Deliverable Ref: D1.1 (D1)

Deliverable Title: Development of genetically modified iPSCs

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Key project information

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Description of work: iPSCs will be genetically modified to generate necessary cell-type specific optogenetic, chemogenetic, calcium, indicators and/or other expression lines to enable all optical interrogation of human neural network activity.

Task 1.1 Develop iPSC lines for all optical interrogation. iPSCs will be genetically modified to generate neuron/glia lines expressing optogenetic actuator and calcium activity indicators. (M01-12). AU**.** UB, LU.

D1.1 Development of genetically modified iPSCs for generation of 'all optical' imaging approaches (M08)

Report

Online meetings and discussions with UB and LU were conducted, where requirements of optogenetic actuator and sensor reporting lines were identified. Latest available technologies for possible implementation were discussed and course of action decided.

To generate specific optogenetic, chemogenetic, calcium, indicators and/or other expression lines to enable all optical interrogation of hNN activity genetic modification of iPSCs or Neural progenitor cells is required. To ensure that 100% neuronal cells expressed the genetic construct iPSCs could be genome edited to incorporate the optogenetic reporter in a safe harbour' site in the cellular genome such as AAVS1. Neuron/glia lines derived from these iPSCs would then express the optogenetic actuator and calcium activity indicators in a cell specific manner.

However, this approach is currently not viable and genome editing service providers are unable to guarantee the success of generating such a large and complex insert for the generation of stable iPSC. We have approached a number of service providers to generate the iPSC lines, however they are also unwilling to take on such a high-risk project.

To overcome this issue, we have developed alternative approaches to interrogate the cell lines using lentiviral vectors that enable the selection of virus expressing neural progenitor cells using antibiotic selection, ensuring that 100% of cells express the construct. Due to the inclusion of an antibiotic resistance cassette, we are able to remove non-transduced cells through the addition of antibiotics to the media. The inclusion of an antibiotic resistance cassette, has enabled the selection of transduced cells and following differentiation these lines have demonstrated cell type specific and long-term expression of the construct.

To further develop this technology, we have also obtained a number of additional (recently published) viral constructs to robustly mediate induction of spiking and monitoring of distinct neural populations in cultures. Upon validation of these constructs, we will modify these additional viral vectors to enable antibiotic selection of cells.

In addition to the use of alternate viral transduction methods we are also comparing alternative methods for the generation of long-term neural progenitor cell (NPC) populations. We have developed cultures using methods adapted from Shi et al (2012) (Fig 1) and Calvo-Garrido et al. (2021) Fig 2. lt-NES cells allow continuous culture for up to 100 passages. This characteristic is crucial for the generation of NPCs that stably express a construct using antibiotic selection, since NPCs should be expanded before differentiation for 'all optical' imaging approaches.

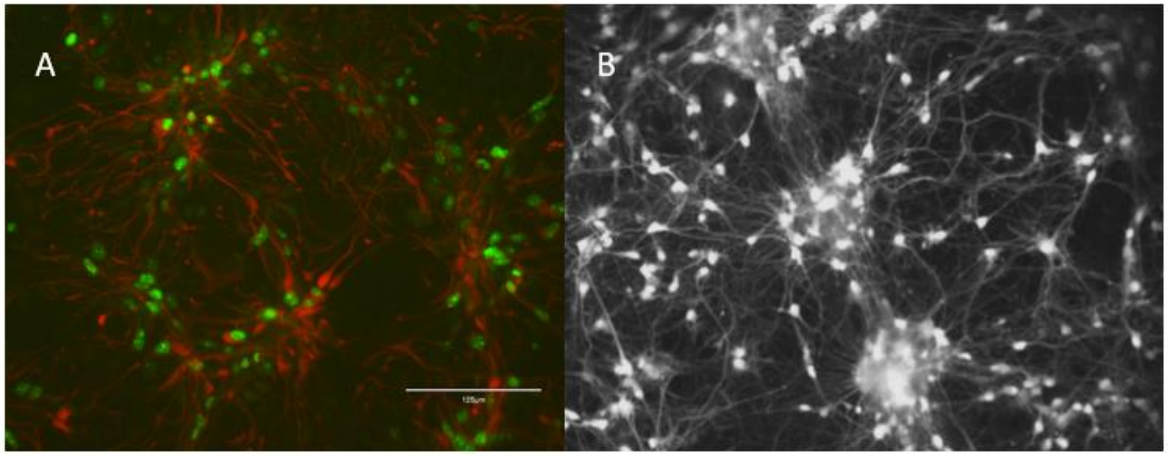

Fig 1. Efficient derivation of human cerebral neocortical neural precursor cells (NPCs) and functional neurons from pluripotent stem cells (Shi et al 2012). A) immunofluorescent image of NPCs Nestin (red) Sox2 (green). B) Fluo-4 loaded cortical cultures after 50 days.

Calcium imaging using GCaMP6 has also demonstrated the feasibility of this approach. Cells transduced with the selection construct demonstrate calcium transients for over 60 days (demonstrating long term expression).

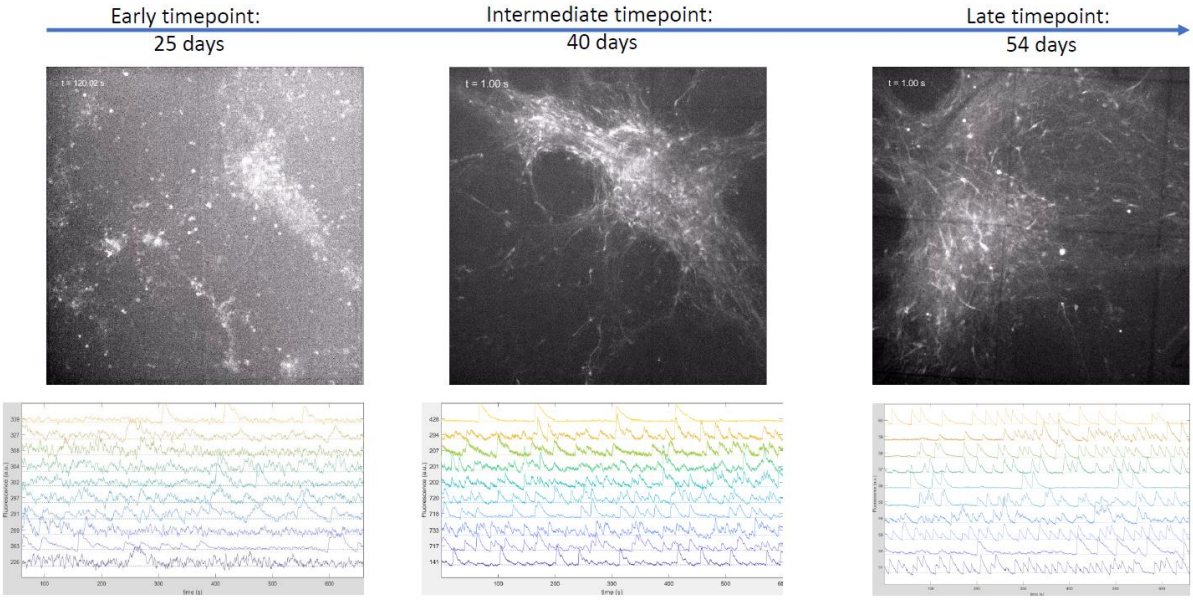

Fig 2. Evaluation of neuronal activity using calcium imaging monitorization of neuronal cultures stably expressing GCaMP6s after infection with Lentiviral vector and subsequent antibiotic selection. Examples of recordings at different timepoints (25, 40 and 54 days of differentiation) of the cultures. Top images show GCaMP6s fluorescence and bottom panels show representative traces of fluorescence intensity over time from several neurons.

Using viral transduction and antibiotic selection we are able to generate genetically modified NPCs that can be used for long term ‘all optical’ experiments. This approach will be used for the interrogation of defined functional neuronal and glial cell networks generated in the project.