hPSC-Derived Maturing GABAergic Interneurons Ameliorate Seizures and Abnormal Behavior in Epileptic Mice

Graphical Abstract

Highlights

- Human-PSC-derived mGIs engraft within mouse epileptic brain
- Human mGIs migrate extensively and integrate within host epileptic circuitry
- The activation of human mGIs induces inhibitory synaptic responses in host neurons
- mGIn grafts suppress seizure and abnormal behavior

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In Brief

In a mouse model of temporal lobe epilepsy, Cunningham et al. use optogenetic approaches to analyze human pluripotent stem cell (hPSC)-derived GABAergic interneurons transplanted into the hippocampus. Engrafted cells suppressed spontaneous seizure activity as well as seizure-associated cognitive deficits, aggressiveness, and hyperactivity.
hPSC-Derived Maturing GABAergic Interneurons Ameliorate Seizures and Abnormal Behavior in Epileptic Mice

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SUMMARY

Seizure disorders debilitate more than 65,000,000 people worldwide, with temporal lobe epilepsy (TLE) being the most common form. Previous studies have shown that transplantation of GABA-releasing cells results in suppression of seizures in epileptic mice. Derivation of interneurons from human pluripotent stem cells (hPSCs) has been reported, pointing to clinical translation of quality-controlled human cell sources that can enhance inhibitory drive and restore host circuitry. In this study, we demonstrate that hPSC-derived maturing GABAergic interneurons (mGINs) migrate extensively and integrate into dysfunctional circuitry of the epileptic mouse brain. Using optogenetic approaches, we find that grafted mGINs generate inhibitory postsynaptic responses in host hippocampal neurons. Importantly, even before acquiring full electrophysiological maturation, grafted neurons were capable of suppressing seizures and ameliorating behavioral abnormalities such as cognitive deficits, aggressiveness, and hyperactivity. These results provide support for the potential of hPSC-derived mGIN for restorative cell therapy for epilepsy.

INTRODUCTION

Epileptic seizures are characterized by unpredictable abnormal electrical discharge, loss of consciousness, and convulsions, and they are experienced by 1 in 26 individuals at some point in their lifetime (Jensen, 2014). One of the most common forms of seizures is temporal lobe epilepsy (TLE), characterized by epileptic abnormalities in the hippocampus, parahippocampal gyrus, and amygdala (Engel, 2001). About one-third of patients with TLE exhibit intractable seizures that cannot be controlled by antiepileptic drugs (AEDs) (Engel, 2002), and surgical resection of the seizure focus may be necessary (Christoph, 2008). Patients who are not candidates for surgery must live with ongoing seizures—in many cases, multiple events in a single day. Although AEDs can reduce or eliminate seizures for the more fortunate patients, these medicines are associated with diverse and troublesome side effects, including weight gain, metabolic acidosis, hepatotoxicity, movement disorders, and mental status changes (Cramer et al., 2010; Wallia et al., 2004). More effective, permanent therapeutic solutions are desperately needed for many of these patients with limited treatment options.

A key pathological feature of human TLE is synaptic reorganization, including neuronal loss and gliosis in CA1 and hilus, granule cell dispersion, and mossy fiber sprouting in the dentate gyrus (Wieser, 2004). Examination of excised epileptic tissue from TLE patients has revealed a loss of interneurons releasing inhibitory neurotransmitter GABA (de Lanerolle et al., 1989; Marco et al., 1996; Spreafico et al., 1998). It is believed that a decrease in GABA-mediated inhibition is a critical contributing factor in epilepsy. Indeed, decreased inhibition has repeatedly been demonstrated in TLE animal models (Cossart et al., 2001; Hirsch et al., 1999; Kobayashi and Buckmaster, 2003). Therefore, one possible therapeutic approach is to increase GABA-mediated inhibition to suppress hyperexcitable neurons during seizure initiation. Early work exploring the potential for inhibitory neural grafts in controlling epileptic activity has shown promise and has inspired further studies (Fine et al., 1990; Lindvall and Björklund, 1992; Löscher et al., 1998). More recent experiments have shown that mouse GABAergic interneuron precursors engrafted into the TLE mouse brain decreased seizure activity (Baraban et al., 2009; Hattiangady et al., 2008; Hunt et al., 2013; Maisano et al., 2012; Southwell et al., 2014).

However, in order to transform such proof-of-principle studies into viable therapeutic approaches for human TLE patients, it is critical to develop optimal human cell sources that can integrate into host circuitry and increase GABA-mediated inhibitory tone, thereby reducing seizure activity in the epileptic brain. Human pluripotent stem cell (hPSC) technologies, including induced PSCs (iPSCs), have the potential to provide an unlimited and
were primarily clustered near the injection site (59,027 ± 560). Analysis showed that, 2 weeks posttransplantation (PT), cells reported previously (Hunt et al., 2013) (Figure 1A). Histological analysis of cells bilaterally with four separate targets on each side, as disseminated throughout most of the hippocampus by deposition at the site of injection (Figure 1K). At 2 weeks PT, most cells expressed GABA and Sox6 as well as Nkx2.1 (Figures 2A–2C), and a minority of cells expressed the more mature neuronal marker NeuN (Figure 2E). However, at 4 months PT, the majority of cells expressed NeuN and β-tubulin as well as GABA and Sox6 (Figures 2G, 2H, 2J, and 2R–2T). The expression of precursor marker Nkx2.1 was significantly diminished at 4 months PT in comparison to 2 weeks PT (Figures 2I and 2W), whereas the mature interneuron marker Lhx6 was significantly increased at 4 months PT in comparison to 2 weeks PT (Figures 2D, 2K, and 2W). In addition, proliferating cell marker Ki67 was significantly decreased after 4 months PT in comparison to 2 weeks PT (Figures 2F, 2L, and 2W). Furthermore, at 4 months PT, some GABAAergic interneurons were found to express somatostatin, parvalbumin, calretinin, neuropeptide Y, and calbindin (Figures 2M–2Q, 2X, and S5A–S5F). As seen during in vivo embryonic development, interneuron maturation was not synchronous, and cells with simple bipolar morphology and cells with more complex neurites coexist at this time point (Figure S5G–S5L). Transplanted cells generated very small numbers of astrocytes (GFAP+; Figures 2U and 2X) or oligodendrocyte lineage cells (Olig2+; Figures 2V and 2X).

### FUNCTIONAL INTEGRATION OF HUMAN mGINs INTO THE EPILEPTIC BRAIN

Electrophysiological and morphological analyses were used to determine whether transplanted human MGE cells develop into functional GABAAergic neurons and integrate into host neural circuitry. Human MGE cells, transduced with lentivirus in order to stably express channelrhodopsin-2 (ChR2) (H134R)-GFP fusion under a synapsin promoter, were transplanted into the hippocampus of TLE mice. Then, 2 to 5 months after transplantation, grafted-MGE-derived cells were identified with green fluorescence in acute brain slices containing the hippocampus (GFAP+ cells; Figure 3A). All 31 GFP+ cells displayed typical ChR2-mediated currents induced by blue light illumination (Figure 3B), indicating that recorded GFP+ cells were indeed human-MGE-derived cells expressing ChR2. Consistently, short pulses of blue light illumination evoked action potential (AP) firings in most GFP+ cells (Figure 3C), suggesting that grafted GFP+ cells can be activated by photostimulation in brain slice preparations. Passive membrane properties of GFP+ human MGE cells, including resting membrane potential (RMP) and membrane resistance (Rm), were similar to those reported previously (Nicholas et al., 2013) (Figure 3D). However, unlike the previous report, we did not observe an increasing trend of the membrane capacitance (Cm) of the grafted cells (Figure 3D). This discrepancy may be due to the different experimental conditions that human MGE cells were transplanted into the brain in our study, whereas they were grown in culture in the previous report. Thus, our findings reflect the membrane properties of human MGE cells under more physiological conditions. In comparison to host hippocampal interneurons in adult mice, RMP was significantly depolarized in grafted mGIN (Figure 3D; p < 0.001), suggesting that grafted cells were not fully mature at this time point. However, there was no significant difference in Rm, and Cm between human and mGIN.
mGINS versus host interneurons. When voltage pulses were applied, grafted human mGINS showed rapidly desensitizing inward currents activated at membrane potential > –40 mV (Figure 3E), indicating the expression of voltage-gated Na⁺ channels. In current-clamp mode, 45% of human mGINS displayed spontaneous AP firings at resting membrane potential at 2.0 ± 0.2 Hz (Figures 3F and 3G), suggesting that some of the grafted mGINS generate tonic firings.

Figure 1. Transplanted Human mGIN Migrate Robustly and Integrate in Adult Epileptic Brain
(A) Overall experimental design. hPSC-derived MGE cells were transplanted into the hippocampus of TLE mice. Behavioral analysis was conducted after 3 months PT and histology analysis at 4 months PT.
(B–C) Two weeks PT, transplanted cells display minimal migration, shown by human cytoplasm-specific antibody staining (B’, B”, and C’ show enlarged photomicrographs of dentate gyrus regions from corresponding pictures).
(D–J) Four months PT, transplanted cells display robust migration and integration into the host brain, as shown by human cytoplasm-specific antibodies (D’–J’ show enlarged photomicrographs of dentate gyrus regions from corresponding pictures, depicting migration from the injection site). White arrows indicate injection sites. Yellow scale bars represent 500 μm. White scale bars represent 100 μm.
(K) Quantification of migration of transplanted cells (mean ± S.E.M.; *p < 0.05, two tailed Student’s t test) 2 weeks PT (n = 3) and 4 months PT (n = 8). See also Figures S1–S4.
Furthermore, the injection of depolarizing currents induced AP firings in all human mGINs examined (Figures 4A and 4B). As for passive membrane properties, grafted mGINs displayed less mature biophysical properties of AP firings in comparison to the host interneurons in terms of posthyperpolarization and AP width (Figure 4B), consistent with their well-known protracted

Figure 2. Transplanted Human MGE Cells Generate mGIN in Adult Epileptic Brains
(A–F) Immunohistochemical analysis of transplanted cells 2 weeks PT.
(G–V) Immunohistochemical analysis of transplanted cells 4 months PT. SST, somatostatin; PV, parvalbumin; Calr, calreticulin; NPY, neuropeptide Y; Calb, calbindin. Green scale bar represents 50 μm. White scale bars represent 100 μm.
(W) Cell-counting analysis of 2 weeks PT versus 4 months PT (mean ± SEM; n = 3, *p < 0.05, two tailed Student’s t test).
(X) Cell-counting analysis at 4 months PT (n = 3).
See also Figure S5.

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Figure 3. Electrophysiological Characterization of Grafted Human mGIn in the Hippocampus

(A) Top, a microscopic image showing the distribution of grafted human mGIn in the hippocampus. Channelrhodopsin 2 (ChR2)/GFP-expressing human MGE cells (green) transplanted into the cornu ammonis region 3 (CA3) of the hippocampus migrate extensively to the CA1 and dentate gyrus (DG). The graft core is indicated by an asterisk. Strata oriens (s.o.), pyramidale (s.p.), and radiatum (s.r.) are also indicated. Bottom, confocal microscopic images showing that the recorded grafted cell labeled with biocytin-streptavidin (red, left) expresses ChR2-GFP (green, middle). (B) Whole-cell patch-clamp recordings were performed with grafted cells expressing ChR2-GFP. Grafted human mGIn were identified with green fluorescence in acute brain slices. Biocytin was included in the pipette solution to label the recorded cells. Left, representative traces of ChR2-mediated currents in a grafted cell. These inward currents were induced by blue light illuminations (470 nm, 1 s pulses, blue horizontal bar) with variable intensities (0.02–0.61 mW/mm²) and recorded at –80 mV in voltage-clamp (V-clamp) mode. Right, a summary graph showing the peak amplitude of ChR2-mediated currents plotted versus light power. ChR2 currents were larger in human mGIn 4–5 months after transplantation (n = 16 cells) than in cells 2–3 months after implantation (n = 9 cells; p < 0.001). (C) Representative traces of AP evoked by short pulses of blue light illumination (1 ms, 12.5 mW/mm², blue vertical line, left). These optogenetically-induced APs (oAPs) were recorded in current-clamp (C-clamp) mode at approximate –85 mV and were detected in most grafted human mGIn examined (n = 18 cells, right). (D) Summary plots of resting membrane potential (RMP), membrane resistance (Rm), and a fast component of membrane capacitance (Cm) of grafted human mGIns, which were examined 2, 4, or 5 months after transplantation (n = 6, 8, and 11 cells, respectively) as well as host adult hippocampal interneurons (adult, > 3 months old, n = 4 cells). ***p < 0.001, adult versus all other groups.
maturation (Nicholas et al., 2013), whereas there was no significant difference in AP threshold. When grouped based on AP firing, most human mGINs displayed repetitive (type A, 52%) or single AP firing (type B, 32%), whereas delayed (13%) or burst firing pattern (3%) was also observed in a small proportion of transplanted cells (Figure 4C). Furthermore, although more frequent AP firings were induced by small current injections (<50 pA) in repetitive-firing type A cells, type B cells generated only one to three AP firings induced by much larger current injections (>50 pA; Figure 4E). As expected, $R_m$ was significantly larger in type A cells than type B cells (Figure 4E), accounting for different firing patterns of these cells.

After recording, we collected the intracellular contents of the recorded cells and performed single-cell RT-PCR in order to examine the RNA profile of transplanted human mGINs (Figure 4F). Most grafted cells expressed glutamate decarboxylase (GAD) and Sox6, whereas some grafted cells also expressed other GABAergic neuronal markers including parvalbumin, calreticulin, somatostatin, vasoactive intestinal peptide, and neuropeptide Y (Figure 4G). We also performed morphological analysis with biocytin-labeled human mGINs and found characteristic neuronal morphologies with various patterns of neuronal processes (Figure 5G). Therefore, these results demonstrate that transplanted human MGE cells develop into mGIN with diverse electrophysiological, biochemical, and morphological properties in the epileptic hippocampus.

Then, we investigated whether grafted human mGIN possessed functional postsynaptic mechanisms allowing synaptic transmission from host neurons. Using confocal microscopic imaging, we observed postsynaptic dendritic spines in biocytin-labeled grafted cells, suggesting that they may receive excitatory synaptic inputs (Figures 5A and 5B). Consistently, in acute hippocampal slices, two-thirds of 21 GFP$^+$ mGINs showed spontaneous postsynaptic currents at ~85 mV in voltage-clamp mode at a frequency > 0.1 Hz (Figures 5C and 5E). Moreover, these currents were inhibited completely by NBQX, an AMPA/kainite-type glutamate receptor antagonist (Figure 5D), indicating that they were mediated by excitatory neurotransmitter glutamate. There were no significant differences in biophysical properties of spontaneous postsynaptic activities between host mGINs and host hippocampal interneurons (Figures 5F–5I). These results suggest that most human mGINs transplanted into the hippocampus have functional postsynaptic machinery and receive excitatory synaptic inputs from host glutamatergic neurons. Immunocytochemistry analysis also showed that many postsynaptic PSD95$^+$ puncta on GFP$^+$ grafted cells were juxtaposed with presynaptic synaptophysin puncta (Figure 5J; 2.18 ± 0.56 PSD95$^+$ puncta per 10 μm GFP$^+$ dendrite, n = 22 dendrite segments), suggesting the formation of host glutamatergic synapses onto transplanted human mGINs. Further confirmation of functional synapse formation between host and transplanted neurons was obtained from ultrastructural analysis by transmission electron microscopy (TEM). Examination of hippocampal brain slices immunostained with dianminobenzidine (DAB) for human cytoplasm (human cytoplasm) showed synaptic connections onto grafted mGINs (Figures 5K–5L). These combined electrophysiological and ultrastructural data demonstrate functional synaptic integration of grafted mGINs into host parenchyma.

**Activation of Human mGINs Induces GABA-Mediated Inhibitory Postsynaptic Responses in Host Hippocampal Neurons**

Next, we investigated whether grafted human mGINs also have functional presynaptic machinery to release GABA and induce inhibitory postsynaptic responses in host hippocampal neurons (Figure 6A). To this end, we used optogenetic approaches to selectively stimulate ChR2-expressing transplanted cells in hippocampal slices (Figure 6B). Blue light illumination induced ChR2-mediated inward current and AP firings in GFP$^+$ grafted cells (Figures 3B and 3C), whereas the same photostimulation did not induce such currents in any GFP$^-$ cells tested (Figure 6B), suggesting that grafted cells can be selectively activated in acute brain slices with this approach. Under these conditions, short pulses of photostimulation, activating ChR2-expressing grafted cells, induced postsynaptic responses in 44% of a total 27 GFP$^-$ cells (Figures 6C and 6D). The recorded postsynaptic currents showed a short synaptic delay, indicating monosynaptic origin (Cho et al., 2013). Furthermore, these synaptic responses were inhibited completely by bicuculline, a GABAA receptor antagonist (Figures 6C and 6D), suggesting that they were mediated by inhibitory neurotransmitter GABA. The current-voltage relationship revealed the reversal potential of these currents at ~70 ± 3 mV (Figure 6E), consistent with the estimated reversal potential of chloride ion (~65 mV under our experimental conditions). In some GFP$^+$ cells, photostimulation induced probabilistic quantal responses (Figures 6F and 6G), confirming their synaptic nature. Moreover, train photostimulation at 1 Hz induced postsynaptic responses without significant reduction in peak amplitude (Figure 6H and 6I), suggesting that the repetitive activation of grafted mGIN can consistently induce GABAergic responses in GFP$^+$ cells. Considering that grafted cells constitutes 30.7% ± 4.7% of total cells at the graft core (n = 6 mice), where the density of GFP$^+$ cells is highest, and that 26.3% ± 4.7% of grafted cells are GFP$^+$, the majority of the recorded GFP$^-$ cells would be host hippocampal neurons. Thus, our results suggest that the activation of transplanted human MGE-derived cells can generate inhibitory postsynaptic responses in host hippocampal neurons.

Imaging studies provided additional evidence for the formation of inhibitory synaptic connections onto host neurons by...
**A** GFP+ grafted cell

- **Biocytin**
- **ChR2-GFP**
- **Merge**

**B**

- **GFP+ grafted cell**
- **AP threshold (mV)**
- **AHP (mV)**
- **AP half-width (ms)**

**C**

- **Repetitive firing (type A)**
- **Single AP firing (type B)**
- **Delayed firing (type C)**
- **Burst firing (type D)**

**D**

- **GFP+ grafted cell**
- **Delayed (4)**
- **Burst (1)**
- **Repetitive (16)**
- **Single (10)**

**E**

- **Number of AP firings**
- **Type A**
- **Type B**

**F**

- **Fus**
- **GAD67**
- **GAD65**
- **PV**
- **CR**
- **GST**
- **VIP**
- **NPY**
- **Sox6**

**G**

- **Single-cell RT-PCR (n = 23)**

(legend on next page)
transplanted human mGlns. Fluorescence microscopy showed that many of the presynaptic VGAT+ puncta on GFP+ mGlns were juxtaposed with postsynaptic gephyrin+ puncta (Figure 6J). TEM ultrastructural studies also identified symmetric synaptic contacts between presynaptic grafted cells and postsynaptic host neurons (Figures 6K and 6L). These combined results suggest that grafted human mGln have presynaptic machinery for releasing GABA and inhibiting host hippocampal neurons as well as postsynaptic machinery for receiving excitatory inputs from host neurons.

**Human mGlns Reduce Seizure Activity in Epileptic Mice and Ameliorate Behavioral Abnormalities**

Our electrophysiological findings suggest that transplanted human mGlns integrate into host hippocampal circuitry and may be sufficient for exerting antiepileptic effects by releasing inhibitory neurotransmitter GABA and increasing inhibitory synaptic responses in host hippocampal neurons. Therefore, we next investigated the therapeutic potential of transplanted human mGlns for preventing seizures in our TLE mouse model. Seizure activity of engrafted TLE mice was analyzed 3 months after transplantation by continuous electroencephalography (EEG) video monitoring. Vehicle-injected control TLE mice with sham surgery (n = 11) showed seizure EEG activity with high-frequency and high-voltage synchronized polyspikes (Figure 7A) and had a seizure event frequency of 1.92 ± 0.45 seizures per day. However, mGln-grafted TLE mice (n = 9) showed significantly reduced seizure event frequency (0.13 ± 0.07 seizure per day); in five animals in this group, seizure activity was eliminated entirely (Figure 7A). Seizure EEG activity was confirmed by simultaneous video recording, which showed clonus and rearing and falling of the mice (Racine stages 3–5; Movie S1). Naive Nod-Scic mice without pilocarpine injection did not show any seizure EEG activity during the monitoring (n = 6). The duration of seizures was not significantly different between control TLE mice and mGln-grafted TLE mice (39 ± 2.7 s versus 42.8 ± 8.7 s, n = 4–10, p = 0.61). These results indicate that transplantation of human mGlns suppresses seizure activity in the TLE mouse model.

Because epilepsy patients frequently suffer from comorbid cognitive impairment and psychiatric symptoms (Brooks-Kayal et al., 2013), we next analyzed the effect of human mGln transplantation on other behavioral abnormalities of TLE mice. Previous studies have shown that these animals, similar to TLE patients, show cognitive deficits (Grötècic et al., 2007), which could be reversed by engraving mouse fetal MGE cells (Hunt et al., 2013). Therefore, we tested whether transplanted mGln can improve cognitive function of TLE mice in a similar manner. In a Y maze test, control TLE mice (n = 10) showed significant deficits in short-term working memory in comparison to naive mice (n = 9). This deficit was abolished after mGln transplantation (n = 8), whereas there was no significant difference in total arm entry among test groups (p = 0.49, Figure 7B). In novel object recognition test, an independent measure of learning and memory, control TLE mice (n = 11) showed significantly decreased time exploring the novel object in comparison to the naive mice (n = 12), whereas this deficit was reversed after mGln transplantation (n = 8; Figure 7C). The frequency of novel object exploration showed a similar trend as the duration of novel object exploration but did not reach statistical significance (p = 0.19). These data suggest that transplantation of human mGlns can reduce cognitive deficits in rodent model of TLE.

In addition to cognitive deficits, hyperactivity and aggressiveness have been reported in the pilocarpine-induced rodent model of TLE (Müller et al., 2009; Rice et al., 1998). Consistently, control TLE mice (n = 11) displayed significantly higher locomotor activity in comparison to the naive mice (n = 14), as measured with a photobeam activity system (PAS). However, animals engrailed with human mGlns displayed a significant attenuation of this abnormality (n = 8; Figure 7D). Moreover, hypervigilance

**Figure 4. Transplanted Human MGE Cells Differentiate into GABAergic Interneurons in the Epileptic Hippocampus**

(A) Left, microscopic images of a recorded human MGE cell in acute hippocampal slices. ChR2/GFP-expressing human MGE cells were identified with green fluorescence and labeled with biocytin-streptavidin (red) with recording pipettes. Right, blue light illumination (470 nm, 0.61 mW/mm², blue horizontal bar) induces ChR2-mediated currents recorded at –80 mV in voltage-clamp mode, confirming that the recorded cell is a grafted cell. (B) Analysis of AP firings in human MGE transplanted into the hippocampus. Top, a representative trace of AP firings in a ChR2/GFP-expressing grafted cell. APs were induced by depolarizing current injection near threshold (500 ms long) and recorded in current-clamp mode at approximately –85 mV. The amount of injected currents is indicated below the trace. For each grafted cell, the first AP (an arrowhead) was analyzed. Bottom, summary graphs showing the average AP threshold (n = 3–5, p = 0.002) and high-voltage synchronized polyspikes (Figure 7A) and had a seizure event frequency of 1.92 ± 0.45 seizures per day. However, mGln-grafted TLE mice (n = 9) showed significantly reduced seizure event frequency (0.13 ± 0.07 seizure per day); in five animals in this group, seizure activity was eliminated entirely (Figure 7A). Seizure EEG activity was confirmed by simultaneous video recording, which showed clonus and rearing and falling of the mice (Racine stages 3–5; Movie S1). Naive Nod-Scic mice without pilocarpine injection did not show any seizure EEG activity during the monitoring (n = 6). The duration of seizures was not significantly different between control TLE mice and mGln-grafted TLE mice (39 ± 2.7 s versus 42.8 ± 8.7 s, n = 4–10, p = 0.61). These results indicate that transplantation of human mGlns suppresses seizure activity in the TLE mouse model.

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Figure 5. Transplanted Human mGIN Receive Glutamatergic Inputs from Host Neurons
(A) A representative image of a human mGIn transplanted into the hippocampus (green). The ChR2/GFP-expressing grafted cell was labeled with biocytin with a recording patch pipette (red). The cell body is indicated as a larger dotted square and is zoomed in below the image. Scale bar represents 20 µm.
(B) Top left, grafted human mGIn were recorded in acute brain slices. Grafted cells, identified with green fluorescence, receive synaptic inputs from host neurons. The recorded cell was labeled with biocytin-streptavidin (red) using patch pipettes. Top right, blue light illumination induced inward currents, confirming that the recorded cell is a grafted cell expressing ChR2-GFP. ChR2-mediated currents were induced and recorded as in Figure 4A. Bottom, confocal microscopic images show a portion of dendrites of the recorded human mGIn and are the zoomed-in images of that indicated as a smaller dotted square in (A). Dendritic spines are indicated by asterisks.
(C) Left, a representative trace of postsynaptic responses recorded in a GFP+ grafted cell. Spontaneous excitatory postsynaptic currents (sEPSCs) were recorded in GFP+ grafted cells at –85 mV in voltage-clamp mode. Right, a trace showing the average of sEPSCs recorded in the same cell. Decay time constant (\( \tau_D \)) of sEPSC was calculated by fitting the decay phase of the trace to a single exponential function (red curve).
(D) The application of 10 µM NBQX inhibited sEPSC completely in the same grafted cell as in (C), indicating that sEPSCs were mediated by AMPA/kainate-type glutamate receptors and that the grafted cell receives functional synaptic inputs from host glutamatergic neurons. \( n = 4 \) cells.
(E) Two-thirds of recorded human mGIn displayed spontaneous postsynaptic responses with the frequency > 0.1 Hz.
(F) Representative images of the cell bodies of GFP+ grafted cells (top) and GFP+ host hippocampal interneurons (bottom). The recorded cells were labeled with biocytin-streptavidin (red) as in (A). Scale bar represents 20 µm.
and aggressiveness normally observed in control TLE mice (n = 11) were completely reversed to levels of naive mice (n = 15) after transplantation of human mGINs (n = 10; Figure S7E and Movie S2). In sum, these results suggest that transplantation of human mGINs suppresses seizure activity in epileptic mice and ameliorates other behavioral abnormalities.

**DISCUSSION**

Although fetal MGE cell transplantation has demonstrated proof of principle for cell-based therapy of epilepsy (Hattiangady et al., 2008; Hunt et al., 2013), clinical application is limited by the lack of standardized and reliable cell sources as well as ethical controversies associated with using fetal cells. hPSC technology offers the potential to provide cell sources that are well-characterized, quality-controlled, and virtually unlimited in supply, so long as efficacious progenies can be proficiently derived. We have utilized optimized differentiation of human PSCs into MGE cells (Kim et al., 2014) and report functional efficacy of mGIN in order to reduce epileptic activity and comorbid behavioral abnormalities in the epileptic brain even before they attain full maturity. Considering full electrophysiological maturation of human GABAergic interneurons could take years (Le Maqueresse and Monyer, 2013; Nicholas et al., 2013), our findings with human mGINs provide a major step toward developing an efficient and cell-based therapy for treating intractable epilepsy.

We have demonstrated that PSC-derived human mGINs migrate extensively within the epileptic hippocampus, integrate into host circuitry and reduce seizure activity and other behavioral abnormalities. The primary mechanisms of the functional effects of grafted mGINs are suggested by our electrophysiological studies. Although they are not fully mature, approximately half of transplanted human mGINs fire spontaneous APs at ~2 Hz, indicating that they are tonically active even without extrinsic synaptic inputs. Moreover, transplanted human mGINs fully integrate into the hippocampal circuitry, receiving excitatory synaptic inputs from host glutamatergic neurons, and are therefore activated by host signals. In turn, our optogenetic studies revealed that grafted human mGINs release inhibitory neurotransmitter GABA in an activity-dependent manner. Therefore, the activation of transplanted mGINs, either by spontaneous activity or by excitatory synaptic drive, causes an increase of inhibitory synaptic responses in host hippocampal neurons, shifting excitation/inhibition balance toward inhibition and suppressing exaggerated neural activity in the epileptic brain. Consistent with previous work (Hunt et al., 2013), we did not observe significant changes in mossy fiber sprouting by human MGE transplantation in comparison to control TLE mice (Figure S7F), suggesting that regulation of inhibitory balance alone by grafted cells may be sufficient to exert the antiepileptic effects observed in this study.

Cell therapy for epilepsy offers a number of advantages over conventional therapies. Distinct cell types can be precisely engrafted into brain substructures (Bjarkam et al., 2010), averting the acute and long-term systemic adverse effects seen with AEDs. Furthermore, neural grafts, with their ability to integrate within the host circuitry, would circumvent the need for daily dosing and sluggish titration required with AED administration. A self-regulating therapeutic system of mGIN grafts would eliminate the need of carrying devices to monitor and control seizures. Temporal lobectomy has been used as a last-resort intervention for intractable epilepsy but is associated with surgical morbidity and permanent dysfunction. However, high-precision stereotactic engraftment of stem cells is less invasive and leaves functional neural tissue undisturbed.

Here, we have demonstrated the biology and utility of hPSC-derived mGIN to ameliorate the symptoms of a prevalent and debilitating neuropsychiatric disease. While the efficacy of mouse fetal interneurons to ameliorate seizure activity has been demonstrated previously (Baraban et al., 2009; Hattiangady et al., 2008; Hunt et al., 2013; Maisano et al., 2012; Southwell et al., 2014), the current investigation is the first to demonstrate the therapeutic efficacy of hPSC-derived interneurons to treat epilepsy, and it represents the potential for a reliable and ethically unimpeded cell source for this purpose. Before transition into the clinical setting, however, the question of ‘dosing’ of MGE cell grafts will need to be addressed. Interestingly, it has been reported that an increase in inhibition reaches a plateau with relatively low numbers of transplanted interneurons (Southwell et al., 2010). This suggests that larger numbers of interneurons are unlikely to result in adverse effects, but at the same time, smaller, less-intrusive deposits of cells may produce an optimal response. In addition, further evaluation of long-term graft survival and safety should be assessed before undertaking clinical applications. Porcine human simulation neurosurgery is presently underway to establish such criteria prior to human trials (M.C., unpublished data). In addition, isolation, and purification of cortical interneuron populations with appropriate cell-surface markers will facilitate the generation of quality-controlled cell sources for human trials. With prudent preclinical testing, this technology holds promise as a therapeutic approach for TLE as well as other intractable diseases of the CNS.

**EXPERIMENTAL PROCEDURES**

**PSC Culture and Differentiation into MGE Cells**

H7 hPSCs were maintained and differentiated into MGE cells as described previously (Kim et al., 2014). Differentiated MGE cells were subject to FACS after staining with anti-ENCAM (BD Biosciences) prior to transplantation. Detailed information can be found in the Supplemental Experimental Procedures.
Figure 6. Optogenetic Stimulations of Transplanted Human mGINs Induce GABAergic Postsynaptic Responses in Host Hippocampal Neurons

(A) Left, a microscopic image showing both a GFP+ grafted human MGE cell and a GFP– host pyramidal neuron in the CA3 of the hippocampus (dotted squares; cell bodies are indicated by arrows). These cells were labeled with biocytin-streptavidin with recording pipettes (red). The grafted cell sends out projections toward the host pyramidal neuron. Right, microscopic images showing the soma and dendrites of the same GFP– pyramidal neuron as in the left image. Projections from grafted human mGINs are shown in the middle (green, ChR2-GFP).

(B) Top, blue light illumination evokes AP firings in GFP+ mGIN expressing ChR2 and induces the release of GABA at axon terminals, generating postsynaptic responses in the recorded GFP– host neuron. Bottom left, blue light illumination (0.61 mW/mm², blue horizontal bar) did not induce ChR2-mediated current in GFP– host neurons at –80 mV in voltage-clamp mode, indicating the lack of ChR2 expression. Bicuculline (30 μM) was added to inhibit GABAergic responses in

(legend continued on next page)
**Induction of TLE in Nod-Scid Mice**
The Animal Care and Use Committee at McLean Hospital approved all animal procedures. For induction of TLE, 7-week-old male and female Nod-Scid mice (Charles River Laboratory) were injected with 400 mg/kg Pilocarpine intraperitoneally (i.p.), 30 min after N-methylscopolamine bromide (1 mg/kg, i.p.) administration to reduce peripheral cholinergic effects (Mazzuferi et al., 2012). To limit the duration of status epilepticus (SE) and extent of damage in the hippocampus, diazepam (10 mg/kg) was injected ip 90 min after seizure induction. The severity of convulsive responses was monitored and classified according to the modified Racine scale (Shibley and Smith, 2002). Ten days after pilocarpine injection, mice that showed stage 3, 4, or 5 seizures were subject to 7 days of continuous video monitoring for SRS. Mice showing SRS with stage 3, 4, or 5 during the 7-day recording period were designated as TLE mice and were randomly assigned for subsequent transplantation and behavioral analysis. Detailed information can be found in the Supplemental Experimental Procedures.

**Transplantation of Human MGE Cells Into Hippocampus of TLE Model Mice**
Differentiated and FACS-sorted human MGE cells were transplanted into TLE model mice at the following coordinates: AP-1.75 mm, L ± 2.3 mm, V −1.7 mm for the rostral CA3 site; AP-3.25 mm, L ± 3.0 mm, V −3.65 mm, −2.9 mm and −2.0 mm for the three caudal sites along the dorso-ventral axis of the hippocampus in this coronal plane. A total of 5 × 10² MGE cells in a 0.5 μl volume were delivered at each of the target coordinates. Sterile, stainless steel bone screw recording electrodes (diameter, 0.5mm; length, 1.1mm; Plastics One) soldered with a lead wire were placed epidurally through rostral burr holes in the skull (AP-1.75 mm, L ± 2.3 mm), and reference electrodes were positioned caudal to lambda. Electrodes were secured with a rapid-curing dental cement (DenMat Holdings). Detailed information is in the Supplemental Experimental Procedures.

**Behavioral Analysis**

**Continuous Video EEG Recording of Transplanted Mice**
Three months after transplantation, seizure activity of control or MGE-transplanted TLE mice was recorded with a MP150 Biopac data acquisition System, and EEG100C EEG amplifier module along with Eco Black Box security camera system (Lorex Technology). EEG seizures with high-frequency, high-voltage synchronized polyspike profiles with amplitudes greater than 2-fold that of background and a duration of greater than 15 s (Hunt et al., 2013) were analyzed with AcqKnowledge 4.0 EEG Acquisition and Reader Software (BIOPAC Systems) by investigators who were blind to treatment conditions. This was followed by confirmation of EEG seizure activity by video recording.

**Y Maze**
We used a three-arm Y maze for this study: each arm was 3 cm wide, 40 cm in length, and had a wall height of 12 cm. Mice were initially placed within one arm, and the sequence and number of entries was recorded for each mouse over a 10 min period.

**Novel Object Recognition Test**
For a training session, each mouse was placed into an open-field box (42 × 42 × 31 cm) containing two identical objects and allowed to freely explore for 3 min. One hour after the training session, one of the familiar objects was replaced with a novel object (defined as the test session). The time that each animal spent exploring the novel object compared to the familiar object was recorded and traced with Ethovision software (Noldus).

**Locomotion Test**
The home cage (7 ½ × 11 ½ × 5 in) containing an individual mouse was placed in the center of a PAS monitoring frame (San Diego Instruments) with 4 × 8 photobeam configuration for 15 min under standard overhead lighting conditions. Total photobeam break numbers were detected by PAS software.

**Handling Test**
Aggressiveness of the mice was assessed as described previously (Hunt et al., 2013) with some modifications. Each of the following three tasks was performed for 15 s: (1) nonstressful handling (stroking slowly along the back of the mouse in the direction of the grain of fur), (2) stressful handling (vigorous stroking against the grain of the fur), and (3) pinching at the tail base with a rubber-ended forceps (Fine Science tools). Reaction to each handling was scored by investigators blinded to treatment conditions with the following rating scale: (1) initial struggle, but calmed within 15 s, (2) struggle for more than 15 s, (3) struggle for more than 15 s and exhibiting one or more defensive reactions (piloerection, flattening of the ears against the head, and attempt to bite or back away from the experimenter), and (4) struggled for more than 15 s and exhibited flight behavior (loud vocalization or wild running). Summation of these three scores provided a total aggressiveness score for each mouse.

More detailed information of behavioral analysis is in the Supplemental Experimental Procedures.

**Immunohistochemistry, Cell Counting, and Statistical Analysis**
Transplanted mice were terminally anesthetized with an i.p. overdose of pentobarbital (150 mg/kg, Sigma-Aldrich) and perfused transcardially with heparin saline (0.1% heparin in saline) followed by paraformaldehyde (4%)
Human Interneurons Ameliorate Seizure Activity

2 weeks or 4 months postgrafting. Brains were removed, postfixed in 4% paraformaldehyde for 12 hr, equilibrated in 20% sucrose/PBS solution, and then sectioned coronally at 40 μm with a freezing microtome. Histological analysis was performed as described previously (Kim et al., 2014), and detailed information is in the Supplemental Experimental Procedures.

Transmission Electron Microscopy
See the Supplemental Experimental Procedures for details.

Statistical Analysis
For statistical analysis, we performed a Student’s t test (α = 0.05) for comparison of two groups with Prism6 software (Graph Pad). For multiple sample comparison, we performed ANOVA with an α level of 0.05 in order to determine possible statistical differences between group means. When significant differences were found, post hoc analysis was performed with Fisher’s least significant difference (α = 0.05) again with the use of Prism6 software. For samples with unequal variances, a nonparametric Kruskal-Wallis test was performed with Prism6 software.

Electrophysiology, Optogenetic Stimulations, and Neurolucida Tracing
For electrophysiological studies, MGE cells were infected with lentivirus that express ChR2 (H134R)-GFP fusion protein under the control of synapsin promoter (University of Pennsylvania Vector Core) at day 14 of differentiation. The cells underwent FACS and were transplanted as described above. Two to five months after transplantation, acute brain slices containing the hippocampus were prepared using a vibrating microtome for electrophysiological analysis. After recovery, brain slices were placed in the recording chamber and continuously perfused at the rate of 1 ml per min with the artificial cerebrospinal fluid containing 130 mM NaCl, 2.5 mM KCl, 2.5 mM CaCl2, 1 mM MgSO4, 1.25 mM NaH2PO4, 26 mM NaHCO3, and 10 mM glucose with 95% O2 and 5% CO2. Whole-cell patch-clamp recordings were performed at 31 °C with an EPC-9 amplifier and Pulse v8.8 software (HEKA Elektronik). For recording grafted MGE-derived neurons (GFP+ cells) and host hippocampal interneurons, the patch electrodes were filled with solution containing 140 mM Cs-methanesulfonate, 5 mM NaCl, 1 mM MgCl2, 5 mM NaHCO3, and 5% glucose with 10 mM HEPES (pH 7.3). Liquid junction potential of 15.5 and 8.9 mV was corrected for the K-gluconate- and caesium-based pipette solutions, respectively. Series resistance (access) was not compensated. Blue collimated light-emitting diode (LED) with 470 nm peak wavelength (M470L2, Thorlabs) was used for photostimulations of grafted MGE-derived cells expressing ChR2-GFP. Brain slices in the recording chamber were illuminated through a 40× water-immersion objective lens (IR-Achromplan, Carl Zeiss). Illumination area was 0.26 mm2 and was centered at the cell using a 50× water-immersion objective lens (IR-Achroplan, Carl Zeiss). Illumination area was 0.26 mm2 and was centered at the cell.
Offline data analysis was performed with Clampfit 9 (Molecular Devices). Reagents were purchased from Tocris Bioscience (OX 314 chloride, bicucullin, and NBOX) or Sigma-Aldrich (ATP, GTP, and bicuculline methochloride). For statistical analyses of electrophysiological data, we used ANOVA with Bonferroni’s simultaneous multiple comparisons. Statistical analysis was performed with Minitab16 (Minitab), and p < 0.05 was considered statistically significant.

After electrophysiological recordings, brain slices were fixed in 4% paraformaldehyde at 4°C overnight. Recorded cells loaded with biocytin were labeled with streptavidin, Alexa 568 conjugate (20 μg/ml in PBS, Molecular Probes) as described previously (Cho et al., 2013). Images of biocytin-loaded and streptavidin-labeled cells were taken with z stack function with a Leica TSC SP8 confocal microscope. The confocal images were then used for neuron tracing with Neuronlucida software (Microbright Field).

**Single-Cell RT-PCR**

See the Supplemental Experimental Procedures for details.

**SUPPLEMENTAL INFORMATION**

Supplemental Information contains Supplemental Experimental Procedures, seven figures, and two movies and can be found with this article online at http://dx.doi.org/10.1016/j.stem.2014.10.006.

**AUTHOR CONTRIBUTIONS**


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