Functional Deficits in Fragile X Neurons Derived from Pluripotent Stem Cells

A. Zhang[1,2], H. Kjeldsen[3], A. Szucs[4], M. Masters[3], M. J. Boland[2,5], K. L. Nazor[2,6], M. Szymanski[3], Y. Wang[2,7], J. Loring[2]

[1]Graduate Program, [2]Department of Molecular Medicine, The Scripps Research Insititute, La Jolla, CA; [3]Truust Neuroimaging, San Francisco, CA; [4]UCSD, La Jolla, CA; [5]Present address: Columbia University, New York, NY; [6]Present address: MYi Diagnostitcs & Discovery, San Diego, CA; [7]Present address: Rush University, Chigaco, IL.

Introduction

Fragile X Syndrome (FXS) is the leading monogenic cause of intellectual disability and autism spectrum disorder. It is caused by expansion of a trinucleotide repeat in the 5'UTR of the Fragile X Mental Retardation-1 (FMR1) gene. We recently reported that induced pluripotent stem cells (iPSCs) derived from FXS patients exhibit profound neurogenic defects early in development, accompanied by genome-wide changes in DNA methylation and gene expression (Brain, 2017). To understand how these early phenotypes affect neuronal function and maturation in FXS neurons, we performed microelectrode array (MEA) recording and whole cell patch-clamp analysis during the timecourse of cortical differentiation on neurons derived from control iPSCs, FXS- iPSCs, and an isogenic pair of control and FMR1-KO-ESCs. Our MEA recordings suggest active network formation in both control and FXS neurons. However, networks in FXS neurons appear to be less dynamic at earlier timepoints, and become more dynamic at later timepoints. Bicuculline treatment suggests that differences in observed network activity in FXS cultures at later points is due to less inhibitory activity compared to control cultures. Consistent with this observation, our patch clamp recording revealed that FXS neurons received much less inhibitory synaptic transmission. Overall, our study provides a proof-of-principle case to bridge cellular phenotype to network level phenotype in neurological disease modeling.

Results

1. *In vitro* differentiation of cortical neurons using hPSCs.

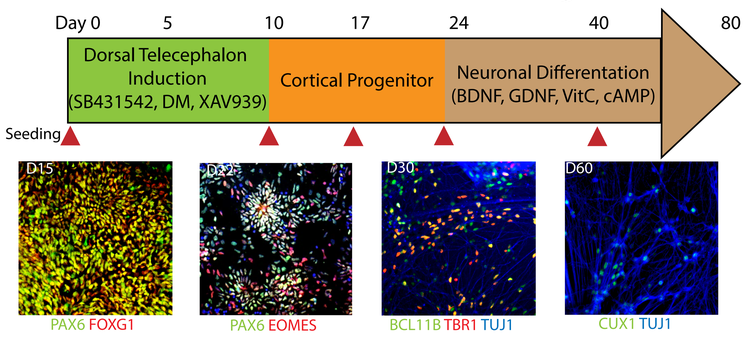

Figure 1. Schematic representation of the strategy for differentiating hiPSCs into cortical excitatory neurons, displaying culture and treatment conditions. This monolayer protocol begins with dual SMAD and WNT inhibition, creates cortical progenitors, and then produces deep and upper layer excitatory neurons. Interestingly, ~10% of inhibitory neurons are produced in our culture system.

Results

2. Multi-Electrode Array recording reveals diverse variabilities within both FXS and control groups using frequency based measurements.

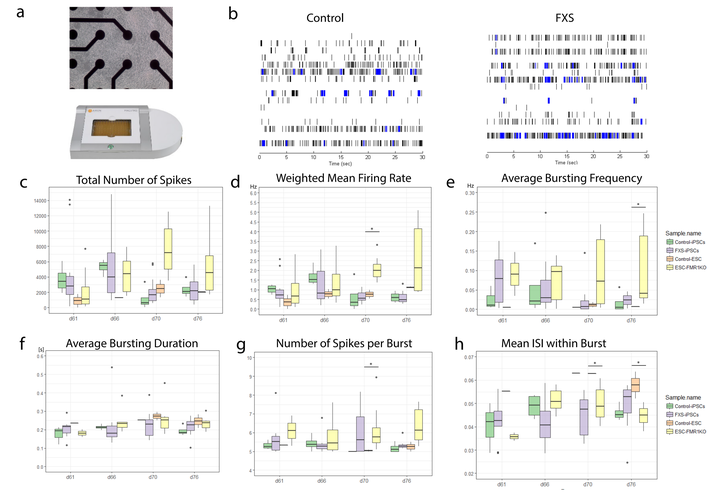

Figure 2. MEA recordings of extracellular spontaneous spike activity. (a) representative image of neurons cultured on electrodes using the maestro system (Axion). (b) representative image of raster plot of a control well and a FXS well. Frequency based measurements such as (c) number of spikes, (d) weighted mean firing rate, (e) burst frequency, (f) burst duration, (g) number of spikes per burst, and (h) mean ISI within burst are done on differentiation days 61, 66, 70 and 76 respectively. Non-parametric t-test were performed, and a few statistically significant changes were observed. Three FXS-iPSCs and one control iPSC, H1 and H1-FMR1-KO lines were included.

3. With vector based measurement, MEA recordings suggest FXS neurons showed less dynamic networks at earlier time points, but more dynamic networks at later time points

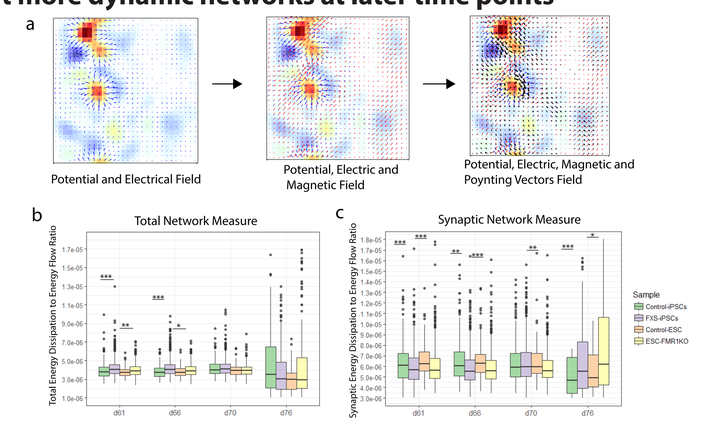

Figure 3. Near-Field Electromagnetic Holography analysis of MEA recordings. (a) Product of potential, electric and magnetic field were calculated to generate a vector representing energy dissipation and energy flow. (b) Total network analysis. (c) Synaptic network analysis suggest FXS networks shows less energy dissipation at earlier time points, but present more energy dissipation at later time points compared to control cultures.

Results

4. FXS neurons are less responsive to Bicuculline treatment, suggesting immature inhibitory network in FXS culture.

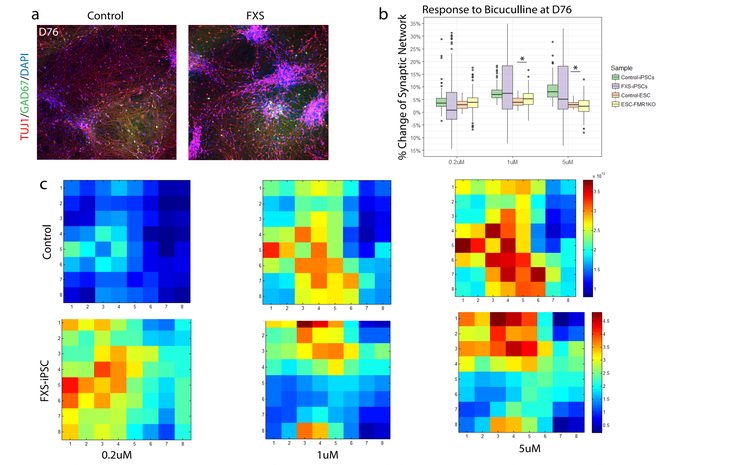

Figure 4. Bicucculine treatment suggest a less mature inhibitory network in FXS culture. (a) representative ICC image of inhibitory neurons. (b) Control networks become more dynamic upon Bicuculline treatment while FXS networks are less responsive to Bicuculline. (c) representative heat map visualization of how networks respond to varying concentrations of Bicuculline.

5. Whole-cell patch-clamp suggest FXS neurons receives less inhibitory synaptic inputs.

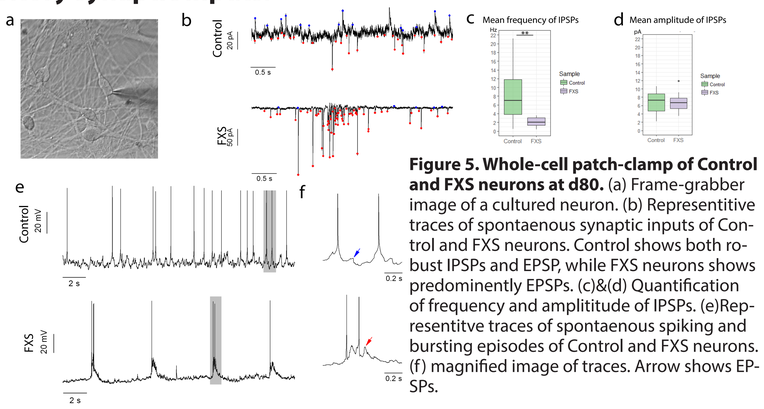

Figure 5. Whole-cell patch-clamp of Control and FXS neurons at d80. (a) Frame-grabber image of a cultured neuron. (b) Representitive traces of spontaneous synaptic inputs of Control and FXS neurons. Control shows both robust IPSPs and EPSP, while FXS neurons shows predominently EPSPs. (c)&(d) Quantification of frequency and amplititude of IPSPs. (e)Representitve traces of spontaenous spiking and bursting episodes of Control and FXS neurons. (f) magnified image of traces. Arrow shows EPSPs.

Conclusions and Future Directions

Consistant with previous *in vivo* studies, we find a less mature inhibitory network in FXS neurons during development. Our study demonstrates inhibitory/excitatory network imbalance can be modeled using iPSCs derived neurons and MEA recording. With human cells and easy to use MEA system, the assays we established here will be invaluable for disease modeling and therapeutic screening.

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[1] Boland MJ et al, Molecular analysis of neurogenic defects in a human pluripotent stem cell model of fragile X syndrome. Brain 2017.
[2] Kjeldsen HD et al, Near-field electromagnetic holography for high-resolution analysis of network interactions in neuronal tissue