Neurofunctional Techniques

Lesson 13

13 November 2024

Review Virus/X-genetics

Calendar

- M 30 Sept: Course introduction
- W 2 Oct: Functional imaging
- F 4 Oct: Statistics (Cesca)
- M 7 Oct: Functional imaging
- W 9 Oct: Biophysics of diffusion
- F 11 Oct: Statistics (Cesca)
- M 14 Oct: Functional imaging
- W 16 Oct: General introduction to the papers for the presentations
- F 18 Oct: Statistics (Cesca)
- M 21 Oct: Modeling in neuroscience
- W 23 Oct: Molecular approaches in neuroscience
- F 25 Oct: Statistics (Cesca)
- F 25 Oct: Laboratory (14:00- 18:00)
- M 28 Oct: Practical exercises on the first part of the course
- W 30 Oct: Genome editing in neuroscience (Dr. Jaudon)
- M 4 Nov: Optogenetics
- W 6 Nov: Papers assignment to the groups; introductions to the specific papers
- T 12 Nov: X-genetics + Practical exercises on the second part of the course
- W 13 Nov: Introductions to the specific papers
- M 18 Nov: Introductions to the specific papers

- 9, 10, 11 Dic: Paper presentation 15:00-19:00)
- Tue 17 Dic: Test (14:00 - 16:00 Room 3A, Building H2bis)

Structure of the exam

1. **Paper presentation in small groups (5 students 1 hour) Maximum score 20 (+1):**

   a) **Presentation (30 min)**
   - **What was known**
   - **What is the gap**
   - **What are the main findings**
   - **Are the techniques appropriate**
   - **What are the broader implications**

   b) **Questioning on individual figures and techniques used (30 min)**
   (9, 10, 11 December)

2. **Questionnaire on the Moodle platform. 10 multiple choice questions (also on statistics). Each question has only one correct answer, and each correct answer is awarded 1 point. Maximum score: 10.**
   (17 December Room 3A Building H2bis
   First turn: 14:00 - 15:00
   Second turn: 15:00 - 16:00

1) What we mean for tropism of a virus? And why do we need to consider it in infection strategies?

2) Stereotaxic surgery for virus delivery:

a) **is currently used only in rodents**

b) **is used in humans but not yet for virus delivery**

c) **There are few successful examples of stereotaxic surgery for virus delivery in humans for medical purposes**

d) **is common practice in medicine**

3) **The retrograde virus rAAV2-retro is a retrograde transynaptic virus: it infects the soma of the neurons in the brain region where it is injected and it crosses the synaptic cleft, thus infecting the neurons projecting to that nucleus.**

TRUE

FALSE

4) Which are the advantages of optogenetics (relative to electrophysiology and pharmacology)?

5) Which are the advantages of chemogenetics (as compared to optogenetics)?

6) **Type I opsins (those most commonly used for optogenetics) are light-activated ion channels**

TRUE
FALSE

7) Are the following statements true or false?

a) rAAVs are bigger than lentiviruses

b) rAAVs have slower expression times than lentiviruses

c) rAAVs have generally higher titers than lentiviruses

8) **The DO Cre-off construct uses recombination between two pairs of loxP and lox2272 sites to confer a permanent recombination event, thus inactivating the expression of the gene of interest in the cells expressing the recombinase Cre. Starting from the scheme below, draw all the possible intermediate and final recombination events, taking care to highlight position and orientation of the loxP and lox2272 sites for each step.**

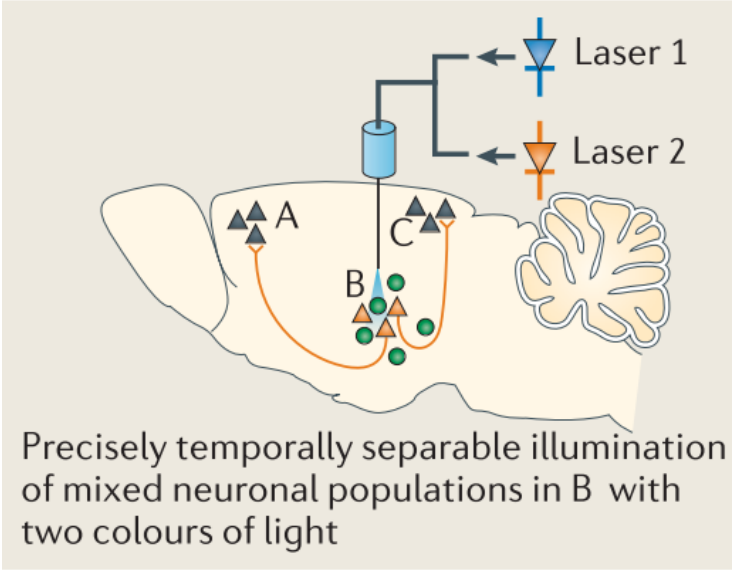
9) **You need to stimulate alternatively excitatory and inhibitory neurons in the nucleus B in mouse. Given the current state of the art,**

a) **Which transgenic mice would you use, if any?**

b) **How would you design the required rAAV constructs?**

c) **Which rhodopsins would you select?**

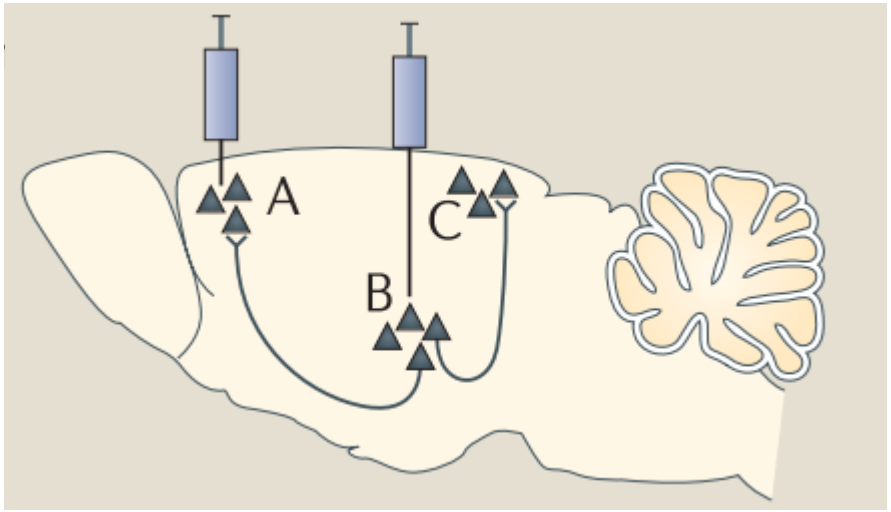
d) **Which lasers would you use?**



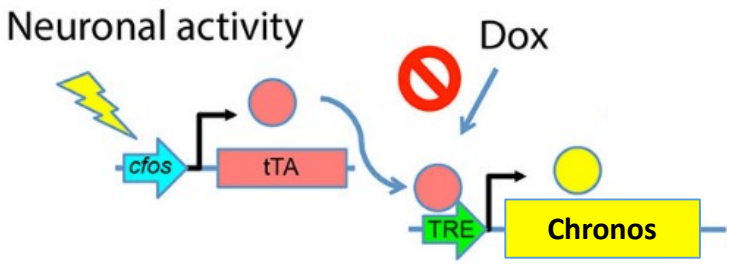
Precisely temporally separable illumination of mixed neuronal populations in B with two colours of light

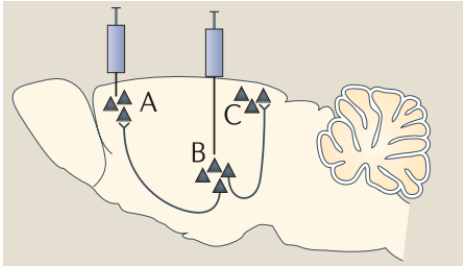
10) You need to target the channelrhodopsin Chronos selectively to the neurons that connect the hippocampus (nucleus B in the figure) to the medial prefrontal cortex (mPFC; nucleus A in the figure)

How would you design the two required rAAV constructs in terms of (i) serotypes, (ii) intersectional expression strategies? (iii) injection sites?



11) The **transgenic mouse line TetTag** (fig. on the left) drives the expression of the tetracycline transactivator (**tTA**) in pyramidal neurons under the control of the **activity dependent promoter c-fos**. The tTA protein binds to the tetracycline-responsive element (TRE) to trigger the expression of a downstream target gene of choice. The binding of tTA to TRE is however blocked by **Dox**, which can be administered through the animal's diet. If Dox is removed from the food (e.g. during the encounter with a familiar mouse), a window for activity-dependent labeling is opened: tTA can bind to TRE to turn on the expression of the gene of interest (i.e. the **channelrhodopin Chronos**).
You have at your disposal this mouse line for a project on social memory. Specifically, you need to target the channelrhodopin Chronos selectively to the neurons that connect the hippocampus (nucleus B in fig. on the right) to the medial prefrontal cortex (mPFC; nucleus A) and that are active exclusively when the test mouse encounter a familiar mouse.





a) How would you design the two required rAAV constructs in terms of (i) serotypes, (ii) promoters (iii) intersectional expression strategies?
b) Which genes need to be expressed and in which rAAV?
c) Which rAAV needs to be injected where?