behavioural tests to evaluate cognitive functions

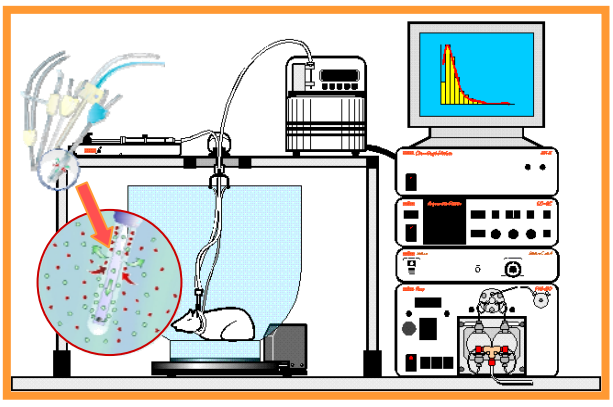
Different types of learning and memory have different mechanisms involving different cerebral areas. We perform the following types of tests to evaluate different aspects of cognitive function:

A.1. Conditional discrimination in a Y maze.



Learning ability is tested as described in (Aguilar et al., 2000) in a wooden Y-shaped maze. The walls of the maze may be white or black. When they are white, food is put on the right; when they are black, on the left. The rats must learn the location of the food depending on the colour of the walls. Rats perform ten trials per day, with an inter-trial interval of approximately 5 min, until the completion of a criterion of ten correct responses in the same day or a maximum of 250 trials.

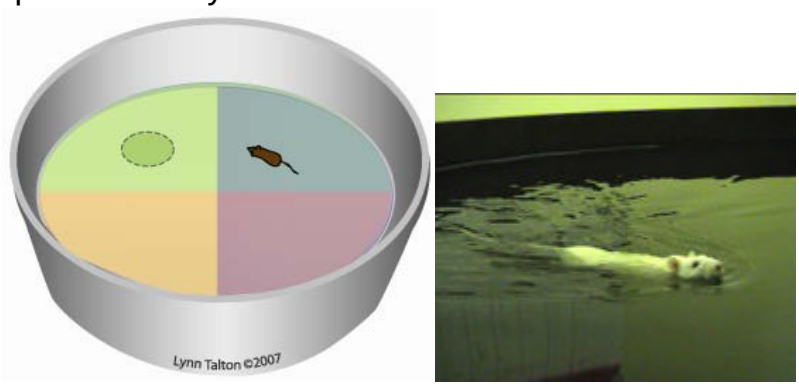
In vivo brain microdialysis



Underlying mechanism: This type of learning is modulated by the function of the glutamate-nitric oxide-cGMP pathway in cerebellum.
We analyze the function of this pathway in brain in vivo by microdialysis in cerebellum of freely moving rats (see for example Hermenegildo et al, 1998; Erceg et al, 2005). We correlate the function of the pathway in vivo with the ability to learn this task in the same rats.

A.2. Spatial learning and memory in the Morris water maze

The Morris water navigation task is a behavioral procedure designed to test spatial memory.



In the typical paradigm, a rat is placed into a small pool of water which contains an escape platform hidden a few millimeters below the water surface. Visual cues, such as colored shapes, are placed around the pool in plain sight of the animal. When released, the rat swims around the pool in search of an exit and on subsequent trials the rat is able to locate the platform more rapidly. This improvement in performance occurs because the rat has learned where the hidden platform is located. The latency to find the platform is measured.

After learning the platform is removed and the memory of the rat is evaluated by measuring the time spent in the quadrant in which the platform was present during learning (Monfort et al, 2007)

A.3. Object recognition memory

This test measures the ability of rats to recognize a novel object in an otherwise familiar environment. This is a very common test to assess object memory.
In the novel object recognition test, the interest of an animal in a novel object versus a familiar one, is measured and compared.

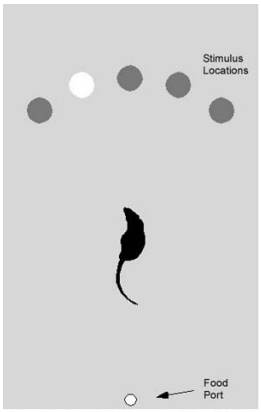
The time spent by the animal exploring each object is measured. If the exploration of the novel and the familiar object is equal, this can be interpreted as a memory deficit.

A.4. Active avoidance

This task provides a simple way to assess associative learning and memory. The active avoidance task is designed to test the ability of the rat to avoid an aversive event by learning to perform a specific behaviour in response to a stimulus cue.

The rat has to learn to predict the occurrence of an aversive event based on the presentation of a specific stimulus, in order to avoid the aversive event by actively moving to a different compartment. The measures recorded, number of avoidances (the rat crossing to the other compartment during the stimulus signal), number of non-responses (the rat failing to cross to the other compartment during the trial), response latency (latency to avoid or escape), serve as an index of learning and allows memory to be assessed (Aguilar et al, 2000).

A.5. Attention in a 5-choice reaction time task



Five Choice Serial Reaction Time Task (5CSRT) in the Floor Projection Maze

The 5 Choice Serial Reaction Time Task (5CSRT), tests specific types of attention, including sustained, divided, and selective attention (Robbins 2002).
It can be used for measuring various aspects of attentional control over performance with its main measures of accuracy, premature responding, correct response latencies and latency to collect earned food pellets.
The 5-CSRTT is implemented in a specially designed operant chamber with multiple response locations using food reinforcers.

The usual paradigm requires the rats to detect brief flashes of light presented in a pseudorandom order in one of five spatial locations over a large number of trials. If they fail to respond, respond in the wrong hole or at an inappropriate time, a short period of darkness (time-out) is presented as punishment and no reward is delivered (Robbins 2002).

B. Behavioural tests to evaluate motor functions

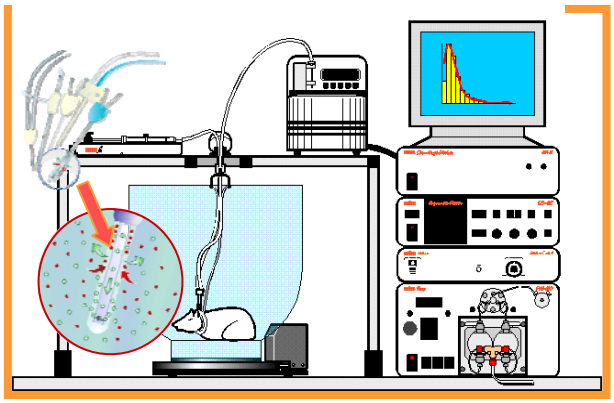
B.1. Motor activity

We determine different parameters of motor activity by using an automatic actimeter (Cauli et al., 2009).
Rats are placed in the actimeter equipped with infrared photocell beams. Rats by moving interrupt the beams. Software allows measuring different parameter of motor activity:

a) ambulatory counts,
b) ambulatory episodes,
c) average velocity,
d) vertical counts,
e) stereotypic counts,
f) jumps, and
g) time spent performing each behaviour

Underlying mechanism: Motor activity is modulated by different mechanisms. A main player in modulation of locomotor activity is dopaminergic neurotransmission in Nucleus Accumbens (NAcc), which, in turn, is modulated by extracellular glutamate through metabotropic glutamate receptors (mGluRs).

In vivo brain microdialysis



We analyze extracellular dopamine and glutamate and modulation of dopamine by mGluRs in NAcc in vivo by microdialysis in freely moving rats.

We correlate these parameters in vivo with motor activity in the same rats (see for example Cauli et al, 2006,2007).

We also analyze by in vivo brain microdialysis in different brain areas, the function of the neuronal circuits modulating motor activity, the sequential activation of the brain areas involved and the neurotransmitters and receptors responsible (see for example Cauli et al, 2006,2007).

B.2. Motor coordination

We are using different tests to assess motor coordination in rats:

B.2.1. Rotarod

The Rotarod Performance test is based on a rotating rod with forced motor activity. The Rotarod Performance test evaluates balance and sensorymotor coordination of the subjects.

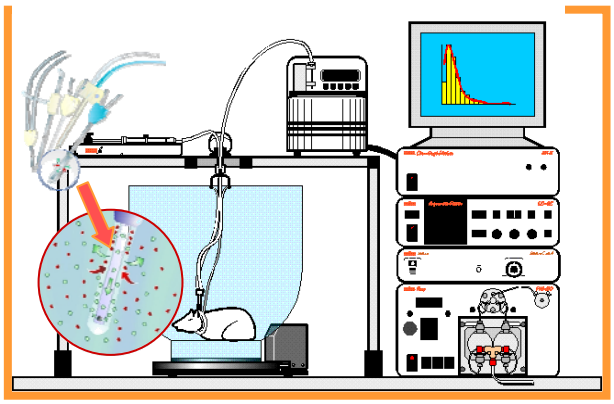
The rat is placed on a horizontally oriented, rotating cylinder (rod) suspended above a cage floor, (not high enough to injure the animal, but high enough to induce avoidance of fall).
Rats naturally try to stay on the rotarod, and avoid falling to the ground.
The length of time that the rat stays on this rotating rod is a measure of their balance, coordination, physical condition, and motor-planning.
The speed of the rotarod is motorically driven, and may either be held constant, or accelerated.

We evaluate the alterations in rotarod performance in animal models of pathological situations compared to control rats (Boix et al, 2010).

Because of concern for impairment in human motor behaviour from the use of prescription medications, the rotarod test is frequently used in early stages of drug development to screen-out drugs that might later cause impairment in human driving, etc., which might not be detected epidemiologically in the human population for a very long time.

Underlying mechanism: Motor coordination is mainly modulated in cerebellum. A main player in modulation of motor coordination is GABAergic neurotransmission in cerebellum. Increased activation of GABA receptors impairs motor coordination.

In vivo brain microdialysis



We analyze extracellular GABA and glutamate in cerebellum in vivo by microdialysis in freely moving rats.

We correlate these parameters in vivo with motor coordination in the same rats (see for example Boix et al, 2010).

B.2.2 Beam walking

We also assess motor coordination, particularly of the hindlimbs, using the beam walking test.
The rats have to traverse an elevated narrow beam which is suspended between a start platform and their home cage.
The difficulty of this task can be varied by using beams with different shapes and widths (Jover et al., 2006).

B.2.3. Footprint analysis

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The footprint test evaluates the walking pattern of rats and thereby allows to detect gait abnormalities.
Footprint patterns of rats are analyzed after the rat was walking along a narrow corridor (Carter et al, 2001). To obtain footprints, the hind and fore paws are coated with non-toxic paints of different colours.

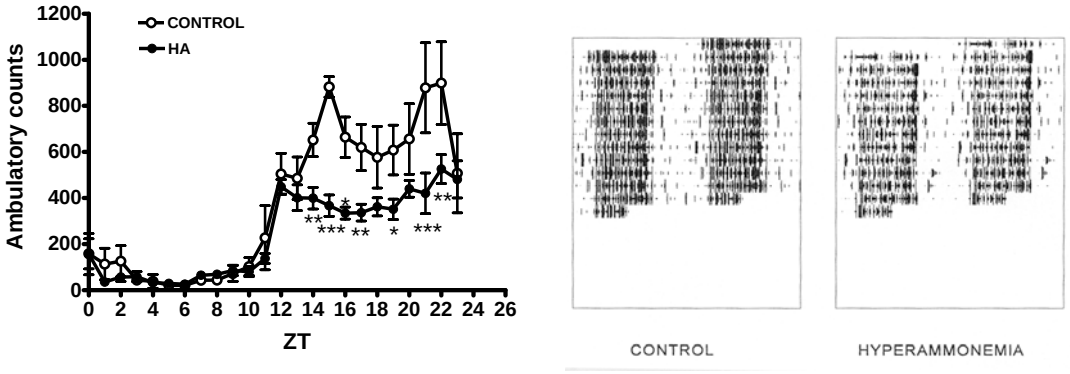
C. Circadian rhythms

We also analyze the circadian rhythms of different parameters, including:

C.1. Circadian rhythm of spontaneous motor activity.

We determine different parameters of spontaneous motor activity by using an automatic actimeter. These parameters are automatically recorded duting around two weeks to analyze their circadian rhythms.
Rats are placed in the actimeter equipped with infrared photocell beams. Rats by moving interrupt the beams. Software allows measuring different parameter of motor activity:

a) ambulatory counts,
b) ambulatory episodes,
c) average velocity,
d) vertical counts,
e) stereotypic counts,
f) jumps, and
g) time spent performing each behaviour

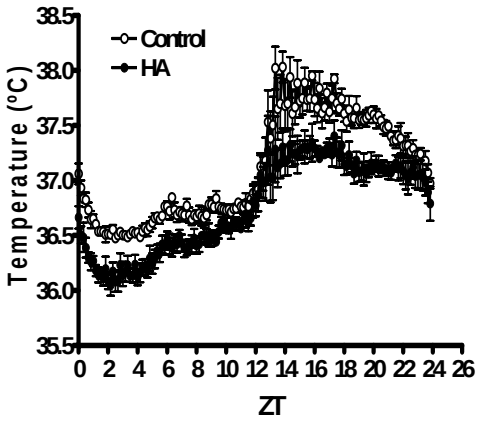


Example of circadian rhythm of ambulatory counts in control and hyperammonemic rats (Ahabrach et al, 2010)

C.2. Circadian rhythms of wheel running

We also analyze circadian rhythms of activity in wheel. A main difference between spontaneous locomotor activity and wheel activity is that wheel activity is voluntary and rewarding, whereas ambulatory activity is “involuntary” and not rewarding in the conditions used with free access to food and water(Ahabrach et al, 2010)

C.3. Circadian rhythms of temperature



To record body temperature we use temperature sensors (from 0 ºC to 45 ºC range) placed in the abdomen of the rat.
The temperature is recorded every 10 min during 10 days.

An example of circadian rhythms of temperature in hyperammonemic and control rats is show in the Figure (Ahabrach et al, 2010)

C.4. Circadian rhythms of concentration and metabolization of different substances in blood or brain.

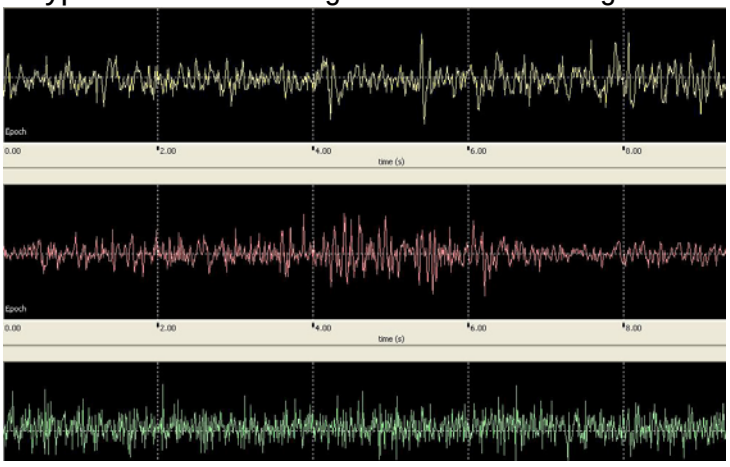
We also perform continuous microdialysis in vivo from blood or extracellular fluid in different brain areas of the rats to measure different parameters, including neurotransmitters, hormones, amino acids, melatonin, ILs, corticosterone, cGMP, nitric oxide, etc.

Samples are taken continuously during 24-48 hours and the parameters of interest are determined using the corresponding procedures.

The same system may be used to follow the concentration in blood or brain of a drug or substance injected into the rat. This would allow assessing the half-life and metabolization of the drug or substance of interest.

D. Electroencephalogram (EEG) in rats

We also analyze the EEG in rats. Recording is performed during 1-4 days.
A typical EEG recording is shown in the Figure.



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