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# **Internship report**

6/6/16 - 7/1/16  
at « l'INSTITUT DE BIOLOGIE DE L'ÉCOLE NORMALE SUPÉRIEURE »

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# Thanks

As a biology student at the University Pierre and Marie Curie, I did between my first and second years of study, an internship of observation at the « Institut de Biologie de l'École Normale Supérieure » (IBENS). From the 6th June till the 1st of July, I followed four groups of researchers, each during one week.

I would like to say many thanks to these teams, and all of their members for their availability, for their goodwill, and the richness of the explanations they gave me.

I would like also to express my gratitude to Ms Danièle Murciano, Director of Scientist Affairs, who gave me the opportunity to do my internship in a such prestigious environment.

This internship gave me the chance to discover the subject of the research these four teams are conducting.

All of them work on neurosciences subjects : the molecular and cellular mechanisms involved in the biology of neural stem cells (Nathalie Spassky's team), the cells divisions and the neurogenesis (Xavier Morin's team), the glutamate receptors and the excitatory synapses (Pierre Paoletti's team), and finally the zebra-fish neuroethology (German Sumbre's team).

So it is to the study of the most (I believe), fascinating and complex object we know in the world : the brain, that these teams contribute to.

It was a great opportunity for me to observed them during their work.

This internship taught me more on how works a research laboratory, and on the cellular and molecular biology technics they use.

I tried to report in here what I saw and learnt during this internship.

As I followed these four teams during one week each, I naturally organized this report into four parts : one per team.

I started this internship following Nathalie Spassky's Team. Marion Faucourt, a research engineer, guided me during this discovery.

For the second week, it is Xavier Morin's team that welcomed me.

The next week, Pierre Paoletti received me in his team, where Lætitia Mony took me with her to follow her works.

Finally, I integrated Germán Sumbre's laboratory, where Adrien Jouary and Véronique Candat entertained me.

I will briefly present the subject of the work of every team. Then, I will talk about the model organisms they use, the experiments I observed, and sometimes, took part of.

Also, seminars are organized at IBENS, where researchers often from outside of IBENS, come to present their work. I list the seminars I sat in.

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# Week 1 - Nathalie Spassky's team

Nathalie Spassky's team study the molecular and cellular mechanisms of the neural stem cells biology. These stem cells can differentiate into neural cells (neurons or glial cells). Their discovery were a revolution in neurosciences because it has long been known that the adult brain could not regenerate its neurons. This discovery lets hope a use of cellular therapy in neurodegenerative pathologies.

Spassky's Team work in particular on the role of the primary cilia during neurogenesis

<http://www.ibens.ens.fr/spip.php?rubrique6>

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## Roles of cilia in neurogenesis and pathologies

Cilia are a cytoplasmic extension of the cells. They can have two roles : a mechanic role, or a sensory role.

They can help for the cell's move, to spread fluid, or to agitate the external medium. Cilia can also receive signals from the outside of the cell to regulate the cell's environment.

In her work to better understand the molecular and cellular mechanisms that rule the neural stem cells, Nathalie Spassky and her team are interested particularly on the role of ependymal cells' cilia, located in the ventricular part of the brain. Ependymal cells are a type of glial cells (that means a type of cells who form the neurons' environment) of the central nervous system. They ensure the interface between the central nervous system and the cerebrospinal fluid. Their mobile cilia allow the cerebrospinal fluid to flow. They are essential for the brain development and for the differentiation of neural cells.

Ciliary defects cause pathologies called ciliopathies. It can cause hydrocephaly : when the cerebrospinal fluid is not correctly drained, because of a ciliary defect, it accumulates in the brain.

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## Model organism

The model organism used by Nathalie Spassky and her team is the mouse. In order to see the effects of the missing of cilia during neurogenesis, they used genetically modified mice, that can't produce cilia on the ependymal cells, that is to say, mice called knock-out (KO) for a necessary gene for cilia's development.

### How to obtain homozygous KO mice

One of the difficulties, is that the homozygous mutation is lethal. So it is not possible to obtain generations of mice without cilia because a such mouse can not reach maturity.

To avoid this problem, and to obtain KO mice, we use a technique called Cre-Lox (« Cre-Lox recombination »), which allows to delete genes (or make insertions, translocations or inversions) in a specific site of the DNA. A pair of Lox sequences is a target site of the « Cre recombinase » which is a restriction enzyme : the enzyme splices the part of the DNA located between them. So this technique consists to frame the gene of interest with two

Lox sequences. And if the DNA of the mice produces also the enzyme Cre, there is a recombination and the framed gene is deleted.

The Lox sequences come from a bacteriophage DNA : it's a sequence we don't find in eucaryote DNA. So this technic will not mess up our mice's DNA.

An important advantage of this technic, is that the Cre enzyme is under the control of a specific promotor, so its expression can be limited to specific tissues (like ependymal cells in this case).

To obtain mice « without cilias » (homozygous KO), the first step is to mate a female lox/lox with a male KO/+cre, i.e a mice that is capable to produce the enzyme Cre and that is heterozygous KO/+ (« + » meaning the wild type allele). Thus, this mate will produce mice lox/+ and mice lox/KO. The lox/KO mice producing the enzyme Cre will become the KO/KO mice desired.

Theoretically, only 25% of the mice coming from this mate won't have cilias.

L'animalerie of IBENS provided mice to the different teams after command. When the litter of mice is available, it is now to see which one of them have the genotype of interest. We realize a polymerase chain reaction of the DNA of these mice, in order to sequence and then revealed the genotype of the mice for the studied genes.

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## Experimental work

Here is experimental works I took part

### PCR

A polymerase chain reaction (PCR) of DNA enable to amplify quickly a sequence of DNA or RNA (« target DNA ») from a small amount of this sequence. A PCR is a cycling of three phases of temperature changes :

- The first step is the denaturation of the DNA : the two complementary stranded of the DNA are dividing.
- The next step is to anneal the specific primers to the target DNA. It is also the initialization of the following step : the elongation. (between 40° and 50° C)
- The elongation is the step during which complementary nucleotides match to the one stranded DNA. This step occurs at 72°C in presence of the Taq polymerase. The Taq polymerase is a thermophil polymerase DNA. This enzyme allows the synthesis of a complementary DNA strand to the template DNA.

Thus, during every cycles, the amount of the target sequence is double, and after n cycles, there is  $2^n$  copies of this DNA. Knowing a cycle last for only few minutes, this technic can make a large amount of copies, in a short time.

## Polymerase chain reaction - PCR

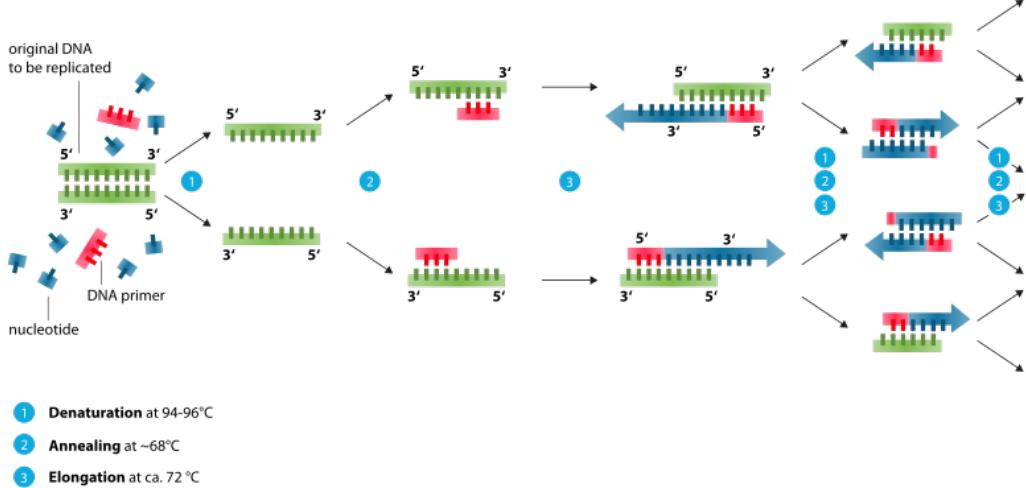


figure 2 PCR

To amplify a DNA sequence, it requires DNA of the specimen, specific primers of the target DNA, dNTP (deoxyribonucleotides, which are elements of synthesis of the complementary DNA), and Taq polymerase.

## Electrophoresis

An electrophoresis is made on agarose gel with the product of the PCR : i.e the DNA will migrate in a gel under the influence of an electrical field. Because the DNA is negatively charged, it goes to migrate towards the positive charge, more or less faster depending on his size (i.e molecular height).

When the DNA migrated, a photo is taken under UV lights. UV revealed the ethidium bromide (EtBr) which is an intercalating agent of the DNA. It becomes fluorescent when it is exposed to UV lights. This last step occurs in a special room to avoid contact with the EtBr which is a dangerous product because it inserts into the DNA.

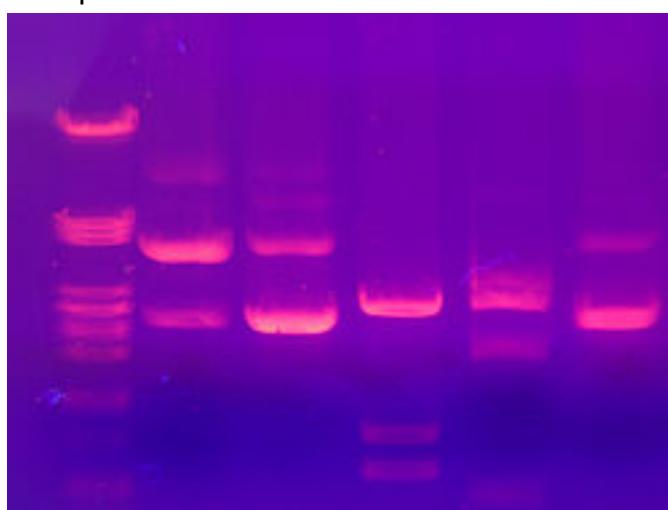


Figure 3 DNA revealed with EtBr

## **Dissection**

To observe the ependymal cells, researchers need to dissect the mice's brain to retrieve the lateral ventricles in where the ependymal cells are. The ventricles are cavities in the brain where the cerebrospinal fluid flows. It exists four ventricles : the lateral ventricles (right and left), and the third and the fourth ventricles.

Ventricles' cells are after grow in culture, to analyze their development.

## **Cell culture**

Cell culture enable to watch and to analyze the metabolism of cells *in vitro* in an artificial media. For example, it is possible to study the differentiation cells mechanisms, growing stem cells in culture.

The media must be prepared under a laminar flow cabinet to avoid contaminations. The media is constitute with a layer of poly-L-lysine at the bottom of the support. It will keep the cells stuck to the support because of ionic interactions. (See annex for protocole)

# Week 2 - Xavier Morin's Team

Xavier Morin's team are interested in the mechanisms controlling the cell divisions of the neural cells.

<http://www.ibens.ens.fr/?rubrique13>

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## Cell divisions and neurogenesis

Neural cells of vertebrates come from the division of neural stem cell that constitute the neuroepithelium.

These stem cells, that are also called progenitor cells can divide in two different ways : symmetrically, or asymmetrically. Thus, they will give birth to tow new progenitor cells (following the symmetrical model) or to a progenitor cell and a neurone (following the asymmetric model).

It is important to better understand the mechanisms that conduct to one or other type of division : this is what this team try to understand.

Samuel Tozer is a researcher working in the same laboratorythan Xavier Morin. He explores the influence of the Notch signaling pathway in the regulation of these divisions. Actually, in this process of differentiation of neurons, this signaling pathway plays an important role. Samuel Tozer noticed that the distribution of the enzyme Mib1, which is a regulating molecule of the Notch pathway, influence the differentiation of cells. When the distribution of Mib1 is asymmetric in the two daughter cells from the mitose of a progenitor, we got 40% of symmetrical divisions (i.e two progenitor cells after division), and 60% of asymmetrical (one progenitor cell and one neurone after division). When the distribution of Mib1 is symmetric, we got 100% of divisions making two progenitors. The presence of Mib1 would involve a double feedback, inhibiting the differentiation of cells ? This is the question Samuel Tozer try to respond.

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## Model organism

Xavier Morin's team use the chicken embryo, as a model organism : fertilized eggs. Chick embryo has been the first organism model to study embryology in the 60s. This model is practical because it is cheap, and because the embryo is easy of access (after making a hole in the eggshell).

The team target the neural tube, where the neural cells are generated in the embryo (the neural tube is the embryonic stage of the nervous system of the chordate).

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## Experimental work

To better understand the mechanisms regulating the neurogenesis, Xavier Morin and his team express in the cells of the chick embryo's neural tube new genes. They analyze the impact of the proteins made from these genes on the embryo.

I followed Chooyoung Baek in her works, a PHD student who were making such experiments. This has been for me the occasion to try to understand how and why these technics can be used. Here I describe the whole process, as implemented by Chooyoung.

Thus, the goal is to integrate a foreign gene in the cells of the tube neural of a chick embryo. Therefor, the sequence of this gene has to be created in enough quantity, and to put it inside of the target cells.

Technically, this process takes place in 3 steps :

- 1) integrate the gene in a plasmid (which will be the vector)
- 2) integrate this vector in bacterias, that are then cultured to obtain a large amount of modified plasmid
- 3) insert the modified plasmid in cells of the neural tube by electroporation

### **« Cloning » : building of a plasmid with the gene of interest.**

A plasmid is a bacteria's DNA (generally), no chromosomal, usually circular, double stranded, and capable of autonomous replication. Plasmids are not essential to the bacteria to survive, but the few genes that it carries can be very useful, in particular circumstances. Plasmids are frequently used as vector for molecular cloning, and it is the case in this experiment.

Thus, the first step is to integrate into a plasmid the foreign gene.

To be able to select the bacterias transfected with the vector during the next step, the vector that will contain the gene of interest, will have also integrate a gene of antibiotic resistance to a specific antibiotic. In this case, the plasmid will contain an ampicillin resistance gene.

How the gene can integrate the plasmid?

This is based on :

- cut the plasmid in a specific locations thanks to a restriction enzyme.
- and the exploitation of the « homologous recombination »

The homologous recombination is a natural genetic mechanism in which sequences of nucleotides are exchanges with similar DNA molecule (this universal mechanism enables to repairs DNA breakage. In eukaryotes, this mechanism also contribute in the meioses to the genetic diversity. It is also occurs during horizontal gene transfers, in bacteria and virus).

So the idea is to cut the plasmid in a specific location, and to add a DNA sequence which its extremities are identical to what is in the plasmid at the ends of the cut, and which also contains the target gene.

A restriction enzyme will cut the plasmid (endonuclease restriction ECO RV in this case). A such enzyme cleaves the DNA at specific nucleotide sequences.

The sequence to integrate into the plasmid has to contain the gene of interest and (at his 5' and 3' extremities), the homologous sequence to a part of the extremities of the plasmid (about twenty nucleotides long).

Therefore, these different elements has to be abutted. The technic enabling this process is « Gibson Cloning ».

The laboratory appeals for this to an outside specialized company, to which they order these « primers » (the company will provide the sequence with the gene of interest, the homologous sequence in its 5' and 3' extremities).

Then the sequence is ready to be integrate into the plasmids - in a small amount. The PCR will amplify the sequence.

Before, the target sequence, and the cleaved plasmid have to be purified from the products of the experiments made before (purified the sequence from the PCR products, and the plasmid from the other products of cleavage thereof).

In Chooyoung protocol, the plasmid cleaved with a restriction enzyme is only purified doing an electrophoresis. But if the vector plasmid was amplified doing a PCR without purifying it with an electrophoresis, the plasmid parent would have been eliminated adding DpN1 which is an endonuclease that can digest the parent DNA.

Finally the sequence is integrated into the plasmid thanks to the homologous recombination : the cleaved plasmids and the sequences are mixed in a buffer that contains dNTPs (the four nucleobases G, A, T, C), a polymerase, an exonuclease, and a ligase.

This preparation stays during one hour at 50°C.

### **Integration of the vector into a bacteria, and culture**

The goal is to obtain the vector (the modified plasmid) in a large amount, thanks to culture of bacteria which contains the vector.

The transfection of the vector into the bacteria can be done with two different techniques used in their laboratory (the use of one of these techniques depends on the bacteria):

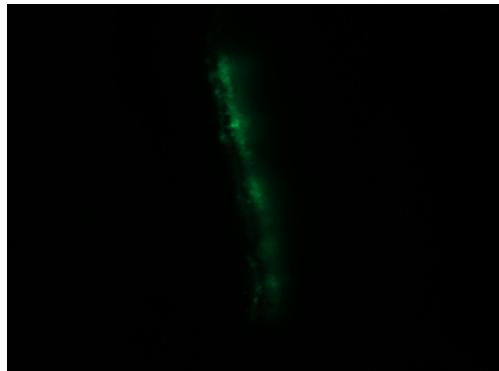
- electrical shock
- heat shock

The bacteria are then spread in Petri dish, with a lysogeny broth or « Luria-Bertani » (LB) nutritionally medium and an antibiotic (ampicillin in our case)

Then, a colony of bacteria is taken with a pipette tips, and the tips is transferred in an erlenmeyer flask which contains LB medium and ampicillin.

The day after the bacterias were incubated, few colonies are picked up to make a mini/midi or maxi preparation (it is a mini preparation in our case, in view of our sample size). It is a technic that allows to recuperate the plasmid's DNA, from the bacterias they are in. For this, this protocol is made of repeated centrifugations and washes. To verify the success of this protocol on the samples, the concentration of DNA is measured by spectrophotometry at 280nm.

### Electroporation



Neural tube of a chick embryo after electroporation  
seen with a fluorescence microscope



Neural tube of a chick embryo seen with a optic microscope

Electroporation is a technic that allows to transfer the DNA in a cell thanks to an electrical fields. DNA is negatively charged, thereby the DNA will migrate toward the positive pole of the electrical field.

The DNA is transferred into the neural tube of chick embryos in the form of a blue liquid with a capillary connected to a tube. A blow into this tube will push out the liquid. Then, the electroporation is made with two electrodes : a current will pass through the neural tube. The DNA will migrate toward the positive pole and penetrate into the cells of the tube.

### Dissection and cryostat-microtome cutting

Few days later, the chick embryos are dissected to recover the neural tube.

The neural tube is fixed in an agarose gel and frozen. Then cuts are made with the cryostat-microtome, (a cryogenic device with a microtome that allows to make very fine

cuts (14 µm) in a very cold environment (-20° C). The cuts have to be conserved at this temperature (-20°).

### **Western blot**

A western blot detects specific proteins in a sample thanks to the binding properties of antibodies. This technic is used on the cryomicrotome cuts to detect proteins produced by the DNA that was transferred in the neural tube of the chick embryos.

First, all the non specific binding sites are blocked with a bovine serum albumin (BSA) to avoid interactions with the specific antibody of the target protein. Then, the sample is incubated in a primary antibodies solution. The primary antibodies will bind with the target protein. Then the sample is rinsed to remove all the primary antibodies unbound with the protein. After, the sample is exposed to another antibody : a secondary antibody who will bind with the primary antibody. The secondary one is linked with a fluorescent enzyme to visually detect the protein.

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### Seminars

- Ehud Isacoff : “Lighting apparence GPRs : circuit to molecular mechanism”
- Tobias Bonhoeffer : “How experiences changes synapses in the mammalian brain”

# Week 3 - Pierre Paoletti's team

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## Glutamate receptors and excitatory synapses

Glutamate is the most excitatory neurotransmitters in the vertebrate (the majority of the ion channels on the post-synaptic receptors work with glutamate receptors). The NMDA (« N-methyl-D-aspartate ») are a ion channels receptors group of the glutamate. They are important for synaptic plasticity related to learning, and memory. Furthermore, dysfunctions of these receptors can be the origin of pathologies such as chronic pains, schizophrenia, and stroke.

Pierre Paoletti's team study the organisation, the molecular architecture, the working and the diversity of these receptors. One of their goal is to better understand their involve in particular pathologies.

These receptors are tetramers organized in dimers of dimers. They are composed of two subunits GluN1 binding glycine and two others GluN2 binding glutamate (in most of the cases). These two antagonists (molecule interacting with a receptor, in this case the NMDA receptor) are necessary to open the ion channel of the NMDAs. Their fixation to the subunits of the receptors activate the channel.

It is Pierre Paoletti's team that, for example determined the spatial arrangement of the subunits GluN1 and GluN2 around the ion channel.

<http://www.ibens.ens.fr/spip.php?rubrique24>

## References

NMDA receptor subunit diversity: impact on receptor properties, synaptic plasticity and disease (Pierre Paoletti, Camilla Bellone & Qiang Zhou)

<http://www.nature.com/nrn/journal/v14/n6/full/nrn3504.html>

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## Influence of azospermine (AzoSp) on NMDARs receptors.

This week with Læticia Mony, we tested the influence of AzoSp on NMDARs receptors. L'AzoSp has different effects on the NMDARs receptors activation, depending on its concentration : potentiation of the receptors at low concentration ( $0,1\text{-}1\mu\text{M}$ ), and inhibition at a higher concentration ( $3\text{-}10\ \mu\text{M}$ ). Furthermore, l'AzoSP exists in two different configurational isomerism *cis* and *trans*. The *cis* configuration of AzoSp is obtained by illuminating at 365nm (UV) and AzoSp prefers the *cis* conformation. After illuminating at 440nm (blue light), AzoSp re-conforms in its *trans* configuration.

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## Model organism

The model organism used by Lætitia Mony is the *xenopus* oocyte (a kind of frog). The oocyte of *xenopus* is a big cell, it is around 1mm of diameter, which allows to inject easily DNA inside. Other advantage, the *xenopus* produces a lot of eggs. These are collected by a surgical intervention : a cut in the abdomen allows to collect the oocyte bags. We can re operate the *xenopus* few months after to collect again its oocytes. This model is economic, because *xenopus* don't need a lot of care, they are very resistance and their eggs dont need to be manipulate in a sterile environment.

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## Experimental work

### **Collect of the oocyte bag of a xenopus**

A cut is made in the abdomen of a *xenopus*, who was anesthesiazied before, and the bags are collected with a clamp.

Then the animal is closed by suturing the muscle, and then the skin. The animal is then isolated from the other *xenopus* during the healing time to avoid complications.

After the operation, the oocyte are sorted, the dead ones are thrown away. Then, the eggs are incubated in collagenase which is going to digest the tissues from the bag.

### **Genetic**

Like in all the research laboratory I have visited, Lætitia Mony does a lot of genetic work : cloning, bacteria culture, PCR, sequencing, etc. The procedures vary depending of the labs, and the goal of the researchers.

PCR was already mentioned in the first week part, and a cloning technic has been described in the second week part. In this third part, the way of sequencing DNA will be described.

Sequencing is used to verify if the DNA sequence amplified after a PCR, or cultured inside of bacteria (in which it was inserted for exemple by a plasmid used as a vector, cf. week 2) is the DNA wanted.

The sequencing is not made directly in the lab. A sample of plasmids containing the DNA took of the transfected bacterias are send to an other laboratory in Germany. The results are send the day after by e-mail. Then these results have to be analyzed.

Lætitia Mony has to compare the DNA sequences sent to sequencing, to the wild type DNA sequence for the NMDARs.

The protein wild type sequence of the wild type NMDARs receptors can be find on <http://www.uniprot.org>.

Now the nucleotide sequence and this amino acid sequence have to be compared together.

To do that, the nucleotide sequence is translated as a protein sequence thanks to the web site [web.expasy.org](http://web.expasy.org). This web site allows to translate a nucleotide sequence into a protein sequence, by setting at different starting point of the sequence that could be an open reading frame. Then the protein sequences as obtained and the wild type protein

sequence are aligned to compare them together on the website [www.ebi.ac.uk/Tools/msa/clustalw2/](http://www.ebi.ac.uk/Tools/msa/clustalw2/). (see annex for an example)

### **Transformation of DNA into *xenopus* oocyte**

The target DNA is injected into the *xenopus* oocyte with a capillary controlled by a machine.

### ***Clampage de l'oocyte***

Now, the goal is to observe the different responses of the oocyte genetically modified to different stimuli. With Lætitia Mony we observed the effects of the AzoSp *cis* and *trans*. The transfection of the oocyte worked if an electrical response to the release of agonists in the oocyte media is observed. This release supplies the two subunits GluN1 and GluN2b, and so opens the ion channels, causing a flow of anion, modifying the electrical potential of the membrane. The electrical potential of the membrane is measured with two electrodes on the cell.

*Results : see annexes*

# Week 4 - Germán SUMBRE's team

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## Neuroethology of zebra-fish

Germán Sumbre's team study the neuroethology of the zebra-fish, i.e how its nervous system controls the behaviors of the zebra-fish.

<http://www.ibens.ens.fr/spip.php?rubrique23>

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## Model organism

The zebra-fish is a model organism often used in neuroethology. It is a little fish, which does not measure more than 5cm in his adulthood. It is found in Indian freshwater. Easy to raise, it lays a lot of eggs during the entire year. It has the particularity to be transparent during the beginning of its development, that allows to observe easily *in vivo* its physiological mechanisms using fluorescent methods. It is also capable of regeneration. For example, to obtain the genotype of a zebra-fish that was tried to be genetically modified, a piece of its tail can be removed and analyzed. The tail will grow again.

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## Experimental work

Like every weeks, genetic is the bases of zebra-fish study in this lab. But, they also use behaviors analysis technics : what are the results of the manipulations made on the animal behavior ?

### **Mixing of zebra-fish**

German Sumbre's team makes the mixing themselves in the animal room. The males and females required for the mixing are placed in an aquarium, but they are separated with a grid. Males and females stay separated all night long : it is a way to excite them to optimize the mating. Therefore, when the light is turned on in the morning and the grid is taken off, the fishes mate quickly, and right after, females lay eggs.

### **DNA Transformation into fish eggs**

Two researchers from « Collège de France » came during this week to realize a transfection of DNA into zebra-fish eggs.

The technic is the same than the one used by Lætitia Mony (week 3).

The zebra-fish eggs can be transfected with DNA only when it is composed of one cell, i.e 0 to 20 min after the laying.

So it is more delicate with zebra-fish eggs than with the *xenopus* oocytes because the cells are much more smaller, and there is a time constraint.

## **Targeted destruction of neurons with a bi-photonic microscope**

This week, Adrien Jouary begun the conducting of a research on the influence of some neurons of the zebra-fish on its movements. He wants to proceed to the ablation of these few neurons to study the impact of their lack on the zebra-fish movements. For that he uses a bi-photonic microscope

The bi-photonic microscope was a big step to the observation of *in vivo* cells, allowing a depth image of live cells.

This microscope is equipped with a laser, that allows to burn cells on which it is focused : therefore neurons can be destroyed one by one.

For that, the zebra-fish has to be immobilized. It is immobilized in a drop of agarose 2% concentrated, and it is enclosed with 4% agarose, to make sure the fish can not escape.



zebra-fish immobilized in a drop of agarose, seen in a optic microscope

After this operation is done, the fish is released from the agarose to observe its movements. A camera linked to a computer is placed right above the fish pond. An informatics program subtract the first image (with the fish pond, and the fish inside) to the other images taken during the recording. After the subtraction, it will only last the position of the fish in the frame. Like this we can calculate the speed, and observe the movements of the fish on an image made by the computer.

### **Fin clip**

This technic consists to cut a little piece of the fish tail, to then conduct the sequencing of its DNA. For that, the fishes are sedated by introducing a sedating solution in their aquarium, and then taken of the water. Then a piece of their tails are cut.

After, they are taking back to an aquarium, with only fishes that add an ablation, like this they will not be in competition to feed themselves for example.

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Seminar

György Buzsaki - Emergence of cognition from action

## Conclusion

During this stage, I had the chance to interact with teams using different model organisms, for different problematics. A particularly interesting aspect for me was to see the uses of biology cellular and molecular technics « day by day » in the context of the research conducted : PCR, electrophoresis, cloning, electroporation... etc.

I have to confess, everything was not always easy to follow for me, even with the goodwill of the people I met there : I had only with me the knowledge of a freshman student. So I tried to understand how and why these different technics were used. There is probably a lot of things I missed, but I also learnt a lot of other things, I would not have seen, or understood this way, learning it in college.

# **Annexes**

## **Annex week 1**

### **procedure for the making of cell's media**

*N SPASSKY Lab*

## **SOLUTIONS AND REAGENTS**

### **Poly-L-Lysine (SIGMA Poly-L-Lysine Hidrobromide P1524):**

Stock 50X (keep at -20C): eppendorfs 1ml, 2mg/ml in sterile water. Filter the solution with a 0.22 µm filter.

Working solution (keep at 4C): dilute 1ml of PLL 50X in 49 ml of sterile MilliQ water.

### **Protocol for cleaning the coverslides:**

Heat 100ml of 68% nitric acid in a beaker under the hood. Put the coverslides (one by one) inside the solution once it is hot and keep the coverslide there during 1 hour (under vigilance) or during 18-36 hours at room temperature.

Rinse several times with distilled water.

Transfer the coverslides one by one with forceps to a Petri dish filled with fresh distilled water (x3).

Dry the coverslides under the hood in a filter paper of at 70C in the oven.

Once dried, sterilize the coverslides (220C during 7 hours, in a glass Petri dish covered by foil).

### **Hank's solution (4C):**

50ml Hanks balanced salt solution 10X (GIBCO, RT)

5 ml HEPES 1M (GIBCO, 15630-056, RT)

5 ml sodium bicarbonate (7,5%; GIBCO, 25080, RT)

5 ml Peni/Strepto (10000 Units; GIBCO 15140, -20C or 4C)

435 ml sterile water

Filter the solution and keep at 4C.

### **DMEM 10% FBS 1% P/S**

450 ml DMEM Glutamax-I (GIBCO 31966, RT or 4C)

50 ml decomplemented FBS (GIBCO 10270, -20C. To decomplement the FBS, once defrosted, heat it in the water bath at 56C during 30 min. Once decomplemented, keep it frozen at -20C.

5 ml Peni/Strepto (10000 Units; GIBCO 15140, -20C or 4C)

### **DMEM 0% FBS 1% P/S**

500 ml DMEM Glutamax-I (GIBCO 31966, RT or 4C)

5 ml Peni/Strepto (10000 Units; GIBCO 15140, -20C or 4C)

**L15:** Liebovitz's L-15 medium (GIBCO 11415, 4C)

**Enzymatic digestion solution** (make fresh when needed)

3 ml DMEM ~~10%~~ FBS 1% P/S (enough 1 ml/centrifuge)

90 µl Papain (Worthington 3126; 100 mg, 4C)

45 µl DNase I (1%; 100 mg in 10 ml water. Worthington 2139, 100mg)

72 µl Cysteine (12mg/ml in water; L-Cysteine Sigma C7352)

Filter the solution

① filter as some DNase  
② filter & complete a 6 ml with DNase.

**Stop Solution** (make fresh when needed)

9 ml L15

(soche ref: 10 109 878 001. 1 inhibits papain)

1 ml trypsin inhibitor (Worthington 3126; 100 mg, 4C). Stock 10mg/ml trypsin inhibitor + 500 µg/ml BSA in PBS or L15°

200 µl DNase I (1%; 100 mg in 10 ml water. Worthington 2139, 100mg)

Filter the solution

**Trypsin-EDTA 0,5%** (GIBCO 25300)

DNase F12 + NC + BSA + 2% FBS      P/S → 50 µl  
mélanger et utiliser pour tester la      BSA 50x → 1x  
plasticité des cellules induit      PBS 2%  
par les acides, minéraux, et oligo      NC 100x → 1x  
DNase F12 + EGF + FGF      DNase F12 9/10 Some  
FGF 20 µl/ml → 10 µl  
EGF " " " "  
P/S → 50 µl  
NC 100x → 1x  
DNase F12 9/10 50 ml

dissous au néurospore: éteindre mélange  
ajouter Hank's + EDTA  
laisser 40' à 37°C  
dissous avec pipette

Malaxage: diluer 1/10 PBS  
mélanger 50 µl  
compter 8 lignes

# Procedure for ependymal cell culture

7/06/2016

N SPASSKY Lab

## EPENDYMAL CELL CULTURE

sox : 1ml + 35ml H2O

1. Coat with **Poly-L-Lysine** (PLL ; 40 $\mu$ g/ml) one 25 cm<sup>2</sup> flask per newborn mice :
  - Add 1ml of sterile PLL per flask.
  - Incubate at 37°C during 1 hour
  - Rinse x3 with sterile MilliQ water
  - Let the flasks dry under the hood, at least for 1 hour.
2. Prepare the **enzymatic digestion solution** (see the solutions protocol). Prepare approximately 1 ml per telencephalon. (*par cerveau*)
3. Dissect the P0-P10<sup>4</sup> mice telencephala (remove the hippocampus, the choroid plexus, the olfactory bulb and meninges) in Hank's or PBS-Glucose solution (keep it on ice once dissected). *sur boîte plastique*.
4. Cut the dissected telencephala into little pieces with the help of a scalpel. Transfer it into a 15 ml tube.
5. Centrifuge 5-7 min at 100G
6. Remove the supernatant and add 1ml of enzymatic digestion solution per mice. (*filtrée*)
7. Incubate 45 min at 37°C (30 - 1h30).
8. Prepare the **stop solution** (see the solutions protocol) (*filtrée*)
9. Centrifuge 5-7 min at 100G. *Aspirer avec pipetman*
10. Remove the supernatant and add 1ml of stop solution per mice. (10ml)
11. Centrifuge 5-7 min at 100G *(au 1000 rpm)* *Aspirer avec pipetman*
12. Remove the supernatant and add 15ml of L15 media.
13. Centrifuge 5-7 min at 100G = *ny*
14. Remove the supernatant, add 1ml of L15 media and dissociate carefully the cells mechanically, with a P1000 (do not pipet the cells more than 10 times). Add L15 to 15 ml 5 ml (*veut pas être trop de bulle*, *ne pas mixer trop vite*, *Dmax de 10 ml*)
15. Centrifuge 5-7 min at 100G (*important faire 5'*) (*mixer doucement*)
16. Remove the supernatant, add 1ml of 15 and redissolve mechanically the cells. Add L15 to 15 ml 5 ml *Aspirer au pipetman*
17. Centrifuge 5-7 min at 100G )
18. During the centrifugation, add 5ml of DMEM 10%FBS 1%P/S per 25 cm<sup>2</sup> flask.
19. Remove the supernatant and add DMEM 10%FBS 1%P/S (1ml per mice) => Pas bonne digestion si *Aspirer au pipetman*
20. Add 1ml of cell solution per flask. *et remettre en suspension*
21. Change the media the following day (no need to rinse)



*cellules vivantes*  
*retirer milieu*  
*au pipetman le*  
*plus possible*  
*(il reste L15 sans*  
*graine).*

- ( 2-5 jours)
22. When cells are confluent, cover the top of the flask with parafilm and shake the flasks at 250rpm over night at room temperature.
- 1h 37 °C
23. Coat round pre-cleaned sterile coverslides with PLL (the same as flask coating protocol) Rinse 3x in H<sub>2</sub>O - Dry → 1h. ( à 5 jours conf on a <sup>1</sup> <sub>1</sub> <sup>1</sup> <sub>1</sub> <sup>1</sup> <sub>1</sub> <sup>1</sup> <sub>1</sub> <sup>1</sup> <sub>1</sub> lamelle / flask )
24. Remove the media of the flasks and rinse with/sterile Ca/Mg free PBS three times.
25. Add 1ml of Trypsin-EDTA solution per flask and incubate for 5 min at 37C. ( 2-5 min ).
26. Look the cells at the microscope to see if they are free floating.
27. Transfer the cells to a 15ml tube and add 1ml of decomplemented FBS per ml of cell solution. ⇒ count the cells (20 µl) → 1ml Trypsin + 1ml SVF & 1ml.
28. Centrifuge 5-7 min at 100G
- ( 29. Remove the supernatant and add 1ml of DMEM 10%FBS 1%P/S per flask. )
30. Count the cells (10 µl of trypan blue + 20 µl of cell solution)
31. Centrifuge 5-7 min at 100G ]
32. Resuspend the cells in the correct amount of DMEM 10%FBS 1%P/S, to make as many drops of 20 µl with  $1.5 \times 10^5$ - $2 \times 10^5$  cells as needed.  $\frac{2 \cdot 10^5}{0.15 \cdot 10^5}$  cells per 20 µl per well
33. Put the drops of 20 µl carefully in the coated coverslides, incubate for 1 hour at 37C (30min minimum). (this allow the cells to adhere at high density)
34. After 1 hour, add 500 µl per well (24 well dish) of DMEM 10%FBS 1%P/S
35. The following day (Day 0):
- ( Rinse the cells x3 (DMEM 1%P/S without FBS) )
- Add 500 µl per well (24 well plaque) of DMEM 1%P/S

Schéma de prép de DMEM P/S 02 SIF

NB : à 5 jours de confluence on a besoin de 12 lamelles / flask pour mettre des gouttes.  
Si + de 5 jours de confluence au si 4 de ces confluences, faire + de lamelle.

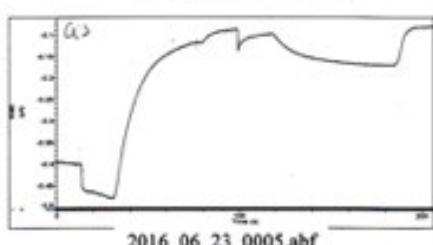
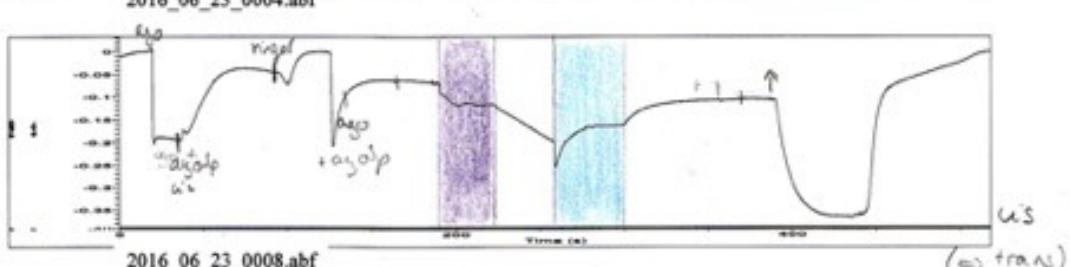
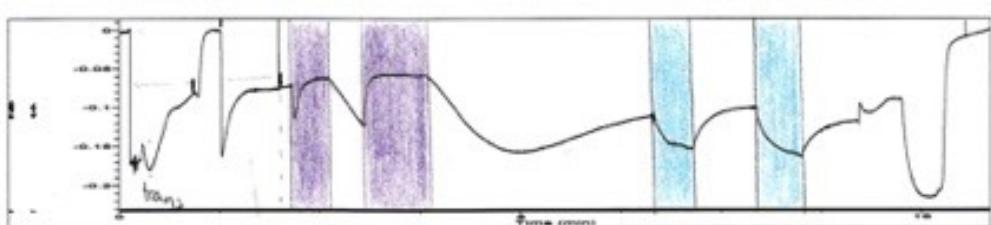
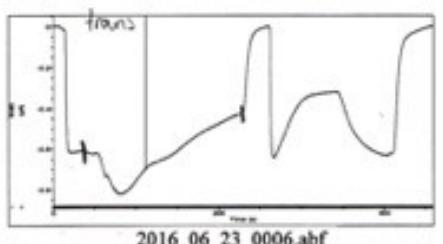
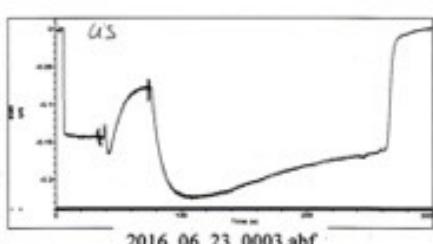
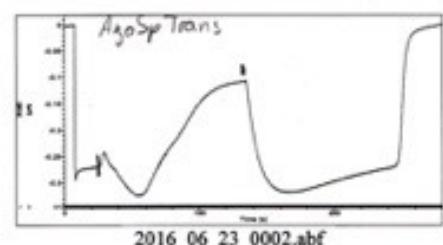
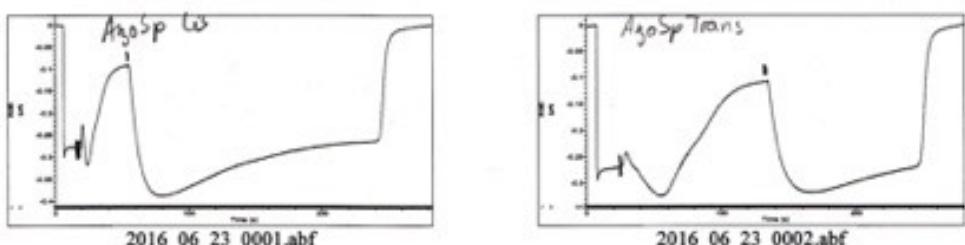
# Annex week 3

## Results of electrophysiology on xenopus oocytes

electrophysiology on xenopus oocytes - 6/22/16

1µg/µL AzoSp - 100µg/µL agonists (Glu, Gly, DNTA)				
-60 mV	current with AzoSp (-µA)	current with AzoSp (-µA)	residual current (µA)	
	<u>Trans</u>			
1	0,44	0,06	0,13636363636363	
2	0,194	0,0384	0,19793814432989	
3	0,31623	0,0804	0,25424532776776	
4	0,26	0,06537	0,25142307692307	
8	0,27	0,07	0,25925925925925	
11	0,03	0,01	0,33333333333333	
average			<b>0,23876046299616</b>	
	<u>Cis</u>			
6	0,2451	0,0848	0,34598123215014	<u>Student test</u> 0,08494728
8	0,26	0,09	0,34615384615384	
10	0,20488	0,05350	0,26112846544318	
11	0,4705	0,1307	0,27778958554729	
average			<b>0,30776328232361</b>	
-30 mV				
	<u>Cis</u>			
5	0,15142	0,165	1,08968432175406	
	<u>Trans</u>			
9	0,6228	0,7853	1,26091843288375	

<b>10µg/µL AzoSp - 100µg/µL agonists (Glu, Gly, DNTA)</b>			
<b>-30 mV</b>	<b>current with AzoSp (-µA)</b>	<b>current with AzoSp (-µA)</b>	<b>residual current (µA)</b>
	<b>cis</b>		
<b>1</b>	0,28	0,09	0,321428571428571
<b>3</b>	0,14	0,08	0,571428571428571
<b>average</b>			<b>0,446428571428571</b>
	<b>trans</b>		
<b>2</b>	0,27	0,11	0,407407407407407
<b>6</b>	0,6	0,409	0,681666666666667
<b>4</b>	0,17	0,08	0,470588235294118
<b>average</b>			<b>0,519887436456064</b>
<b>5 (mutant ΔNTD)</b>	0,07486	0,02963	0,395805503606733
	<b>current with AzoSp (- µA) (trans)</b>	<b>current with UV(345nm) (cis)</b>	<b>residual current (µA) (trans=&gt;cis)</b>
<b>4</b>	0,08	0,07	0,875
<b>4 after UV radiations</b>	<b>current before other UV radiations</b>	<b>current after</b>	
	0,139	0,04	0,287769784172662
	<b>courant avec AzoSp (-µA)</b>	<b>current with blue light radiations (440 nm) (trans)</b>	
	0,059	0,15	2,54237288135593
	<b>current before other blue light radiations</b>	<b>current after</b>	
<b>4 after blue light radiations</b>	0,105	0,155	1,47619047619048



markant: GluN<sub>1</sub> WildT GluN<sub>2B</sub> ΔNTD

~ 30mV

vendredi 24 juin 2016, 15:39:47

Name: \_\_\_\_\_

## Treatment of a sequencing of three sequences (sequences 2.1, 2.2, and 2.4) that are mutated for the sequences coding for GLUN2B

### sequence 2.1

```
XHWXIFSRXTYFPGYQDFVNKIRSTIENSFGWELEEVLLDMSLDDGDSKIQNQLKKL  

QSPIILLYCTKEEATYIFEVANSVGLTGYGYTWIVPSLVAGDTDTVSEFPTGLISVSYD  

EWDYGLPARVRDGIAIITTAASDMLSEHSFIPEPKSSCYNTHEKRIYQSNMLNRYLINV  

FEGRNLNSFSEDGYQMHPKLVIILLNKERKWERVGKWKDKSLQMKYYVWPRMCPETEEQED  

DHLSIVTLEEAPFVIVESVDPLSGTCMRNTVPCQKRIISENKXDEEPGYIKXCCKGFCID  

ILKKISKSVKFTYDLYLVTNGKHGKINGTWNGMIXEVVMKRAYMVGSLTINEERPEVV  

DFXXPFXXTGISM
```

### sequence 2.2

```
FXIVXPXTSPATRTS-TRSAALLRTALCAGSSRKSSC-TCL-TMATLRFRIS-RSCRAPS  

SSTAQRKKYDXVXLLHXRXYFPGYQDFVNKIRSTIENSFGCELEEVLLDMSLDDGDSK  

IQNQLKKLQSPIILLYCTKEEATYIFEVANSVGLTGYGYTWIVPSLVAGDTDTVSEFPT  

GLISVSYDEWDYGLPARVRDGIAIITTAASDMLSEHSFIPEPKSSCYNTHEKRIYQSNML  

NRYLINVTFEGRNLSFSEDGYQMHPKLVIILLNKERKWERVGKWKDKSLQMKYYVWPRM  

PETEEQEDDHLSIVTLEEAPFVIVESVDPLSGTCMRNTVPCQKRIISENKXDEEPGYIKX  

CCKGFCIDILKKISKSVKFTYDLYLVTNGKHGKINGTWNGMIGEVVMKRAYMAVGSLTI  

NEERX
```

Protein sequences of four samples sent to sequencing. The part highlighted in pink matches open reading frames.

### sequence 2.4

```
LVXLLHRXTYFPGYQDFVNKIRSTIENSFGWELEEVLLDMSLDDGDSKIQNQLKKLQS  

PIILLYCTKEEATYIFEVANSVGLTGYGYTWIVPSLVAGDTDTVSEFPTGLISVSYDEW  

DYGLPARVRDGIAIITTAASDMLSEHSFIPEPKSSCYNTHEKRIYQSNMLNRYLINVTE  

GRNLNSFSEDGYQMHPKLVIILLNKERKWERVGKWKDKSLQMKYYVWPRMCPETEEQEDDH  

LSIVTLEEAPFVIVESVDPLSGTCMRNTVPCQKRIISENKTDEEPGYIKXCCKGFCIDIL  

KKISKSVKFTYDLYLVTNGKHGKINGTWNGMIGEVVMKRAYMAVGSLTINEERSEXDF  

SXPFXXTGIXXMXVRSGNTXXP
```

2.2  
2.4  
**GLUN2B**  
2.1

QDFVNKIRSTIENSFGCELEEVLLDMSLDDGDSKIQNQLKKLQSP**IILLYCTKEEATY**  
**QDFVNKIRSTIENSFGWELEEVLLDMSLDDGDSKIQNQLKKLQSP**IILLYCTKEEATY  
**QDFVNKIRSTIENSFGWELEEVLLDMSLDDGDSKIQNQLKKLQSP**IILLYCTKEEATY  
**QDFVNKIRSTIENSFGWELEEVLLDMSLDDGDSKIQNQLKKLQSP**IILLYCTKEEATY

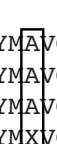
it is the wild type sequence, which the others sequences are compared with

2.2           **IEVANSVGLTGYGYTWIVPSLVAGDTDTVSEFPTGLISVSYDEWDYGLPARVRDGIAI**  
2.4           **IEVANSVGLTGYGYTWIVPSLVAGDTDTVSEFPTGLISVSYDEWDYGLPARVRDGIAI**  
**GLUN2B**     **IEVANSVGLTGYGYTWIVPSLVAGDTDTVSEFPTGLISVSYDEWDYGLPARVRDGIAI**  
2.1           **IEVANSVGLTGYGYTWIVPSLVAGDTDTVSEFPTGLISVSYDEWDYGLPARVRDGIAI**  
\*\*\*\*\*

2.2           **ITTAASDMLSEHSFIPEPKSSCYNTHEKRIYQSNMLNRYLINVTFEGRNLSFSEDGYQMH**  
2.4           **ITTAASDMLSEHSFIPEPKSSCYNTHEKRIYQSNMLNRYLINVTFEGRNLSFSEDGYQMH**  
**GLUN2B**     **ITTAASDMLSEHSFIPEPKSSCYNTHEKRIYQSNMLNRYLINVTFEGRNLSFSEDGYQMH**  
2.1           **ITTAASDMLSEHSFIPEPKSSCYNTHEKRIYQSNMLNRYLINVTFEGRNLSFSEDGYQMH**  
\*\*\*\*\*

2.2           **PKLVIIILLNKERKWERVGKWKDKSLQMKYYVWPRMCPETEEQEDDHLSIVTLEEAPFVIV**  
2.4           **PKLVIIILLNKERKWERVGKWKDKSLQMKYYVWPRMCPETEEQEDDHLSIVTLEEAPFVIV**  
**GLUN2B**     **PKLVIIILLNKERKWERVGKWKDKSLQMKYYVWPRMCPETEEQEDDHLSIVTLEEAPFVIV**  
2.1           **PKLVIIILLNKERKWERVGKWKDKSLQMKYYVWPRMCPETEEQEDDHLSIVTLEEAPFVIV**  
\*\*\*\*\*

2.2 2.4 GLUN2B 2.1	ESVDPLSGTCMRNTVPCQKRIISENKXDEEPGYIKXCKGFCIDILKKISKSVKFTYDLY ESVDPLSGTCMRNTVPCQKRIISENKTDDEPGYIKXCKGFCIDILKKISKSVKFTYDLY ESVDPLSGTCMRNTVPCQKRIISENKTDDEPGYIKKCKGFCIDILKKISKSVKFTYDLY ESVDPLSGTCMRNTVPCQKRIISENKXDEEPGYIKXCKGFCIDILKKISKSVKFTYDLY ***** ***** *****
2.2 2.4 GLUN2B 2.1	LVTNGKHGXKINGTWNGMIGEVVMKRAYMAVGSLTINEERX----- LVTNGKHGKKingTWNGMIGEVVMKRAYMAVGSLTINEERSEXXDFSXPFXXTGIXXMVX LVTNGKHGKKingTWNGMIGEVVMKRAYMAVGSLTINEERSEVVDFSVPIETGISVMVS LVTNGKHGKKingTWNGMIXEVVMKRAYMXVGSLTINEERPEVVDXFXXPFXXTGISXM-- ***** ***** ***** *****


 the whitespaces  
 shows a difference  
 of one amino acid  
 between the  
 sequences

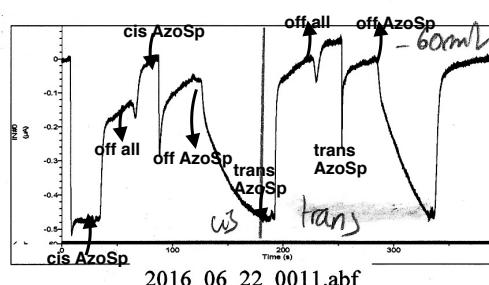
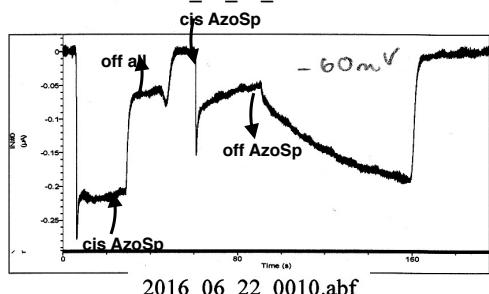
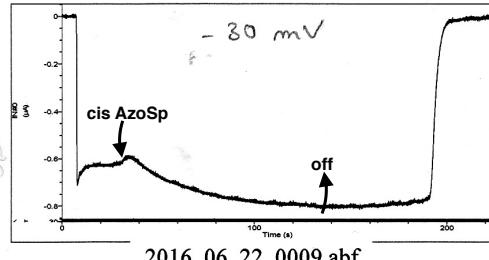
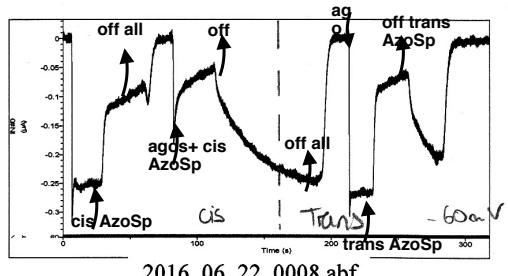
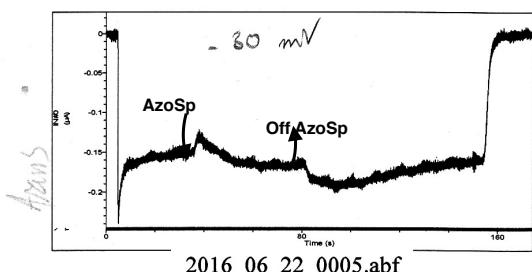
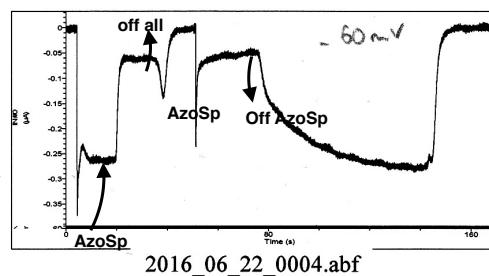
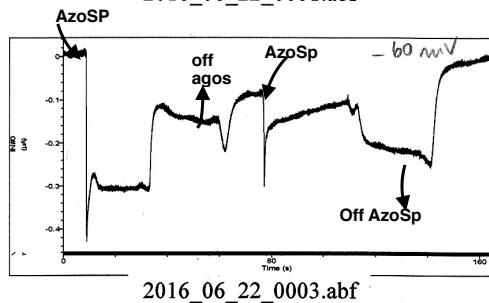
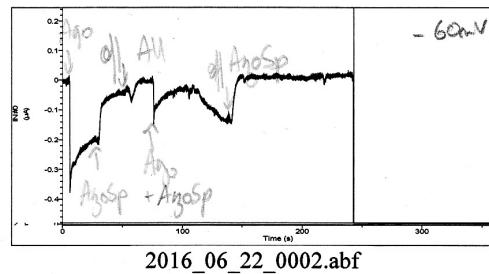
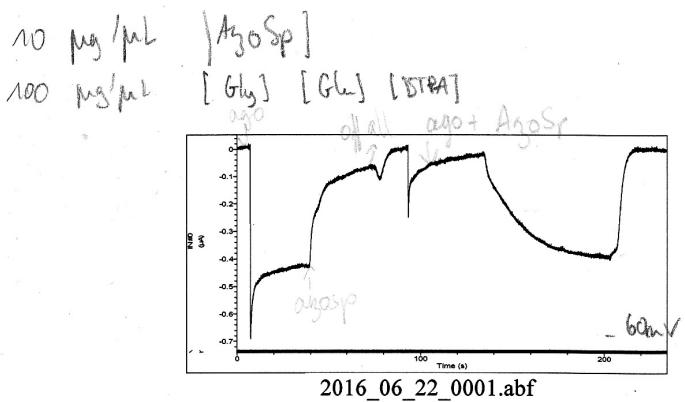
the whitespaces  
shows a difference  
of one amino acid  
between the  
sequences











électrophysiologie sur orocyte de xénope

22/06/16

vendredi 24 juin 2016, 13:58:13

Name: \_\_\_\_\_

GluN1 WT  
GluN2 WT