Application of patient generated iPSCs derived neurons to define the pathophysiology of FXS neurons

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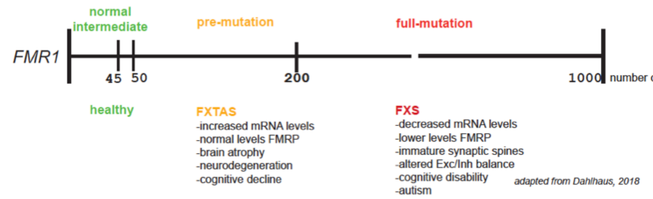
Introduction

The Fragile X Syndrome (FXS)

- Most frequent form of monogenetically determined mental retardation
- Affects approximately 1 in 7,000 males and 11,000 females
- Patients display a variety of intellectual deficits that range from mild learning impairments to severe cognitive disabilities and autistic spectrum disorders
- In most cases, FXS, arises from the loss of expression of the Fragile X Mental Retardation gene, *FMR1*, due to methylation of CGG triplet expansion in the 5' untranslated region of the gene
- Fragile Mental Retardation protein (FMRP) is an RNA-binding protein with important roles in mRNA metabolism including transport, stability, and translation. Its absence leads to excessive accumulation of certain proteins and reduction of others.
- FMRP appears to be involved in developmental decisions at the level of neurite extension, guidance, and branching. The absence of FMRP leads to longer and thinner/immature dendritic spines



Limitations of using non-human FXS model systems

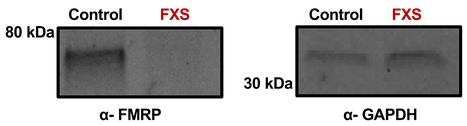
- Epigenetic silencing of *FMR1* gene only occurs in humans
- Mice engineered with the CGG repeat expansion in *Fmr1* do not show the characteristic gene methylation and silencing. Additionally, studies support that in mice, 200–350 repeats still represent a pre-mutation situation
- Inconsistencies in the results obtained from *Fmr1-KO* mice behavior such as standard learning and memory tasks
- Although preclinical studies conducted in *Fmr1-KO* mice have helped to delineate signal pathways that couple neural activity to FMRP-regulated protein synthesis, among others, the translation of the findings to clinical applications has been unsuccessful

Human pluripotent stem cells as a model to study FXS

- Patient-derived hiPSCs provide a paradigm to understand neurological FXS pathogenesis in human genetic background
- Differences between patients, iPSC reprogramming methods, and neuronal differentiation paradigms can introduce variability. Reproducible identification of specific cellular phenotypes requires large numbers of samples or, better yet, isogenic cell lines
- Although FXS hESCs and hiPSCs have been established, there is limited data on the development and phenotypic characterization of human FXS neurons

Goal: Characterize Excitatory and Inhibitory neuronal cells derived from FXS patient iPSCs carrying *FMR1* full-mutation (SC-135*)

- SC-135 FXS iPSCs derived iNs do not express FMRP



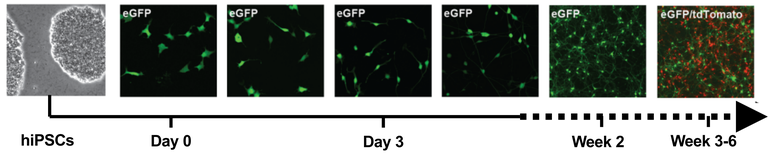
FXS SynFire® provides a well defined translatable neuronal platform for understanding the signal pathways that underlie FXS pathophysiology

*National Human Neural Stem Cell Resource Children's Hospital of Orange County Research Institute

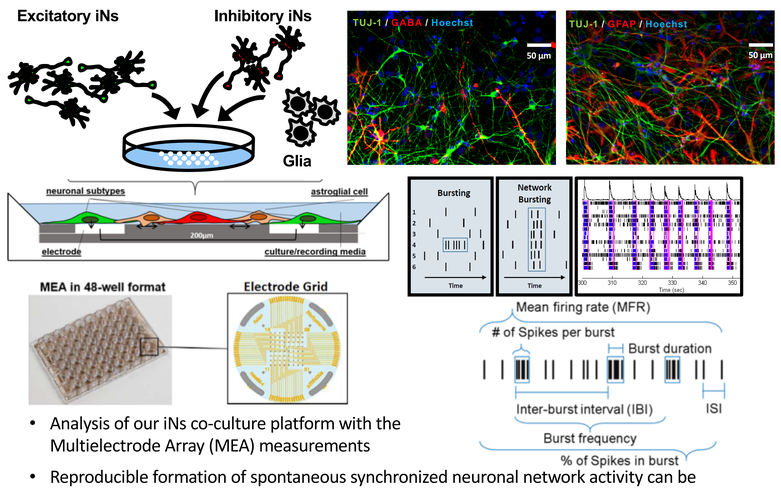
SynFire® iPSC-Derived Neural (iN) Cell Technology

NeuCyte's proprietary neuronal differentiation approach

- Direct reprograming of iPSCs into highly functional iNs of defined subtypes, i.e., glutamatergic and GABAergic neurons



- Established a pure human neuronal/glial co-culture platform with defined ratios of glutamatergic to GABAergic iNs and primary human astrocytes
- Rapid neuronal maturation and network formation. Ability to differentially label various iN subtypes

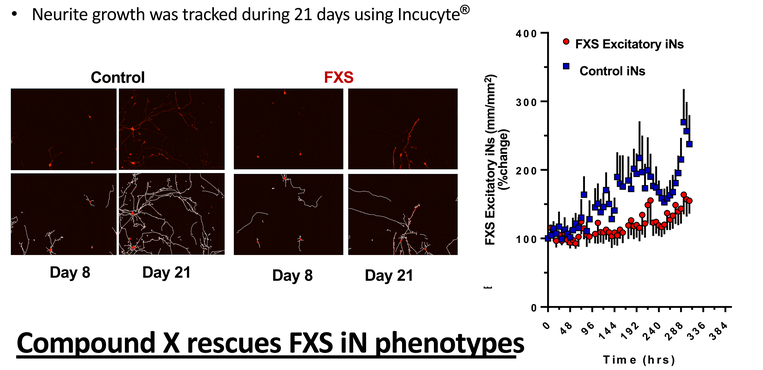


- Analysis of our iNs co-culture platform with the Multielectrode Array (MEA) measurements
- Reproducible formation of spontaneous synchronized neuronal network activity can be detected 3-4 weeks after plating. MEAs permit analysis of complex phenotypes

Characterization of FXS iNs

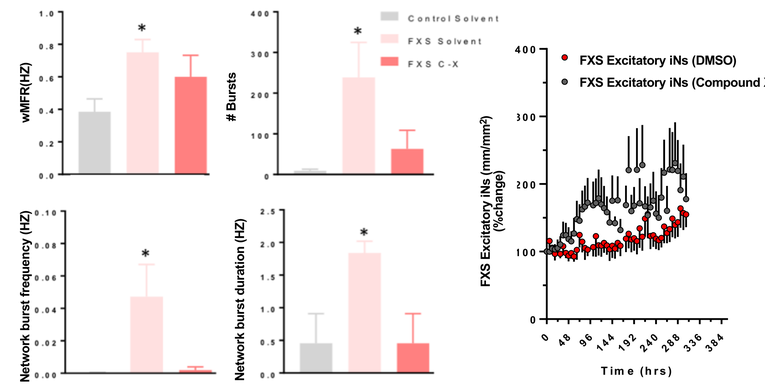
FXS glutamatergic iNs display distinct neurite growth kinetics

- Labelled FXS Excitatory and Inhibitory iNs were spiked into a control iN co-cultures
- Neurite growth was tracked during 21 days using Incucyte®



Compound X rescues FXS iN phenotypes

- FXS iN co-cultures were treated with 50 mM of tool compound X (C-X), involved in epigenetic regulation. Treated cultures were analysed by MEA recordings and neurite outgrowth assay

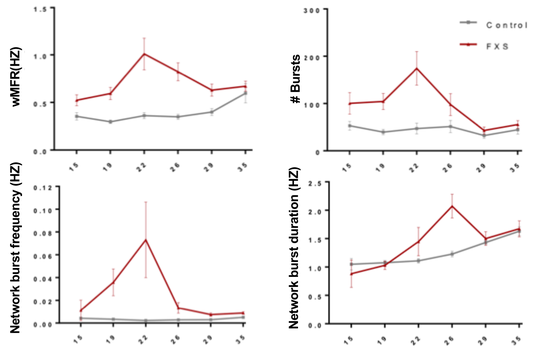


- Compound X restores neuronal activity and morphology of FXS iNs to healthy control

Characterization of FXS iNs

MEA readout of FXS iN co-culture shows an increased activity

- hiPS cell line from FXS patient carrying 450 repeats (SC-135*)
- Reprogrammed into glutamatergic or GABAergic iNs and seeded on MEA plates with human glia, 70% glutamatergic vs. 30% GABAergic iNs. Neuronal activity recorded Day 15 -35



Conclusions & Ongoing work

- NeuCyte's SynFire® provides a fully defined human neural co-culture platform ideal for the study of CNS diseases such as FXS
- FXS iN co-cultures displayed an increased network activity pointing towards an imbalance in the E/I ratio and altered neurite growth dynamics. These phenotypes are consistent with the clinical pathophysiology of FXS
- Treatment of FXS iN co-cultures with a tool compound-X, restored the network activity and neurite growth levels observed in the Control iN co-cultures
- **Currently these phenotypes are being validate with isogenic lines generated from 6 individual mosaic patients**