Characterization of the activity-dependent development of IPSC-derived neurons from Fragile X patients

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1. Generation of cortical neurons from iPSCs

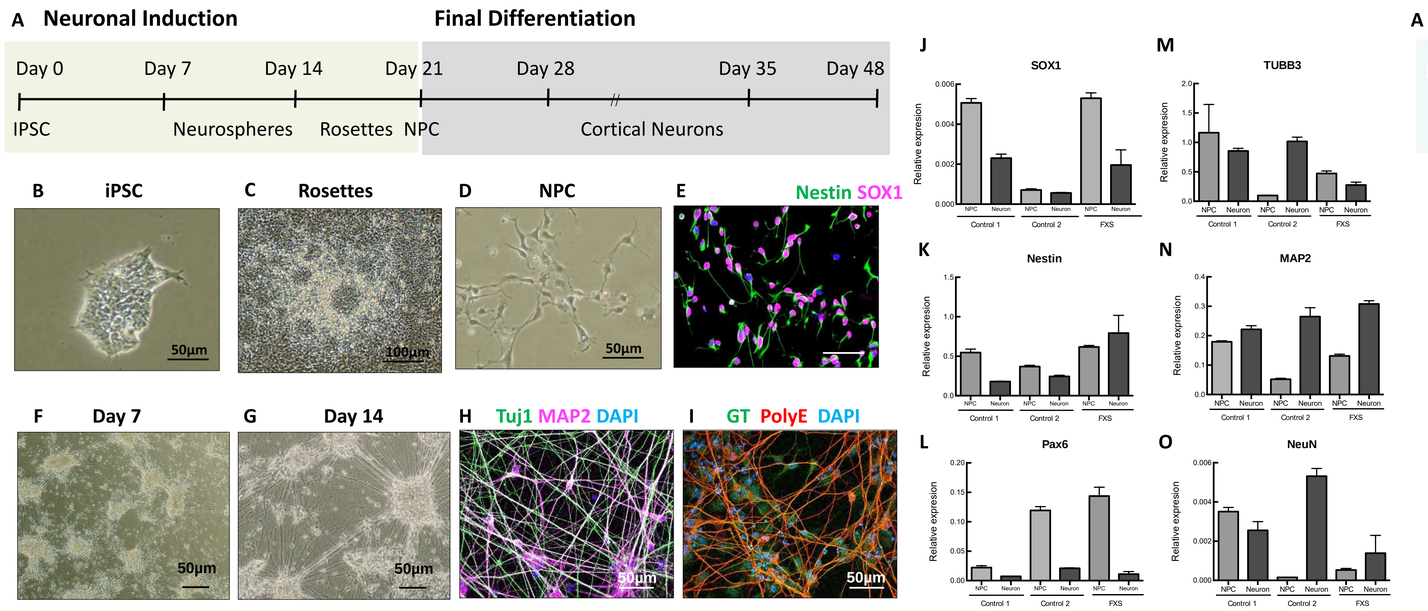

Figure 1- Generation and characterization of iPSC-derived cortical neurons. (A) Experimental workflow for differentiation of cortical neurons from multipotent neural progenitor cells (NPCs) generated from control iPSCs. Images show the generation of neuronal progenitors using human iPSCs. Brightfield image of (B) control human iPSCs that were cultivated in neural induction media for fourteen days to obtain (C) neuronal rosettes and (D) neuronal progenitor cells (NPCs) at the end of 21 days of induction. Immunostaining of NPC cultures confirming expression of neuronal progenitor markers Nestin and SOX (E). (F-G) Cortical neurons after 7 and 14 days of differentiation. Confocal images demonstrate the expression of neuronal markers Tuj1 and MAP2. (H) The presence of monoglutamylated (GT antibody in green) and polyglutamylated (PolyE antibody in red) was assessed by confocal microscopy and showed that microtubules in human cortical neurons are highly modified. qPCR analysis to access expression of neural progenitors (J-L) and (M-O) neuronal genes.

2. IPSC-derived forebrain spheroids

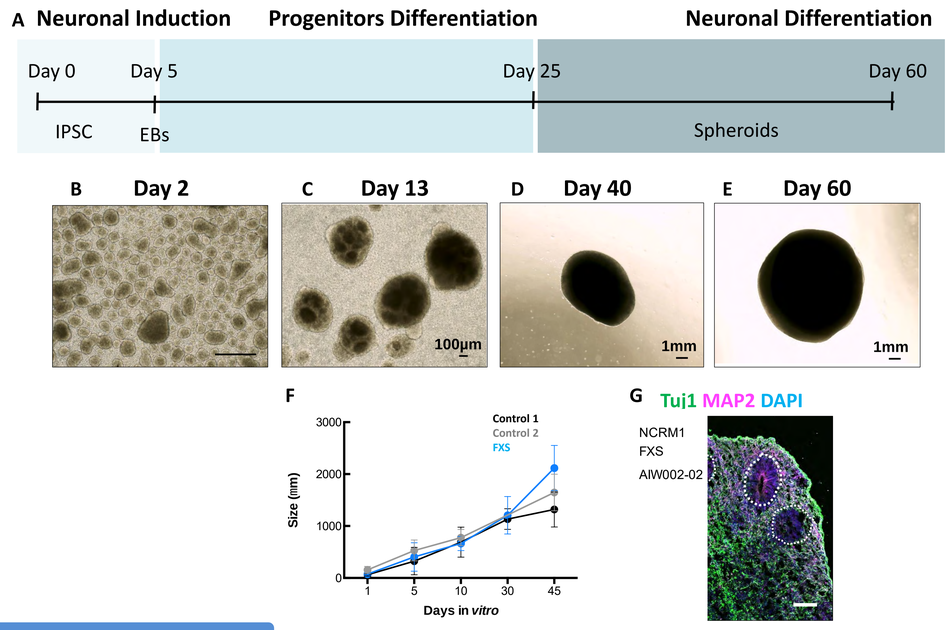

Figure 2. Forebrain organoids generated from human iPSCs. (A) Experimental workflow for forebrain organoid generation using control human iPSCs. (B) Brightfield images of human embryoid bodies (B) and cortical spheroids after 13, 40 and 60 days in culture (C-E). Size of spheroids from two different control cell lines and one FXS line was measured for 40 days (F). (G) Confocal images of neuronal markers in 60 days-old showing corticogenesis in ventricular-like zones delimited by dashed circles in white.

INTRODUCTION

Fragile X syndrome is a form of syndromic autism whose genetic causes have been relatively well uncovered. It is actually mainly caused by a CGG triplet expansion in the 5' UTR sequence of *FMR1* gene, affecting mostly men. *FMR1* encodes a mRNA binding protein which is involved in the regulation of local translation at the synaptic level. The mechanisms leading from such gene mutations to a neurodevelopmental disorder still need to be investigated. While several studies have shown that the neuronal development is driven by cellular activity and connectivity, we aim to further investigate the effect of *FMR1* repression on the neuronal activity taking advantage of IPSC-derived neurons from patient's cells. IPSC-derived neurons will be investigated through calcium imaging to characterized their pattern of spontaneous activities, as well as their capability to respond to neurotransmitter through extra-synaptic receptors. A multielectrode array approach is going to be used to analyse the overall network activities. Those studies should provide further information on the impairment of activity-dependent neuronal development in Fragile X syndrome.

3. Spontaneous activity of cortical progenitors from controls and FXS patients

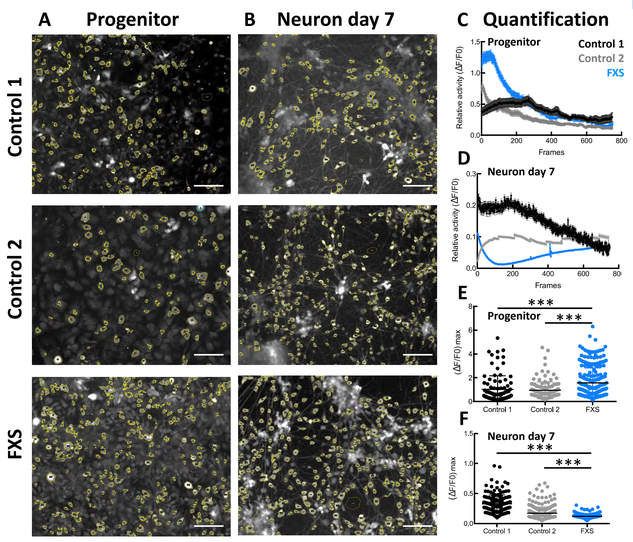

Figure 3- Spontaneous activity of cortical progenitors from controls and FXS patients. Calcium flux was visualized by FLUO4 live staining. Acquisition was made for 5 minutes with 400ms intervals between frames. Data were expressed as ΔF/F0 where ΔF is, for each region of interest and after background subtraction, the difference between the averaged pixel value at a specific time minus the smallest pixel value (F0) across all the timepoints. (A-B) Automatic segmentation of regions of interests of neural progenitor cells and of D7 cortical neurons.(C-D) Graphical representation of the averaged spontaneous activity expressed as ΔF/F0 across all the regions of interest for each timeframe. (E-F). Dot plot of the maximum ΔF/F0 observed for each region of interest over all the timeframe of the acquisition. We observed an increase in ΔF/F0max ratio in FXS NPCs compared to controls, which reflects an increase of calcium flux in the cells. At day 7, a decreased ΔF/F0max ratio is observed. ***:p<0.001. Scale bar: 10µM

4. NMDA Response in 2D cultures of cortical progenitors and neurons from controls and FXS patients

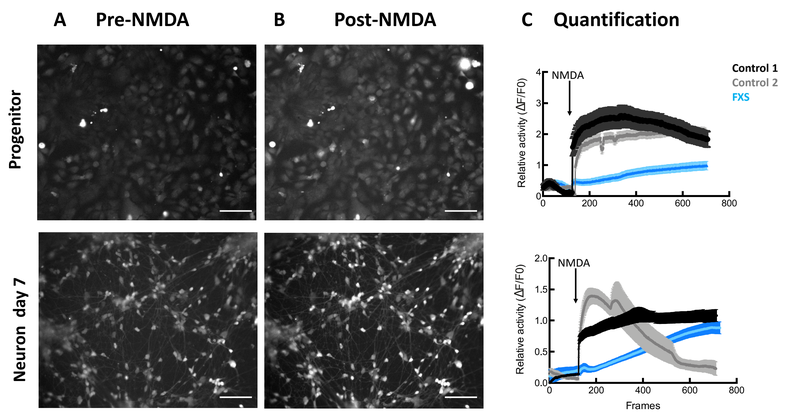

Figure 4-NMDA Response in 2D cultures of cortical progenitors and neurons from controls and FXS patients. Data acquisition and treatment were performed as in Figure 3. NMDA was applied at the timepoint 120 (black arrow). (A-B) Manual segmentation of the regions of interests before and after NMDA application. (C) Graphical representation of the averaged relative activity, expressed as ΔF/F0 during the time of the acquisition. In control condition, an important increase of ΔF/F0 ratio following the NMDA application is observed whereas smaller variation are observed in the FXS line at both stages Those data suggest a decrease of NMDA response in Fragile X lines compared to controls. Scale bar: 10µM

5. NMDA response of dissociated cultures from 3D spheroids.

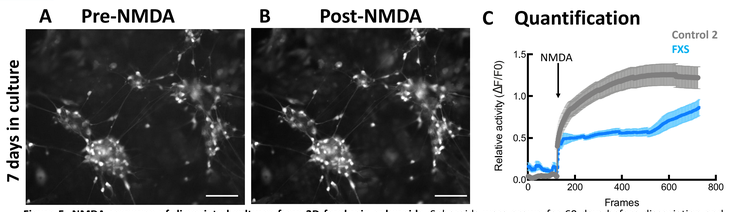

Figure 5- NMDA response of dissociated cultures from 3D forebrain spheroids. Spheroids were grown for 60 days before dissociation and cells were cultured for 7 days prior to calcium imaging experiment. Data acquisition and treatment was performed as in figure 3 and 4. (A-B) Manual segmentation of the regions of interests before and after NMDA application. (C) Graphical representation of the averaged relative activity, expressed as ΔF/F0 during the time of the acquisition. NMDA was applied at the timeframe 120 (black arrow). A significant difference of the ΔF/F0 ratio observed between control and FXS line after NMDA application, what suggests a decreased NMDA response in dissociated neurons of 60 days spheroids from the FXS line compared to control lines, as in the 2D cultures. Scale Bar: 10µM

CONCLUSION

- Spontaneous activities are observed in NPCs and Neurons from controls and FX patients.
- The amplitude of spontaneous activities are higher in the FXS NPCs compared to controls.
- FXS NPCs and cortical neurons show a reduced response to NMDA application.
- Observations were confirmed in 3D model.
- Change in calcium flux and in NMDA-dependent signalling may affect the neuronal maturation processes in the brain from FX patients.

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