Neuroscience BIO-311 - Ramdya

Exercise solutions: Techniques in neuroscience

Question 1 – Animal research in neuroscience:

i) List 3 advantages and 3 disadvantages of using an animal model rather than humans to investigate a neuroscience research question.

Advantages of animal models:

- We can use invasive techniques that we cannot use on humans (e.g. microelectrode recording, genetic manipulation, optogenetics, pharmacological manipulation…)
    - Those techniques generally yield signals with better spatial and/or temporal resolution
    - We can study deep brain regions that we cannot easily access in humans
    - We can investigate the safety of a potential drug (e.g. in the context of a clinical trial)
- We can gain insights from the perspective of evolution across species
- We have a higher control over experimental conditions (e.g. genetic manipulation such as knock-out, control of the environment, diet…)
- Animal models typically have a short lifespan and allow researchers to study development or the progression of a disease in a reasonable timeframe

Disadvantages of animal models:

- We cannot ask animals what they perceive or how they feel, we can only interpret their behavior
- Animals can only perform simple tasks and they require extensive training
- It is not straightforward to generalize animal results to humans due to physiological, anatomical, and behavioral differences
    - This is particularly true for complex diseases such as psychiatric or neurodegenerative conditions
- Animal experimentation requires careful ethical considerations
- Animal facilities are expensive to maintain (electricity, specialized personnel, constant attention…)

ii) Why is it relevant to also use simple organisms (e.g. fruit fly) in addition to more complex animals (e.g. mouse, monkey, human) for neuroscience research? What is comparable in fruit flies and in mammals nervous systems?

Fruit flies and worms have smaller and simpler nervous systems (302 neurons in C. elegans and ~100'000 in Drosophila) but still share many structural and functional fundamental principles with mammals and humans. Like in mammals, fly neurons communicate using electrical (action potentials) and chemical (GABA, acetylcholine, dopamine, glutamate…) signals via synapses. Flies nervous systems evolved to carry out the same general functions than mammals nervous systems: collect and process sensory information (visual, olfactive, mechanosensory), send motor commands to muscles (locomotion, reflex) and allow for some adaptability (synaptic plasticity, learning).

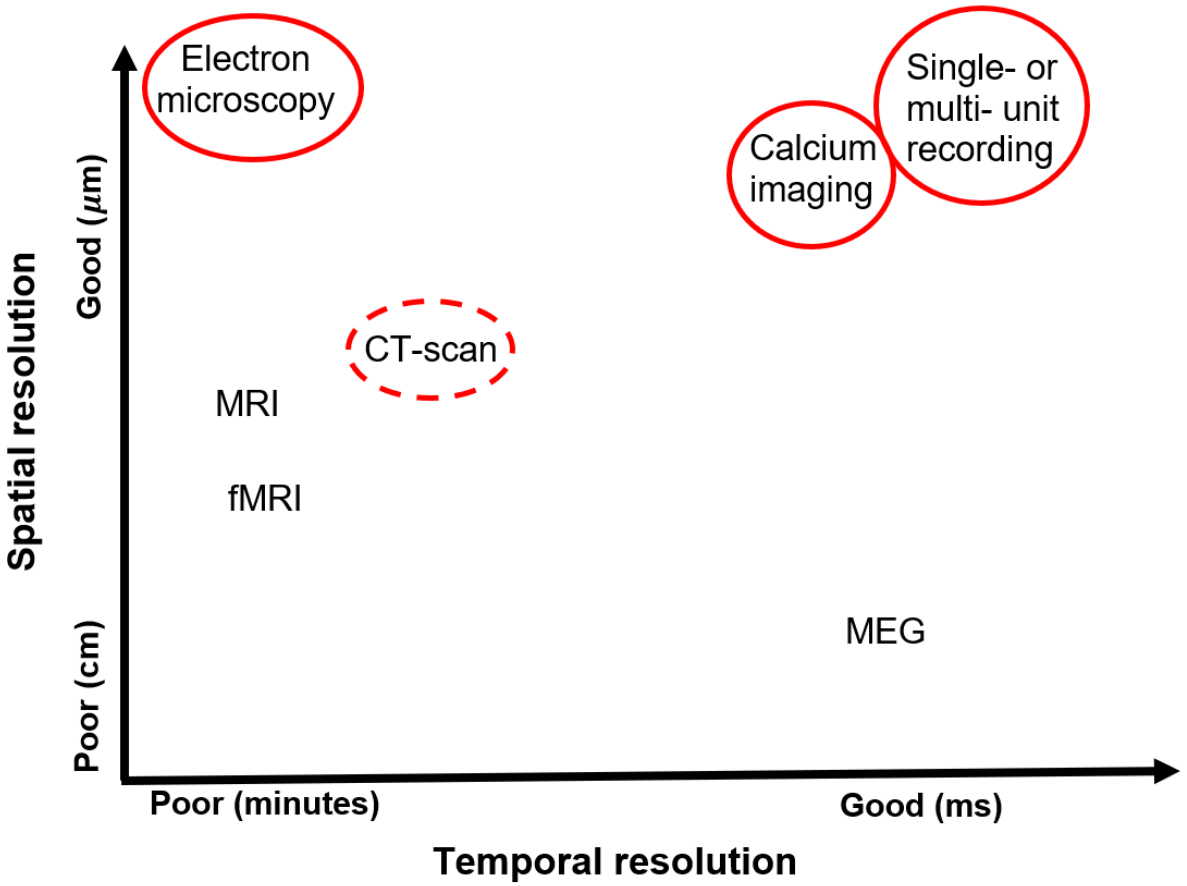
Question 2 – Neuroimaging methods:

i) Place the following neuroimaging modalities on the axis system below: CT-scan, MRI, electron microscopy, calcium imaging, fMRI, MEG, electrophysiology (single- or multi-unit recordings)

ii) Draw a red circle around the invasive methods. What can you conclude from what you obtain?

A CT-scan requires the subject to be exposed to a certain dose of X-ray. Too many CT-scan in a short amount of time can increase the risk of cancer. Electron microscopy can only be performed on dead samples that underwent an extensive preparation procedure. Calcium imaging requires the presence of a calcium sensitive dye in the cells of interest via genetic manipulation or direct injection. Additionally, the tissue of interest must be optically accessible to the microscope (e.g. craniotomy). Electrophysiological recordings require the insertion of a microelectrode in the brain and thus requires direct access to it (craniotomy). One of the rare contexts that allow such measurements in humans is during brain surgeries (e.g. epileptic patients).

There is a trade-off between invasiveness and optimal spatial and/or temporal resolution.



Question 3 – Optogenetics:

i) Your lab investigates motor control in fruit flies and discovered a specific neuron type called X that is active every time the fruit fly grooms its antennae. Is this observation enough to claim that the activity of X is required (*necessary*) for antennal grooming? Why or why not?

No, because this observation only establishes a correlation between the activity of X and grooming behavior. For instance, X neurons may be active as a downstream consequence of grooming rather than its cause. Establishing causation requires controlled experiments to manipulate the variable of interest (X neuron activity) and demonstrate direct effects on behavior.

ii) To better understand the role of the X neurons, you decide to use optogenetics. How would you proceed if your goal would be able to selectively control the depolarization of the X neurons?

- Selectively express in the X neurons an opsin that depolarizes neurons in the presence of light (e.g. channelrhodopsin-2). You identify a genetic marker specific to the X Neurons. For example, you could find a protein Y that is only expressed in the X neurons. Then you would identify the promoter of the gene coding for protein Y and insert the genetic sequence of the opsin right after (e.g. using CRISPR-Cas9 or creating a fly line using the GAL4/UAS system).
- Design an optogenetic setup to deliver light of the appropriate wavelength (e.g., blue light for ChR2) to the brain region where X neurons are located.
- Observe whether light stimulation of X neurons induces antennal grooming in the genetically modified fruit flies.

iii) Using optogenetics, you find that stimulating neurons X leads to antennal grooming. Can you now conclude that the activity of X is required (necessary) for antennal grooming? Why or why not?

No, this experiment does not demonstrate *necessity*. It only shows that X neuron activity is *sufficient* to drive grooming behavior because artificial activation of X neurons alone is enough to induce grooming, suggesting that these neurons can initiate the behavior. For instance, it could be that a redundant set of neurons (neurons Y) can also elicit grooming independently of neurons X activation.

iv) You decide to run one more optogenetic experiment, but this time using halorhodopsin. You add dust on fruit flies' head to force them to groom their antennae. You find that stimulating the halorhodopsin channels in the X neurons causes the flies to stop grooming. Explain what happens in the X neuron during optogenetic stimulation. Can you now conclude that X neuron activity is required (necessary) for antennal grooming?

When halorhodopsin is activated by yellow light, it hyperpolarizes the X neurons, making it more unlikely that the neurons X will fire action potentials.

Yes, your results now strongly supports the conclusion that X neuron activity is *necessary* for antennal grooming.

v) What are the strengths of optogenetics compared to other techniques for testing causality?

- Specificity: Genetic targeting ensures that only the neurons of interest are manipulated.
- Temporal Precision: Light stimulation allows for precise timing of activation or inhibition.
- Bidirectional Control: Both activation (e.g., using channelrhodopsins) and inhibition (e.g., using halorhodopsins) are possible, enabling comprehensive testing of necessity and sufficiency.
- Minimal Invasiveness and precision: Compared to traditional electrical stimulation (microelectrode), optogenetics reduces the risk of non-specific activation of surrounding tissue.