# Astrocyte Involvement in Learning, Memory and Synaptic Plasticity

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#### 1. Introduction

Classical neuroncentric view of brain function is being challenged by mounting evidence claiming in favour of a hand-to-hand cooperation between neurons and astrocytes in the tripartite synapse model. Therefore, the goal of this project research proposal is a more comprehensive description of the relative participation of astrocytes in processes such as learning and memory.

Bearing in mind that cAMP response element-binding protein (CREB) is a transcription factor which regulates the expression of plasticity-related genes essential for synaptic long-term changes, and taking into account that the hippocampus is an essential structure for declarative memory and that CA1 region is the output area of the processed information from this brain structure, in this project a study of the astrocytic CREB-dependent molecular contribution from the CA1 area to learning and memory is proposed.

### 2. Objectives

#### **General aims:**

- 1. To establish astrocytes as an active part of the cellular substrate sustaining the computational power of the brain.
- **2.** To further characterize the function of CREB in synaptic plasticity and associated cognitive processes.
- **3.** To gain molecular understanding of CREB-related activity in astrocytes.

#### Specific aims:

- 1. To develop an astrocyte-directed gene delivery strategy.
  - a) Based on lentiviral vectors pseudotyped with Mokoloa G protein.
  - b) Aiming the silencing of CREB activity in such cell type by means of using a shRNA targeting Rattus novergicus CREB.

#### 2. In vitro studies.

- a) To test the silencing efficiency of the construct obtained in Specific aim 1 on secondary astrocyte cultures.
- **b)** To test the cell survival rate after lentiviral infection and CREB silencing on secondary astrocyte cultures.
- c) Proteomic characterization of CREB-silenced secondary astrocytes.

#### 3. In vivo studies.

a) Behavioural assays after stereotaxic injection of the shRNA-bearer vector into the CA1 area of Rattus novergicus hippocampus.

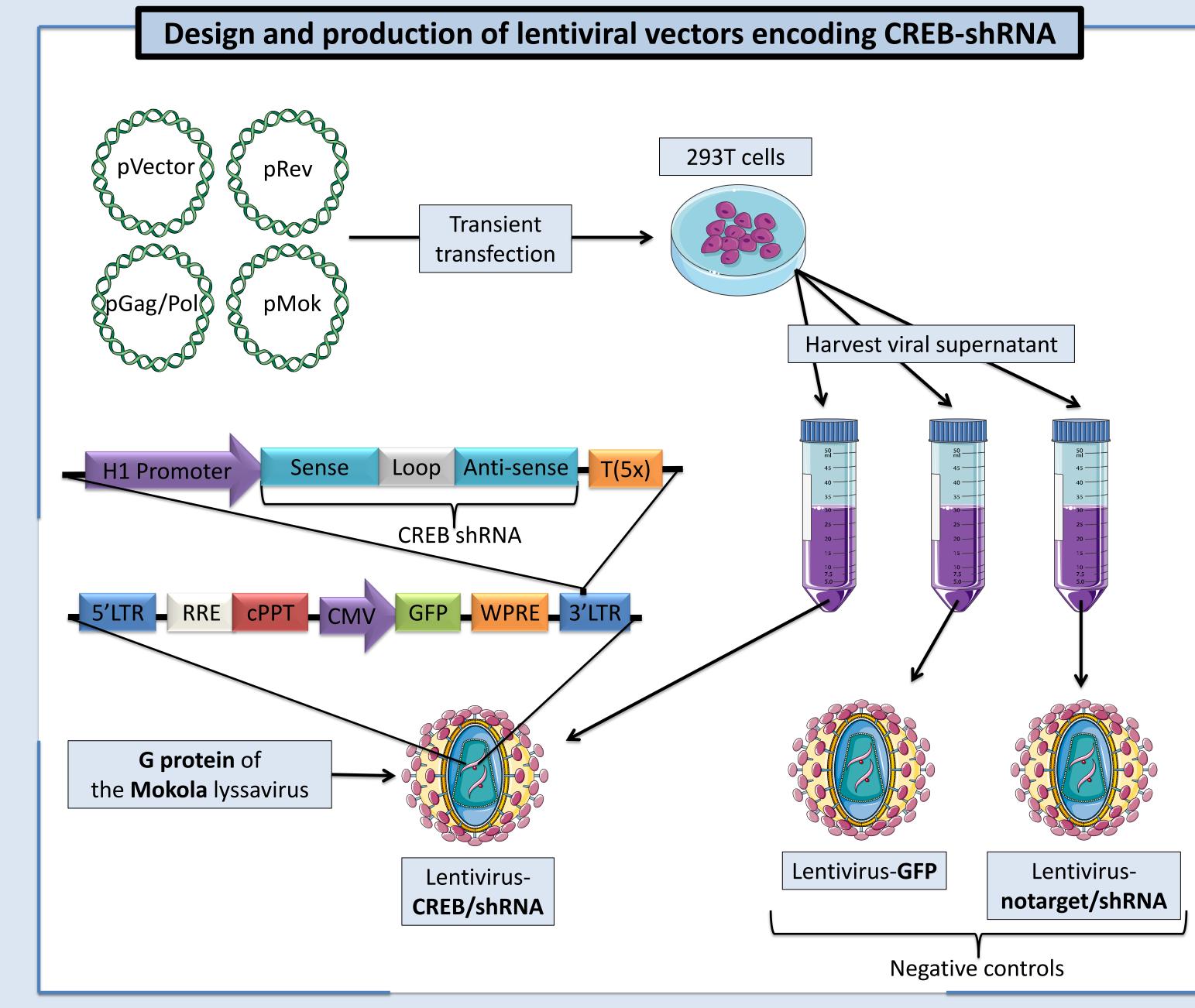
exposure to LV-shRNA is determined by

measuring the metabolic activity, which is

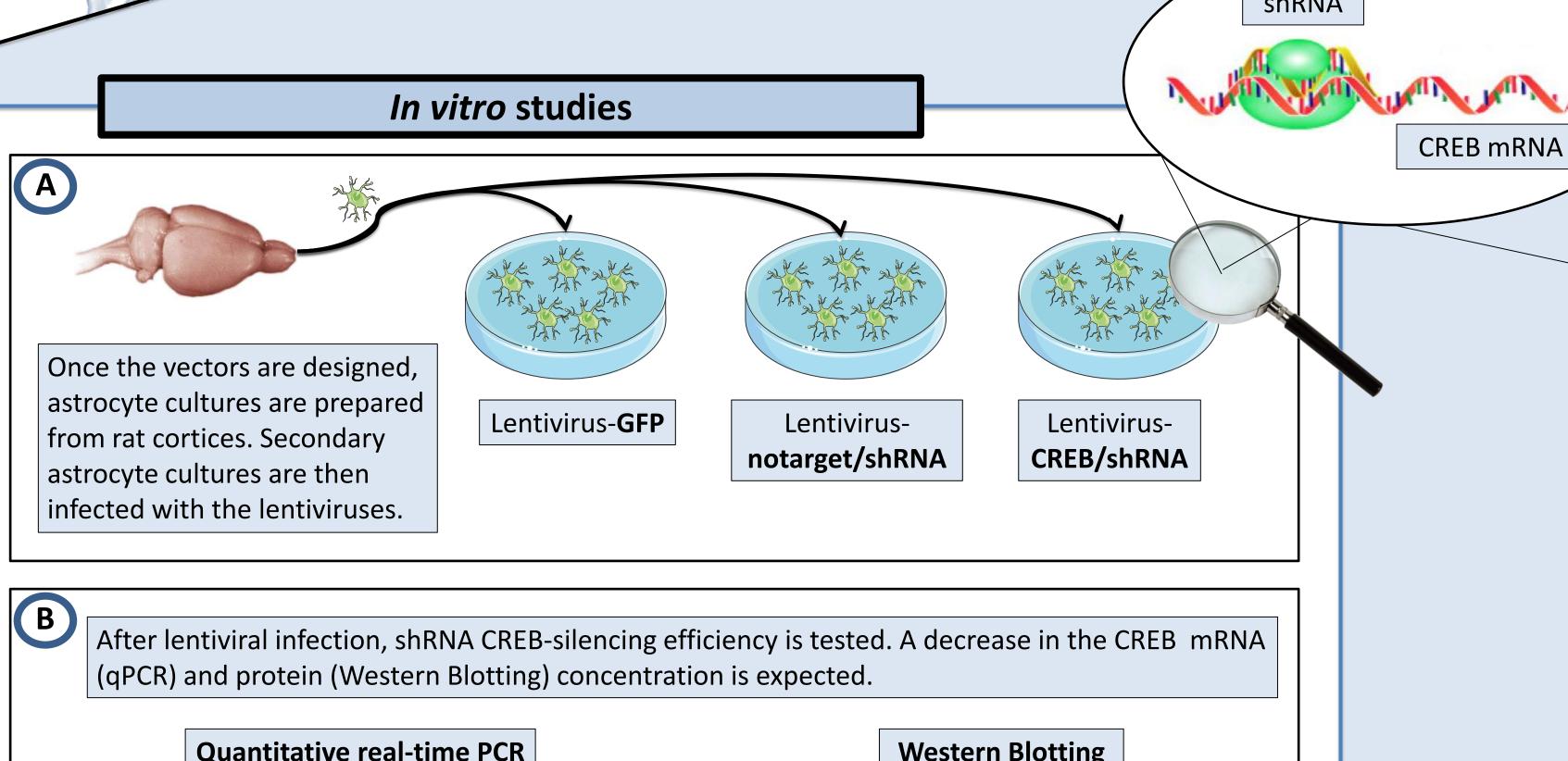
quantified at different multiplicity of infection

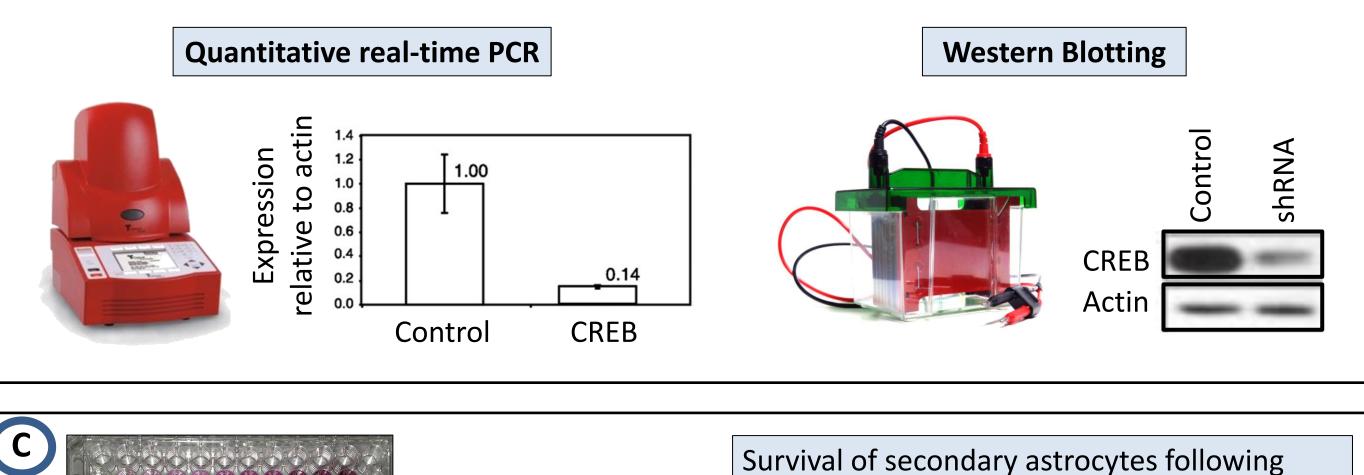
b) Proteomic characterization of CREB-silenced extracted astrocytes after behavioural analysis.

## 3. Methodology

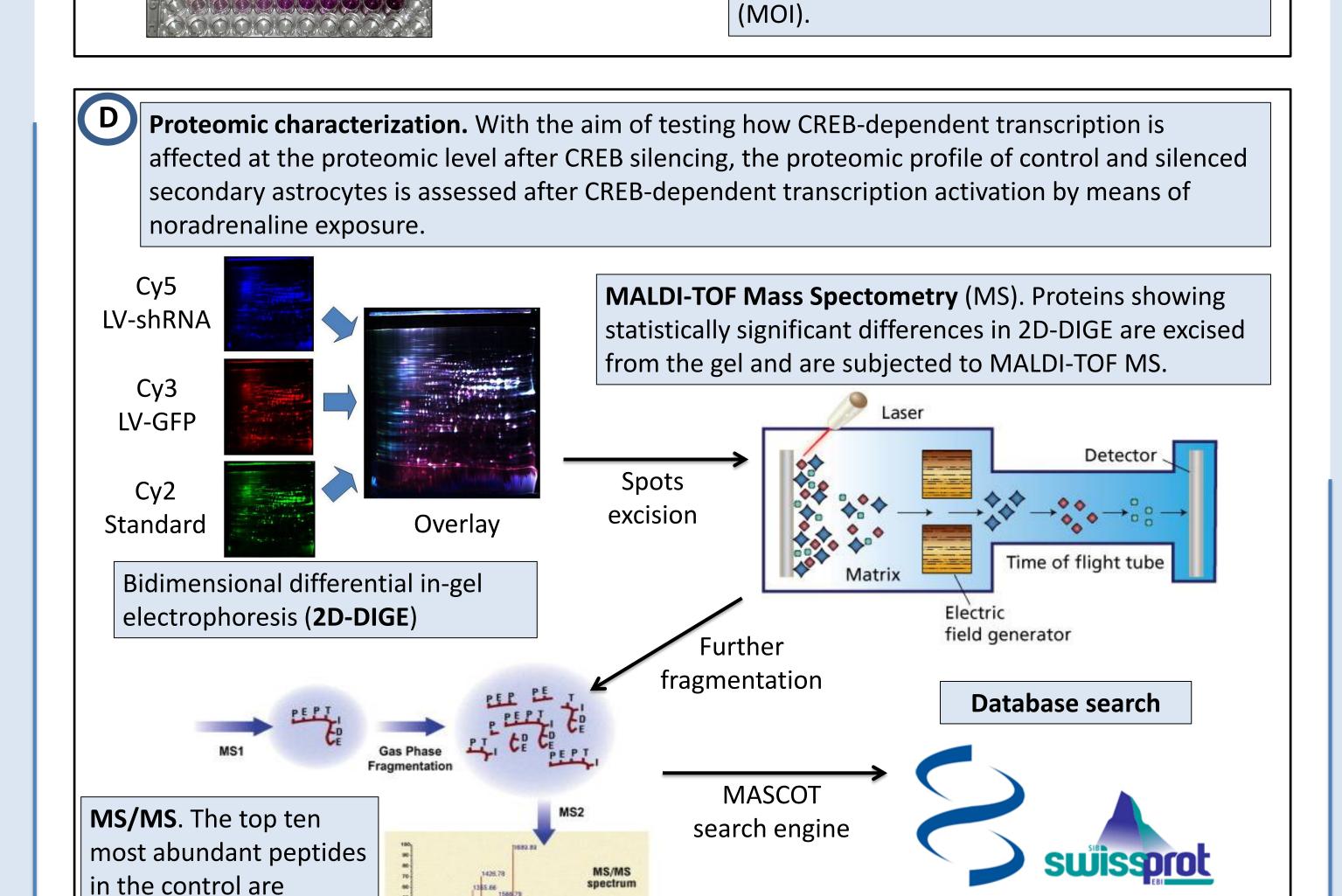


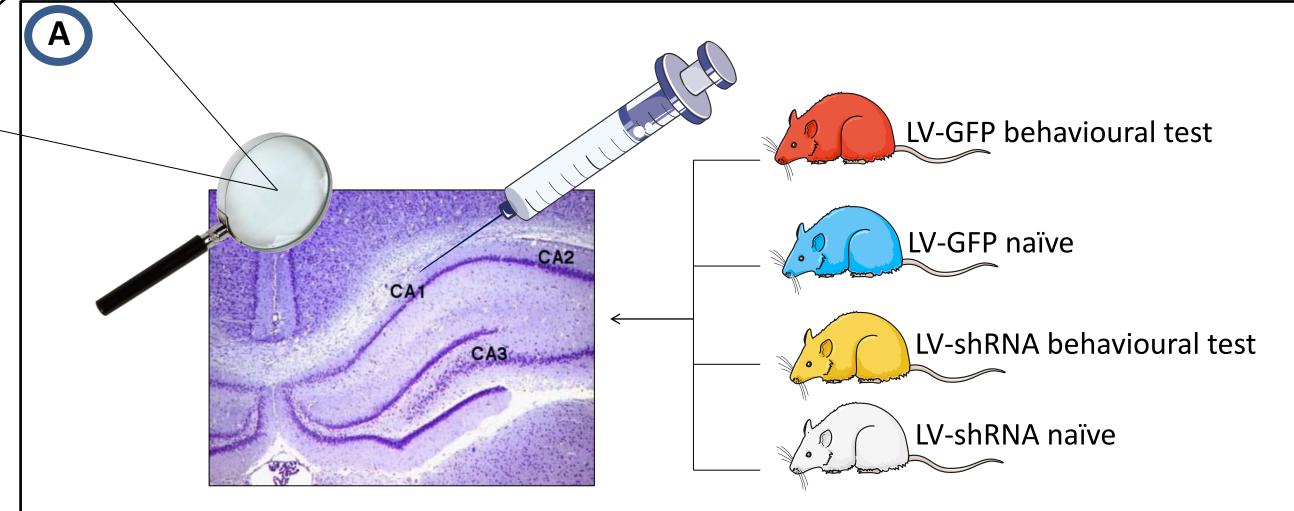
*In vivo* studies



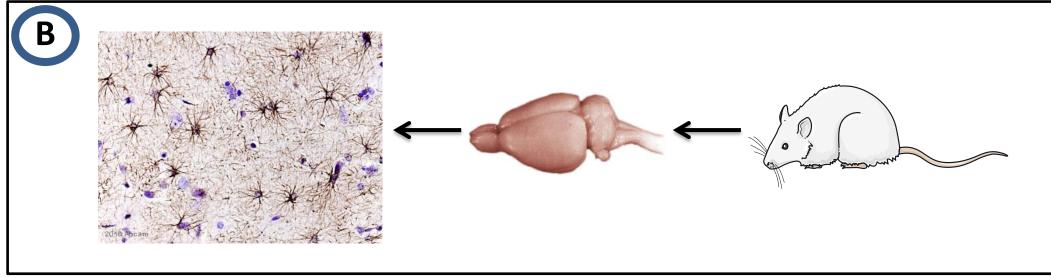


MTT assay

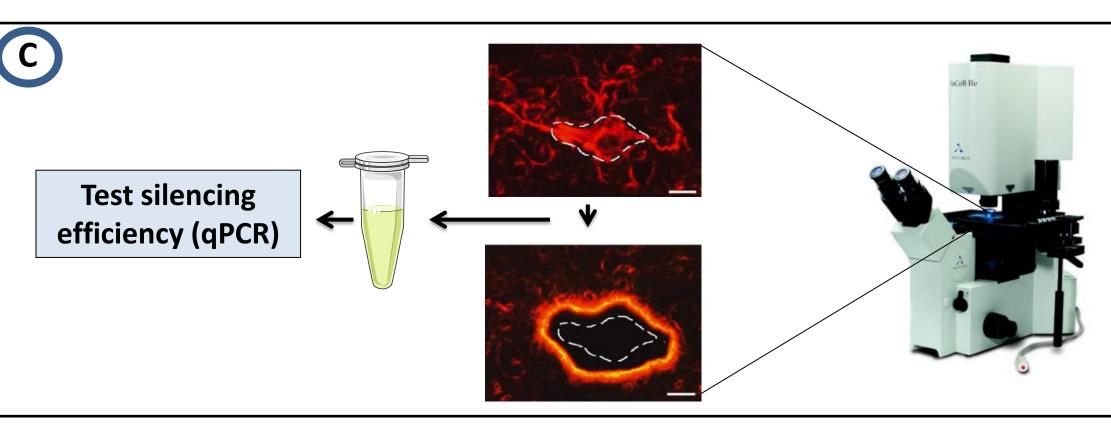




Adults male Sprague-Dawley rats which are 6-8 months old are classified into **four groups**: two groups infected with LV-GFP and two groups infected with LV-shRNA. In each of them, one group will undergo the behavioural tests and the other will be the naïve control. The stereotaxic injections are bilaterally carried out in the CA1 hippocampal region.

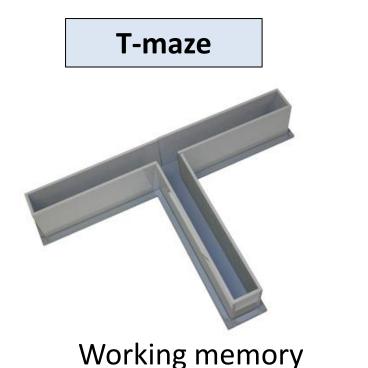


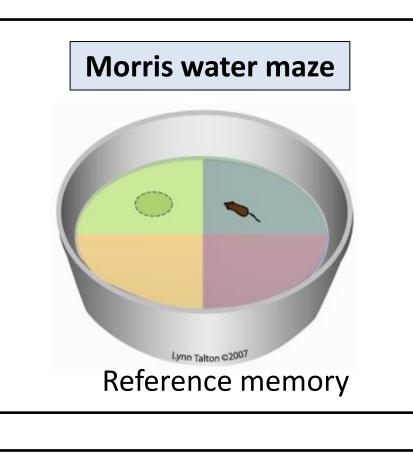
Immunohistochemistry and Stereology. In order to check the astrocyte-specificity of the vector and the transduction efficiency in vivo, brain hippocampal sections are analyzed by way of these two techniques.



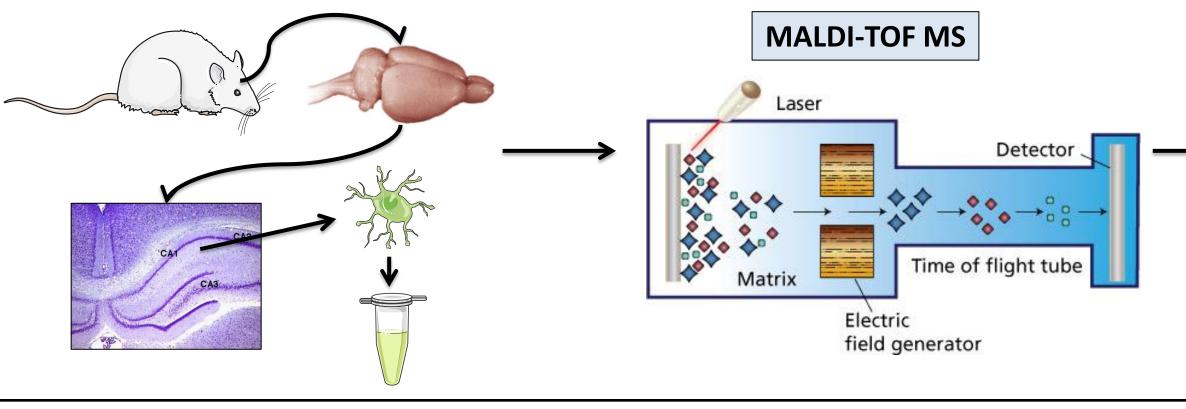
Laser capture microdissection of astrocytes. With the aim of testing the CREB-silencing efficiency of the transduced shRNA in vivo, astrocytes are microdissected by means of LCM so as to avoid tissue background and subjected to RNA extraction and posterior qPCR.

#### D **Behavioural tests** To asses the hippocampal function after astrocytic CREB silencing, the indicated behavioural tests are carried out. On the one hand, the T-maze allows us to test the exploratory behaviour and on the other, the Morris water maze analyzes spatial learning. If astrocytic CREB activation is a key happening for the proper development of such activities, statistically significant differences between controls and CREB-silenced rats are expected.





Laser capture microdissection of astrocytes and Proteomic characterization. If significant differences where observed in the behavioural tests, it would be interesting to look for the proteins which underpin the downstream synaptic changes after astrocytic CREB silencing. **MALDI-TOF MS** 





## 4. Expected results

further subjected to gas

phase fragmentation

- 70-80% of astrocytic transduction with the pseudotyped lentiviral vector (immunohistochemistry and stereology).
- 70% of astrocytic CREB knockdown effect both in vitro and in vivo (qPCR, WB).
- Minimum of 80% cell survival rate in vitro (MTT).
- Statistically significant differences between control and CREB-silenced rats in both Morris water maze and T-maze.
- Pool of proteins showing statistically significant differences between control and CREB-silenced rats (Proteomic characterizations).

## 5. Discussion

The herein project proposal, if carried out and if its results were the expected ones, would shed light on the cellular underpinnings of learning and memory, two of the most breathtaking processes that have always mesmerized human curiosity. Furthermore, and based on morphological and physiological aspects of astrocytes, this study would provide the behavioural evidence to finally piece up all the data and proclaim this cell type as an indispensable component to bear in mind when postulating mind theories.

On the other hand, and by way of the proteomic characterization, proteins involved in synaptic plasticity downstream the activation of astrocyte-specific CREB-dependent transcription could be unravelled.

Hence, this project proposal would, jointly with other studies in the Gliobiology field, pave the way for the advent of astrocytes as a key piece in the puzzle of cognition.

## 6. References

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