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.

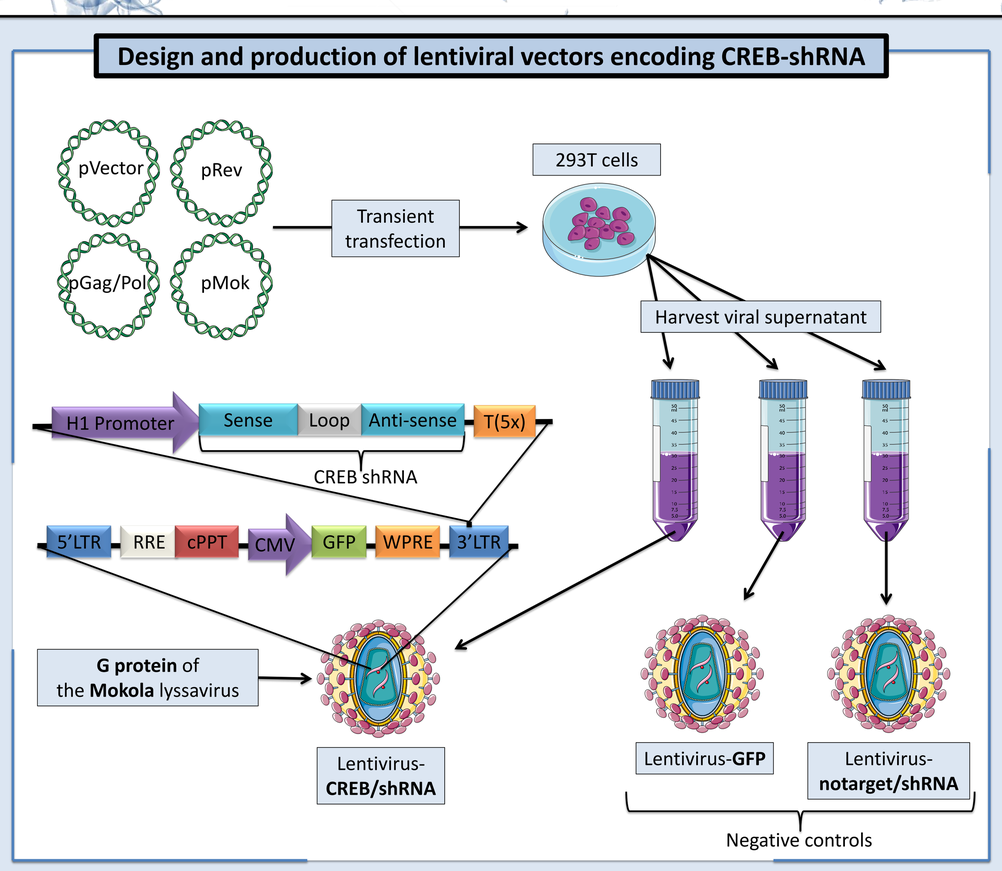
Specific aims:

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.

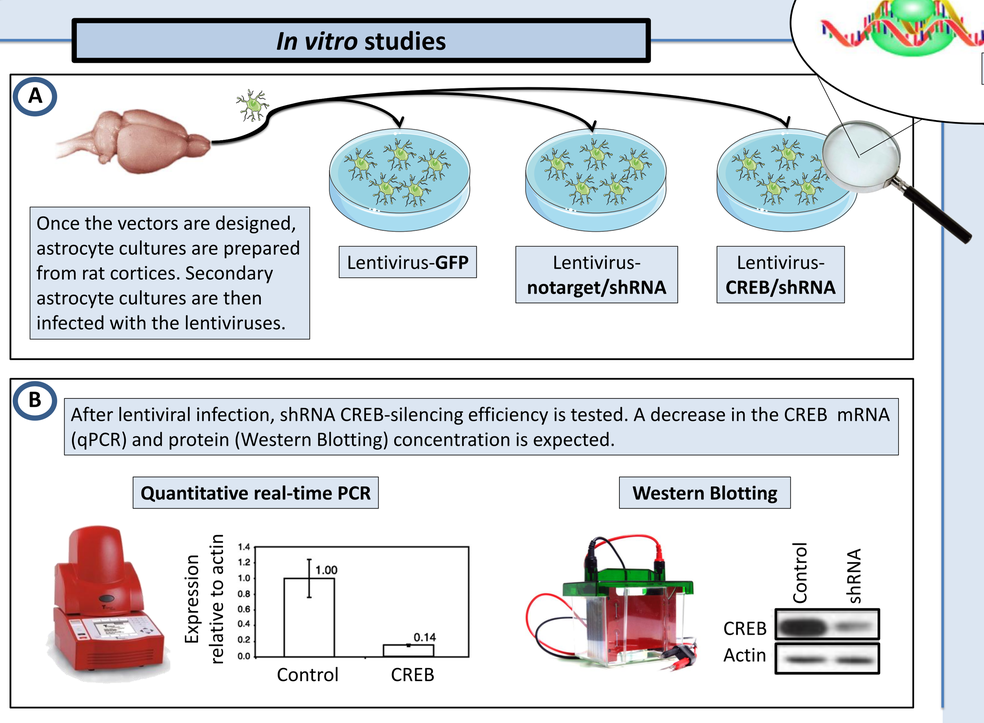
2. *In vitro* studies.
- **a)** To test the silencing efficiency of the construct obtained in Specific aim 1 on secondary astrocyte cultures.
- **b)** To test the cell survival rate after lentiviral infection and CREB silencing on secondary astrocyte cultures.
- **c)** Proteomic characterization of CREB-silenced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

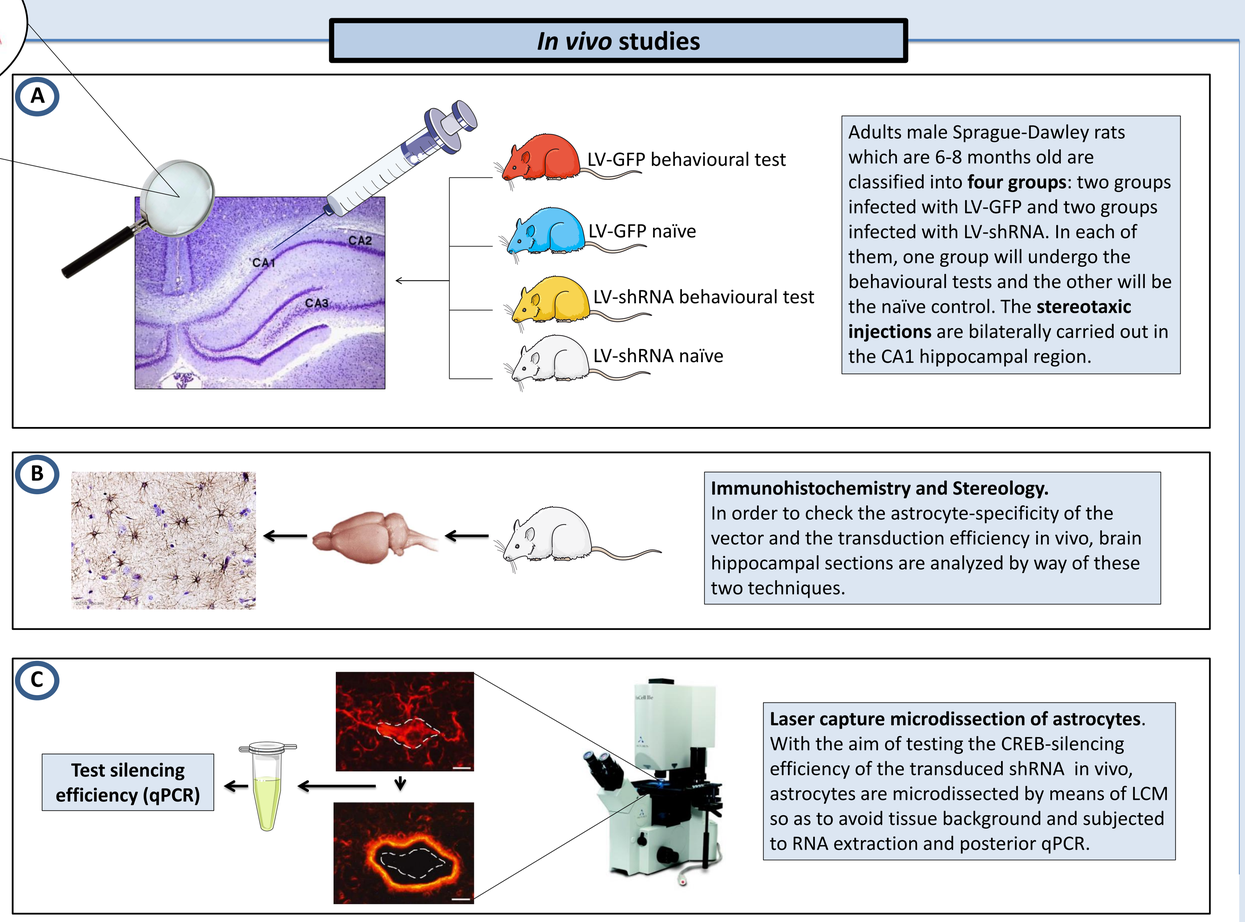
3. Methodology



In vitro studies



In vivo studies



4. Expected results

- 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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