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**REVIEW ARTICLE**

# Therapeutic effects of stem cells in rodent models of Huntington's disease: Review and electrophysiological findings

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**Summary**

The principal symptoms of Huntington's disease (HD), chorea, cognitive deficits, and psychiatric symptoms are associated with the massive loss of striatal and cortical projection neurons. As current drug therapies only partially alleviate symptoms, finding alternative treatments has become peremptory. Cell replacement using stem cells is a rapidly expanding field that offers such an alternative. In this review, we examine recent studies that use mesenchymal cells, as well as pluripotent, cell-derived products in animal models of HD. Additionally, we provide further electrophysiological characterization of a human neural stem cell line, ESI-017, which has already demonstrated disease-modifying properties in two mouse models of HD. Overall, the field of regenerative medicine represents a viable and promising avenue for the treatment of neurodegenerative disorders including HD.

**KEYWORDS**

animal models, electrophysiology, Huntington's disease, stem cells

## 1 | INTRODUCTION

Huntington's disease (HD) is an inherited, progressive, and fatal neurodegenerative disorder caused by an autosomal dominant mutation in at least one copy of the huntingtin (*HTT*) gene.<sup>1</sup> HD arises from an expanded repetition of the CAG codon, which codes for glutamine in the huntingtin (htt) protein. In healthy individuals, the CAG triplet repeats 10–35 times while mutant alleles containing >36 CAG repeats predispose individuals to develop HD with time of onset depending mostly on repeat length, but also on genetic modifiers<sup>2–4</sup> and environmental factors.<sup>5–7</sup> The hallmark symptoms of HD include involuntary movements (chorea), cognitive

deficits, and psychiatric disturbances including, prominently, depression.<sup>8–10</sup> HD presents in two main forms; the most common is adult or late-onset HD and a less common form (only about 6% of all HD cases) known as juvenile-onset HD (JHD), the latter having higher CAG repeat lengths (>55 CAGs).<sup>11</sup> In the adult form, symptoms generally begin in midlife and worsen over a period of 15–20 years. In JHD, symptoms typically appear during the teenage or young adult years and progress more rapidly and aggressively than adult HD,<sup>12,13</sup> and similar to adult cases, individuals affected by JHD experience gradual neuronal degeneration in particular brain regions and display severe motor incoordination. However, while chorea occurs as a prominent feature of adult HD, it is rarely seen

in the juvenile form.<sup>13,14</sup> Instead, for unclear reasons, in JHD dystonia, rigidity, mental retardation, and epileptic seizures occur.<sup>15-17</sup> There is no cure or disease-modifying treatment for either form of HD, and most pharmacological treatments are palliative; thus, there is an urgent need to find alternative therapies.

The main histopathological feature of HD is the loss of striatal medium-sized spiny neurons (MSNs).<sup>18</sup> However, with disease progression, neurons in the cerebral cortex, hippocampus, hypothalamus, and thalamus also are lost.<sup>19</sup> Normal brains have two striatal projection pathways, each with distinct MSN populations expressing different types of dopamine receptors and neuropeptides.<sup>20</sup> The direct pathway projects to the substantia nigra pars reticulata and the internal segment of the globus pallidus, whereas the indirect pathway projects to the external segment of the globus pallidus which, in turn, projects to the subthalamic nucleus.<sup>21,22</sup> It is believed that MSNs giving rise to the indirect pathway are more vulnerable and degenerate before MSNs giving rise to the direct pathway, leading to an imbalance between both pathways and the emergence of involuntary movements.<sup>23,24</sup> In addition, although HD mainly affects projection neurons in striatum and cortex, recent studies have reported that parvalbumin interneurons also are vulnerable to the disease and eventually degenerate.<sup>25</sup> Other types of GABAergic interneurons, including those expressing somatostatin and calretinin, are protected. The large striatal cholinergic interneurons, although preserved in HD, display morphological and functional alterations.<sup>26-28</sup> At present, it remains unknown why striatal MSNs are the most susceptible to degeneration although several hypotheses have been proposed.<sup>29-33</sup> Most likely, MSN degeneration occurs due to lack of neurotrophic support,<sup>34,35</sup> as well as alterations in glutamate release along the corticostriatal pathway or abnormal sensitivity and localization of N-methyl-D-aspartate (NMDA) receptors.<sup>36-42</sup> Ultimately, the important question is how can we prevent and/or replace cell degeneration and loss in HD brains.

Grafting of mouse and human fetal striatal tissue obtained from animal and human embryos as a potential treatment has been tested in animal models of HD<sup>43-46</sup> and in human patients (for a review, see Ref. 47), respectively, but the success of this approach has been variable and ethical issues as well as tissue availability raise important concerns.<sup>48,49</sup> The emergent field of regenerative medicine offers renewed hope for cell replacement therapies and provides new approaches for a potential cure. In particular, the use of human embryonic stem cells (hESCs) and induced pluripotent stem cell (iPSC)-derived products has generated widespread interest for neurodegenerative diseases including HD.<sup>50-52</sup> In this review, we present recent advances in the use of various stem cell types in animal models of HD. In addition, we provide further electrophysiological characterization of ESI-017 human neural stem cells (hNSCs), a good manufacturing practice (GMP)-grade cell line which has already shown promising results in two HD mouse models.<sup>53</sup> Before reviewing the use of stem cells in HD research, we will briefly describe the animal models used in these studies.

## 2 | RODENT MODELS OF HD USED FOR STEM CELL RESEARCH

### 2.1 | Lesion models

Many of the early rodent models used for studying HD involved the injection of chemical toxins. Intra-striatal injection of quinolinic acid (QA), an NMDA receptor agonist, became a popular model due to its ability to replicate the striatal histopathology of HD. In high doses, QA destroys local MSNs while sparing axons and some types of interneurons.<sup>54</sup> Although lesioned rats did not display chorea, they exhibited hyperkinesia, dystonia, and dyskinesia. Moreover, QA-lesioned rats showed spatial and cognitive impairments when tested in the Morris water maze and the T-maze.<sup>55</sup> The advantage of the QA model is largely related to the glutamatergic excitotoxic method of striatal death thought to happen in patients with HD.<sup>56</sup>

3-nitropropionic acid (3-NP) induces cell death via mitochondrial impairment. It inhibits the enzyme succinate dehydrogenase, leading to the accumulation of reactive oxygen species.<sup>57</sup> This impairment is also seen in patients with HD and thus provides another method for studying the disease in rodent models.<sup>58</sup> The toxin is injected subcutaneously and induces hyperkinesia at low doses and hypokinesia at high doses, as well as cognitive impairments related to memory and attention.<sup>57</sup> Toxin-induced models helped to elucidate the process of cell death in HD, but they are limited in that they do not allow the study of disease progression or dysfunctional changes before any obvious neurodegeneration.

### 2.2 | N-terminal transgenic models

In comparison with toxin-induced models, genetic rodent models of HD provide a more specific and deeper understanding of the behavioral and physiological progression of the phenotype.<sup>59</sup> N-terminal transgenic HD mice carry a fragment of the 5' N-terminal human mutant *HTT* gene in exon 1. The most commonly used transgenic mouse model is the R6/2 with ~150 CAG repeats, creating an aggressive and rapidly progressing phenotype, and a short survival time of up to 15 weeks.<sup>60</sup> At about 5 weeks of age, these mice start to show irregular gait, hindlimb claspings, weight loss, increased grooming, and cognitive decline. Furthermore, as the transgenic mice age, they become increasingly susceptible to seizures. Thus, this model may better represent JHD.

The N171-82Q model includes an N-terminal fragment of the gene, with exons 1 and 2, expressing the first 171 amino acids with 82 glutamines.<sup>61</sup> Similar to the R6/2, this model shows striatal atrophy and modest MSN degeneration at the late stage of the disease, ventricular enlargement, and a failure to gain weight.<sup>62,63</sup> Yet, these mice do not display hyperkinesia or seizure activity and survive approximately 18-25 weeks. Some drawbacks to using N-terminal models are that they cannot be studied long term and do not carry the full-length gene, therefore do not have all of the additional regulatory elements. However, these models produce rapidly progressing

symptoms and are therefore beneficial in studying JHD and late-onset HD. Their development of symptoms in a short period of time allows for a rapid analysis of potential treatments. Furthermore, N-terminal transgenic models form nuclear inclusions and mutant huntingtin (mhtt) aggregates, which also are found in postmortem brains of patients with HD.<sup>64,65</sup>

### 2.3 | Full-length transgenic models

Full-length models, such as the YAC128 or BACHD, carry the entire human mutant *HTT* transgene and provide alternative benefits when studying the disease. The YAC128 mouse model has 128 CAG repeats from human *HTT*.<sup>66</sup> Affected mice do not display abnormal behaviors until 6 months of age and selective degeneration of striatal neurons at around 12 months of age. The progression of symptoms generally runs the course of initial and temporary weight gain at 2 months, hyperactivity, difficulty walking along a rotating rod from 6 to 12 months, and then hypokinesia. Loss of MSNs in this model is evident at 12 months, while cognitive dysfunction and mhtt aggregates are also present by 18 months. The full-length models provide a means to study the disease long term, which is conducive to prolonged therapeutic studies. The presence of the entire gene means that all of the human regulatory elements are included. Furthermore, research using the YAC128 model has shown the presence of mhtt inclusions after the onset of behavioral and neuropathological changes.<sup>66</sup>

### 2.4 | Knock-in models

Knock-in (KI) models differ from transgenic models in that they express the mutation within the endogenous genomic locus. Many KI models have been produced that vary based on their CAG repeat length, in which the allele for the mouse *HTT* exon 1 is replaced by the human mutant variant. For example, the CAG140 has 140 polyglutamine repeats added to the mouse *HTT* gene. By 1-4 months of age, these mice show many motor and behavioral deficits, with loss of striatal volume by 2 years.<sup>67,68</sup> Moreover, homozygotes for the mutation show more severe symptoms than those heterozygous for the mutation. The similarity in phenotype to human HD, their longer life span, and the gradual progression of disease-related symptoms make KI models useful for studying HD, as well as in evaluating long-term grafting of stem cells.

## 3 | STEM CELL GRAFTS IN HD MODELS

Although some drug therapies for HD have been approved, for example, tetrabenazine to reduce chorea,<sup>69</sup> not all individuals respond well to them, and over time, they can lose their effectiveness. Further, to date there are no approved drugs that modify disease age of onset or disease course. Cell-based approaches for treatment of degenerative brain diseases are emerging as a therapeutic strategy having the potential to modulate neuropathology, as suggested

by promising studies in Alzheimer's disease, Parkinson's disease, and HD (reviewed in Refs. 70-74). A variety of stem cells have been implanted in HD rodent models (Table 1) to assess their potential therapeutic ability, including mesenchymal stem cells (MSCs), fetal neural stem cells, or neural cell types differentiated from induced pluripotent stem cells (iPSCs) and embryonic stem cells (ESCs) (see also recent reviews in Refs. 52, 75-78).

### 3.1 | Mesenchymal stem cells

The use of adult multipotent stem cells, which includes adipose- and bone marrow-derived MSCs, has been a widely used approach for stem cell transplantation therapy for HD due to their ability to differentiate into a wide variety of cell types,<sup>79-81</sup> are readily accessible, are relatively safe as they are not pluripotent and therefore not tumorigenic, are thought to be immune privileged, and do not pose ethical concerns over their use in treatment of disease.<sup>82</sup> However, their effects may also be relatively transient due to cell survival limitations and altered in vivo performance following long-term exposure to ex vivo culturing environments prior to transplantation.<sup>83</sup> MSCs secrete many bioactive substances that assist in the repair and regeneration of damaged tissue by reducing inflammation, inhibiting apoptosis, and stimulating angiogenesis.<sup>84</sup> Injection of MSCs into the striatum of 3-NP lesioned rats improved their motor function and extended their life span,<sup>85</sup> while transplantation into QA-lesioned rats resulted in a reduction in cognitive deficits shortly following implantation.<sup>86</sup> Human adipose-derived MSCs implanted bilaterally into the striata of R6/2 mice showed improved motor function and increased survival time.<sup>87</sup> Following transplantation, the presence of mhtt aggregates and the rate of striatal apoptosis declined. The same type of MSCs was later implanted in YAC128 mice to study their long-term effects on disease progression.<sup>88</sup> When transplanted prior to the presence of disease-related symptoms, no therapeutic improvement was apparent. When transplanted after the presentation of symptoms, the mice showed motor improvement on the rotarod test. One of the beneficial actions of this method lies in the fact that MSCs release multiple trophic factors, including brain-derived neurotrophic factor (BDNF), which is decreased in patients with HD.<sup>35</sup> When MSCs engineered to overexpress BDNF were implanted into the striatum of YAC128 mice, some behavioral and pathological deficits were reduced.<sup>88</sup> When these cells were introduced into R6/2 mice, they increased their life span and promoted endogenous neurogenesis.<sup>89,90</sup> While increased levels of trophic factors by MSCs encourage the growth of axons and cell attachment, the ability of these cells to differentiate into mature neurons and establish synaptic communication with other neurons in vivo remains controversial.<sup>91</sup>

### 3.2 | Pluripotent stem cells

PSCs, which can be differentiated into each of the three germ line lineages, provide an alternative therapeutic option for the treatment of HD. Human PSCs, both embryonic and induced patient fibroblasts, can be differentiated in culture to neurons with

**TABLE 1** Summary of stem cell grafts implanted in HD rodent models

| HD model  | Stem cell graft   | Treatment outcome   | References |
|---|---|---|------------|
| <i>Mesenchymal stem cells (MSCs)</i>  |   |   |            |
| 3NP-lesioned rats   | Low dosage (200 000) and high dosage of cells (400 000) bilaterally in striatum   | Motor improvements only seen at low dosage<br>Reduction in the size of striatal lesion seen in both groups  | 85         |
| QA-lesioned rats  | 500 000 cells bilaterally in striatum   | Reduction in cognitive deficits<br>Reduced spatial working memory deficits<br>No reduction in size of lesion<br>No changes in sensorimotor impairments                              | 86         |
| R6/2 mice   | Human adipose-derived cells<br>500 000 cells bilaterally in striatum  | Improved rotarod and limb claspings<br>Reduction in mhtt aggregates   | 87         |
| YAC128 mice   | Patient-derived adipose MSCs<br>500 000 cells bilaterally in striatum   | No rotarod performance improvement at 8 mo but significant improvement at 12 mo   | 88         |
| R6/2 and YAC128 mice  | Human BDNF-MSCs<br>500 000 cells bilaterally in striatum  | Increased life span in R6/2 mice<br>Increased endogenous neurogenesis<br>Increased immune response<br>Decreased striatal atrophy in YAC128 mice                                     | 90         |
| <i>Pluripotent stem cells (PSCs)</i>  |   |   |            |
| QA-lesioned mice  | Patient-derived (72 CAG repeats) iPSC-NSCs<br>200 000 cells unilaterally in striatum  | Behavioral improvements for weeks following transplantation<br>Stem cells showed signs of HD pathology (mhtt aggregates) after 33 wk  | 97         |
| R6/2 mice   | Normal CAG expansion cassette in HD patient-derived iPSCs<br>100 000 cells bilaterally in striatum  | In vitro, cells expressed DARPP-32. Following implant, cells survived 2 wk and continued to express DARPP-32<br>Normalized cadherin, TGF beta, BDNF, and caspase signaling pathways | 94         |
| YAC128 mice   | Healthy mouse iPSC-NSCs<br>200 000 bilaterally in striatum  | Motor improvements<br>iPSCs survived and differentiated into MSNs<br>Increased BDNF protein and receptor levels   | 96         |
| <i>Embryonic- or fetal-derived neural precursor/stem cells (NPCs or NSCs)</i> |   |   |            |
| QA-lesioned, R6/2, and N171-82Q mice  | Mouse ESC-derived NPCs<br>100 000 cells unilaterally in striatum in QA-lesioned mice<br>100 000 cells bilaterally in striatum of R6/2 and N171-82Q mice | Improved motor abilities (rotarod, gait performance)  | 102        |
| QA-lesioned rats  | Mouse ESC-derived NPCs and NSCs<br>500 000 cells (2 sites) unilaterally in striatum   | Reduced inflammatory response<br>Reduction in striatal lesion<br>Prevention of MSN cell loss when stimulated by growth factors  | 103,104    |
| N171-82Q mice   | Mouse ESC-derived GDNF-NPCs<br>300 000 cells (2 sites) bilaterally in striatum  | Improved rotarod performance<br>Increased survival of striatal neurons<br>Continued decreases in body weight<br>Fewer mhtt inclusions at the site of implant and around it          | 105        |
| QA-lesioned mice  | 20 000 human ESC-derived neural precursor cells unilaterally in striatum  | Significant behavioral improvement in the apomorphine-induced rotation test   | 101        |
| QA-lesioned rats  | 75 000 human ESC neural precursor cells (2 sites) unilaterally in striatum  | Cells survived 8 wk post-transplant but did not differentiate into MSNs<br>No DARPP-32 expression but positive expression of Nestin and TUJ1  | 100        |
| R6/2  | Fetal-derived human NSCs (undifferentiated and differentiated into DARPP-32 positive cells)<br>225 000 cells (2 sites) bilaterally in striatum          | Continued decreases in body weight<br>No improvements in behavior or motor performance  | 106        |

(Continues)

**TABLE 1** (Continued)

| HD model             | Stem cell graft  | Treatment outcome   | References |
|----------------------|--|---|------------|
| 3-NP lesioned rats   | Fetal-derived human NSCs<br>400 000 cells (4 sites) unilaterally in striatum | Reduced inflammatory response<br>Reduction in striatal lesion<br>Prevention of MSN cell loss when stimulated by growth factors  | 107        |
| R6/2 and CAG140 mice | ESC-017-derived human NSCs<br>100 000 cells bilaterally in striatum          | Improvements in motor and cognitive behaviors<br>NSCs were electrophysiologically active and found to make synaptic connections with host cells<br>Increased BDNF production<br>Decreased mhtt aggregates | 53         |

MSN characteristics and stain for MAP2, CTIP2, and GABA.<sup>92,93</sup> Electrophysiological recordings from differentiated PSCs showed the presence of GABA-evoked currents and typical firing patterns of mature MSNs, although interneurons also were present in the cultured samples.<sup>92</sup> Human iPSCs have helped elucidate disease mechanisms in HD and provide a human stem cell platform for screening new candidate therapies. In addition, human iPSCs have created the opportunity for transplantation of patient-derived autologous cells that can be differentiated into any cell type, thereby obviating the need for immunosuppression.

Several groups have implanted cells differentiated to various stages during the MSN differentiation process into mice and observed survival and further differentiation,<sup>94-97</sup> supporting the potential for transplantation. Transplantation of iPSCs derived from somatic cells has demonstrated behavioral improvement in rodent models of HD. For instance, when mouse iPSCs from normal, healthy mice were differentiated into neural stem cells (iPSC-NSCs) and implanted into striata of 10-month-old YAC128 mice,<sup>96</sup> there was an improvement in motor abilities over the span of 10 weeks following implantation. In addition, the stem cells survived and differentiated into mature MSNs. Moreover, BDNF protein and receptor levels significantly increased in the striatum of the treated mice. However, for diseases such as HD, the mutation is present in all cells; therefore, transplantation would require modification of the cells as they could promote disease or more readily take on HD phenotypes. One such study showed that HD patient-derived iPSCs could be differentiated into GABAergic striatal neurons, *in vitro*. These cells were then transplanted into unilateral QA-lesioned mice.<sup>97</sup> Initially, the cells did not form mhtt aggregates. By 33 weeks, however, after stem cell grafts were fully integrated into the host tissue, the iPSC-NSCs showed signs of pathology including mhtt aggregates. Nevertheless, the QA-lesioned mice exhibited behavioral and motor recovery for weeks following transplantation. An alternative strategy of replacing the CAG expansion cassette in HD patient-derived iPSCs with one of normal repeat length *via* homologous recombination showed potential for correcting the recurring pathology seen in iPSCs derived from HD patients.<sup>94</sup> The cells grew *in vitro* into mature DARPP-32-expressing MSNs that were then implanted into R6/2 mice. These cells survived 2 weeks post-transplantation, continued to express DARPP-32, and normalized cadherin, TGF beta, BDNF, and caspase signaling pathways,

supporting feasibility of this type of gene correction approach of patient-derived iPSCs.

### 3.3 | Embryonic stem cell (ESC)-derived products

Other studies have evaluated the use of differentiated ESCs in rodent models of HD. Both ESCs and iPSCs have the potential for tumorigenesis, although iPSCs may have a reduced likelihood of forming tumors following transplantation, which may provide additional clinical benefit.<sup>98,99</sup> ESC-derived products can also face ethical dilemmas in their use; however, extensive work has been carried out monitoring the stability and differentiation properties of ESCs. In one study, the implantation of human neural precursors differentiated from hESCs in mice with QA lesions in the striatum showed that the cells grew and survived, but they did not differentiate into specific cell types of the particular environment.<sup>100</sup> An additional study showed that hESC-derived neural precursor cells (NPCs) failed to express the striatal marker DARPP-32, when analyzed at 8 weeks post-transplantation. Despite their inability to differentiate into MSNs, their presence still improved the motor and behavioral deficits observed in QA-lesioned mice, as early as one week post-transplantation.<sup>101</sup>

Recently, ESC-derived neural stem cells (NSCs), which can differentiate into neurons, glia and oligodendrocytes, or NPCs have been evaluated in mouse models of HD and show cell survival for many weeks, can differentiate into mature neurons and astrocytes, and relieve many behavioral and motor symptoms as well as the electrophysiological alterations seen in rodent models of HD.<sup>53,102</sup> *In vitro*, ESC-derived NSCs have the ability to modulate inflammation, particularly by reducing the responses that follow T-cell receptor, IL2, and IL6 activation.<sup>103</sup> One HD study showed that the secretion of growth factors from mouse-derived embryonic NSCs plays a vital role in their ability to support QA-lesioned rat striatal neurons. When stimulated by epidermal growth factor (EGF), human nerve growth factor (hNGF)-releasing NSCs reduced the size of the striatal lesion, prevented the loss of GABAergic MSNs, and thus helped maintain the overall circuitry of the striatum.<sup>104</sup> The presence of hNGF-secreting NSCs likely provides neuroprotection to the neurons most greatly affected in individuals with HD. When ESC-derived mouse NPCs engineered to overexpress BDNF were implanted into QA-lesioned and transgenic HD mice (R6/2 and

N171-82Q), clear improvements in motor function were only seen in lesioned mice while only mild improvements were reported in transgenics.<sup>102</sup> The likely cause of this difference in greater efficacy in lesioned mice versus transgenic models may be the higher stem cell survival in QA-lesioned mice than in the R6/2 or N171-82Q mice. Although it is unclear why stem cell survival was increased in the QA model, enhanced survival would likely result in higher levels of secreted BDNF, thus enhancing neuroprotection and increasing endogenous neurogenesis. Further, there may be something unique to the lesioned niche that provides signals for the transplanted cells to survive and differentiate.

Interestingly, mouse NPCs can be used to deliver growth factors such as glial cell-derived neurotrophic factor (GDNF).<sup>105</sup> These GDNF-secreting mouse NPCs migrated and expanded as endogenous cells died. In addition, they also rescued impaired motor performance in N171-82Q mice. This suggests that secretion of other types of known cytoprotective factors released from NPCs may be beneficial in the treatment of symptoms associated with HD.<sup>105</sup> While there have been several studies showcasing the potential benefits of ESC-derived NPCs or NSCs as a potential therapy for HD, most have used stem cells of murine lineage. Human ESC-derived products have the potential to provide an unlimited cell source for transplantation therapy. To move forward with clinical studies, cells will need to be of human origin. Few studies have examined the efficacy of human NSCs in HD models. El-Akabawy et al<sup>106</sup> showed poor stem cell survival and no behavioral performance improvements in R6/2 mice implanted with undifferentiated or predifferentiated human NSCs expressing DARPP-32, although the time of implantation may have been a factor. In a different study, when rats were implanted with hNSCs (fetal-derived) prior to receiving 3-NP administration, motor performance was enhanced compared to rats that received stem cells after 3-NP administration or those that received vehicle only.<sup>107</sup> A recent study by Reidling et al<sup>53</sup> is the first report illustrating the therapeutic benefits of a human ESC-derived NSC line in two mouse models of HD, the R6/2 and the CAG140. In both models, hNSCs were implanted during the presymptomatic stage, supporting the idea that the time of implantation may influence therapeutic outcomes.

### 3.4 | ESI-017-derived hNSCs

ESI-017 is one of the six clinical-grade hESC lines generated from supernumerary embryos by the Singapore Stem Cell Consortium.<sup>108</sup> Their use for therapeutic application was approved by multiple stem cell research councils and adheres to US FDA regulations for use of human cells. Of those lines, four (including ESI-017) were chosen for the generation of GMP and Good Laboratory Practice-grade hESC banks for preclinical research based on the absence of human and nonhuman pathogens.<sup>108,109</sup> Subsequently, an hNSC line was differentiated from the GMP-grade hESC line ESI-017.<sup>53</sup> ESI-017 hNSCs stain for CD24, SOX1, Nestin, and Pax6 hNSC markers, as well as the proliferation marker SOX2, but do not stain for the pluripotent marker SSEA4.

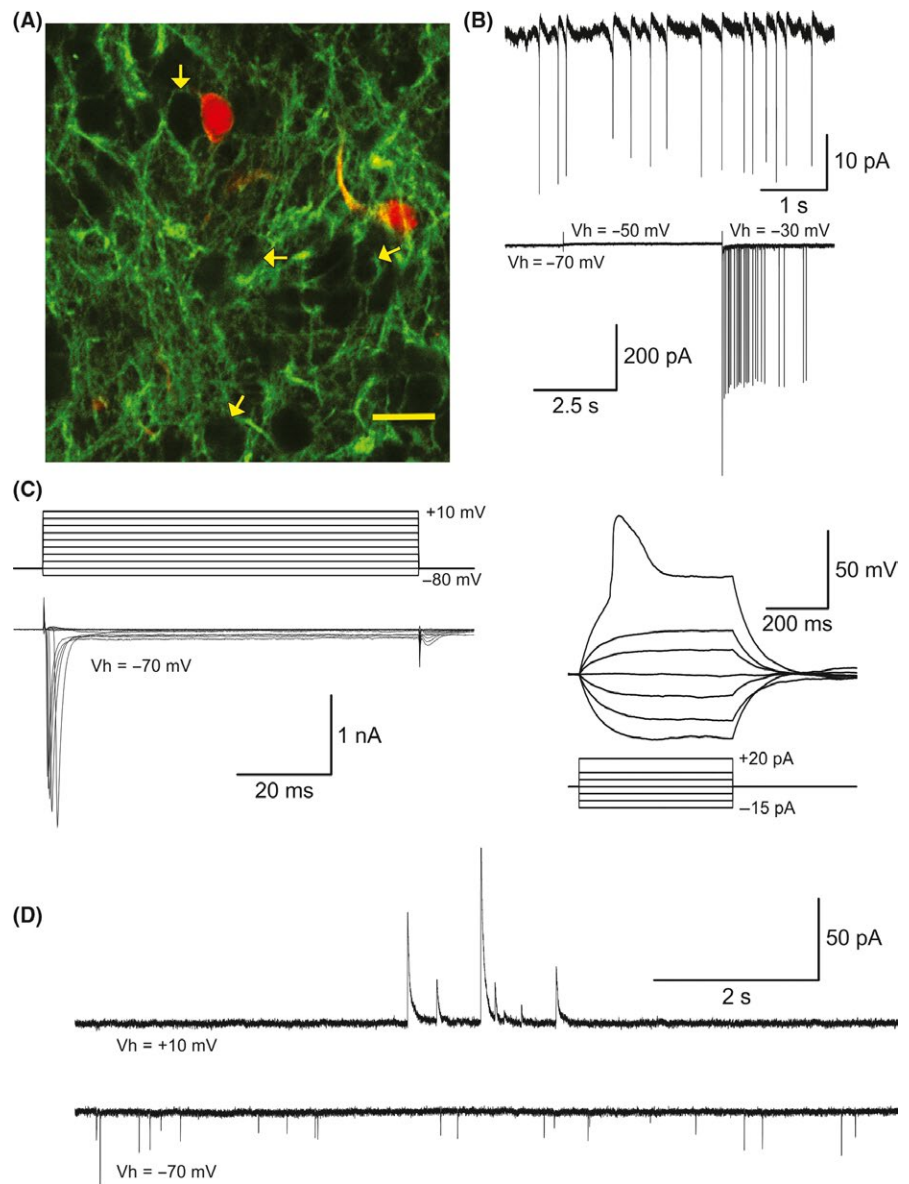
### 3.5 | hNSCs improve the behavioral and neurological phenotype of R6/2 mice

R6/2 mice display a number of behavioral and neurological abnormalities which start at ~5 weeks of age and become more severe as disease progresses.<sup>60</sup> These include stereotypical hindlimb grooming movements, claspings, and irregular gait. Reidling et al demonstrated a significant delay in the onset of R6/2 hindlimb claspings in hNSC-treated mice. In addition, R6/2 mice implanted with hNSCs showed a statistically significant improvement in rotarod performance and the pole test. Neuromuscular function (grip test) and motor coordination also were significantly improved 4 weeks after implant.<sup>53</sup> In this publication, it was shown that mice treated with ESI-017 hNSCs in the striatum exhibited a significant change in some of the electrophysiological alterations seen in MSNs. Furthermore, implanted hNSCs remained viable for weeks following implantation and formed synaptic connections with host and/or other stem cells.

We next undertook a series of electrophysiological studies aimed at further characterizing the passive and active membrane properties of ESI-017-derived hNSCs implanted into R6/2 mice, as well as their effects on host striatal neurons. For this, we used the same ESI-017-derived hNSCs approved by the University of California Irvine, the UCLA, and the University of California Davis Human Stem Cell Research Oversight Committee. R6/2 mice and their wild-type (WT) littermates were obtained from breeding colonies maintained at UCLA (JAX line 2810,  $\sim 150 \pm 5$  CAG repeats). Male and female mice were implanted with hNSCs in dorsolateral striatum (100 000 cells/site) or cerebral motor cortex (50 000 cells/site) at 5 weeks of age. To prevent rejection of the xenograft, mice were immunosuppressed by daily injections of cyclosporin A (10 mg/kg, sc) and CD4 antibody weekly and sacrificed 4–6 weeks after implantation. At this stage, R6/2 mice were fully symptomatic. Details for electrophysiological recordings in ex vivo brain slices have been published.<sup>53,110</sup>

### 3.6 | ESI-017-derived hNSCs survive and are electrophysiologically active in striatum and cerebral cortex

Infrared videomicroscopy and differential interference contrast (IR-DIC) optics allowed visualization of the needle track and the implant. hNSCs clustered along the needle track and were well-circumscribed to the injection site. The demarcation between implant and host tissue was also distinct due to the myelin presence in the host tissue, which produces a darker image under IR-DIC microscopy. The localization of hNSCs within the implant was further corroborated by immunostaining with the human stem cell marker, SC121 (Figure 1A). hNSCs were densely packed, and most cell bodies appeared healthy under IR-DIC optics. Short processes were sometimes visible. hNSCs recorded in striatum displayed basic neuronal properties characteristic of immature neurons. Cell membrane capacitance was small, the input resistance was very high, and the decay time constant was relatively fast, <1 ms (Table 2). In contrast, host MSNs displayed much



**FIGURE 1** hNSCs in striatum: A, Image of hNSCs (green, immunostained with the human stem cell marker, SC121) in the striatum of a mouse. Recordings were obtained from hNSCs and filled with biocytin (red). Arrows indicate some hNSCs not labeled with biocytin. Calibration bar is 20  $\mu\text{m}$ . B, In cell-attached mode, a few hNSCs fired action potentials spontaneously. Although most hNSCs in the slice were silent, holding the membrane at more depolarized potentials (eg,  $-30\text{ mV}$ ) consistently induced cell firing (bottom trace). C, In voltage clamp recordings (Cs-methanesulfonate in the patch pipette, left panel), depolarizing step voltage commands generated small  $\text{Na}^+$  and  $\text{Ca}^{2+}$  currents in hNSCs. In current clamp mode (K-gluconate in the patch pipette), depolarizing current pulses generated small-amplitude, long-duration action potentials. D, In voltage clamp mode, hNSCs displayed infrequent sEPSCs and sIPSCs

**TABLE 2** Passive membrane properties of hNSCs in the striatum and cerebral cortex

|                 | Capacitance (pF) | Input resistance ( $\text{M}\Omega$ ) | Time constant (ms) |
|-----------------|------------------|---------------------------------------|--------------------|
| Striatum        |                  |                                       |                    |
| WT (n = 30)     | 29.6 $\pm$ 1.9   | 2237 $\pm$ 156                        | 0.69 $\pm$ 0.06    |
| R6/2 (n = 42)   | 23.1 $\pm$ 1.5** | 2835 $\pm$ 168*                       | 0.64 $\pm$ 0.05    |
| Cerebral cortex |                  |                                       |                    |
| WT (n = 18)     | 18.9 $\pm$ 2.7   | 2271 $\pm$ 202                        | 0.4 $\pm$ 0.07     |
| R6/2 (n = 21)   | 18.3 $\pm$ 1.6   | 2975 $\pm$ 232*                       | 0.4 $\pm$ 0.06     |

Statistical significance determined using Student's *t* tests, where \* and \*\* indicate  $P < 0.05$  and  $P < 0.01$ , respectively, for comparisons made between genotypes for each structure.

larger capacitances, smaller membrane input resistances, and time constants  $>1\text{ ms}$ .<sup>53</sup>

In cell-attached mode, some hNSCs generated action potentials spontaneously (Figure 1B), but most of them were silent. However, in the whole-cell configuration (voltage clamp mode), steps to  $-50$

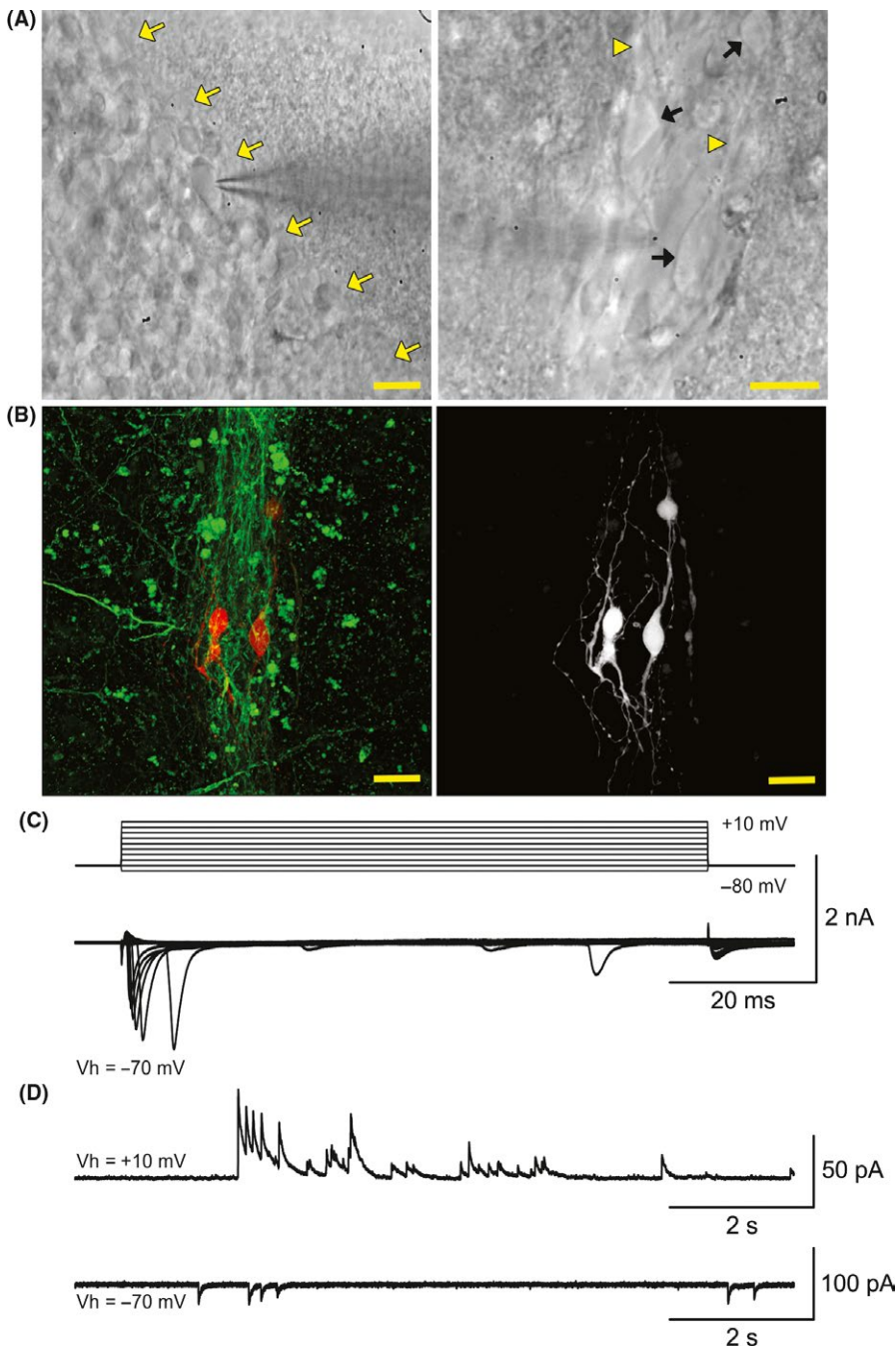
or  $-30\text{ mV}$  induced repetitive  $\text{Na}^+$  currents in the majority of hNSCs (Figure 1B,C). These currents were relatively small ( $\sim 0.1\text{--}3\text{ nA}$ ) compared with host MSNs ( $\sim 3\text{--}15\text{ nA}$ ). In current clamp mode, action potentials evoked by depolarizing current steps were incipient and with long durations, typical of immature neurons (Figure 1C, right



panel). hNSCs displayed spontaneous excitatory and inhibitory postsynaptic currents (sEPSCs and sIPSCs), indicating that they received synaptic inputs from the host tissue or from other implanted hNSCs (Figure 1D). However, compared with MSNs, frequencies were much lower: <0.1 Hz on average for NSCs (range 0.01–0.7 Hz) vs ~3 Hz on average for MSNs (range 0.6–10 Hz). To further assess whether hNSCs receive synaptic inputs, the striatum was electrically stimulated with a monopolar electrode placed in the slice ~150  $\mu\text{m}$  away from recorded hNSCs. A short duration stimulus (0.5 ms, 0.1 mA) yielded a small outward current at positive holding potentials that was subsequently blocked completely by the GABA<sub>A</sub> receptor antagonist Bicuculline (10  $\mu\text{mol/L}$ ), confirming that these cells receive GABAergic synaptic inputs. It remains unknown whether these

inputs come from other hNSCs and/or cells from the host tissue as the stimulation of most striatal cells produces a GABA response. Nevertheless, it is clear that grafted hNSCs have the ability to receive inputs from local circuits. In addition, Reidling et al.<sup>53</sup> showed that using electron microscopy analysis, synaptic connections were being formed with the host tissue for a subset of transplanted cells.

In HD, cortical pyramidal neurons (CPNs) also degenerate. Accordingly, in our studies we tested the properties of hNSCs implanted in the cerebral cortex. Similar to hNSCs implanted in striatum, those implanted in motor cortex could be easily identified by their reduced capacitance, high membrane input resistance, and fast time constant (Table 2). Figure 2A shows a cortical graft and illustrates the clear demarcation between implanted and host tissue



**FIGURE 2** hNSCs in cerebral cortex: A, A patch electrode is attached to an hNSC in the cerebral cortex of a mouse (left panel). Note the clear demarcation between the graft and the host tissue (arrows). Right panel is from another mouse showing high-magnification image of hNSCs in cortex (arrows). Note the elongated appearance of the cell somata and the presence of an apical dendrite pointing towards the dura in the lower two hNSCs (yellow arrowheads). B, Confocal images showing, in green (SC121 immunostaining), the area of the implant and the presence of hNSC processes extending out of the implant. In red, three hNSCs (same as right panel in A) that were patched, recorded, and filled with biocytin (left panel). The right panel shows a more complete reconstruction of the biocytin-filled cells. Calibration bar is 20  $\mu\text{m}$  for all panels in (A) and (B). C, Voltage clamp recording from an hNSC shows the presence of both Na<sup>+</sup> and Ca<sup>2+</sup> currents in response to increasing depolarizing voltage commands. D, Recordings of sEPSCs and sIPSCs from an hNSC in an R6/2 mouse 4 wk after stem cell transplantation in motor cortex

**TABLE 3** Passive membrane properties of MSNs in the striatum

|                         | Capacitance (pF)  | Input resistance (M $\Omega$ ) | Time constant (ms) |
|-------------------------|-------------------|--------------------------------|--------------------|
| WT + hNSC (n = 5)       | 120.13 $\pm$ 14.9 | 76.7 $\pm$ 14                  | 2.48 $\pm$ 0.35    |
| R6/2 + vehicle (n = 23) | 87.31 $\pm$ 4.0*  | 205 $\pm$ 17**                 | 1.79 $\pm$ 0.10*   |
| R6/2 + hNSC (n = 13)    | 94.51 $\pm$ 5.5   | 142 $\pm$ 13 <sup>†</sup>      | 1.99 $\pm$ 0.12    |

Statistical significance determined using a one-way ANOVA followed by Holm-Sidak post hoc analyses, where \* and \*\* indicate  $P < 0.05$  and  $P < 0.01$ , respectively, for comparisons made between R6/2 treatment groups and WT. <sup>†</sup>Indicates  $P < 0.05$  for comparisons made between R6/2 treatment groups.

(left panel). In another mouse, a higher magnification IR-DIC image (Figure 2A, right panel) shows three hNSCs that were patched, recorded, and subsequently stained for biocytin that was present in the internal recording solution (Figure 2B). Interestingly, some hNSCs in the cerebral cortex appeared to acquire a pyramidal-like morphology (Figure 2A, right panel), suggesting that the location of the graft site in the host could affect hNSC morphology. Similar to hNSCs in the striatum, hNSCs in the cortex displayed Na<sup>+</sup> currents (Figure 2C) and spontaneous EPSCs and IPSCs (Figure 2D). Their frequencies were much lower than those in host CPNs [ $<0.1$  Hz on average for hNSCs (range 0.01–0.8 Hz) vs  $\sim 7$  Hz on average for CPNs (range 1–24 Hz)].

One important question is whether or not the disease process affects the development and properties of hNSCs. We noticed the cell capacitance was reduced and the input resistance was increased in hNSCs implanted in the striatum of R6/2 mice, suggesting reduced membrane area. In the cortex, there were no differences in cell capacitance or membrane time constants of hNSCs implanted in WT and R6/2 mice, but there was an increase in the input resistance of hNSCs in R6/2 mice (Table 2). This probably indicates that the local environment in the striatum of R6/2 mice is less favorable than in control mice or in cerebral cortex. There also was a significant difference in the capacitance between hNSCs in the striatum versus the cortex ( $P < 0.001$ , two-way ANOVA).

Overall the data indicate that hNSCs 4–6 weeks following implantation in WT and R6/2 mice are viable, integrate well in the host striatum and cortex, and display neuronal, although still immature, passive, and active membrane properties. In addition, they receive excitatory and inhibitory synaptic inputs and have the potential to modulate the activity of other hNSCs and probably also host neurons as they are capable of firing action potentials. The question is whether this is sufficient to modulate disease progression and rescue some of the electrophysiological changes in HD models.

### 3.7 | Effects of hNSCs on membrane properties of host striatal projection neurons

MSNs recorded in symptomatic R6/2 mice display reduced membrane capacitance and increased input resistance, caused in part by reduced somatic area, loss of spines, and reduced K<sup>+</sup> channel expression.<sup>111–113</sup> Implantation of hNSCs did not prevent alterations in cell membrane properties of MSNs recorded near the injection site (Table 3). Cell membrane capacitances of R6/2 MSNs were smaller

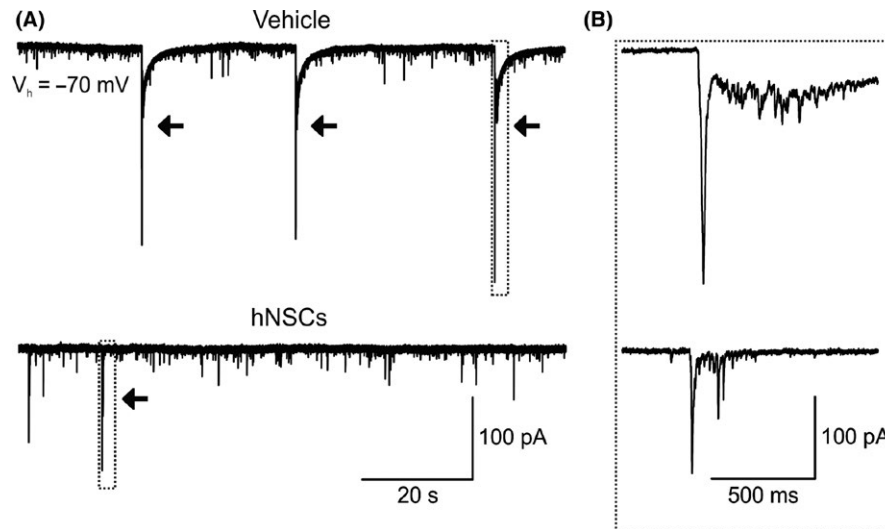
than values recorded in WT MSNs although only significantly smaller between R6/2 MSNs from vehicle-injected mice and WTs ( $P = 0.012$ ). Likewise, membrane input resistances in R6/2 MSNs were increased compared to WT MSNs although only significantly between MSNs from vehicle-injected mice and WTs ( $P = 0.002$  for WT vs R6/2 vehicle and  $P = 0.076$  for WT vs R6/2 hNSC). However, it is important to note that input resistances were significantly smaller in MSNs from R6/2 mice implanted with hNSCs when compared with values obtained in MSNs from R6/2 mice injected with the vehicle only ( $P = 0.018$ ). These results suggest there is a partial rescue of some MSN membrane properties in R6/2 mice implanted with ESI-017 hNSCs.

### 3.8 | hNSCs attenuate epileptiform activity in R6/2 mice

One distinctive feature of JHD is the susceptibility to develop epileptic seizures.<sup>15</sup> R6/2 mice also display seizures which could be related to the increase in cortical excitability.<sup>114</sup> Cortical hyperexcitability is manifested in striatal MSNs by the occurrence of large-amplitude EPSCs and high-frequency bursts, particularly evident after extended blockade of GABA<sub>A</sub> receptors and coinciding with an increase in the frequency of sEPSCs.<sup>111,114</sup> A smaller proportion of MSNs exhibited increased corticostriatal excitability in hNSC-implanted mice (20.5%) compared with vehicle-injected mice (28.6%) (Figure 3). Moreover, the increase in sEPSC frequencies within this population did not occur in the R6/2 mice implanted with hNSCs, indicating that the hNSCs can reduce facilitated input from cortex to striatum when GABA<sub>A</sub> receptors are blocked.<sup>53</sup>

### 3.9 | Possible mechanism of action

One of the possible mechanisms whereby MSNs degenerate is the loss of trophic support provided by CPNs. In particular, studies in animal models have demonstrated that BDNF, released from corticostriatal terminals, is reduced in HD striatum and external delivery can rescue some aspects of the phenotype.<sup>34,115</sup> Similar to the rescue induced by GDNF delivery using ESCs and NSCs, one mechanism invoked to contribute to the rescue by ESI-017 hNSCs was an increase in BDNF.<sup>53</sup> In hNSC-implanted R6/2 mice, immunostaining for BDNF colocalized with doublecortin-positive hNSCs while the striatum of vehicle-treated R6/2 mice showed no positive staining



**FIGURE 3** Implantation with hNSCs in striatum reduced paroxysmal discharges induced by Bicuculline: A, Bath application of the GABA<sub>A</sub> receptor antagonist Bicuculline (10–20  $\mu\text{mol/L}$ ) induced epileptiform activity in striatal MSNs. This activity was expressed in the form of large-amplitude synaptic currents followed by transient bursts of low-amplitude EPSCs (arrows). In mice implanted with hNSCs, the amplitude and frequency of these events were reduced and the transient low-amplitude EPSCs displayed reduced frequencies. B, Inset shows a magnified view of the paroxysmal discharges taken from the dotted rectangles in A

for BDNF. This suggests that these differentiated stem cells produced the neurotrophic factor(s) that aid in providing a supportive local environment for neurons. In addition, ESI-017 hNSCs reduced aberrant accumulation of a high molecular weight, potentially toxic form of mhtt protein in the striatum of transplanted animals.

#### 4 | CONCLUSIONS, LIMITATIONS, AND FUTURE PERSPECTIVES

We have presented an extensive, but not exhaustive, review of studies using stem cells that have demonstrated positive effects on neurological, cognitive, and electrophysiological alterations in rodent models of HD. In addition, we provide additional characterization of the GMP-grade human ESI-017 line of NSCs that when implanted into HD mice are able to remain viable and electrophysiologically active for weeks in vivo. They also have the ability to establish synaptic connections.<sup>53</sup> With so many stem cell options, it is difficult to determine which type would serve as the best therapy for neurodegenerative diseases, such as HD. A limitation to using ESCs or PSCs as starting material is the potential for derived cells from these lines to have undifferentiated cells that could form teratomas. For use of PSCs, an alternative method may be to bypass the PSC state utilizing direct conversion of fibroblasts to cells of specific lineages such as neurons.<sup>116</sup> One of the safest and less ethically controversial stem cell-based therapies is the use of iPSCs derived from patients with HD. Although induced cells can eventually take on diseased characteristics such as the presence of mhtt aggregates,<sup>97,117</sup> their implantation can potentially aid in the suppression of disease-related phenotypes. Furthermore, innovative technologies have rapidly emerged that will likely improve the effectiveness of these

and other types of stem cell-derived products for the treatment of neurological diseases. The CRISPR/Cas-9 system<sup>118,119</sup> can be used to edit patient-derived iPSCs or induced NSCs (iNSCs) to have a normal CAG length expansion cassette within the *HTT* gene.<sup>96</sup> These modified stem cells, particularly iNSCs, can be further differentiated into distinct cell types (eg, GABAergic MSNs) which may potentially serve as replacements for degenerating cells.<sup>77</sup>

However, concerns that even healthy stem cells in a diseased environment will eventually become dysfunctional pose a challenge to this sort of therapeutic approach. Here, we show the ESI-017 hNSCs implanted in striatum of R6/2 mice display some alterations compared with those implanted in WT mice, for example reduced cell membrane capacitance, while still rescuing HD phenotypes including behavior and some electrophysiological deficits. This could indicate that the environment in R6/2 mice delays the process of maturation compared to WT animals. More experimentation in longer-term knock-in models is warranted. Incidentally, the bulk of stem cell research for the treatment of HD points to the need to not only replace cells that are lost during disease progression, but also to better support and enrich the local environment of surviving cells. As HD is a slowly progressing disease, it may be more beneficial to use stem cells that survive in vivo for a significant amount of time to avoid the continued need for reintroducing new cells and to allow for sufficient increases in baseline levels of protective trophic factors. As some classes of striatal interneurons are relatively spared in HD, such as the somatostatin- and calretinin-expressing types,<sup>25</sup> stem cell differentiation strategies aimed at generating these interneurons may increase stem cell life expectancies and further extend their neuroprotective abilities on host MSNs. Additionally, astrocytes and oligodendrocytes are widely known to release trophic factors that support the growth and repair of partner neurons<sup>120–124</sup>

and should also be considered as potential cell differentiation candidates in stem cell-based therapies for HD.<sup>125</sup>

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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