Sveučilište u Rijeci
University of Rijeka

BIOtech
Odjel za biotehnologiju
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Syllabus:

Laboratory apprenticeship - *Drosophila melanogaster* in the neuroscience of addiction

Academic year: 2020/2021

Program: Master program: Biotechnology for the life Sciences

Course number: EBLS101

ECTS points: 3

Language: English

Contact hours: 280

Prerequisite: Completion of the courses: Introduction to Research Methods and Introduction to Laboratory Work & Safety

Course coordinator and contact:
Associate Professor Rozi Andretić Waldowski
Address: R. Matejčić 2, Rijeka
tel: 051 584 553
e-mail: randretic @ uniri.hr

Individual consultations: Course coordinator is available at any time upon previously agreed date and time.

Other personal in the Laboratory for Behavioral Genetics:
Dr.sc. Ana Filošević – posdoctoral fellow
Mr.sc. Franka Rigo – PhD student

Required reading:

Sanchis-Segura C, Spanagel R. 2006. "Behavioural assessment of drug reinforcement and addictive features in rodents: an overview." Addiction Biology 11:2-38.

Kaun KR, Devineni AV, Heberlein U. 2012. "Drosophila melanogaster as a model to study drug

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addiction." Hum Genet. 131(6): 959-975.

Filošević A, Al-samarai S, Waldowski RA. 2018 High Throughput Measurement of Locomotor Sensitization to Volatilized Cocaine in Drosophila melanogaster Front. Mol. Neurosci. 11:25. doi: 10.3389/fnmol.2018.00025

Kaun KR, Azanchi R, Maung Z, Hirsh J, Heberlein U. 2011. "A Drosophila model for alcohol reward." Nat Neurosci. 14(5):612–619.

Pereira SP and Cunha-Oliveira T., Role of Mitochondria on the Neurological Effects of Cocaine. In: Victor R. Preedy, editors, The Neuroscience of Cocaine. : Academic Press, 2017, pp. 205-218. ISBN: 978-0-12-803750-8

Reccomended reading:

- Other relevant and recent reserach articles

Course description:

Drug addiction is a complex behaviour that is commonly studied in animal models as a series of simple behaviours (endophenotypes) associated with addiction. Acute psychostimulant (PS) exposure increases locomotor activity, while repeated exposure to the same dose lead to locomotor sensitization, a behaviour that is based on neuroplastic changes in the nervous system. PS self-administration leads to preferential PS consumption when there is a choice between a solution without and with the addition of PS.

At the molecular level, PS administration induces dopamine release from presynaptic neurons, an event that mediates both endophenotypes. Excess of free dopamine is susceptible to degradation resulting in an increase in the level of reactive oxygen species (ROS). Recent scientific research suggests that enzymes involved in the generation and removal of free radicals also participate in cellular signalling that is involved in the development of addiction. Based on these findings, the focus of the study in the Laboratory for Behavioral Genetics is on changes in redox balance that regulate PS-induced neuroplasticity. Our hypothesis is that locomotor sensitization, as one form of neuroplasticity, depends on redox status.

Students who choose this course will spend 8 weeks in the Laboratory for Behavioral Genetics. During this time, in agreement with the mentor and the immediate mentor in the laboratory (postdoctoral fellow and or PhD student), they will master some practical knowledge and skills in measuring behavioural, genetic and biochemical parameters associated with the development of addiction. They will then be assigned to an independent project to be carried out in pairs or individually (depending on the number of students in the lab). The mentor and immediate tutor, in agreement with the students, will offer several possible topics

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related to the identification of genes involved in redox regulation and addiction development or the measurement of the oxidative status by quantifying enzymatic activity of antioxidant enzymes and the amount of ROS.

Upon completion of the apprenticeship period, students will be able to choose or propose a topic of their own research for the Graduate thesis which will be conducted under mentorship of assistant prof. Rozi Andretic Waldowski, or with the co-mentoring with head of other Laboratories at the Department.

Study outcomes:

General study outcomes:

1. Acquire theoretical knowledge about the neurobiological bases of the development of addiction.
2. Acquire practical knowledge and skills in measuring behavioural, genetic and biochemical parameters associated with the development of addiction.
3. Acquire practical knowledge and skills in using the model organism Drosophila melanogaster in the field of addiction research.
4. Acquire knowledge of planning experimental work in accordance with proposed scientific hypotheses.
5. Gain experience of analysing and interpreting the results of their own research in the context of assigned project and available literature.

Specific study outcomes:

Students are expected to gain practical laboratory skills in some of the following activities that are used in the Laboratory for Behavioral Genetics, and gain theoretical knowledge in most of other listed skills:

- basics of working with the model organism Drosophila melanogaster (fruit fly):
  - preparation of different cultivation medias regarding planned experimental protocol (supplementation with chemical substances) which flies can ingest orally;
  - methods for anesthetizing and separating flies based on sex (males, females, virgins) or phenotypic characteristics (change in morphology due to gene mutations);
  - basics of fly anatomy and brain dissection.
- implementation and analysis of behavioral essays to study genetic basis of addiction development:
  - inducing and quantifying the locomotor activating influence of psychostimulants using commercially available locomotor activity measurement system - Drosophila Activity Monitoring System (DAMS);
  - use of the FlyBong method to measure the genetic influence on development of locomotor sensitization after exposure to volatilized psychostimulants. FlyBong method was developed in our laboratory and is based on the DAMS system;

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- use of the FlyCafe method to measure preferential consumption of psychostimulants when offered a choice between a sugar solution and a psychostimulant solution. FlyCafe method was developed in our lab;
- testing of neurotoxicity and neurodegeneration induced by the administration of psychostimulants using a negative geotaxis test.

• tools and techniques for gene and protein manipulation:

- the basics of crossing and inheritance in flies;
- the use of mutants and the binary expression system UAS-Gal4 to increase or decrease the expression of the target protein in different types of central nervous system cells;
- use of gene reporters to monitor oxidative changes at the cellular level.

• methods for monitoring biochemical changes after psychostimulant administration in flies:

- tissue and organ collection, isolation and quantification of total (native and denatured) proteins in protein extract samples;
- measuring the activity of native catalase and superoxide dismutase enzymes;
- SDS-PAGE protocol for the separation of proteins from extracts;
- measuring the relative amount of reactive oxidative species (superoxide anion, hydrogen peroxide, hydroxyl radical and singlet oxygen) using fluorescent dyes in protein extracts and fixed microscopic preparations;
- measurement of AGE (Advanced End Glycationproduct) production.

Requirements, scoring and grading:

Student activities:

Students will be introduced to methods and techniques used in the Laboratory for Behavioral Genetics at the beginning of the educational period. Student will gain practical skill by performing simple exercises to measure the behavioural, genetic and biochemical parameters associated with the development of addiction. At a later stage, when they gain sufficient practical knowledge, they will be assigned a an independent task that is part of the research focus of the Laboratory for Behavioral Genetics.

Students are required to keep a detailed laboratory notes for the entire 8 weeks in which they will record protocols, experimental conditions, and test results. At the end of each working day, the immediate mentor in the laboratory will review the laboratory notes, while they are required during weekly meetings with the mentor to report about their work progress and consult about the future tasks. In addition to the compulsory literature received at the beginning of the educational period, students are expected to look for current scientific publications relevant to the field of research. Students may also be expected to present part of work or scientific publication to other members of the Laboratory or Department staff.

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At the end of the educational period, the student (each student for him/herself) is expected to submit a report no less than 5 pages in which they will discuss the field in which they have completed apprenticeship, the techniques they have gain and used, and the details of their own project results.

Exam dates:
The report is due no later than one week after finishing the appreticeship.

Grading (according to Pravilnik o studijima Sveučilišta u Rijeci):
50% of the grade will be based on the continuous monitoring of the student throughout the educational period, which will be based on laboratory notes quality, proficiency in understanding and implementing techniques and methods, independence in conclusion making based on their own results and motivation for scientific work. Students will be able to consult with the mentor and the immediate mentor in the laboratory on a daily basis regarding their work and results, and will be able to intervene independently when and if required. This part of the grade is provided by the mentor in writing and numerical grade form.

The remaining 50% of the grade is a written report of no less than 5 pages in which they will discuss the field in which they have completed apprenticeship, the techniques they have gain and used, and the details of their own project results. This report will be evaluated numerically by a mentor and another teacher at the Department of Biotechnology who will jointly give the final grade.

Percent of knowledge and competencies	**ECTS grade**	**National grade**
90% do 100%	A	Excellent (5)
75% do 89,9%	B	Very good (4)
60% do 74,9%	C	Good (3)
50% do 59,9%	D	Sufficient (2)
0% do 49,9%	F	Fail (1)

Final grade is the sum of pointsachieved during classes and on the final exam and the passing grades are: excellent(5), very good (4), good (3) and sufficient (2).

Additional information:
Ethical code:
Students are expected to behave according to the ethical code as defined in the documents of the University of Rijeka: *Etički kodeks Sveučilišta u Rijeci* and *Etički kodeks za studente.*

Satisfaction survey:

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In the interest of improving this course we politely ask all students to complete the survey about their satisfaction with the course and with mentor's performance. The survey is anonymous, and the results are important for analysing the quality of the program and of the teaching staff at the Department of biotechnology.