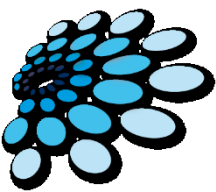


“ Techniques in Cellular and Molecular Neurobiology ”

International Master's Degree in Neuroscience

Lesson 1



DIPARTIMENTO DI
SCIENZE DELLA VITA

Gabriele Baj
gbaj@units.it



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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

TOPICS – in progress

- 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

5) Experimental “target”

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

6) Experimental results and “readout”

- Morphological vs Biochemical

provisional

7) Virtual / Real Laboratory

- Video
- Hands ON
- Laboratory tour

8) Scientific research revision

Technics in Cellular and Molecular Neurobiology



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Course handouts and learning materials on moodle

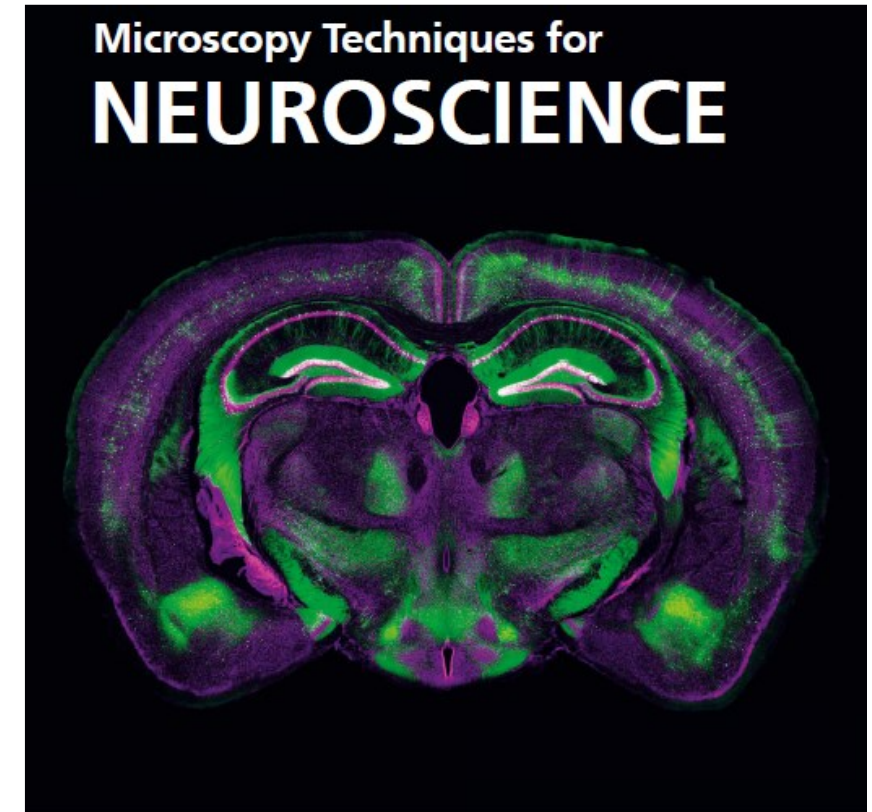
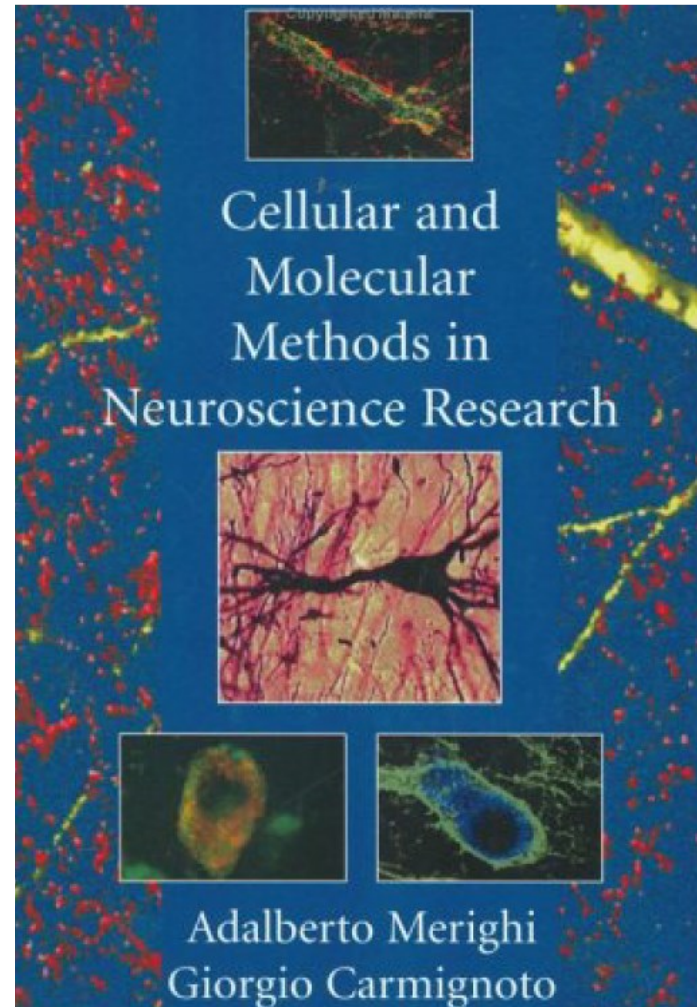
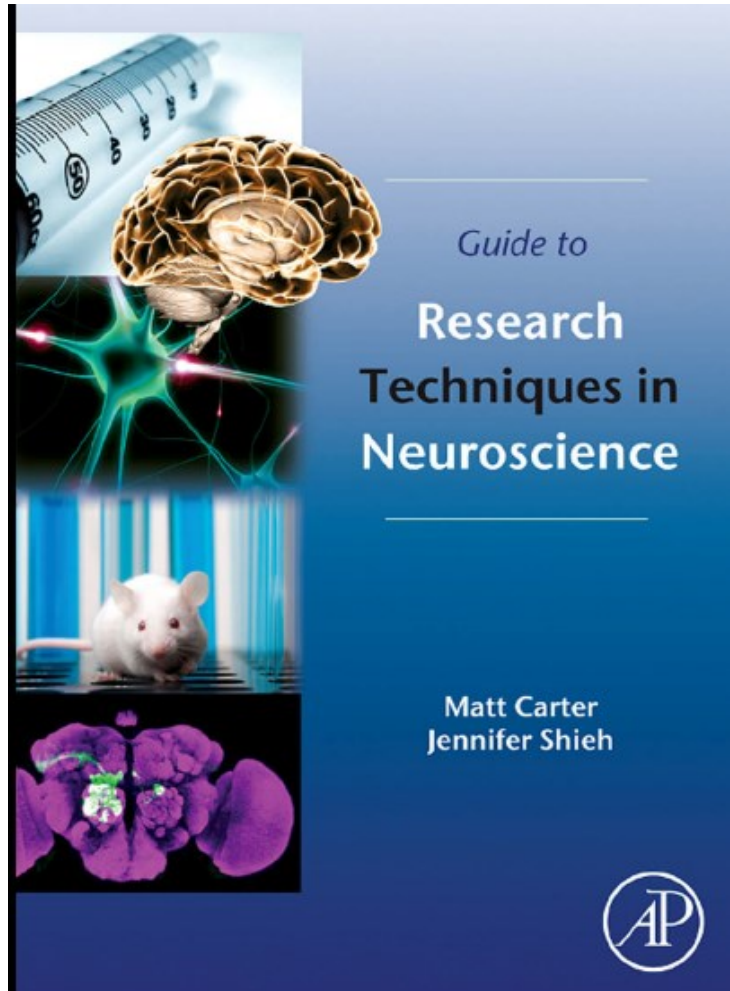
- 1) Lesson handouts
- 2) Video of the lessons (link)
- 3) Ebook chapters / Research papers

Technics in Cellular and Molecular Neurobiology



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Selected chapters from



WILEY

Neuroscience and neurobiology



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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



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

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: tc-s1@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.

Examination Procedures

provisional

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

Methods:	Text with 22 questions with open or multiple choice answers
Evaluation	Correct 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 together 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 total result)

SCIENTIFIC METHOD



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PURPOSE

State the problem.

RESEARCH

Find out about the topic.

HYPOTHESIS

Predict the outcome to the problem.

EXPERIMENT

Develop a procedure to test the hypothesis.

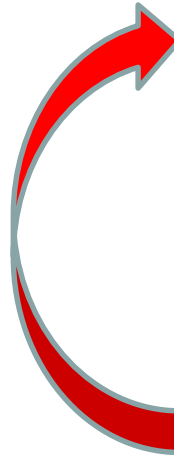
ANALYSIS

Record the results of the experiment.

CONCLUSION

Compare the hypothesis to the experiment's conclusion.

hypothesis
rejected



hypothesis
confirmed



PURPOSE

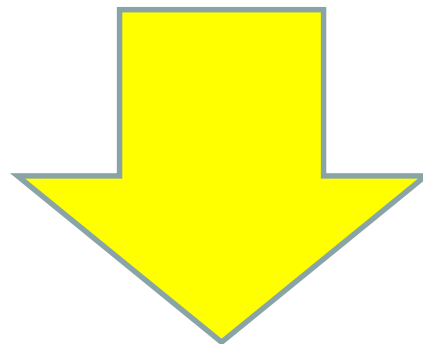
State the problem.



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example

How it works?



characterization at molecular level

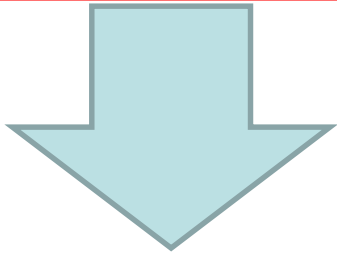
PURPOSE

State the problem.



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Pathology cure



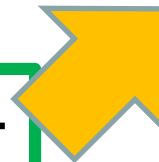
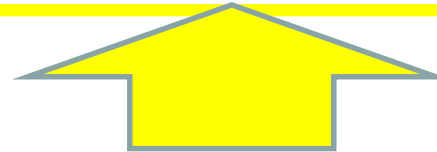
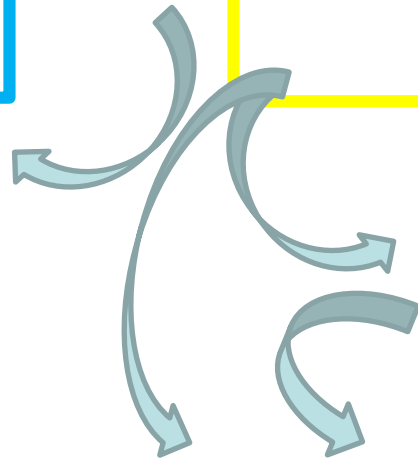
Therapy



pharmacological target

What's Wrong??
pathology
characterization at
molecular level

target identification

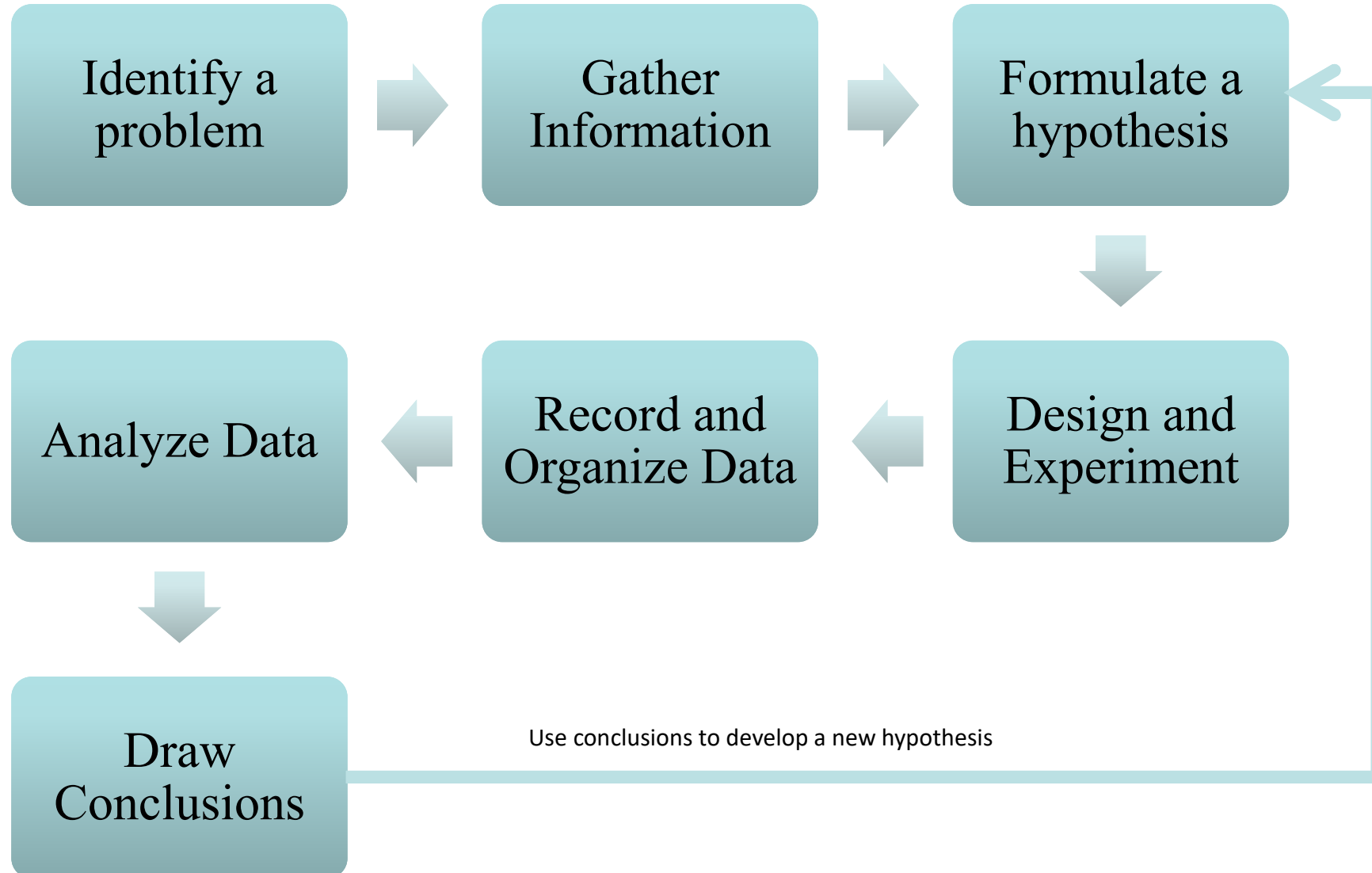


example

Introduction to the Scientific Process



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

A light blue hourglass-shaped diagram. The top narrow section contains the text 'TIME & EFFORTS'. The bottom wide section is empty.

TIME & EFFORTS

The Scientific Method



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



lipartof.com

Is there only one “scientific method”?



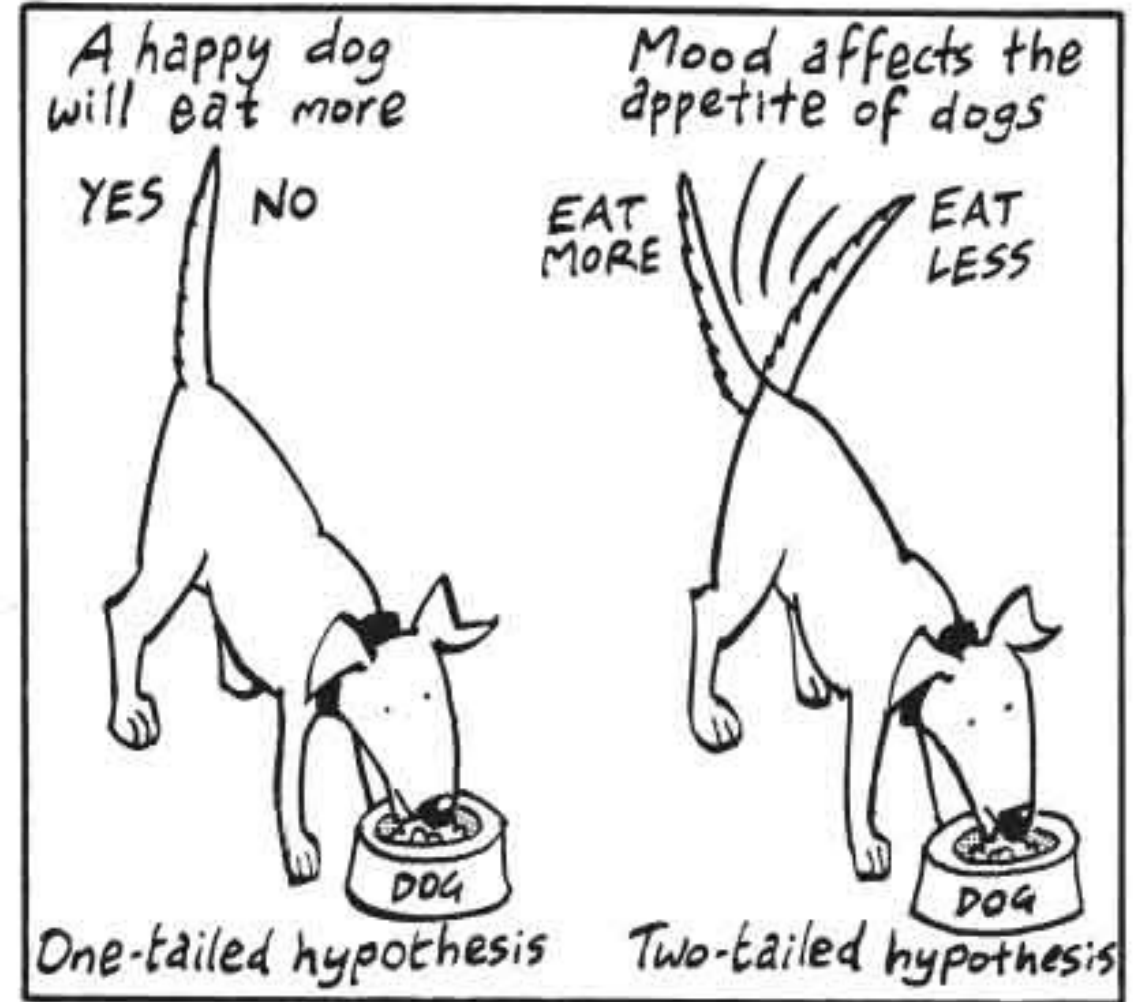
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- No, there are several versions of the scientific method.
- Some versions have more **steps**, while others may have only a few.
- However, they all begin with the identification of a **problem** or a **question** to be answered based on **observations**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

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



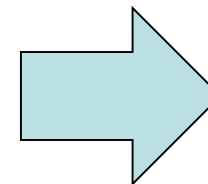
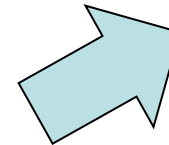
- What is a hypothesis?
 - It is an **educated guess** based on observations and your knowledge of the topic.
 - You state it as a possible answer to a question.



• What is data?



- It is **information** gathered during an experiment.
- It is organized into a data **table** and displayed visually as a **graph**.



Math Insight: Rockin' Roller Coasters! - USE TOOLS AND ...

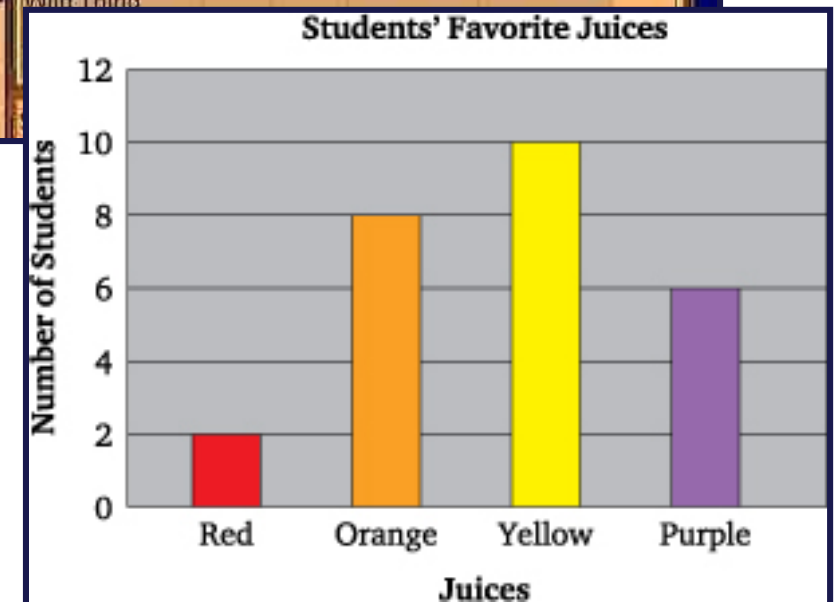
File Edit Tools Linked-Tools Help DataTable

Roller Coaster Data Table #1

Data Statistics

new case new field

Roller Coaster	Park	State	AvgSpeed	TopSpeed
Apollo's Chariot Bus...				
California Scr...	Call...	CA	25	55
Desperado	Buff...	NV	24	80
Magnum XL-2...	Ced...	OH	29	72
Mamba	Wor...	KS	21	75
Millenium Force	Ced...	OH	43	93
Nitro	Six ...			
Raging Bull	Six ...	IL	23	73
Ride Of Steel	Six ...	NY	25	70
Steel Force	Dor...	PA	21	75
Superman	Six ...	MA	25	80
Wild Thing				



Steps of the Scientific Method



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



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

Identifying Variables



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

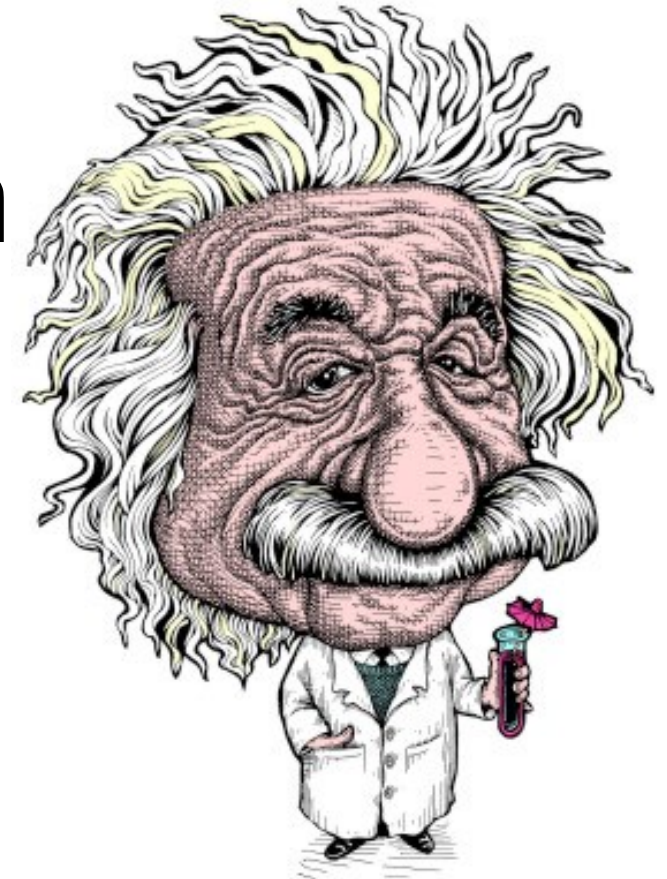


Do you know the difference



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between the independent and
dependent variables?



3 Kinds of 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 **measured**
 - 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

Procedure



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



Control Group



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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



- **Conclusion**: your results or findings based on data collected during the experiment
- Answer your **problem**/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 **question** to ask?
- If you **repeat** this project, what would you change?

Science experiments use...



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

What experiments are you planning?

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

*We will be back here
in final lessons*

Biological problems in Neuroscience



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Trends Cogn Sci. 2015 April ; 19(4): 173–175. doi:10.1016/j.tics.2015.01.007.

The unsolved problems of neuroscience

Ralph Adolphs

California Institute of Technology, Pasadena, CA, USA

Note that some future questions build on prior ones: we need to understand psychiatric illnesses before we can cure them, and whole-brain microscopic-resolution imaging of the zebrafish brain (100 000 neurons; done, although temporal resolution will improve [4]) needs to come before we do the same for the mouse brain (70 000 000 neurons), let alone the human brain (80 000 000 000 neurons).

The unresolved problems of neuroscience



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The unsolved problems of neuroscience

Problems that are solved, or soon will be:

- I How do single neurons compute?
- II What is the connectome of a small nervous system, like that of *Caenorhabditis elegans* (300 neurons)?
- III How can we image a live brain of 100 000 neurons at cellular and millisecond resolution?
- IV How does sensory transduction work?

The unresolved problems of neuroscience examples... personal perspective



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Problems that we should be able to solve in the next 50 years:

- V How do circuits of neurons compute?
- VI What is the complete connectome of the mouse brain (70 000 000 neurons)?
- VII How can we image a live mouse brain at cellular and millisecond resolution?
- VIII What causes psychiatric and neurological illness?
- IX How do learning and memory work?
- X Why do we sleep and dream?
- XI How do we make decisions?
- XII How does the brain represent abstract ideas?

The unresolved problems of neuroscience examples... personal perspective



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Problems that we should be able to solve, but who knows when:

- XIII** How does the mouse brain compute?
- XIV** What is the complete connectome of the human brain (80 000 000 000 neurons)?
- XV** How can we image a live human brain at cellular and millisecond resolution?
- XVI** How could we cure psychiatric and neurological diseases?
- XVII** How could we make everybody's brain function best?

The unresolved problems of neuroscience examples... personal perspective



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Problems we may never solve:

XVIII How does the human brain compute?

XIX How can cognition be so flexible and generative?

XX How and why does conscious experience arise?

Meta-questions:

XXI What counts as an explanation of how the brain works? (and which disciplines would be needed to provide it?)

What are Scientific Models?



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- Models are a representation or description designed to make a particular part/feature of the world easier to understand, define, quantify, visualize, or simulate by using multiple forms of data.
 - Models help us understand.
 - Explain or analyze concepts.
 - Represent things too small or too large.
 - Explain past or the present; predict the future.

What are Scientific Models?



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Physical Models

- Models you can see and touch. (3D or 2D)
- Used to represent very large (organism) or small (cell) objects.
- Limitation: Looks similar, but does not function in the same way as the original.

What are Scientific Models?



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Mathematical Models

- Models are made up of mathematical equations and data. They help us process large amounts of data.
 - Simple models – formula for how fast phenome occurs
 - Complex models (computer) – population growth
- Limitation: Only as accurate as the data inputted.

What are Scientific Models?



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Conceptual (Diagram) Models

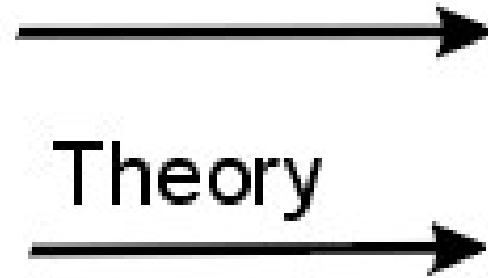
- Used to understand large and complex processes and how they work.
 - Biochemical Pathways
 - Organs – tissue description
 - Nervous system representation
- Limitations: Gives limited information, shown in 2D.

Scientific MODEL



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Real World Out There



Identification of details
relevant to description,
translation of 'real' objects
into variables of the model

Model



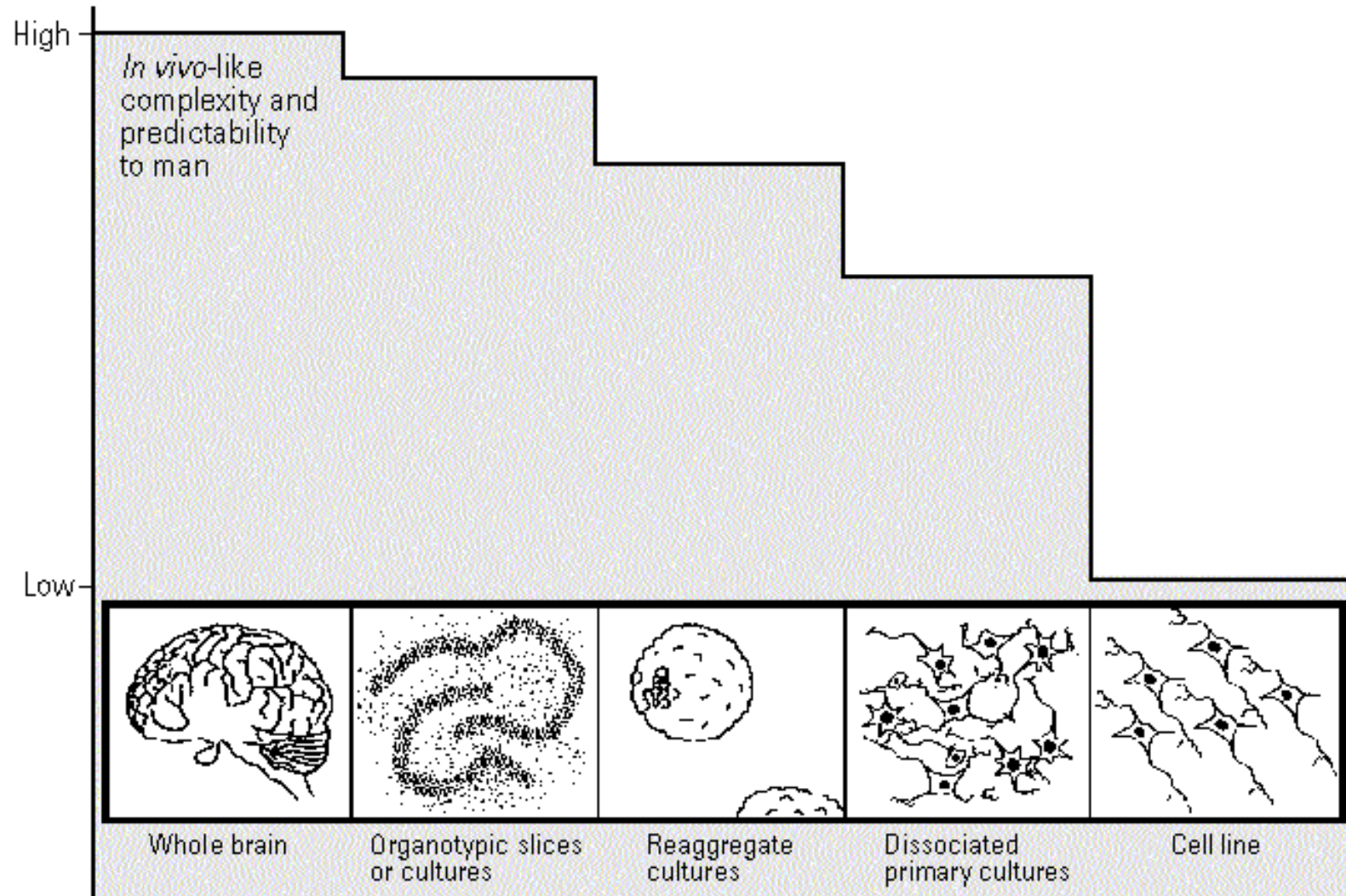
The model is a simplified version of the real world out there, simplified in the sense that it deals only with a limited amount of details.



MODELs IN NEUROBIOLOGY



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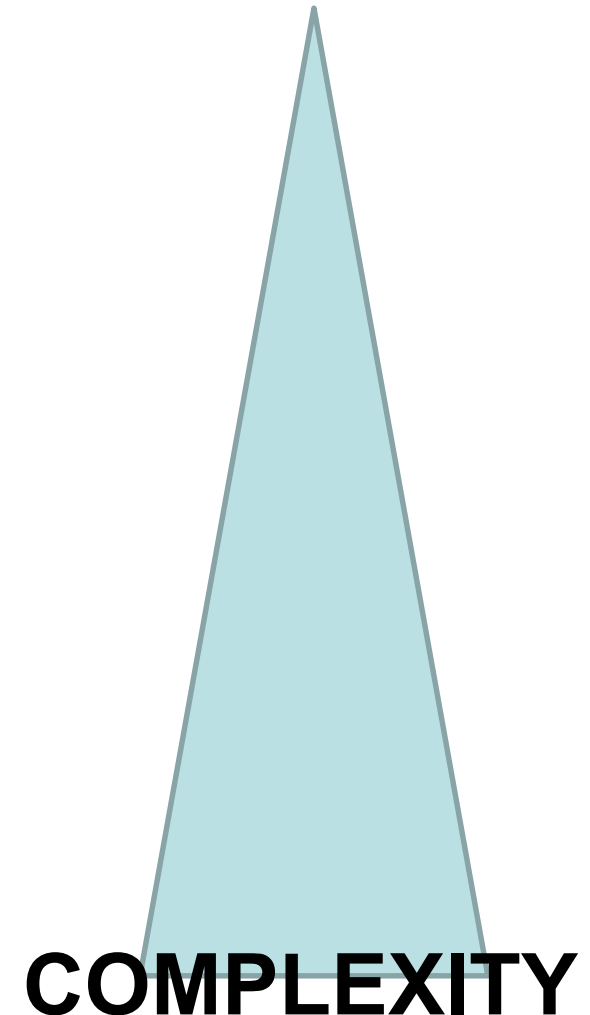


Classification of tissue cultures based on the origin of the cells



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- Extended culture (multipassage culture) – cell strain and or (transformed) cell lines
- STEM CELLS and iPSCs
- Primary culture (directly from animal tissue)
- STEM CELLS and iPSCs
- Organotypic cultures
- Animal Model



Neuroscience



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

Neuroscience is a field of study that deals with:



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

Methods Mol Biol. 2013 ; 1078: 1–8. doi:10.1007/978-1-62703-640-5_1.

General overview of neuronal cell culture

Jennifer Gordon, Shohreh Amini, and Martyn K. White

Department of Neuroscience, Temple University School of Medicine, Philadelphia, PA 19140

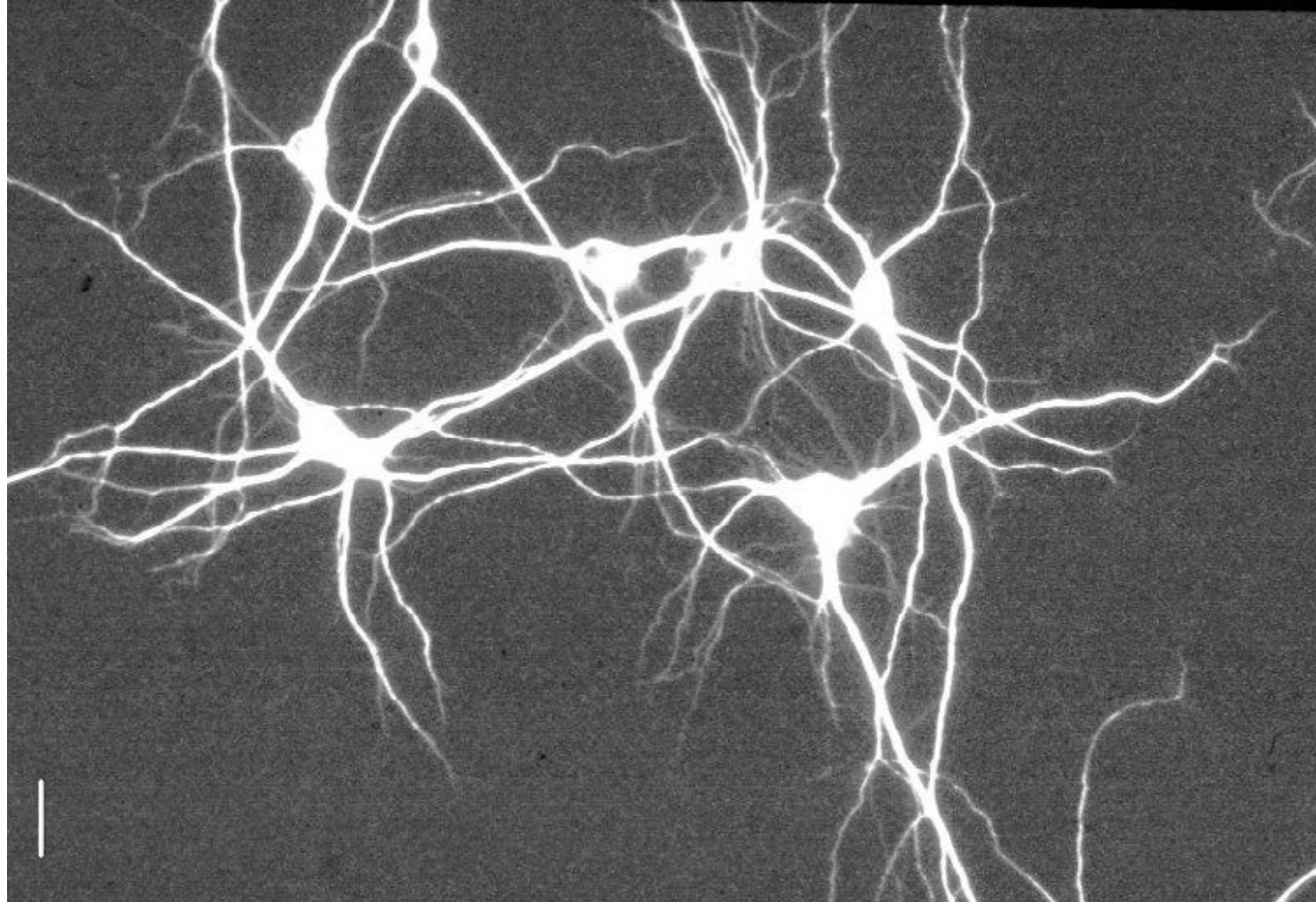
Experimental Read-OUT in cellular neurobiology



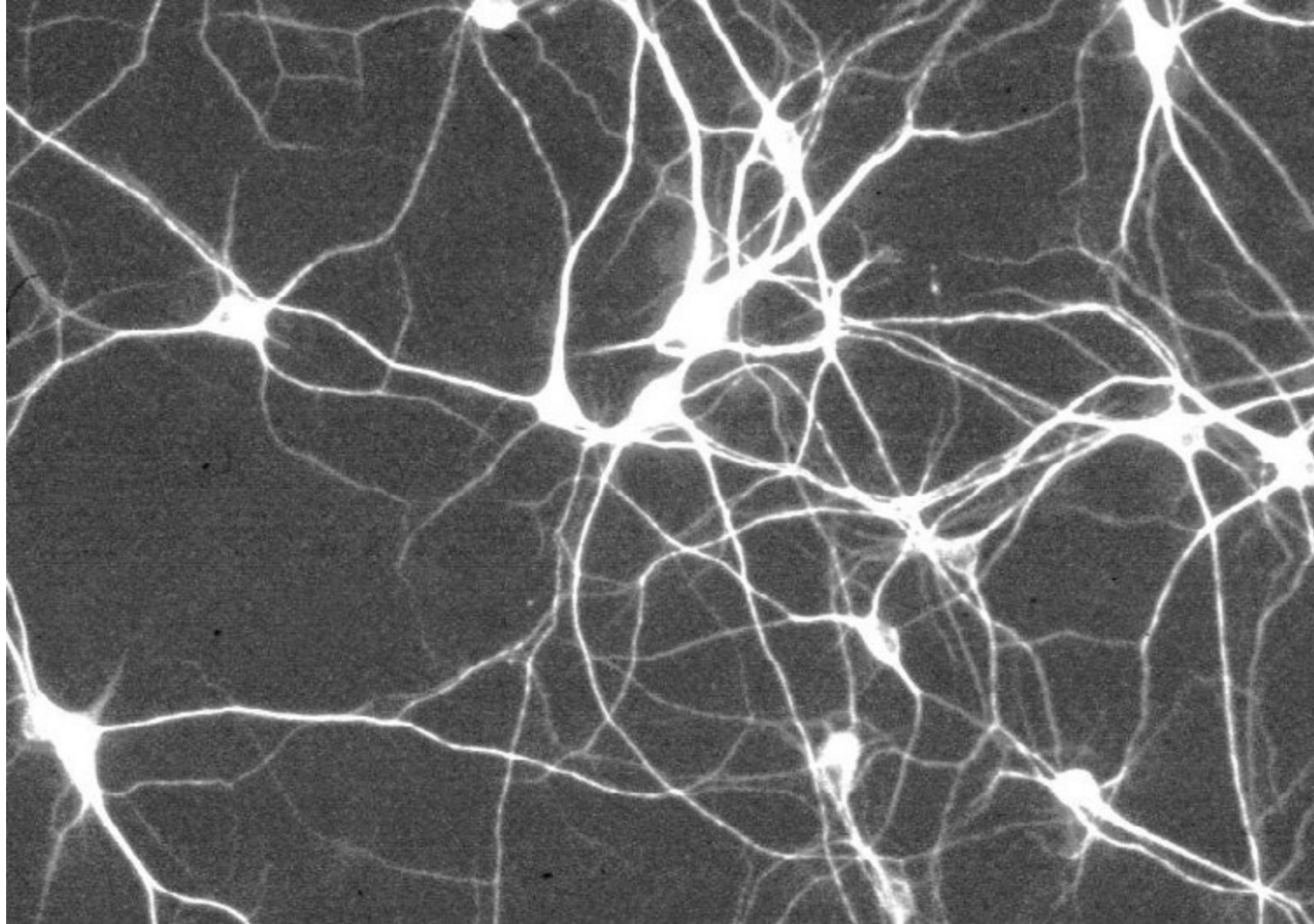
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- Primary culture (directly from animal tissue)
- Extended culture cell strain and/or established (transformed) cell lines
- Stem cells / iPSCs
- Organotypic cultures

- Primary culture (directly from animal tissue)



- Primary culture (directly from animal tissue)



- Primary culture (directly from animal tissue)



COMMON READ OUT primary cell line

Protein

1. Western BLOT (limitation due to cell amount)
2. Immunocito (localization and morphology)
3. ELISA (minor limitations due to cell amount)
4. Overexpression (limitation due to transfectability)
5. Downregulation (limitation due to transfectability)

- Primary culture (directly from animal tissue)



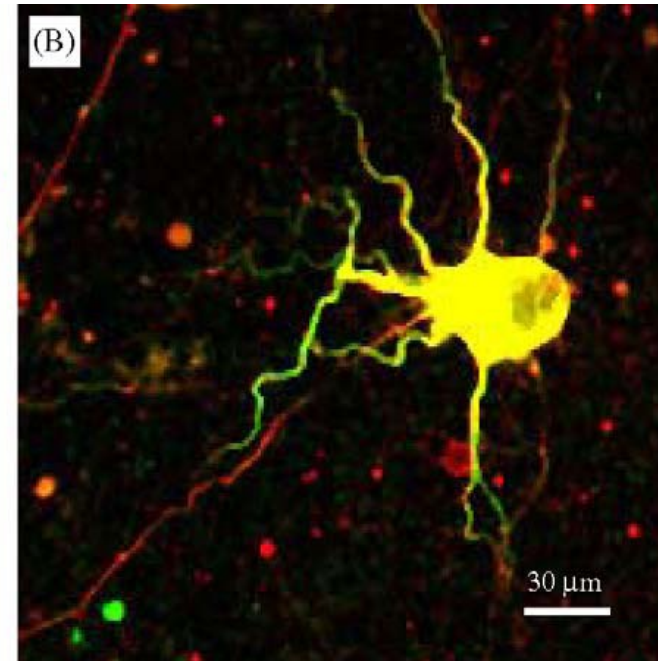
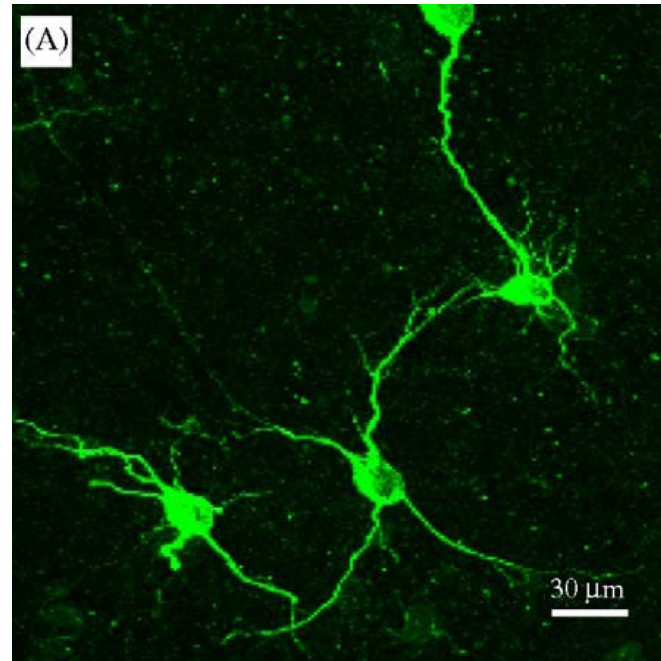
RNA

1. PCR
2. Real Time-PCR
3. Northern Blotting
4. InSitu Hyb

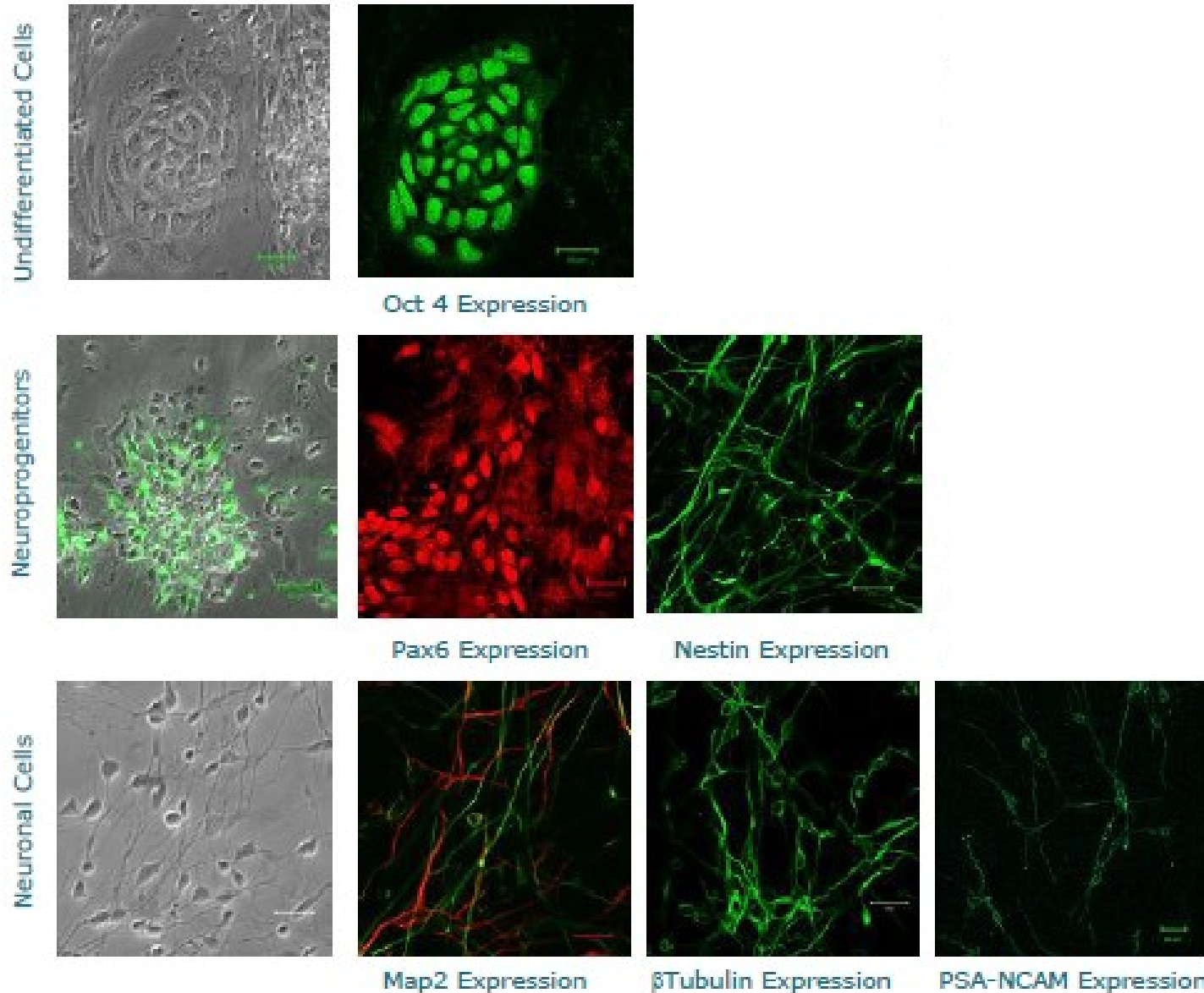
- Primary culture (directly from animal tissue)



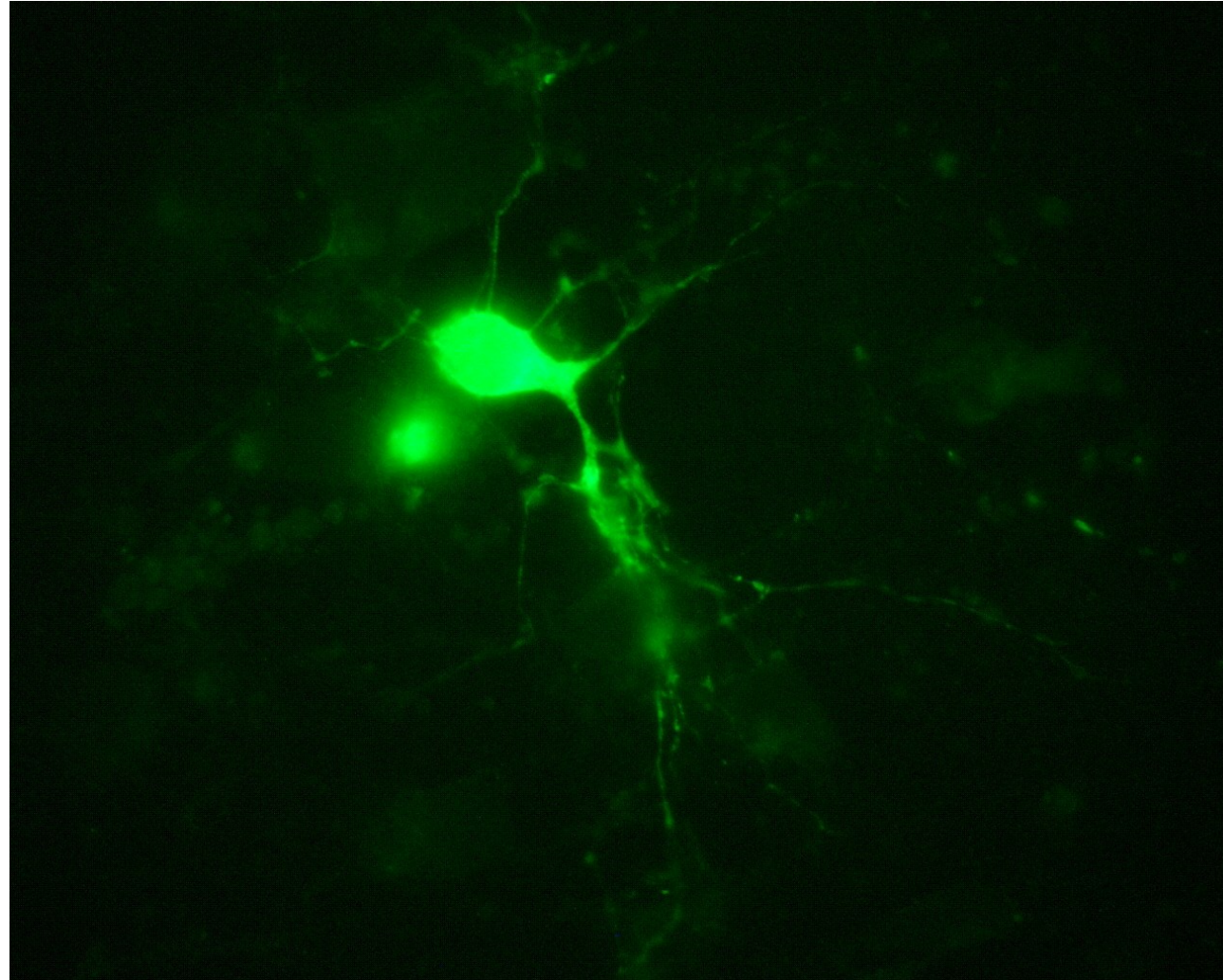
Electrophysiology on “fake” network
single cell
field potential



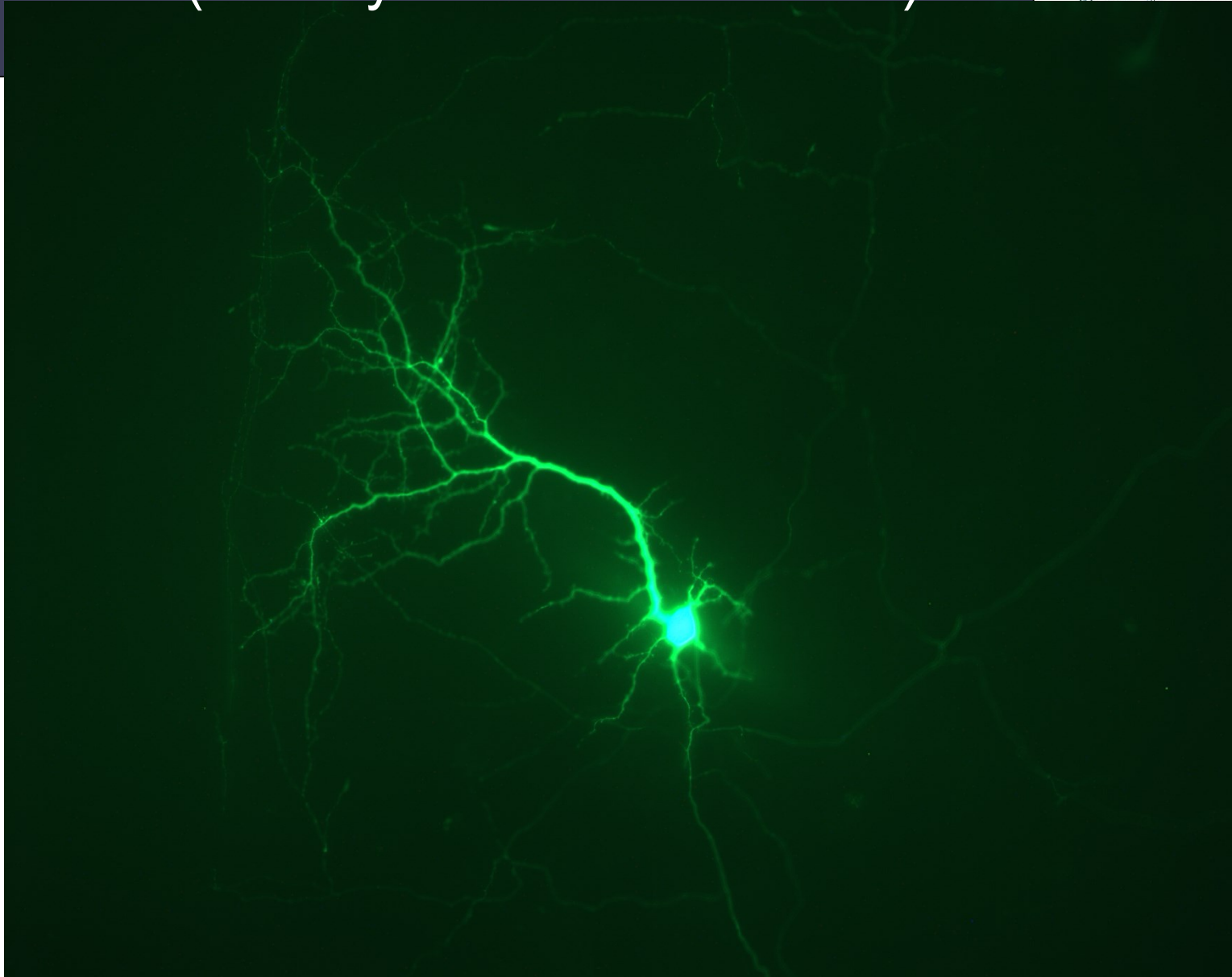
- Primary culture (directly from animal tissue)



- Primary culture (directly from animal tissue)



- Primary culture (directly from animal tissue)



Technics in Cellular and Molecular Neurobiology



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MOLECULAR NEUROBIOLOGY – TECHNIQUES

Electrophysiology

(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)

