Agryha proposes a technique 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 first creates an experimental group of rats which undergo an injury of the sciatic nerve and she also has a control group of rats 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 (OS) 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 gene expression uses AAV transduction, which may induce immune response leading to death of neurons, however optogenetic stimulation has the determinant advantage to be specific spatially (single-cell level), temporal (spike frequency and pattern modulation), and phenotypical (cell type and even neuron subtype) [1].
- For axonal regrowth, Agryha method is overall relevant but at same time lacking some guidelines to assess visually and quantitively in-vivo neural regeneration progress during the 1- or 2-months observation period.
- However, her goal is also to design a technique that, in addition of axonal growth, promotes proliferation of Schwann cells (SCs) and the connection between the two is not clearly exposed.
- Inspired by a recent study showing that optogenetic stimulation (OS) promotes in-vitro SC proliferation, differentiation and myelination, we replace ChR2 opsin with a ChR2 mutant, CatCh, with enhanced Ca^{2+} permeability. The light-stimulated specific neurons (at 473 nm wavelength), are induced by an elevated Ca^{2+} influx through CatCh [2]. Another benefit of CatCh is that it is ultra-light sensitive which enables neural activity in transfected neuron cells to be increased by low-intensity light (1 mW/mm^2).
- We also change the settings of the study to evaluate how different factors can impact 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. It is also more difficult to execute 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 since age is an important factor of neuronal regeneration.
  - We refine the methodology of light simulation and 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
  - 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.
- In uninjured neurons, myelin Schwann express various proteins including pro-myelin transcription factor Krox20, myelin protein zero (MPZ) and myelin basic protein (MBP). We will sample some issues closed to the crushed area, and we will measure levels of Krox20 and MBP, which are SC transcription regulatory factors driving the transition from non-myelinated to myelinated status in SCs and analyze how they progress over time after light stimulation (for ex. through western blot analysis).
- In a cut or crush, in the distal stump, both types of SCs are reprogrammed in transient repair Schwann cells which promote healing and form regeneration tracks (Bungner bands). It has been established that deterioration of repair cells is an important reason for regeneration failure. We want to quantify the impact of OS on repair Schwann cells consequently we propose to measure expression of key transcription factors like STAT3 and c-Jun involved in repair Schwann cells [3][4].
- We select randomly axons in the nerve transverse sections, compute the g-ratio (diameter of axon/diameter of axon plus myelin sheath) and average them, to quantify the impact of OS on myelination. We want to show that OS not only promotes axon regrowth, but sSCs differentiation, and eventually axon myelination.

Overall, the study stays similar to the technique described by Agryha with more content related to Schwann cells since it was her initial intent to include them in the study with additional details provided to assess over time the success of the methodology on axonal regrowth and myelination of a pre-identified subset of neurons.

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