

Agryha proposed to validate previous studies which showed that optogenetics can selectively stimulate specific neurons like dorsal root ganglion (DRG) cells to enhance neurite growth accompanied by an increase expression of neurotrophic factors NGFs and BDNFs. She proposes to create an experimental group of rats which undergo a neurotmesis of the sciatic nerve and have a control group with no nerve injury. She decides to use the most severe type of nerve injury, a neurotmesis, level IV in Sunderland injury classification, with the assumption that a success with this type of injury will translate favorably to less severe injury. The experimental group is injected with AAV-ChR2 targeting DRG cells which upon light stimulation express ChR2. After few months, the two groups of rats are compared on motor and sensory tests to evaluate functional recovery of the sciatic nerve.

- Optogenetic stimulation has various limitations: 1) it is invasive, 2) it is very localized due to the weak tissue penetration of light, 3) there is concern to heat accumulation from light illumination which can cause cell damage and inhibits neuron activity, 4) its most common of gene expression using AAV transduction may induce immune response leading to death of neurons, however optogenetic stimulation has the determinant advantage to be highly accurate and selective.
- For axonal regrowth, Agryha method is overall relevant but at same time lacking some guidelines to assess visually and quantitatively in-vivo neural regeneration progress during the 1- or 2-months observation period.
- However, the goal of her methodology is to design a technique that, in addition of axonal growth, also promotes proliferation of Schwann cells (SCs) and the connection between the two is not explicitly detailed.
- Inspired by a recent study showing that optogenetic stimulation (OS) promotes in-vitro SC proliferation, differentiation and myelination, we will replace the ChR2 opsin with a ChR2 mutant, CatCh, with enhanced  $\text{Ca}^{2+}$  permeability. The light-stimulated DRG neurons are induced by an elevated  $\text{Ca}^{2+}$  influx through CatCh.
- We also change the settings of the study to evaluate how different factors can impact the neuronal regeneration:
  - Neurotmesis is a complete transection of the nerve and recovery of function is extremely unlikely and requires some level of experience in surgery and it is more complex compared to an axonotmesis. Therefore, instead, we decide to perform an axonotmesis.
  - Within the two groups of mice, we introduce mice of different age group as age is an important factor of recovery
  - We include different pattern of stimulation: [1, 2, 3] hour(s), 5ms pulse, [1,2,3] seconds stimulation followed by [1,2,3] seconds of rest to refine the methodology of light stimulation
  - Time is of essence in nerve regeneration, so we propose to apply OS at varying time delays after injection of AAV.
- In addition, we introduce in the opsin-promoter construct a genetically protein EYFP and use c-Fos labeling to detect activity neuronal with immunofluorescence.
- We propose to analyze the levels of myelin-associated proteins such as Krox20 and MBP, which are SC transcription regulatory factors driving the transition from non-myelinated to myelinated status in SCs after OS and how they progress over time after light stimulation (for ex. through western blot analysis).

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