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Direct in vivo assessment of human stem cell graft–host neural circuits

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ABSTRACT

Despite the potential of stem cell-derived neural transplants for treating intractable neurological diseases, the global effects of a transplant's electrical activity on host circuitry have never been measured directly, preventing the systematic optimization of such therapies. Here, we overcome this problem by combining optogenetics, stem cell biology, and neuroimaging to directly map stem cell-driven neural circuit formation in vivo. We engineered human induced pluripotent stem cells (iPSCs) to express channelrhodopsin-2 and transplanted resulting neurons to striatum of rats. To non-invasively visualize the function of newly formed circuits, we performed high-field functional magnetic resonance imaging (fMRI) during selective stimulation of transplanted cells. fMRI successfully detected local and remote neural activity, enabling the global graft–host neural circuit function to be assessed. These results demonstrate the potential of a novel neuroimaging-based platform that can be used to identify how a graft's electrical activity influences the brain network in vivo.

Introduction

Stem cell mediated therapies aim to directly restore the neural circuitry lost during disease progression of the central nervous system (CNS). Applications of interest include Parkinson's disease (Zeng and Couture, 2013), Alzheimer's disease (Lee et al., 2010a), amyotrophic lateral sclerosis (Dimos et al., 2008), retinal degenerative disease (Tucker et al., 2011), stroke (Lee et al., 2010c), and spinal cord injury (McDonald et al., 1999). Yet despite the potential of stem cell based CNS repair, successful therapy development has been hindered by the lack of methods that can assess the functional connections and causal interactions between a neural transplant and host circuitry in an intact animal. For example, recent efforts to characterize transplant integration include in vivo tracking of labeled stem cells (Hoehn et al., 2002; Jasmin et al., 2011; Modo et al., 2004), visualization and electrophysiological recordings of synapse formation (Benninger et al., 2003; Czupryn et al., 2011; Espuny-Camacho et al., 2013; Oki et al., 2012), and behavioral monitoring of the host organism (Ben-Hur et al., 2004; El-Akabawy et al., 2012; Hargus et al., 2010; Kriks et al., 2011; Parish et al., 2008). These techniques provide important information on stem cell survival and engraftment, but are limited by their inability to directly assess the whole-brain functional impact of the graft on host neural networks in vivo. The ability to selectively stimulate engrafted cells with optogenetic techniques provides a unique opportunity to interrogate the functional integration of neural grafts and identify functional graft–host synapses (Tonnesen et al., 2011; Weick et al., 2010, 2011). Here, we report a novel method that enables direct optogenetic stimulation of stem cell-derived human neurons, combined with whole-brain high-field fMRI, to directly evaluate the causal influence of a graft's electrical activity on the global brain network as it integrates into the nervous system of a living subject.

Materials and methods

Human stem cell preparation

To maximize translational potential, we used a human induced pluripotent stem cell (iPSC) line (Huf6) previously shown to possess

Abbreviations: iPSC, (induced pluripotent stem cell); hESC, (human embryonic stem cell).

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hallmark characteristics of pluripotency both in vitro and in vivo (Byers et al., 2011; Nguyen et al., 2011). To evaluate the generalizability of our methods, we also performed experiments with neurons derived from the H9 human embryonic stem cell line (WiCell Research Institute). We engineered the cells to express the light-sensitive ion channel channelrhodopsin-2 (Boyden et al., 2005; Deisseroth et al., 2006; Nagel et al., 2005) (ChR2) prior to their transplantation in rats, which enabled selective, temporally precise control over the electrical activity of neural grafts in vivo (Fig. 1). Cells were transfected overnight using a concentrated EF1a-ChR2-EYFP lentivirus construct carrying the opsin (ChR2) and an enhanced yellow fluorescent protein (EYFP) reporter with the titer tightly controlled to ensure cell survival. The EF1a promoter was chosen since it can achieve long-term expression of transgenes in stem cells. Cells with high EYFP expression were selected manually or with fluorescence activated cell sorting (FACS) at one week post-transfection.

Both cell lines were differentiated following an optimized dual SMAD inhibition protocol based on Chambers et al (Chambers et al., 2009), for which the expression profiles of resulting neurons had previously been characterized by gene expression analysis, immunostaining, in vitro spontaneous differentiation, and in vivo teratoma assay (Nguyen et al., 2011). Briefly, pluripotent stem cells were manually plated on matrigel-coated plates and allowed to expand in iPSC media (mTeSR1, StemCell Tech) until ~30% confluency was reached. Media was then changed to 15% KSR DMEM/F12 and supplemented with Noggin and SB431542, a TGF-β small molecule inhibitor, to achieve dual SMAD inhibition. Neural rosettes were manually transferred to matrigel-covered wells with Sonic hedgehog (SHH) and fibroblast growth factor 8 (FGF8) patterning approximately 3–5 days after their appearance. Cells were then treated with SHH, FGF8, brain-derived neurotrophic factor (BDNF), and ascorbic acid to promote differentiation,

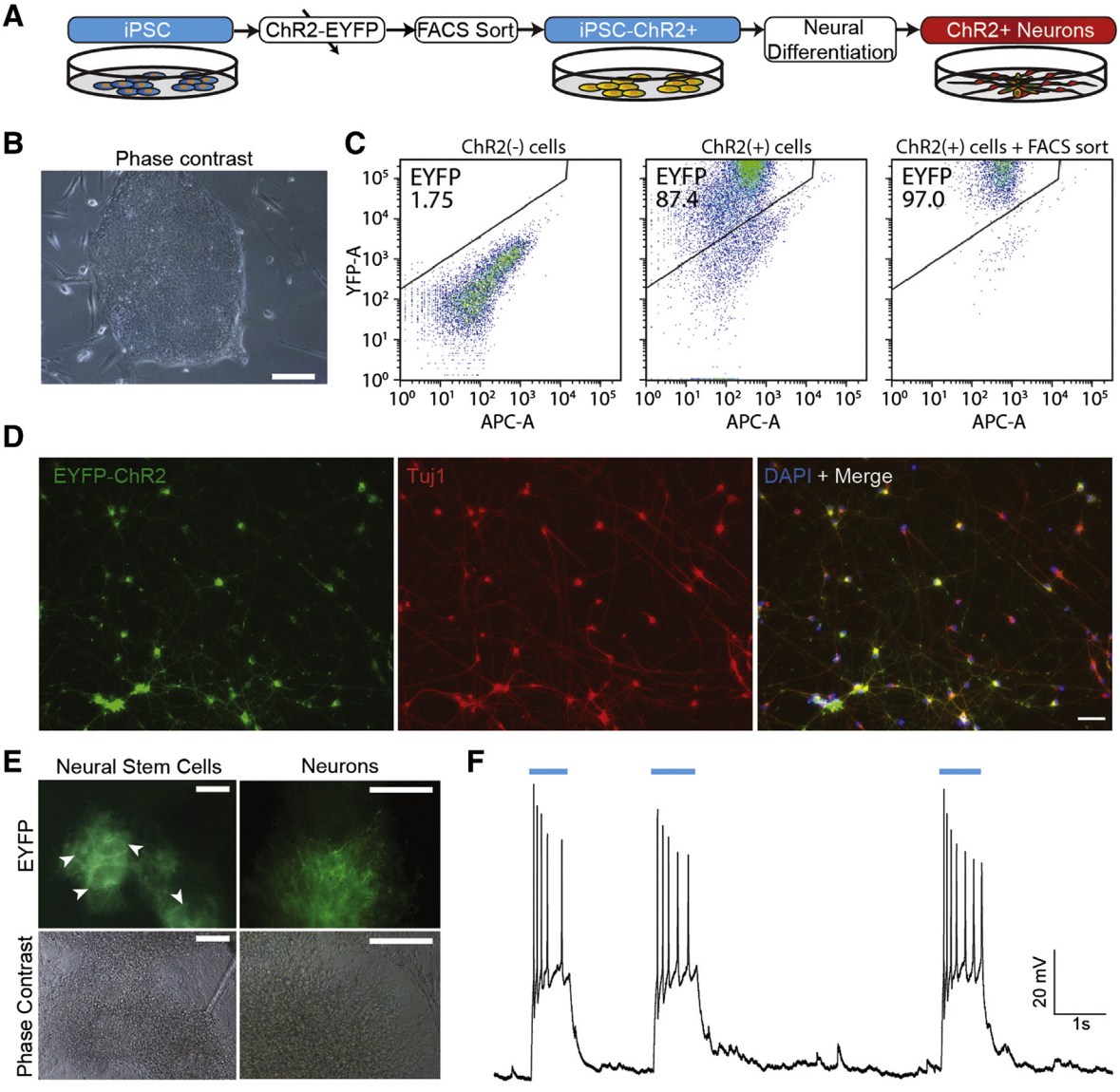

Fig. 1. Human iPSC-derived neurons stably express ChR2 and are optically excitable in culture. (A) Diagram illustrating the generation of ChR2-expressing neurons from human induced pluripotent stem cells (iPSCs). iPSCs were cultured on matrigel (B), transfected with ChR2-EYFP, FACS purified, and differentiated to neurons. Scale bar, 200 μm. (C) Robust ChR2 expression was selected based on high EYFP expression through FACS purification. Numbers in the upper left corner of each panel indicate the percentage of samples above the diagonal line. (D) After 23 days of in vitro differentiation through growth factor patterning, iPSC-derived cell cultures co-express ChR2 and the neuron-specific marker β3-tubulin (Tuj1). Morphologically, the cells have many projections and form networks with neighboring neurons, suggesting that they are progressing to maturation. Scale bar, 100 μm. (E) Neural stem cells (NSCs) and neurons were manually isolated from culture for transplantation. White arrowheads indicate neural rosettes, self-organizing clusters of neural stem cells. Scale bars, 200 μm. (F) In vitro current clamp recordings show robust, selective action potential excitation of isolated neurons in response to repeated ~1 s pulses of continuous photostimulation with 473 nm light. Among the 9 cells that were tagged and recorded, 4 generated action potentials and 5 generated voltage deflections in response to light.

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