# Functional imaging of human iPSCs-derived neurons integrated in mouse cortex using 2-photon microscopy *in vivo*

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## INTRODUCTION

Over the past few years, induced pluripotent stem cells (iPSCs) have emerged as a promising tool for the modeling of human brain diseases and for the rational design of treatment strategies. Using this technology, human neurons are generated from cells isolated form the patient's peripheral tissues (e.g., skin's fibroblasts) opening unprecedented opportunities for investigation of human brain disorders given the precise constellation of genetic variants in a specific individual. However, the lack of the natural brain microenvironment in a cell culture dish can influence the phenotype and maturation. One potential strategy to overcome these limitations is transplantation of iPSC-derived neuronal precursor cells (NPCs) into the mouse brain. Previous studies have proved the feasibility of this "chimeric" approach and demonstrated that the transplanted neurons form synaptic connections using electrophysiological recordings in brain slices (1-3). Ideally, however, activity of human neurons should be measured in the intact mouse brain. To this end, we have an ongoing effort to enable selective, quantitative, and high resolution study of human neurons' function using *in vivo* 2-photon microscopy.

1.Muotri AR, et al. (2005) PNAS 102(51):18644-18648.
2.Espuny-Camacho I, et al. (2013) Neuron 77(3):440-456.
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## APPROACH

- NPCs have been obtained through differentiation of iPSCs derived from skin fibroblasts .
- NPCs expressing fluorescent structural markers are transplanted into the cerebral cortex of newborn NOD/SCID mice.
- Adult chimeric mice are anesthetized with isofluorane during surgical procedures and α-chloralose during data acquisition. ~4x4 mm cranial window is covered with a round glass coverslip cut straight on one side. The cut side was aligned with the lateral edge of the exposure and a gap was left in the seal on the lateral side to allow insertion of glass micropipettes.

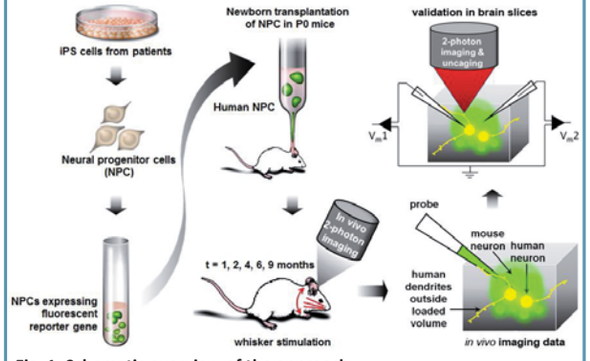


**Fig. 1. Schematic overview of the approach.**

- Fluorescent indicators (e.g., Oregon Green BAPTA-1 AM (OGB1))are delivered at the location of a cluster of human somas.
- Images are obtained using an Ultima 2-photon laser scanning microscopy system from Prairie Technologies (Bruker Nano Fluorescence Microscopy). Excitation light is delivered by Ultra II Ti:sapphire femtosecond laser (Coherent). Green and red fluorophores are imaged using a cooled GaAsP and a multialkali PMT detectors, respectively.
- Data analysis is performed in Matlab.

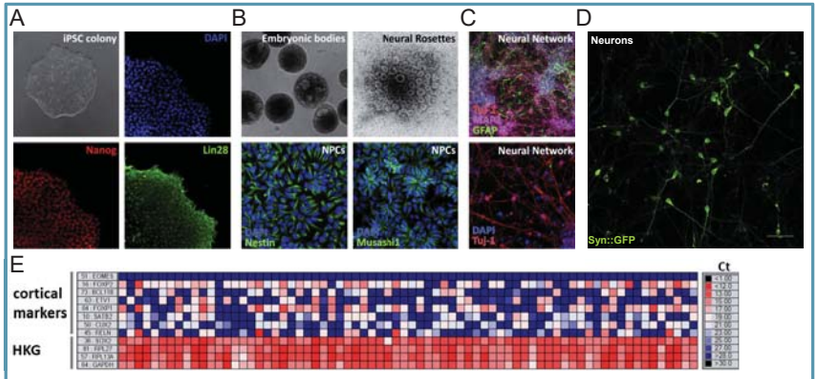


**Fig. 2. Human iPSC cell line generation.** NPCs and neurons are generated and characterized *in vitro*. A. Morphology of cells after reprogramming and colony formation. iPSC express pluripotent markers such as Nanog and Lin28. B. Neural induction and morphology of embryonic bodies and neural rosettes. NPCs express neural progenitor markers such as Nestin and Musashi1. C. Cultured neuronal network derived from hiPSC. Mature neurons express MAP2 and Tuj-1. D. Differentiated human neurons expressing GFP. The scale bar is 50 μm. E. Single-cell gene expression profiling reveals the expression of cortical markers in iPSC-derived neurons.

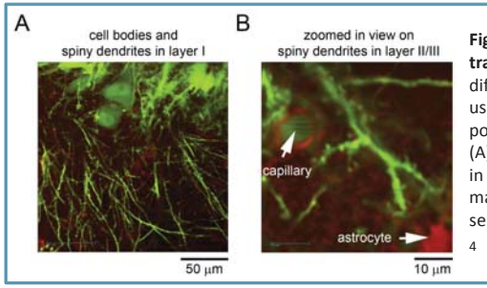


**Fig. 3. Spiny dendritic morphology of the transplanted human neurons.** iPSCs were differentiated into glutamatergic NPCs using the protocol described in (4). EGFP-positive spiny dendrites (green) in layer I (A) and layer II/III (B) were imaged *in vivo* in a 9 months old chimeric mouse. The red marker is sulforhodamine 101 (SR101) that selectively labels astrocytes.

4 Marchetto, M. C. et al. (2010) Cell 143, 527-539.

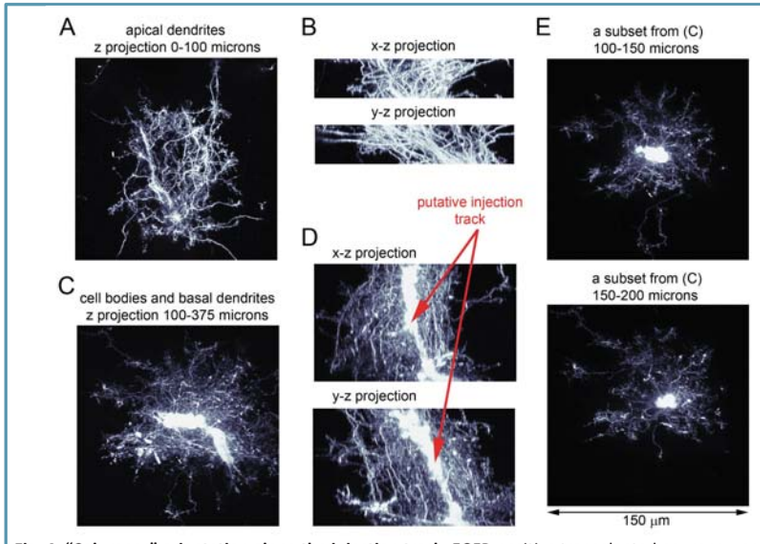


**Fig. 4. "Columnar" orientation along the injection track.** EGFP-positive transplanted neurons were imaged *in vivo* in a 12 months old chimeric mouse. A-E. 2-photon maximal intensity projections; individual images were obtained by stepping 2 μm throughout the depth (z-axis).

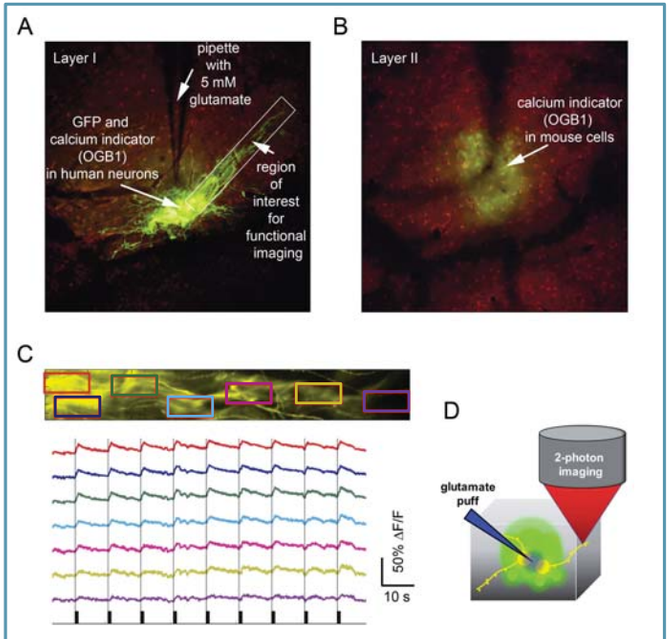


**Fig. 5. *In vivo* functional imaging of the transplanted human neurons.** A-B. Two-photon field of view with a cluster of EGFP-positive human neurons labeled with calcium indicator OGB1 and a micropipette used to deliver glutamate puffs (5 mM). Although both mouse and human neurons uptake OGB1, human dendrites extend outside the volume of loaded mouse tissue allowing selective measurements of their activity. C. Top: Regions of interest with human dendrites used for functional imaging. Bottom: Time-courses of OGB1 response from regions color-coded on top. D. Schematic illustration of the experimental paradigm.

## DISCUSSION & CONCLUSIONS

- In our experience, the cell bodies of human neurons in the adult mouse brain are typically found in clusters along the injection track. That means that when transplantation is performed in neonates, migration of the transplanted human neurons within mouse brain is insignificant. In future, we will take advantage of this property and will transplant NPCs into the whisker-barrel cortex. Response of human neurons to whisker stimulation would unequivocally indicate synaptic communication between human and mouse neurons.
- Transplanted iPSC-derived neurons exhibit spiny morphology and upward orientation of the apical dendrites but lack the main apical dendrite characteristic for Pyramidal neurons.
- Glutamate produced clear increases in OGB1 fluorescence in the human dendrites indicating that human iPSC-derived neurons in a chimeric mouse brain *in vivo* have functional glutamate receptors and calcium channels.
- *In vivo* imaging of the chimeric mouse brain may become a translational tool for studying mechanisms of human disease.

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