Three hours inside the European training network “FLiACT”

Systems neuroscience of Drosophila:
From genes to circuits to behaviours

A protocol for a role game and laboratory experiments to discover the FLiACT project.

Workshop designed for teenagers between 14 and 16 years old (ESO3-ESO4)

Group size:
2 leaders by group of 30 students

Spaces required:
1 meeting room that can host 2 groups of 15 students; 4 experiments stations in lab or office space

http://www.fliact.org/home

Contents

SEVENTH FRAMEWORK PROGRAMME

MARIE CURIE ACTIONS

CRG Centre for Genomic Regulation

General guidelines:

0min	Meeting room	**Role Game part 1: Introduction** The leader/moderator welcomes the students, acting as the coordinator of the project, he/she: • gives accreditation card to all the group members (See annex) • propose to the members of the network to place their coat and bags in a specific place, take their notebook and pencil General introduction: • Goals of this European network • Remind rules/scientific attitude • Goal of this "emergency" meeting: concrete problem to solve Formation of groups of "experts" who are specialized in different techniques (2/3 people per group)
40min	4 lab spaces	**Role Game part 2: Experiments** Anatomy/Microscopes; observe different phenotypes in adult fly Computers/Microscope/Calcite imaging: Analyse neurons´ images done with Calcite imaging technique. Correlate them with samples observation on the microscope Molecular biology/genetics: electrophoresis gel with food colour to simulate the presence or absence of a specific gene (Orco) Behaviour: check if larvae can smell a specific odour Coordination, supervision: check all the experiments and take pictures of all of them
1h40min	Meeting room	**Role Game part 3: Sharing results, conclusion, discussion** Reforming the initial group of 15 people, • Experts put all the results together (table format) • Conclusions by the project coordinator
2h15min	End	Extra visits to real lab can be proposed And/or more explanation on concrete FLiACT project And/or interview of FLiACT fellows.

Detailed guidelines

Material in the meeting room per group of 15 students

- 1 digital camera (ask students to come with one)
- 1 big poster with the result table that needs to be filled in part 3 , + 1 Sharpi pen
- 1 computer connected to FLACT website
- 15 notebooks and pencils
- 4 tubes with flies without identification to present the case
- 5 maps of the installations with the localization of the 4 lab stations, inside one file hanging on the wall next to evacuation sign
- Lab coats/ glasses for the project coordinator

Material in the 4 lab stations

Anatomy/Microscopes station

- 2 loupes or microscopes to observe adult flies
- CO2 fly station
- 2*4 different tubes with flies labelled A,B, C, D
- 2 brushes

Computers/Microscope/Calcite imaging station

- 2 computers
- With 4 different neurones pictures set (A, B, C, D) installed on it (see references)
- 1 microscope with fluorescent light
- with 4 different set of slides and larvae to observe

Molecular biology/genetics station

- 2 agar gels at 1% ready to load in 2 different electrophoresis chambers
- 2* 4 DNA samples (A, B, C, D)
- 2* 1 micropipette at 20µl + tips
- 2 Electrophoresis Gel protocols

Behaviour station

- 2 behaviour protocols
- 2 * 4 agar plates
- 2* 4 ring stickers
- 2*1 odour in concentrate dilution + 2*1 2µl micropipette
- 2*4 tubes with larvae (A, B, C, D) + 2*1 small brush + 2*4 plastic plates
- 1 bottle of sugared water

Material for the Communication /supervision team

- 2*1 digital camera

Role Game part 1: Introduction

The leader/moderator welcomes the students, acting as the coordinator of the project, Matthieu Louis:
He/she is giving accreditation card to all the group members (See annex)
All names are based on real researches profiles, that can be found on the FLiACT website: www.FLiACT.eu
Students can change the little papers on the front of the cards, in order to be identified with a male or female first name

The coordinator proposes to the members of the network:

- to place their coats and bags in a specific place,
- to take their notebook and pencil

Goals of this European network

Hi good morning everyone.
As you all know, I am Matthieu Louis/Maya Louis.
I am working here at the CRG as a group leader since 2008. I am from Belgium I have a background in Physic, Computer Science.
My lab is working on olfaction, the sense of smell, and the connection between this sense and the brain:
How does the nose detect odours? How this information computed/processed in the fly brain?
For our research we used larvae as a model (baby flies).
Studying the brain of the fly and manipulating its function represents a challenge that is too big for one lab. Like the physicists at CERN who were trying to find the boson X, we decided to work in a group and to create a network. As we need to have access to more knowledge and resources, I really wanted to put in place this network. Thanks to a successful application to a European grant, this network is now a reality since January 2012.
We chose to call this project FLiACT for Fly and Action. The project mainly focuses on behaviour, studying the brain as a circuit, a computational circuit;. Another important aim is to share knowledge and expertise, and to trainall the FLiACT scientists.
The questions we aims to solve are all related to the senses: How is behaviour controlled by the brain? How do we receive this sensory information, where and how do we store them, how do we connect the different sensory pieces of information together? In addition, we want to model all processes, to understand the basis of perception and link it to decision making. This means that we want to describe these processes not only with words but with physical, mathematical laws.

Why do we use flies as a model organism: adult or larvae

- They have a relatively short life cycle: in 2 weeks we can have the next generation
- No sophisticated equipment are needed to keep them, only space to put the tubes at a controlled temperature,

- They do not need a lot of space and food: one tube with some yeast and sugar can contain easily 40 flies
- They do not need an intense care to survive. You don't need to have someone giving them food every x hours, but only to change their tubes every 4 weeks
- The fly genome has been sequenced and well described. We know the genome of the fly but also the gene expression, the proteins expression.
- We have a lot of biotechnological tools to change the gene expression: switch on and of specifics genes
- Flies have a small number of neurones, 21 in the larvae's nose, compared to millions in vertebrates.

Role of the CRG

The CRG is leading this European project.

About CRG: The CRG intends to play in the "Champions League" of research in the field of biology: that means the science that is done here is "very well done", and internationally recognized for its importance in the field of biology and health. The CRG believes the medicine of the future depends on the current innovative science. This requires an interdisciplinary scientific team focused on understanding the complexity of life, from the genome to the cell to a complete organism and its interaction with the environment, which provides a comprehensive view of genetic diseases. The CRG pioneered Catalunya. It was the first research center with public money has a way of "private" function: none indefinite contract, demand for results, etc.

So we have an obligation to behave properly to continue at this level. **Reminder rules/scientific attitudes**

- Respect the others, no loud discussions or noise in the lab, corridors, all Installations
- When you are walking in the Centre, look around and do not block/stop anyone; leave space in order for researchers to pass by you; and that their samples do not fall.
- Do not touch anything without gloves in the lab
- Do not touch anything in the lab, except what is specified in the written protocol
- People who cannot respect these rules, will come back with the teacher in the meeting room, doing and seeing nothing.

Goal of this "emergency" meeting: concrete problem to solve

Flies species we want to study are coming directly from Germany.

During the transport process, probably at the custom inspection, all flies tubes were taken out of their boxes and put back in again. We are not sure that each tube is back at its initial place.

These tubes did not have any stickers on them, but their individual references were only on the box; which was a big management mistake…

So today, our mission is to find out which tube is which, and be sure about each tube's specific identify: A, B, C, D
For that we will need all your knowledge, expertise, and at least 4 experiments.

All the species were sent to us because they have a specific genotype that will help us to study olfaction receptors (ORs) connected to the brain circuit in order to explain their specific function. Leslie Vosshall first discovered how OR proteins work together to enable a fly to sense odours. When a fly is exposed to a particular odour, specific ORs which form an ion channel with ORCO pick up the scent and activate the sensory neuron. When ORCO is absent, the OR protein can no longer reach the cell membrane, making the neuron unable to sense odors.
In short, ORCO is an essential co-receptor for the olfactory neurons to function in the fly: with his help, receptors can be activated and the fly can smell. Without ORCO's help, olfactory receptors become dysfunctional and the fly is unable to smell.

Calcium imaging functions thanks to a genetically encoded calcium indicator called **GCaMP**. GCaMP is a molecule that becomes fluorescent after its binding to Ca^{2+} ions.

Gal4 is a transcription factor originating from yeast and not present in wild type flies. This transcription factor recognizes the UAS promoter where it activates transcription of the coding sequence coming downstream from the promoter (here GCaMP)

Here is the description of flies A, B, C and D that we should identify in the tubes we received:

- Flies that express a wild type version of the ORCO gene (C)
- Flies lacking the ORCO gene after its substitution by a piece of DNA carrying the red eye pigment that provides a visible phenotype (no ORCO promotor or gene) (B)
- Flies with the wild type ORCO gene and two additional transgenes, one with the promoter of the ORCO gene fused to Gal4 and another with UAS promoter fused to the calcium sensor GCaMP. These two transgenes are essential to conduct calcium imaging experiment in order to study the activity of all olfactory neurons. (A)
- Flies with the Or42a promotor fused to Gal4 and UAS-GcAMP transgene to monitor the activity of one olfactory sensory neuron – the one expressing the Or42a receptor – with the calcium imaging technique) (**D)**

Formation of groups of "experts" that are specialized in the different techniques (2/3 people per group)

- Explain the four different tools that we will use to solve the problem:
  - Anatomy/Microscopes; observe differences in phenotypes in adult fly

- Computers/Microscope/Calcite imaging: Analyse neurons´ images done with Calcite imaging technique, see how many neurones express fluorescence.
- Molecular biology/gene: electrophoresis gel to see if we have the ORCO gene or not
- Behaviour: check if larvae can smell or not,, do they have orco gene expressed or not
- The last group, the Communication group, will be in charge of the general supervison and communication. As a researcher you need to have bright ideas and questions, a lot of energy to try to solve them, but you also need to communicate your results to other scientist thanks to scientific publications… so your team is in charge of the final redaction of today experiments.

- Give 2 minutes to each student in order that they read the information at the back of their accreditation card (see annex). They can discus together but need to form 5 sub-groups of 3 people, and stay close to the sign that mention their field of "expertise"

- Check the groups:

All accreditation cards have a letter in bold that corresponds to the one on the sign:

- **M**: Microscopy/Anotomy: Si**m**on/**M**arie Weinberger, **M**artianthi Karageorgi,
- **I**: Informátic/Image: Ajianka, Sercan Say**i**n, **I**van/**I**lona Larderet
- **E**: G**e**n**e** ORCO,Molecular biology/electroforesis: Samuel/Sonia Walk**e**r, Rajyashr**e**e S**e**n, Val**e**ntina/Valentino F**e**rlito
- **N**: Behavior: Ibrahim Tasek**i**n, Gua**n**dga Liu, Ta**n**may **N**ath
- **A**: comunic**a**ción/supervisión: M**a**tthieu/**A**licia Louis, G**a**brielle/G**a**briel **A**nna Bertier, L**a**ia/P**a**ul Cendros, **A**nnick/**A**lbert Labeeuw

- Double check that each sub-group has a map of the building and knows where they need to go (key: Laia/Paul cards says that maps are hanging up close to the exit sign of the room)

- Double check that the experiments groups are complete and ready to go
  M + **I** = 6 students + 6 students = 1 leader → go to Teaching lab 2
  E + **N** = 6 students + 6 students = 1 leader → go to Teaching lab 1
  C = 3 students = 1 teacher → go to teaching lab 2 and 1

- Groups can enter in the building through the main entrance. The leader stays at the back, giving the impression to students that they are leading the process
- The communication group can come back early to the meeting room in order to put all pictures in order, learn more about the FLiACT Project thanks to the website and documentation www.FLiACT.eu

Role Game part 2: Experiments

The experiments will be run on 4 stations which are distributed over two teaching labs, with all the necessary equipment. The detailed protocols can be found in the annex 2.

Teaching lab 2:

- **Anatomy/Microscopes**;

Switch on microscope, adjust the lens.
Switch CO2
Let the flies fall asleep
Observe differences in the phenotype in adult fly: specimens that have red eyes or not

- **Computers/Microscope/Calcite imaging**:

Open computer and files.
Look at the picture to see if neurons express some fluorescence; count how many neurons express it.
Prepare slide and larvae to look at them on the fluorescent microscope

Teaching lab 1:

- **Molecular biology/gene: electrophoresis gel**

Load 4 samples
Run the gel, put the electric voltage (100V – 5 minutes)
Look at the color and place of the migrated band

- **Behavior:**

Add sugared water in the tubes with larvae
Put larvae and water in a plastic plate
Load the odor sticker ring
Put larvae in the plate
Count how many larvae are in the “odor circle”

Role Game Part 3: Sharing results, conclusion, discussion

- By investigation group (15 students), the one from the communication group leads the discussion, with the help of the pictures they took, they fill in the table with each sub-group’s result

- Each sub-group present:

    - The technique they used
    - Their results

      Expected results:

	M - Microscope/ phenotype adult	I - Image / neurones activity	E - Gene ORCO/ electrophoresis gel	N- Behavior experiment
Especia A ORCO Gal4 + UAS-GCaMP	= (red eyes)	≠ (see 21 fluorescent neurons)	= (1 blue band)	= (majority of larvae in the odour side)
Especia B ORCO nul	= (red eyes)	= (no fluorescent neurons)	≠ (1 smaller red band)	≠ (larvae repartition 50/50)
Especia C ORCO	≠ (White eyes")	= (no fluorescent neurons)	= (1 blue band)	= (majority of larvae in the odour side)
Especia D OR42a-Gal4 + UAS-GCaMP	= (red eyes)	≠ (see 1 neurone)	= (1 blue band)	= (majority of larvae in the odour side)

- Conclusion done by the leader
  - With one experiment we cannot differentiate all the 4 species
  - With 3 different experiments we can make a hypothesis and differentiate all 4 species
  - With 4 experiments we can say that :
    - A: ORCO + GCaMP
    - B: ORCO nul
    - C: ORCO
    - D: OR42 + GCaMp
  - Nowadays scientific problems are very complex, we need that scientists with very different backgrounds and knowledge work in teams: every one bring their own knowledge and skills to help to solve the problem: exactly as we did it today!

Annex1: Individuals accreditation

(2 names per accreditation card: masculine one and feminine one)
All first names, last name, profiles are freely based on the researchers team description available on the FLiACT.eu website)

Matthieu Louis – Project **c**oordinator

Born: Belgium 

FLiACT localisation:

CRG- Barcelona, Spain

Maya Louis – Project coordinator

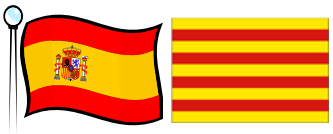
Born: Belgium
University Master: Physics, ULB, Belgium
PhD: computer and system biology, Cambridge, UK
Postdoc: Sytem neurogenetic (olfaction system), Rockfeller University, NYC, USA

- Supervision
- Writing the grant proposal, following all the deadlines for the grant justification report.
- Creativity, management, facility to understand quickly a problem, team building, supervision networking.
- Scientific paper writing skills, facility to orally present scientific research.

Sonja Reiland – Project Manager

Born: Germany 

Working place:

 CRG- Barcelona, Spain

Sonja Reiland – Project Manager

University Master: Biochemistry, Potsdam, Germany
PhD: Plant Biotechnology/Proteomics, Zurich, Switzerland
Postdoc: EMBL, Heidelberg, Germany
Info Professional: BASF, Ludwigshafen, Germany

- I facilitate communication between everyone, students like PIs.
- I help coordinate the training activities.
- I report all FLiACT activities and our progress towards the training objectives to the European Commission.
- I manage the FLiACT website in order to communicate the achievements of the students to the general public.

Paul Cendros – Press Officer

Born: Barcelona 

Working place:

 CRG- Barcelona, Spain

Laia Cendros – Press Officer

University: Biology, Barcelona
Communication, Barcelona

- I write press releases for CRG, including the ones for FLiACT.
- I have a digital camera to take pictures for my press release.
- I follow the work of every scientists at the CRG and I obviously know where they are located.
- I printed maps of the building with some location you can be interested in, I hang them closed to the exit sign of this meeting room.

Albert Labeeuw – Outreach Manager

Born: Belgium 

Working place:

 CRG- Barcelona, Spain

Annick Labeeuw – Outreach manager

University: Pedagogy, Belgium
Working experience: in science divulgation, Belgium and New York, USA

- Organizing activities for kids, teenagers and general public in order that they have a better idea about the type of research that it is done at the CRG.

Si**m**on Weinberger – PhD Student

Born: Austria

Working place:

VIB- Leuven, Belgium

Marie Weinberger – PhD Student

University degree & Master: Genetics and Microbiology, Vienna, Austria

- I work on the evolution of the developmental gene regulatory network.
- I am trying to understand how changes in the coding sequence of transcription factors can affect the development of sensory organs in different fly species.
- Flies that do not bear a transgene have white eyes.

SEVENTH FRAMEWORK PROGRAMME

MARIE CURIE ACTIONS

CRG Centre for Genomic Regulation

Marianthi Karageorgi – PhD student

Born: Crete

Working place:

IBDML - Marseille, France

Marianthi Karageorgi – PhD student

University Degree: biology, Crete
University Master: Molecular biosciences, Heidelberg, Germany

- Understanding mechanisms explaining evolutionary changes in the micro- and macro- evolutionary level.
- Flies that carry the ORCO null allele (mutant) have red eyes. Watch out that part of the flies with white eyes have curly wings. Flies with the curly wing phenotype should be discarded.

SEVENTH FRAMEWORK PROGRAMME
MARIE CURIE ACTIONS
CRG Centre for Genomic Regulation

David Garcia – AL**M**U

Advanced Light-Microscopy Unit

Born: Barcelona 

Working place:

  CRG- Barcelona, Spain

Raquel Garcia- AL**M**U

University: Biology, Barcelona

- I am working for the Microscopy Unit, helping all researchers at the CRG (and PRBB , and more) to use the best microscope for his research needs.
- Flies carrying the GCaMP fluorescent indicator have red eyes.
- Use the microscope, put the light on
- Use brush to manipulate flies in order to not hurt them.
- To observe flies with the microscope you need to follow a specific protocol in order that they fall asleep.
- The anaesthesia protocol can be found in a red folder.

Ivan Chaplin – PhD student

Born: India 

Working place:

 MPI-CE - Jena, Germany

Ines Chaplin – PhD student

University degree: Bioscience, Vellore, India
University Master: Biotechnology, TIFR, Bangalore,, India

- I will investigate the function of different types of local interneurons in the formatting odour representation in the antennal lobe of the adult and the larva.
- In flies expressing GCaMP under the control of the ORCO and Or42a promoter, we can observe the activity of olfactory neurons by using the functional imaging technique.
- Files containing the image sequences acquired by the microscope were saved on the Desktop of the computer.
- Flies that carry the transgene with the Or42a promotor fused to the coding sequence of Gal4 can be used to monitor the activity of 1 olfactory sensory neurones using the calcium imaging technique.

Sercan Sayin – PhD Student

Born: Turkey 

Working place:

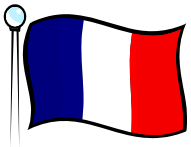
 MPIN – Munich, Germany

Sercan Sayin – PhD Student

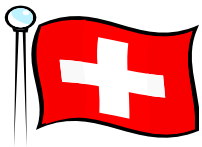
University Degree: Molecular biology, Turkey
internship: In neurogenetics model, Belgium
Univesity master: Sensory neurogenetic, Turkey

- Interested in biology and history.
- Uses in vivo Ca2+ imaging.
- Single fly or population behaviour study.
- Calcium imaging takes advantage of so-called calcium indicators, **GCaMP**, fluorescent molecules that can respond to the binding of Ca^{2+} ions by changing their fluorescence properties. The expression of GCaMP is controlled by the UAS (**U**pstream **A**ctivation **S**equence) promoter to which GAL4 transcription factor binds.
- Computer password is: “darwin”.
- Protocols for microscope observation are in the red folder.

Ivan Larderet – PhD student

Born: France 

Working place:

 UNIFR- Fribourg, Switzerland

Ilona Larderet – PhD student

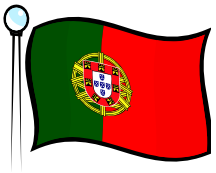
University degree: Biomedical sciences, Paris, France
University master: neurobiology, Ottawa, Canada

- Why the responses at sensory stimuli are changing during late larval stages.
- I will use genetic tools to express fluorescent proteins in distinct domains of genetically traceable neurons.
- Flies that carry the transgene with the ORCO promotor fused to the coding sequence of Gal4 can be used to monitor the activity of the 21 olfactory sensory neurones by means of the calcium imaging technique.
- We can double check our first results thanks to some larvae sample and the fluorescent microscope.

Samuel Walker – PhD student

Born: UK 

Working place:



CF- Lisboa, Portugal

Sonia Walker – PhD student

University degree: Neuroscience, Cambridge, UK
University Master: NCBS, Bangalore, India

- Characterises how chemosensory behaviour are modulated by specific internal state such as mating status.
- Use state-of-the-art genetic techniques.
- Using the gel electrophoresis technique we can distinguish the flies strains that the ORCO gene from those with the ORCO null mutant.

Rajyashr**ee** Sen – PhD Student

Born: India

Working place:

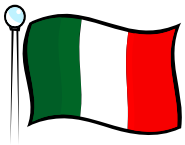
Janelia Research Campus, USA

Rajyashr**ee** Sen – PhD Student

University degree: Microbiology, Kolkata, India
University Master: Biotechnology, Hyderabad, India

- Investigate the neuronal basis of directed locomotion with flies as model system.
- Conducting screens in which we activated or silenced several subpopulations of neurons looking for locomotion defects.
- The protocols to run electoforesis gel is in a red folder.
- DNA is naturally negatively charged, and migrates in the agar gel to the positive side. DNA can be separated by size: larger DNA sequences move slower and shorter sequences move faster.

Valentino Ferlito – PhD student

Born: Italia 

Working place:

Brainwave Discovery Ltd,
Edinburgh , UK

Valentina Ferlito – PhD student

University Master: Molecular and cellular Biology,
Catania, Italia

- Focusing my research efforts on the development and validation of new medical model like flies/larvae brain to study Alzheimer's disease.
- The DNA of the flies was amplified by PCR, we amplified the region where we should find the fragment corresponding to the ORCO gene, or the ORCO mutant that yields a much shorter fragment.

Ibrahim Tastekin – PhD student

Born: Turkey

Working place:

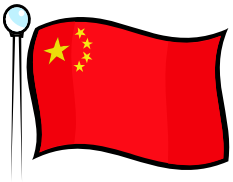
CRG- Barcelona, Spain

Nadia Tastekin – PhD student

University Master: Neuroscience, Turkey

- Identifying and functionally characterize the subset of neurons in the larval brain that are involved in the chemotactic behaviour. (recognize o odour gradients/intensity)
- Use the well-established behavioural assays of my host lab.
- The protocol can be found in a red folder.

Guandga Liu – PhD student



Born: China

Working place:

Peira/VIB – Leuven, Belgium

Guandga Liu – PhD student

University degree: medicine, Shangai, China
University master biomedical imaging, biotechnology, Shangai, China

- I will study social network of flies in group level using new tracking methods.
- Flies that don't have the gene ORCO cannot smell. They won't show any attraction to food odours.

Tanmay **N**ath – PhD student

Born: India 

Working place:

 DCILABS – Antwerp, Belgium

Tanmay **N**ath – PhD student

University: Biomedical Engineer, Varanasi, India
Working experience: in MRI imaging in Iowa, USA

- Use a new software to track multiple flies in the flyworld looking at the group and individual behaviour, trying to quantify both.
- Larvaes like to eat fruits. If you put a droplet of fruit odour they will be attracted by it. Quickly they will move toward the odour and accumulate in its vicinity.

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