Real-time calcium imaging of Niemann-Pick disease type A human iPSC-derived glutamatergic neurons

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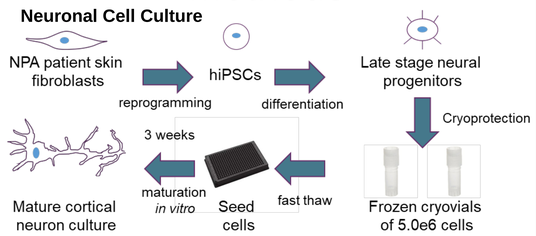
Introduction

Advancement of induced pluripotent stem cell (iPSC) technology has enabled modeling the neurological disease *in vitro* using iPSC differentiated human neurons[1]. These human neurons recapitulate the genetic background of the patient, and can therefore better mimic the disease phenotype and pathophysiology. Here, we demonstrate real-time live-cell imaging of human iPSC-derived cortical glutamatergic neurons derived from Niemann-Pick disease type A (NPA)[2] patient fibroblasts. In this rare lysosomal storage disease, the deficiency in sphingomyelinase function due to mutations in the *SMPD1* gene leads to the accumulation of the lipid substrate sphingomyelin in lysosomes. The neurological symptoms of this fatal childhood disease develop at a young age. We performed the live-cell calcium imaging experiments including the single cell recording (IN Cell 2500HS) and whole-well calcium response (FDSS µCell) in 96- and 384-well plate formats. The real-time imaging experiments indicated an increased frequency of spontaneous calcium transients in NPA disease neurons compared with the wildtype (WT) control neurons. Therefore, the calcium imaging experiments can be used for further study of disease pathophysiology and compound screening to identify lead compounds for drug development.

Methods

Neuronal Cell Culture

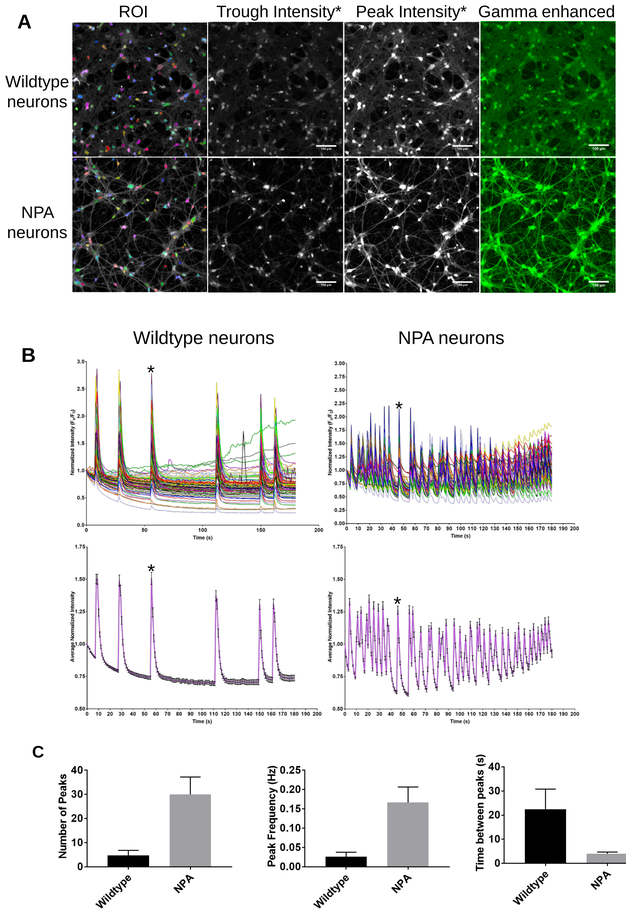


Imaging of spontaneous calcium transients

WT and NPA neural progenitors (from BrainXell) were seeded in multi-well plates (30,000 for 96-; 8,000 for 384-well plate) and cultured for at least 21 days until maturation before experiments. Cells were loaded with calcium dye Calbryte 520-AM (AAT Bioquest) for 1 hour at 37°C. Cells were washed and media was replaced with Live Cell Imaging Solution (Invitrogen). The IN Cell 2500HS (GE Healthcare) automated imaging system was used with a 20X objective lens to capture the spontaneous calcium transients for single cell recording as indicated by increases in green fluorescence intensity at 0.5 to 1 Hz frequency. The plates were then moved to the FDSS µCell (Hamamatsu) for whole well acquisition of calcium signals. IN Cell 2500 HS images were loaded into Columbus (Perkin Elmer) for analysis.

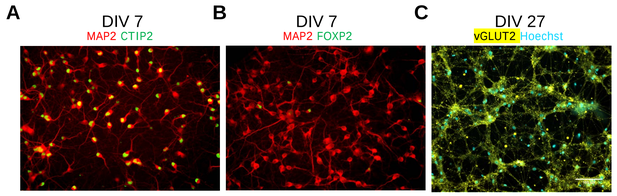
Results

Single-cell imaging and analysis of calcium transients



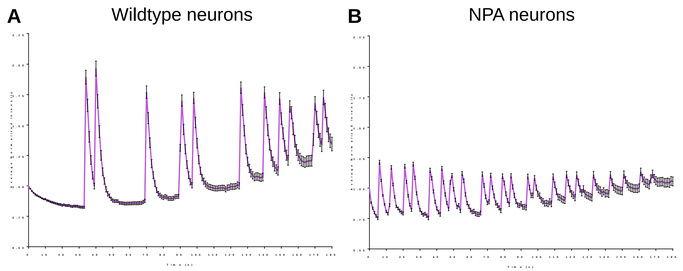
(A) DIV 30 WT (n=102) and NPA neurons (n=83) loaded with Calbryte 520-AM and imaged on IN Cell 2500 HS using a 20x objective at 1 Hz. Automated analysis using Columbus generates ROI on the soma of each neuron in the field of a 96-well plate. Green fluorescence images of the trough, peak, and gamma enhanced green pseudocolor peak timepoint as indicated by asterisk. **(B)** Single cell and average traces of each neuron from A (with asterisk *) indicated peak used to demonstrate increases in green fluorescence intensity (calcium transits). Error bars indicate S.E.M. **(C)** Average number of peaks, peak frequency, and time between peaks for WT (n=4 wells), and NPA (n=3 wells). Error bars indicate S.D.

Immunofluorescence analysis of neuronal markers



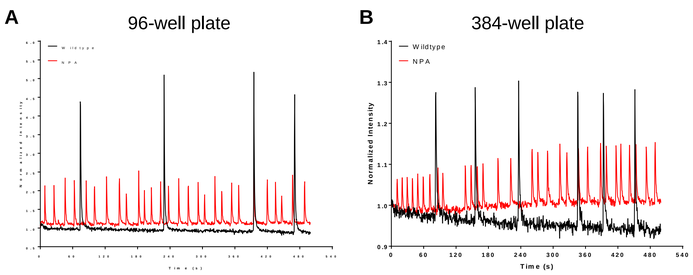
(A) Immunofluorescence staining for dendrites using MAP2 (red), and cortical neurons CTIP2 (green) at Day 7 *in vitro*. **(B)** Immunofluorescence staining for dendrites using MAP2 (red), and pluripotent marker FOXP2 (green) at DIV 7 *in vitro*. **(C)** Immunofluorescence staining for glutamatergic neurons using vGLUT2 (yellow) with nuclear marker Hoechst 33442 at DIV 27.

384-well plate single-cell imaging



(A) WT (n=74) and NPA neurons (n=122) loaded with Calbryte 520-AM and imaged on IN Cell 2500 HS using a 20x objective at 1 Hz. Cells were cultured in a 384 well plate. Error bars indicate S.E.M.

Whole-well recording of calcium transients using FDSS



DIV 37 WT (black traces) and NPA neurons (red traces) cultured in **(A)** 96- and **(B)** 384-well plate, loaded with Calbryte 520-AM, and imaged on the FDSS µCell at 0.5 Hz.

Conclusions

- The glutamatergic neurons (provided by BrainXell) differentiated from NPA iPSCs exhibited mature neuron markers.
- Live-cell calcium measurements revealed the spontaneous calcium response in these neurons.
- A hyperactive phenotype of spontaneous calcium transients was found in NPA neurons compared to WT neurons.

Future Directions

- Calcium responses to various stimuli and conditions will be further studied in the NPA neurons
- Multi-electrode array (MEA) experiments will be carried out to evaluate the electronic activities in the NPA neurons
- A drug screen using the µCell calcium assay will be performed in NPA neurons to identify compounds that ameliorate the hyper active calcium phenotype.

References

1. Tao Y, Zhang SC. Neural Subtype Specification from Human Pluripotent Stem Cells. *Cell Stem Cell*. 2016;19(5):573-586.
2. Long Y, Xu M, Li R, et al. Induced Pluripotent Stem Cells for Disease Modeling and Evaluation of Therapeutics for Niemann-Pick Disease Type A. *Stem Cells Transl Med.* 2016;5(12):1644-1655.

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