University of Trieste

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Techniques in Cellular and Molecular Neurobiology

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International Master's Degree in Neuroscience



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Lesson 1

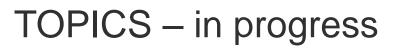


Course Description

The course aims to provide a synthetic overview of the major principles and techniques associated with cellular and molecular neurobiology.

The subject matter includes the different approaches that can be used to address biological questions in cellular and molecular Neurobiology Technics in Cellular and Molecular Neurobiology





- 1) Presentation and Scientific Method
- 2) The biological problem
- 3) Scientific Model in Neurobiology
 - Descriptive Neurobiology and/or mechanisms research
- 4) Experimental manipulations
 - Genetic
 - Pharmacological

provisional





- Structure
- DNA / RNA
- Proteins
- Mechanism and interactions

6) Experimental results and "readout"

• Morphological vs Biochemical



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provisional



- Video
- Hands ON
- Laboratory tour

8) Scientific research revision



Course handouts and learning materials on moodle

- 1) Lesson handouts
- 2) Video of the lessons (link)
- 3) Video of laboratory activities (link)
- 4) Ebook chapters / Research papers

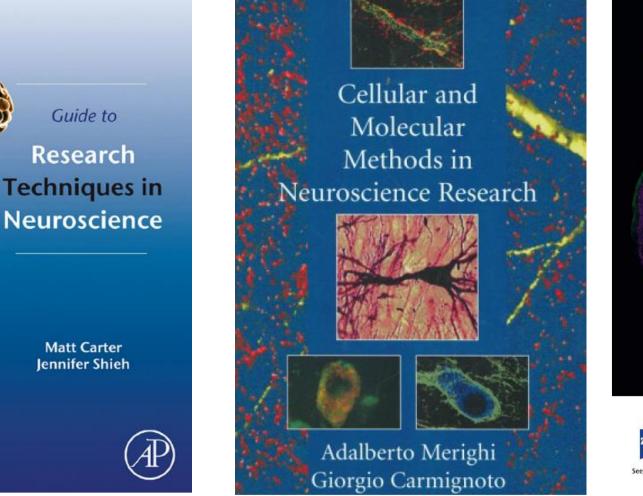
Technics in Cellular and Molecular Neurobiology

Guide to

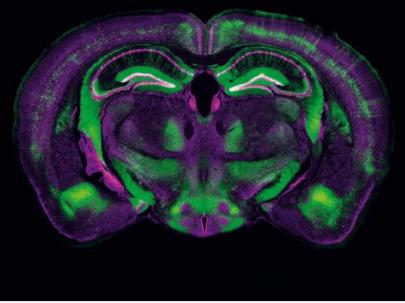
Matt Carter



Selected chapters from



Microscopy Techniques for NEUROSCIENCE







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Examination Procedures

1st choice = Written Exam + Oral discussion (if requested)

Methods: Text with 22 questions with open or multiple choice answers

EvaulationCorrect answer = -1.5 point, partial correct answer = +0.75 point,
no answer = 0 point, wrong answer = -0.5 points

Oral discussion The student can ask to discuss the written exam and that can produce a variation on the previous note of + or – 4 points

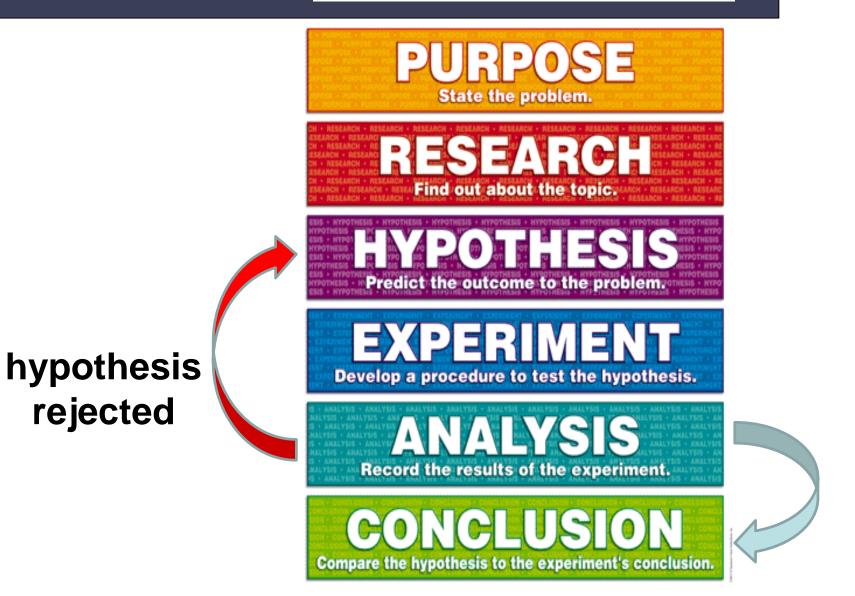
2° choice = Oral discussion

Methods and evaluation 1 question for each main argument up to 6 or 7 questions.

Notes: The «Baj» part of the exam can be performed all togheteher with the main part «Tongiorgi» or in a different exam date. The «Baj» part of the exam produce a mark that will be «weighted» on the final note (1/4 of the totat result)







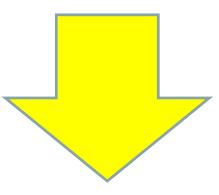
hypothesis confirmed







How it works?

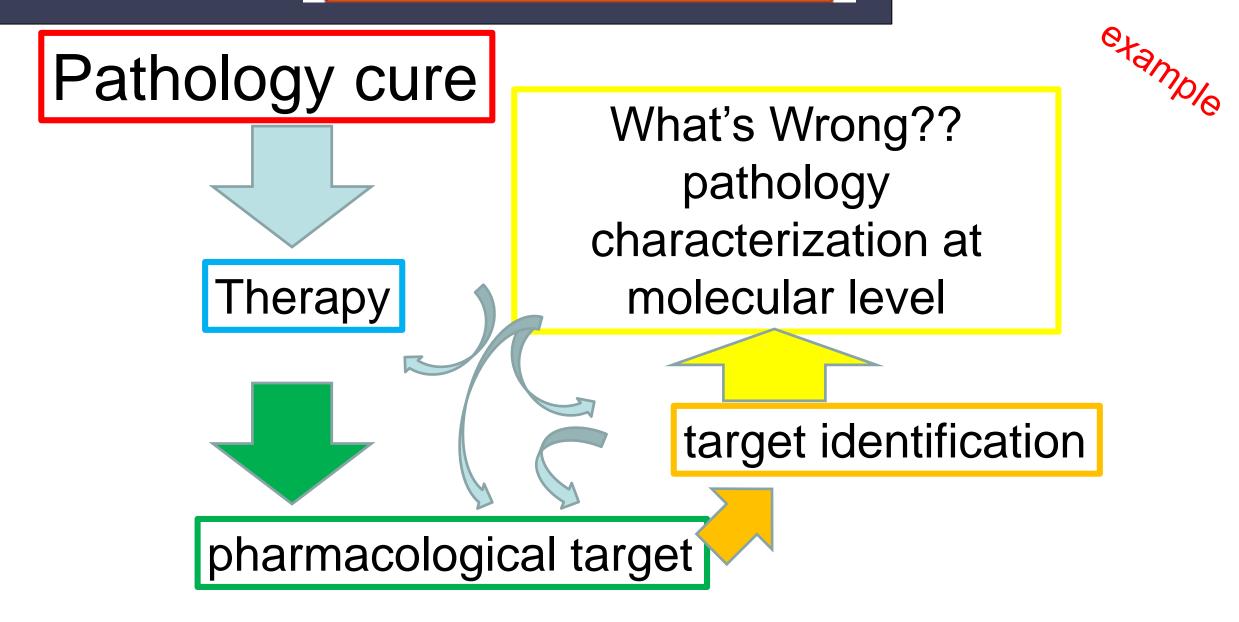


characterization at molecular level

PURPOSE State the problem.

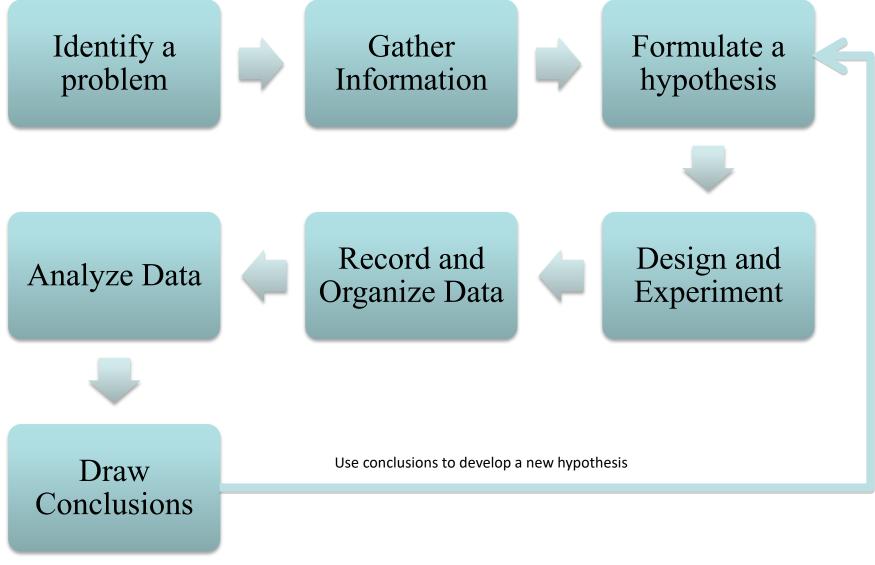






Introduction to the Scientific Process

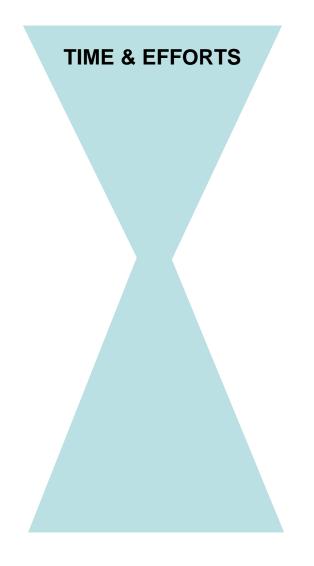




scientific Methog



Problem/Question Observation/Research Formulate a Hypothesis Experiment **Collect and Analyze Results** Conclusion **Communicate the Results**



The Scientific Method



- What is the scientific method?
 - -It is a **process** that is used to find **answers** to questions about the world around us.





- -No, there are several versions of the scientific method.
- Some versions have more <u>steps</u>, while others may have only a few.
- However, they all begin with the identification of a **problem** or a **question** to be answered based on **observations** of the world around us.



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-They provide an organized method for conducting and analyzing an experiment.



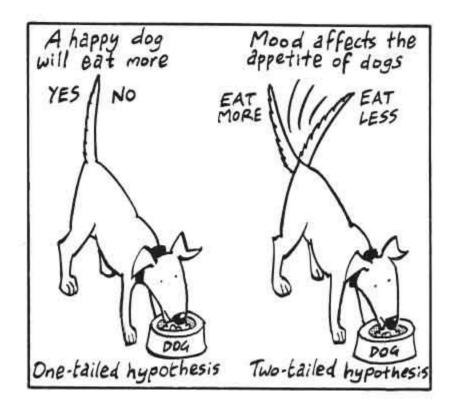


• What is a hypothesis?

-It is an educated guess

based on observations and your knowledge of the topic.

-You state it as a possible answer to a question.





- Stated in the "If...., then...." format
- Example: IF I water three plants with different sodas, THEN the plant that receives Sprite will grow the tallest.



• What is data?

-It is information gathered

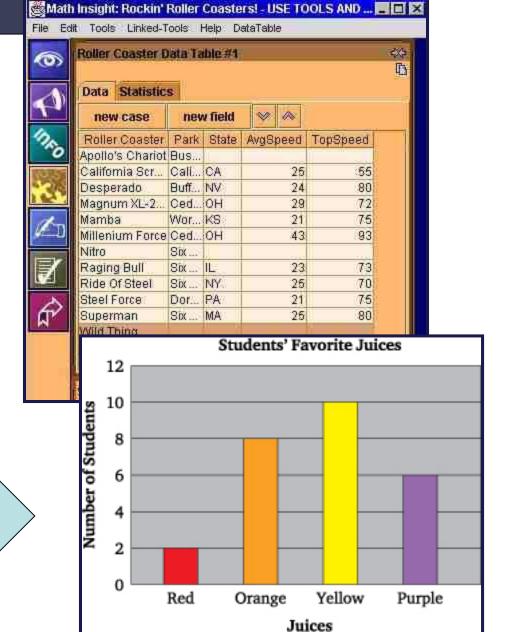
during an experiment.

It is organized into a data

table and displayed visually

as a graph.







- 1. <u>Choose a problem</u>: State the problem as a question.
- 2. <u>Research your problem</u>: Read, get advice, and make observations.
- 3. <u>Develop a hypothesis</u>: Make a prediction about what will happen.
- Design an experiment: Plan how you will test your hypothesis.



- <u>Test your hypothesis</u>: Conduct the experiment and record the data.
- 6. Organize your data: Create a chart or graph of your data.
- 7. <u>Draw conclusions</u>: Analyze your data and summarize your findings.

Identifying Variables



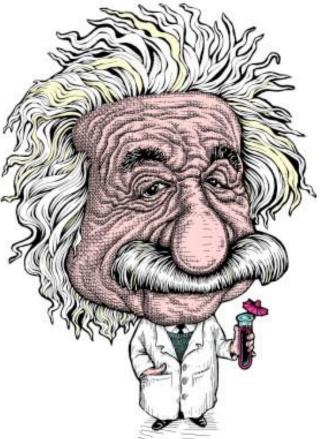
- Independent Variables
- Dependent Variables
- Controlled Variables (Constants)



Do you know the difference



between the independent an dependent variables?







• Independent Variable - something

that is **changed** by the scientist

- What is tested
- -What is manipulated (changed)



• **Dependent Variable** – something that might be affected by

the change in the independent variable

- What is observed
- What is <u>measured</u>
- The data collected during the investigation
- "the **numbers**"
- Example: how tall the plant grew, how far the paper airplane flew



- **Controlled Variable** a variable that is not changed
 - Also called **CONSTANTS**
 - Allow for a "fair test"
 - Everything in the experiment except for the IV should be kept constant



- Give a detailed explanation of how you will conduct the experiment to test your hypothesis
- Be clear about the <u>variables</u> (elements you change) versus your
 <u>constants</u> (elements that do not change)
- A <u>control</u> is the group that you use as a comparison to see if change has occurred.







In a scientific experiment, the control is the group that serves as the standard of comparison. The control group may be a "no treatment" or an "experimenter selected" group.





- Conclusion: your results or findings based on data collected during the experiment
- Answer your <u>problem</u>/purpose statement
- What does it all add up to? What is the value of your project?
- What further study do you recommend given the results of your experiment? What would be the next <u>question</u> to ask?
- If you repeat this project, what would you change?

Science experiments use...



- **Independent Variable:** the one factor that is changed by the person doing the experiment
- **Dependent Variable:** the factor which is measured in the experiment ۲
- **Constants:** all the factors that stay the same in an experiment •

We will be back here in final lessons What experiments are you planning?

- What are your variables?
- How will you keep your experiment fair? •

Neuroscience and neurobiology



a field of study that deals with:

- structure, function,
- development, genetics,
- biochemistry, physiology,
- pharmacology,
- pathology of the nervous system,
- study of behavior and learning is also a division of neuroscience

Neurobiology



- biological study of the brain
- \succ interdisciplinary field that involves many levels of study from the
 - ➤ molecular level
 - cellular level (individual neurons)
- small assemblies of neurons like cortical columns
- Iarger subsystems : subserves visual perception
- Iarge systems : cerebral cortex or cerebellum
- > the highest level the nervous system as a whole



Neuron Perspective

Molecular Neuroscience in the 21st Century: A Personal Perspective

Thomas C. Südhof^{1,*} ¹Department of Molecular and Cellular Physiology and Howard Hughes Medical Institute, Stanford University Medical School, 265 Campus Drive, CA 94305-5453, USA *Correspondence: tcs1@stanford.edu https://doi.org/10.1016/j.neuron.2017.10.005

Neuroscience is inherently interdisciplinary in its quest to explain the brain. Like all biological structures, the brain operates at multiple levels, from nano-scale molecules to meter-scale systems. Here, I argue that understanding the nano-scale organization of the brain is not only helpful for insight into its function, but is a requisite for such insight. I propose that one impediment to a better understanding of the brain is that most of its molecular processes are incompletely understood, and suggest a number of key questions that require our attention so that progress can be achieved in neuroscience beyond a description of the activity of neural circuits.



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VIDEO AUDIO TEST



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MOLECULAR NEUROBIOLOGY – TECHNIQUES

Electrophyisology

(incl. whole-cell, voltage-clamp, current-clamp, single-channel, perforated patch, loose-patch, cell-attached, field potential recordings);

Tissue preparation and tissue culture (acute slices of olfactory tissue, dissociated neurons, cell lines)

Live cell imaging (incl. confocal and two-photon imaging)

Molecular & Genome Biology (incl. in situ hybridization, single cell/small tissue isolation and gene analysis)

Behavioral assays (resident-intruder assay, Bruce effect,)

Histology (incl. immunohistochemistry, neuroanatomy)

