SIS Biologie - Main Discipline 2017
Second part (1.5h)

Alzheimer's disease is a neurodegenerative disease. Post-mortem observation of brains from patients with this disease typically identify extracellular aggregates (or plaques) of β-amyloid protein, as well as intracellular tangles of hyperphosphorylated Tau protein. Neuronal loss is also observed in the most affected structures.
In this examination you will have to interpret a series of experimental results on this latter aspect.

Post-mortem hippocampal slices of tissue from patients (AD, Alzheimer's disease) or healthy controls have been immunostained with different antibodies. Figure 1 shows an example of this staining. The related table shows the results with different antibodies.

B1- The staining is exclusively found in neurons. Propose a simple way to check it.
B2- The proteins detected by the antibodies are involved in an important cell process. Which one? What do you know about Cyclin B?
B3- Why there is no expression of these proteins in healthy tissue?
B4- Amongst the detected proteins, which one is most strongly expressed in AD tissue? Propose an hypothesis on the meaning of this observation.

An in situ hybridization with a specific sequence of chromosome 17, performed on tissue slices from AD is shown in figure 2.

B5- What can you say about A-type and B-type cells identified this way?
B6- What can you conclude from this experience?
B7- From figure 2, can you state that there is a cytokinesis problem?

B-type cell quantification has been done on tissue slices from different patients deceased at different phases of the illness. The total number of neurons has been quantified in these slices (figure 3).

B8- Describe the results presented in the graph. What can you conclude?

In a different context, researchers from a different lab have studied the effects of Nerve Growth Factor (NGF) deprivation on cultured neurons. In these conditions, neuronal morphology resembles the one shown in figure 4.

B9- What kind of cellular process is evoked by the images in figure 4? Why? Imagine a technique or experience that could confirm such hypothesis.

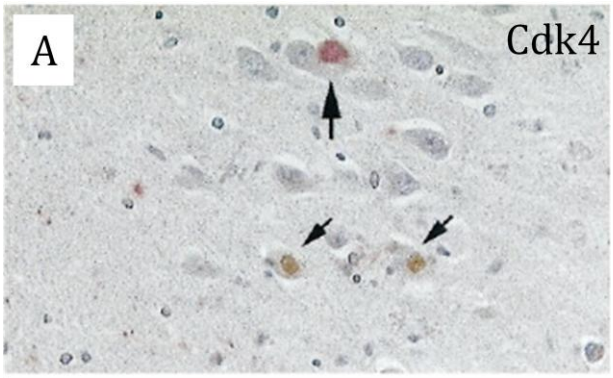
The result of a RT-PCR analysis of cultured cells after different times of NGF deprivation is shown in figure 5.

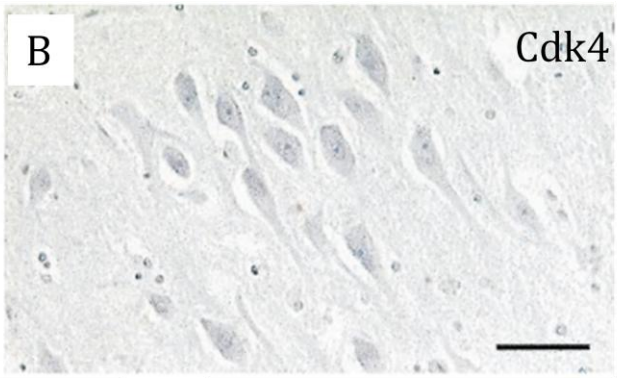
B10- Interpret fig. 5. Why the only signal persisting at 72h is S-100β?

A lab is working on AD, with a mouse transgenic model (B6-R1.40). These mice develop the disease and plaques of β-amyloid protein can be detected between 12 and 14

months. Cyclin D immunocytochemical detection at two different ages is shown in figure 6.

B11- What can you notice? See the arrows in fig 6C and 6F, what do they indicate? Interpret the result shown in this figure.
B12- Propose a mechanistic scheme taking into account the results presented in the 6 figures.





	Cyclin D	cdk4	Cdk2	Cyclin B1
Normal				
% positive	0	0.1	0.5	0.1
AD				
% positive	0.6	3.9	9.0	8.8

Figure 1. Immunocytochemistry against Cdk4 protein on brain slices from AD patient (A) or healthy control (B). Arrows indicate stained cells. Immunostaining has also been performed against CyclinD, Cdk2 and CyclinB. Quantification is shown in the Table as % of stained cells over total number of cells.

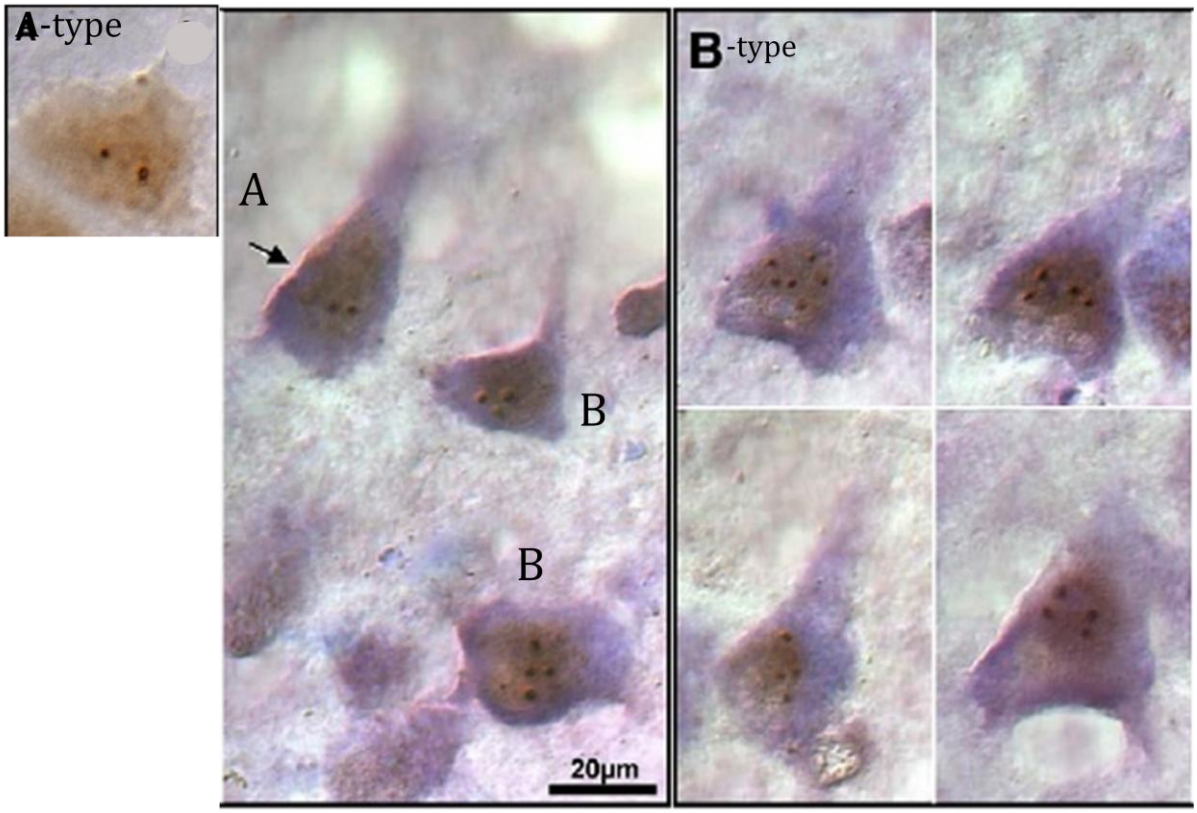


Figure 2. In situ hybridization with a specific sequence of chromosome 17, performed on brain slices from AD patient. Staining has been performed with a colorimetric technique that yields a brown-violet colour. Left, example of A-type cell. Right, examples of B-cell type cells. Middle, a field with A-type and B-type cells.

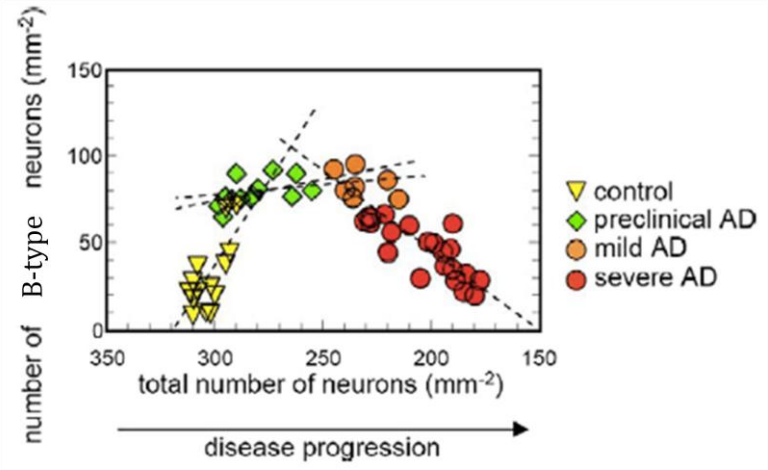


Figure 3. Quantification of the number of B-type cells on slices from patients deceased at different phases of the disease (control, pre-clinical, mild, severe).

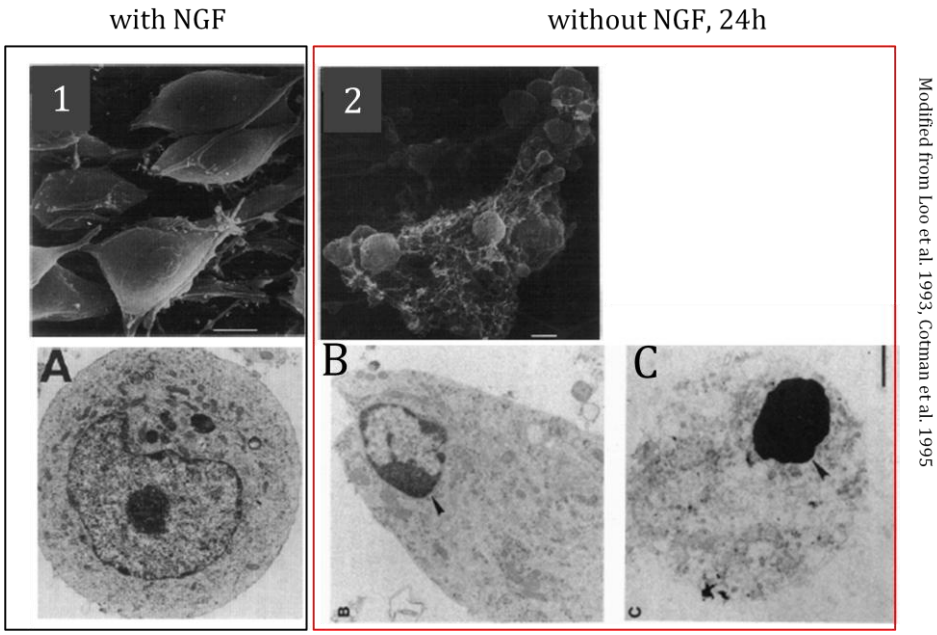


Figure 4. (1-2) Images from cultured hippocampal neurons obtained by scanning microscopy. (A-C) Images from cultured hippocampal neurons obtained by electron microscopy.

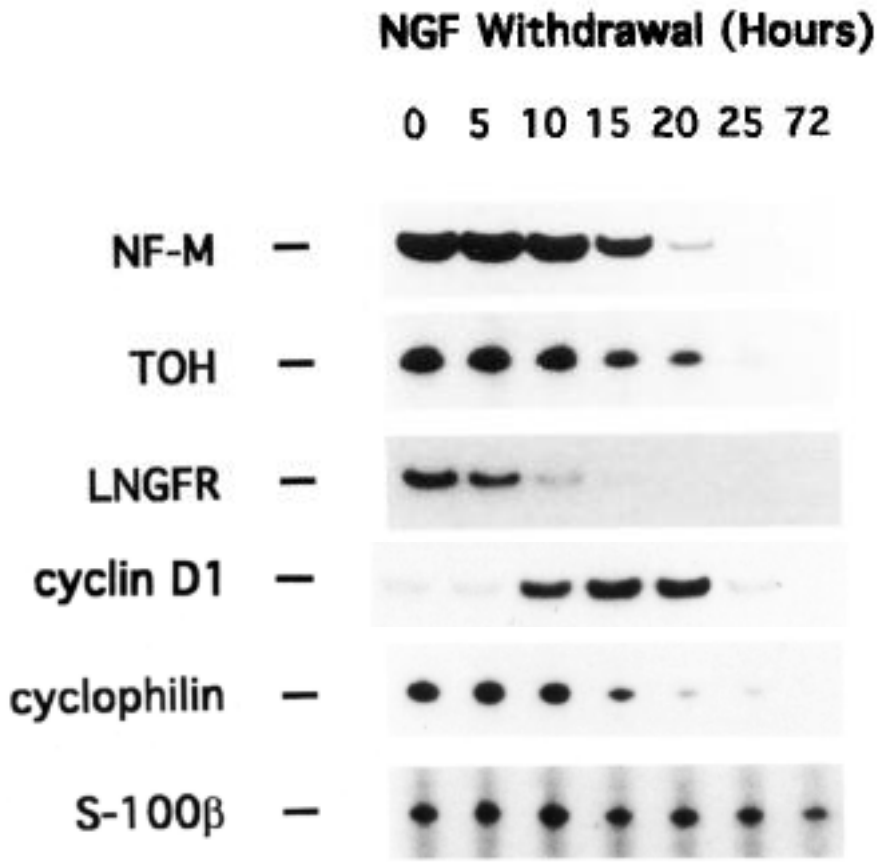


Figure 5. Neurons in culture have been deprived of NGF and analyzed at different times (0-72h). The expression of several markers has been followed by Western Blot: NF-M (neurofilament), TOH (tyrosine hydroxylase), LNGFR (low NGF receptor), cyclinD, cyclophilin (ubiquitous gene), S-100β (gene expressed by glial cells).

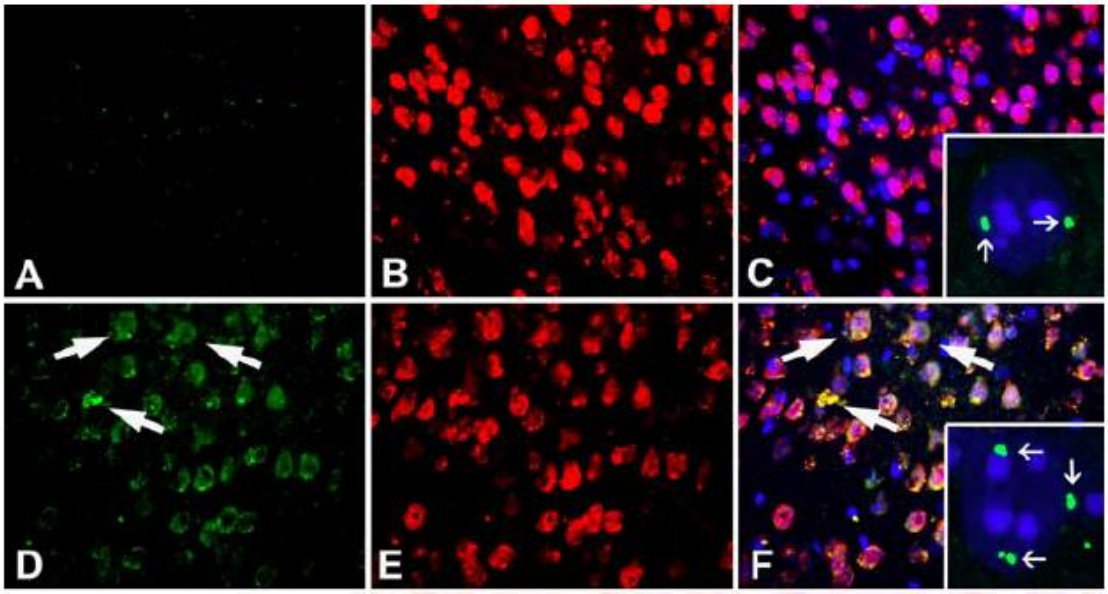


Figure 6. Cyclin D expression in the cortex of B6-R1.40 mouse at 10 months (A-C) and 12 months (D-F). Green: cyclinD; red: NeuN (neuronal marker), blue: DAPI (DNA intercalating agent). C and F correspond to images A-B and D-E superposed, respectively. Insets in C and F show magnification of the nucleus.

Adapted from Busser et al. JNeurosci 1998 ; Arendt , Am Journal of Pathology 2010; Freeman et al. Neuron 1994; Varvel et al. JNeurosci 2008.