

- Fernando model investigates if optogenetic stimulation of the medial prefrontal cortex (mPFC) in SAMP-P/8 mice injected with AAV-CHR2 compared to non-stimulated similar mice show improved cognitive performance measured on delayed non-match-to-sample tasks (DNMS). The experimental group is exposed twice a day to an excitation light every 2 minutes over a 30-minute period, each testing phase being separated by an hour allowing the mice to rest. The next day, both control and experimental groups are subjected to DNMS tasks. Fernando's expectation is to observe better performance on the tests for the optogenetically modified mice.
- The experiment designed by Fernando is to show that cognitive decline observed in aging mice could be mitigated by promoting oligodendrogenesis which could have broad clinical implications.
- However, I will make few clarifications and recommend changes so analysis of the results will be less prone to ambiguity.
- Traditionally delayed matching (DMS) and non-matching-sample tasks involve short-term memory process and the execution of a choice response [1]. Albeit, mPFC is likely needed for both recent and remote memory, it has been demonstrated that the hippocampus plays a role complementary to the mPFC, in that it is strongly activated during retrieval of recent memories but not remote memories which require stronger cognitive control provided by the mPFC [2]. Neurons in mPFC are very sensitive to changes in position or trajectory, and experiments with rats with mPFC lesions, showed that the deficit is not in memory storage but rather in the capability of implementing mediating strategies. Additionally, there has been evidence that mPFC supports working memory. Therefore, the cognitive tests to show improved performance in the neurons of the mPFC have to be carefully designed so to identify that the observed deficits or improved performance for the experimental group are due to trial-specific working memory, mediating strategies and not a deficit in reference memory which involves the hippocampus. Also, it will be important to test the two groups of mice on visual categorization tasks since in rodents the prefrontal cortex (PFC) plays an important role for such tasks including tasks involving rule learning and cognitive flexibility [3][4].
- It is also important to determine if regeneration of oligodendrocyte progenitor cells (OPCs) and oligodendrocytes in vivo happened after optogenetic stimulation.
- Hence, we will divide our study in two steps using two animal models: 1) the Senescence Accelerated Mouse-Prone 8 (SAMP-P/8) following the same methodology described in Fernando model, the only major differences will be a more carefully designed test sets to target more specifically mPFC neuronal functions (working memory) and 2) a Sox10-EGFP mice, which identifies oligodendroglia cells at all stages of differentiation injected with a demyelinating agent lyssolecithin (LPC) and the AAV-Chr2 in the mPFC [5].
- With the SAMP-P/8 we will perform the similar experiment detailed by Fernando: two group of same mice with one injected with AAV-Chr2 in the mPFC and a control group. Since it is difficult to design tests to target only the frontal cortex independently for example of the hippocampus, these mice will be submitted to NDMS tests, but also, other types of tests. For example, they will be submitted to precisely delayed tasks, not too short but long enough so the mice are able to perform the tests but show more significant impairment as the delay is increased. We will also use spatial dependent tests. As an illustration, train the mice on a spatial win-shift task in which the correct choice depends on which maze arm was rewarded two trials back [4]. We will follow a similar schedule to the one described in Fernando experiment: a day of exposition to excitatory light followed by a day of rest for the stimulated mice then tests for both group of mice. We will adopt this pace for at least 2 weeks¹.
- We will then proceed with the second animal model, the Sox10-EGFP mice, which will also have neurons in the mPFC ontogenetically stimulated. We will wait about 6 months because this is the inflexion point after which there has been a significant decline in the rate of remyelination, we will then proceed to the battery of tests used in step 1. We will administer to these mice the cell proliferation marker Edu and the pan-oligodendroglia marker Olig2 to identify and quantify the total number of OPCs at the beginning of each optogenetic stimulation session before the test session and after the test session, keeping track of these numbers and various statistics related to these measures for the next two weeks. We will also keep track of the myelin index (MI) which is a measure of the numerical density of myelin sheaths that cross the z-plane in the mPFC. These metrics will complement various visual examinations of confocal z-sections of induced mice vs. non-induced mice that we will perform to assess the changes in oligodendrogenesis.
- Although we want to compare mice with similar genome, we may integrate a third group of wild-type mice which could act as our reference for the different quantification and statistical analysis we are planning to perform.
- In conclusion, we will follow well-defined cognitive tests [6] and compiled various summary statistics to quantify our measurements on the tests but also in term of oligodendrogenesis allowing to establish a cognitive impairment baseline measurement and its improvement after excitation of the neurons in the mPFC.

¹ Other tests may be investigated like water maze.

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