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## Human Neural Progenitor Transplantation Rescues Behavior and Reduces $\alpha$ -Synuclein in a Transgenic Model of Dementia with Lewy Bodies

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**Key Words.** Synucleinopathy • Glial progenitor • Astrocyte • Transplantation • Dopamine • Glutamate • Memory • Pathology

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### ABSTRACT

Synucleinopathies are a group of neurodegenerative disorders sharing the common feature of misfolding and accumulation of the presynaptic protein  $\alpha$ -synuclein ( $\alpha$ -syn) into insoluble aggregates. Within this diverse group, Dementia with Lewy Bodies (DLB) is characterized by the aberrant accumulation of  $\alpha$ -syn in cortical, hippocampal, and brainstem neurons, resulting in multiple cellular stressors that particularly impair dopamine and glutamate neurotransmission and related motor and cognitive function. Recent studies show that murine neural stem cell (NSC) transplantation can improve cognitive or motor function in transgenic models of Alzheimer's and Huntington's disease, and DLB. However, examination of clinically relevant human NSCs in these models is hindered by the challenges of xenotransplantation and the confounding effects of immunosuppressant drugs on pathology and behavior. To address this challenge, we developed an immune-deficient transgenic model of DLB that lacks T-, B-, and NK-cells, yet exhibits progressive accumulation of human  $\alpha$ -syn (h- $\alpha$ -syn)-laden inclusions and cognitive and motor impairments. We demonstrate that clinically relevant human neural progenitor cells (line CNS10-hNPCs) survive, migrate extensively and begin to differentiate preferentially into astrocytes following striatal transplantation into this DLB model. Critically, grafted CNS10-hNPCs rescue both cognitive and motor deficits after 1 and 3 months and, furthermore, restore striatal dopamine and glutamate systems. These behavioral and neurochemical benefits are likely achieved by reducing  $\alpha$ -syn oligomers. Collectively, these results using a new model of DLB demonstrate that hNPC transplantation can impact a broad array of disease mechanisms and phenotypes and suggest a cellular therapeutic strategy that should be pursued. *STEM CELLS TRANSLATIONAL MEDICINE* 2017;6:1477–1490

### SIGNIFICANCE STATEMENT

$\alpha$ -synuclein accumulation is a hallmark of Dementia with Lewy Bodies (DLB) and other synucleinopathies like Parkinson's disease. Current therapies, however, fail to impact  $\alpha$ -synuclein pathology or provide long-term benefits. This paper describes the use of human neural progenitor cells (hNPCs) from a line that has also recently been manufactured under clinically relevant cGMP conditions, hNPC-CNS10. We show that when transplanted into an immune-deficient mouse model of DLB, hNPCs survive and differentiate toward a preferentially glial fate, stimulating changes in neurotransmitter markers and reduce pathogenic  $\alpha$ -synuclein oligomers. Furthermore, we find that hNPC-CNS10 transplantation leads to substantially improved cognitive and motor deficits, and therefore represents a promising therapeutic approach to treating synucleinopathies.

### INTRODUCTION

Dementia with Lewy Bodies (DLB) is thought to be the second leading cause of age-related dementia, and shares clinical features with both Alzheimer's and Parkinson's diseases, including memory loss, visual hallucinations, and extrapyramidal symptoms [1, 2]. In DLB, these symptoms

are closely associated with the accumulation of insoluble aggregates of the presynaptic protein,  $\alpha$ -synuclein ( $\alpha$ -syn) within neurons of the cortex, hippocampus, and brainstem. In turn,  $\alpha$ -syn and Lewy body pathology can lead to widespread cellular stress and disruption of multiple neurotransmitter systems including dopamine and glutamate [3, 4]. Although aggregated  $\alpha$ -syn is a hallmark of

several synucleinopathies, the physiological and pathological states of this protein remain unresolved.  $\alpha$ -syn is an intrinsically disordered protein with a propensity for folding and oligomerization into multiple conformers and larger Lewy body-like aggregates (reviewed in [5]). Hypotheses distinguishing physiological from pathological  $\alpha$ -syn remain subject to debate, but increasing evidence suggests that small, soluble oligomers may contribute to cell toxicity while Lewy bodies may be part of a more neuroprotective response to cellular stress [5–7]. It is this variety of  $\alpha$ -syn species and lack of consensus as to the protein's pathological states that makes it critical to examine multiple forms in disease and in response to therapeutic approaches.

Although the precise causes of DLB remain unclear, multiple mechanisms have been implicated suggesting that therapies designed to target a single pathway may be insufficient. However, recent studies have shown that neural stem cell (NSC) transplantation can impact a broad array of disease mechanisms and phenotypes in related disease models, thus stem cell transplantation should be further examined in the context of DLB [8, 9]. Recently, our group demonstrated that striatal transplantation of mouse NSCs (mNSCs) can ameliorate both motor and cognitive deficits in a transgenic model of DLB [10]. Transplanted mNSCs displayed a preferentially glial fate, suggesting that human NSC lines that are particularly gliogenic may provide an ideal cell type that could potentially target multiple disease mechanisms as a neuroprotective strategy for DLB [11, 12]. Some groups have also begun to show that glial transplantation may be more effective for rescuing cognitive deficits compared to other cell types [13–15]. In addition, glia distinctly express the glutamate reuptake transporter, GLT-1, whose cell surface expression can be reduced by accumulation of amyloid- $\beta$  [16] and may also be perturbed by  $\alpha$ -syn [17]. These attributes make glial progenitors an attractive candidate cell type for transplantation in these proteinopathies, particularly where glutamate neurotransmission is disrupted.

Clinically relevant human neural progenitor cells (hNPCs) have the potential to treat multiple neurodegenerative diseases [8]. Importantly, hNPCs have been shown to survive transplantation and migrate throughout the striatum in both rodents and primates [18–20]. A well-characterized, genetically unmodified hNPC line derived from the fetal cortex, termed CNS10-hNPC, has been shown to expand in vitro for up to 50 passages with stable karyotype and to have high viability post-transplantation with no tumor formation [21, 22]. Owing to its unique expansion methods [23], this cell line also mimics the course of proliferation observed in developing human cortex without chromosomal disruption [24] making it a relatively safe source of neural cells for clinical translation. The CNS10-hNPC line that is genetically modified to secrete GDNF is currently being used in an FDA approved trial for ALS through transplantation into the spinal cord (NCT02943850). The bipotent (neuronal and astrocytic) progenitors display preferential differentiation toward glial fate following transplantation [22, 25].

Immune-rejection of transplanted xenograft cells is a major challenge to studying the safety and efficacy of CNS10-hNPCs in animal models [26]. Commonly used immune-suppressants such as cyclosporine A or tacrolimus (FK506) have been shown to modulate  $\alpha$ -syn pathology, thereby complicating the potential design and interpretation of xenotransplantation studies [27, 28]. To resolve this challenge, we generated an immune-deficient transgenic model of DLB by back-crossing with a Rag2/il2r $\gamma$  double knockout line. Using this new model, we now examine the effects of clinically relevant human neural progenitor cells (specifically,

line CNS10-hNPC) on multiple phenotypes in the DLB model. Preferentially gliogenic CNS10-hNPCs migrate throughout the striatum, leading to a dramatic improvement in both motor and cognitive function by 1 month that is sustained for at least 3 months post-transplantation. Interestingly, these benefits are associated with changes in striatal dopamine and glutamate regulation and are accompanied by a reduction in soluble  $\alpha$ -syn monomers and oligomers. Taken together, these data suggest that CNS10-hNPCs can effectively ameliorate motor and cognitive dysfunction and associated impairments in dopamine and glutamate neurotransmission in xenotransplantation-compatible DLB mice. Moreover, the ability of CNS10-hNPCs to alter  $\alpha$ -syn oligomer and monomer levels indicates their potential to disrupt the underlying pathological phenotype and perhaps even slow disease progression.

## MATERIALS AND METHODS

### Generation of Immune-Deficient Transgenic DLB Mice

All procedures were performed in strict accordance with the UC Irvine and NIH animal use regulations. The Rag-ASO immune-deficient DLB mouse model was created by backcrossing previously characterized hemizygous wild-type (WT) human- $\alpha$ -syn (h- $\alpha$ -syn) overexpressing (ASO) transgenic mice (PDGF $\beta$  promoter, line D) [29] onto a Rag2/il2r $\gamma$  double knockout background (Strain #4111-Taconic, Hudson, NY, USA, <http://www.taconic.com/>) [30]. This cross was repeated for three generations (Supporting Information Fig. 1) to create immuno-deficient DLB mice that are heterozygous for the ASO transgene and lack both copies of the Rag2 and il2r $\gamma$  transgenes. All treatment groups (Rag-WT and Rag-ASO littermates) were sex- and age-matched, and were group housed on a 12 hours/12 hours light/dark cycle with access to food and water *ad libitum*.

### Cell Preparation and Striatal Transplantation

Human NPCs (specifically, line CNS10-hNPC) derived from the fetal cortex are maintained as free-floating clusters, termed neurospheres, in fibroblast growth factor and epidermal growth factor and are passaged by mechanical chopping in order to maintain cell-cell contact as previously described (Supporting Information) [21, 23]. Cells at passage 26 were thawed, dissociated to a single-cell suspension, washed with vehicle transplantation media, and resuspended at a concentration of 50,000 cells per  $\mu$ l in transplantation media (Supporting Information). Seven-month old Rag-ASO and Rag-WT mice were randomly assigned to a treatment group and either vehicle (transplantation media) or 50,000 cells per site (1  $\mu$ l volume, 2 sites per side) were transplanted bilaterally into the dorsal striatum (Bregma +0.03 AP,  $\pm$  2.0 ML, –3.0 and –3.5 DV).

### Cognitive and Motor Behavioral Assessment

Thirty days (1-month group) or 90 days (3-month group) following transplantation, behavioral assessments began, lasting 10 days. All behavioral testing and analysis was performed blinded to treatment and genotype groups using an identification system decoded during statistical analysis as previously described [10]. Cortical- and hippocampal-dependent memory tasks followed standard protocols for novel object recognition (NOR) and novel place recognition (NPR) [10, 31] (Supporting Information). Mean distance traveled, mean ambulatory speed and time spent

investigating objects during the training period were not altered by either genotype or treatment (Supporting Information Fig. 2E). To assess striatal-associated motor coordination and control, mice were tested on the fixed speed Rotarod (Ugo Basile, Italy, <http://www.ugobasile.com/>) at both 1-month and 3-month time points [32]. Subsequently, mice were tested on the beam traversal challenge as modified from Fleming et al. (2004) [33] in order to detect motor initiation and control (Supporting Information).

### Flow Cytometry

Splenocytes were isolated following standard protocol. Briefly, spleens were mashed and treated with standard ammonium-chloride-potassium lysis buffer to remove erythrocytes. Isolated splenocytes were first incubated with anti-Fc CD16/32 block (1:100; BD Biosciences; San Jose, CA, USA, , USA, <http://www.bdbiosciences.com/>) for 10 minutes at 4°C. After blocking, cells were incubated with antibodies against specific immune cell surface markers (Supporting Information).

### Biochemistry

Six weeks after transplantation and 1 day after the end of behavioral testing, mice were sacrificed by Euthazol and transcardial perfusion with 0.01 M phosphate-buffered saline (PBS). The left hemisphere was post-fixed in 4% paraformaldehyde and sectioned on a microtome (40  $\mu$ m, coronal) for immunohistochemical analyses. The right side of the brain was flash frozen for subsequent biochemical analysis. The dorsal striatum was then microdissected, homogenized in liquid nitrogen and split into two fractions for protein or mRNA isolation. Soluble and insoluble protein fractions were then prepared as detailed in the Supporting Information.

### Immunofluorescence and Confocal Microscopy

Fluorescent immunohistochemistry followed standard protocols, as detailed in Supporting Information. Sections were imaged in a blinded manner using an Olympus FX1200 confocal microscope, with identical laser and detection settings across a given immunolabel. Immunoreactive cells and  $\alpha$ -syn inclusions were hand-counted by a blind observer, and sample coding was revealed once all values were obtained.

### RNA Isolation and Quantitative RT-PCR

mRNA was isolated using Trizol extraction (Thermo Fisher Scientific, Cambridge, MA, USA, <http://www.thermofisher.com/>). Taqman quantitative real time PCR was modified from [34]. In brief, first-strand cDNA was synthesized from 1  $\mu$ g of total RNA by reverse transcription using a High Capacity RNA-to-cDNA Kit (Thermo Fisher Scientific). Proprietary Taqman primer sequences to h- $\alpha$ -syn (Hs01103383\_m1) and mouse  $\alpha$ -syn (Mm01188700\_m1) were analyzed relative to mouse Rn18s (Mm03928990\_g1), using a mixed WT-VEH sample as an internal reference control.

### Statistical Analysis

All animals were randomly assigned to treatment groups based on genotype and sex. All researchers were blind to genotype and treatment throughout the behavioral testing, scoring, and statistical analysis. Comparisons between multiple groups within one time point utilized two-way analysis of variance (ANOVA, in text) followed by Fisher's PLSD post hoc tests (within figures). Behavioral data was analyzed by repeated measures ANOVA as the same animals were assessed at 1-month and 3-month post-

transplantation (Fig. 1A). Correlation analyses were within-animal comparisons, with behavior and protein of animals sacrificed at 1-month post-transplantation, and behavior and protein of animals sacrificed at 3-month post-transplantation. Differences are considered significant when  $p < .05$  for both ANOVA and post hoc tests.

## RESULTS

### Validation of Immune-Deficient ASO Mice as a Model for Xenotransplantation

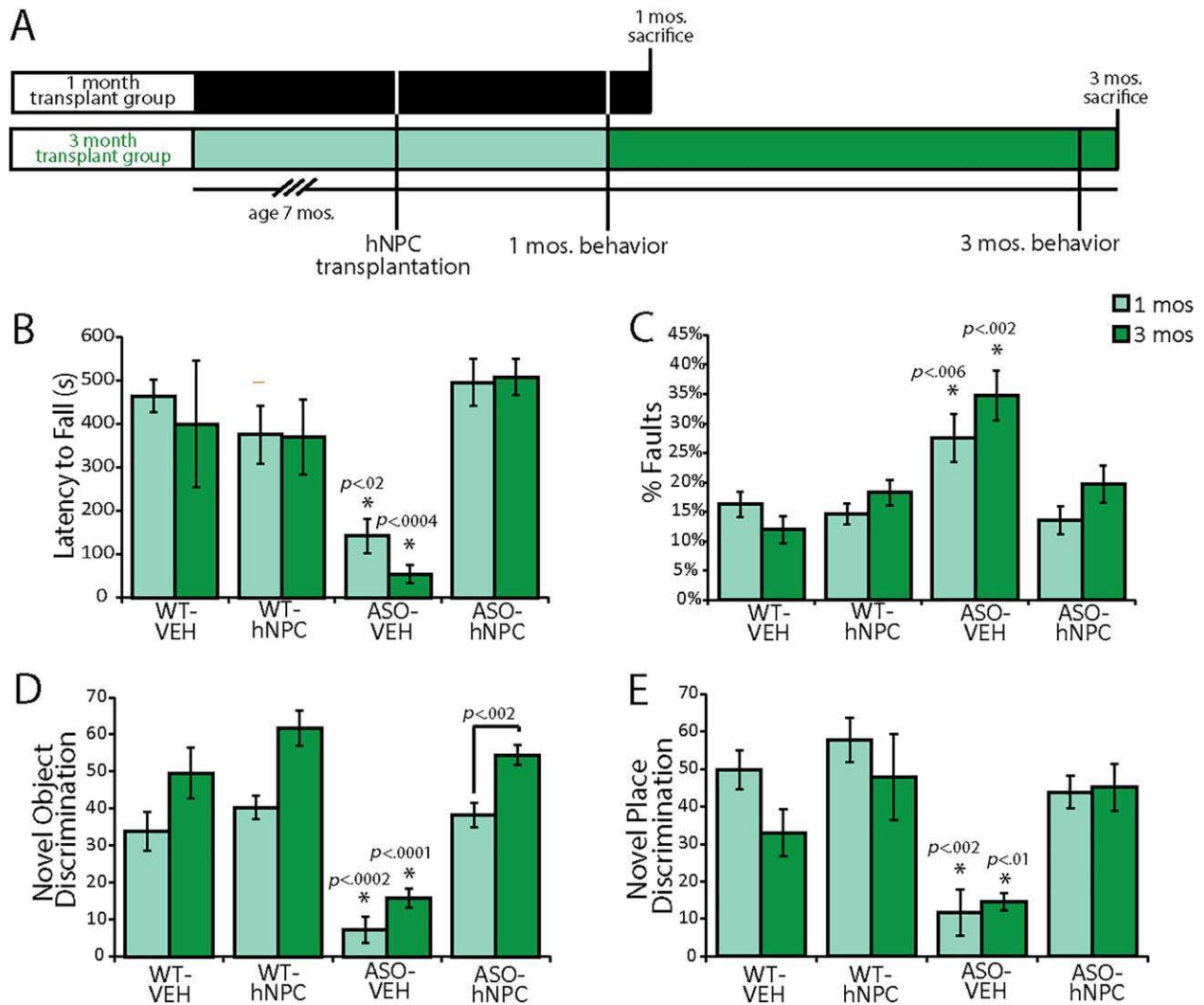
Immuno-deficient DLB mice were developed by backcrossing  $\alpha$ -syn (ASO) transgenic mice onto a Rag2/il2r $\gamma$  double knockout background (Supporting Information Fig. 1A). To confirm that the resulting Rag-ASO mice lacked B-, T-, and Natural killer (NK) cells, flow cytometry was performed on splenocytes isolated from 6-month old mice and compared to immune-intact WT and ASO mice ( $n = 6$ ). Analysis verified that all immune-deficient Rag-ASO animals lacked B cells, CD4 and CD8 T cells, as well as NK cells independent of WT or ASO genotype (Supporting Information Fig. 1B).

Immune-deficient Rag-ASO mice develop equivalent h- $\alpha$ -syn (Syn211) inclusions to age-matched immune-intact ASO mice up to 1 year in age (Supporting Information Fig. 2A). Likewise, Western blot analysis revealed similar levels of soluble h- $\alpha$ -syn and total (human and mouse)  $\alpha$ -syn monomers (Supporting Information Fig. 2B). Finally, Rag-ASO mice develop significant motor (Supporting Information Fig. 2C;  $F_{(1, 9)} = 14.3$ ; ANOVA  $p = .004$ ) and cognitive (Supporting Information Fig. 2D;  $F_{(1, 9)} = 16.7$ ; ANOVA  $p = .0035$ ) deficits compared to Rag-WT mice. Importantly for the interpretation of cognitive assessment, Rag-WT and Rag-ASO displayed similar total activity as measured by mean velocity (Supporting Information Fig. 2E;  $F_{(1, 9)} = 0.43$ ; ANOVA  $p = .52$ ) and total distance (Supporting Information Fig. 2E;  $F_{(1, 9)} = 0.02$ ; ANOVA  $p = 0.9$ ) during the training period.

### hNPC Transplantation Rescues Motor and Cognitive Deficits in Immune-Deficient ASO Mice

To assess the impact of hNPC transplantation on DLB-associated motor and cognitive impairments, the hNPC line CNS10-hNPC or vehicle control were delivered to 7-month old Rag-WT (hereafter, WT) or Rag-h- $\alpha$ -syn transgenic (hereafter, ASO) immune-deficient mice ( $n = 6-8$ ). Bilateral injections of 50,000 cells per site targeted the dorsal striatum, a region known to be involved in both motor function and learning/memory through connectivity within the basal ganglia and with multiple cortical regions. Behavioral outcomes were assessed 1-month or 3-month following transplantation (Fig. 1A). As predicted based on observations in immune-intact ASO mice [10], immune-deficient ASO-VEH mice displayed significant impairment on the striatal-associated Rotarod (Fig. 1B) and Beam Traversal (Fig. 1C) motor tasks at both 1-month and 3-month time points, suggesting deficits in motor coordination, fluidity, and control. In contrast, CNS10-hNPC transplantation resulted in a robust and significant rescue of these deficits in ASO-CNS10 mice at both 1-month and 3-month following transplantation. Significant interactions of genotype and treatment were observed for both motor tasks (Rotarod  $F_{(1, 52)} = 33.7$ ,  $p < .0001$ ; Beam  $F_{(1, 52)} = 18.1$ ,  $p < .0001$ ), with no main effect of time point, confirming a lasting impact of CNS10-hNPCs on these behaviors over time.

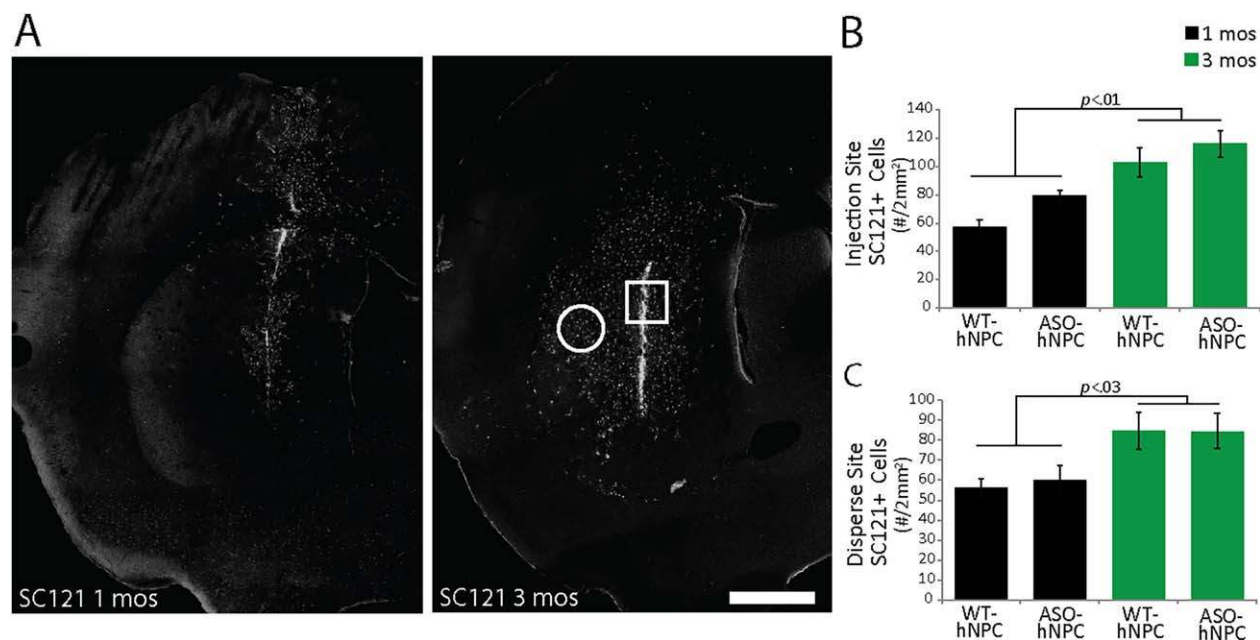
Examining cognition in a model with motor impairments requires the use of tasks that are not heavily influenced by motor function. Novel Object Recognition (NOR) and Novel Place



**Figure 1.** Human neural progenitor cells (NPCs) rescue cognitive and motor deficits. Seven-month (mos) old Rag-wild-type (WT) and Rag-ASO mice received bilateral intrastriatal transplants of CNS10-hNPCs or vehicle. One group (black bars) underwent behavioral testing at 1 mos and was then sacrificed for histological analysis, whereas the second group (green) was tested at both 1-mos (light green) and 3-mos (dark green) time points, and were sacrificed for histological analysis after the 3-mos behavioral analysis (A). ASO-VEH mice exhibited significant deficits in Rotarod (B), beam traversal (C), novel object recognition (D), and novel place recognition (E) tasks compared to both Vehicle and CNS10-hNPC transplanted WT groups. In contrast, treatment of ASO mice with CNS10-hNPCs led to a significant rescue of performance in all four of these tasks at both 1-mos and 3-mos time points  $n = 6-8$  per group. All values are means  $\pm$  SEM. \* $p$  values are significantly different from all other groups. Abbreviations: hNPC, human neural progenitor cells; mos, month; WT, wild-type.

Recognition (NPR) tasks were therefore utilized, which are low-stress paradigms that measure cortical-dependent object and hippocampal-dependent spatial memory, respectively. All four groups were habituated, trained, and tested following standard protocols. Twenty-four hours after training, mice were exposed to a novel object or novel object placement, and the discrimination ratio between exploration time of old and new objects was calculated. A significant interaction of genotype and treatment was observed for both NOR ( $F_{(1, 52)} = 17.4, p = .0001$ ) and NPR ( $F_{(1, 52)} = 3.8, p < .05$ ), but these were independent of time point, suggesting a sustained positive impact over time. As shown, ASO-VEH mice exhibited significant impairments in both NOR (Fig. 1D) and NPR (Fig. 1E) compared to WT-VEH and WT-CNS10 groups at both 1-month and 3-month time points, demonstrating that h- $\alpha$ -syn overexpression can mimic these important aspects of DLB-

related cognitive impairment. In contrast, CNS10-hNPCs considerably improved both cortical- and hippocampal-related cognitive function at 1-month and 3-month following striatal transplantation (Fig. 1D, 1E). Although not significant, cortical NOR discrimination also appeared to improve from 1 to 3 months in WT-VEH and WT-CNS10 mice (Fig. 1D). CNS10-transplanted ASO mice, however, did show significant further improvement in performance between 1 and 3 months. In addition, while hippocampal NPR discrimination slightly declined from 1 to 3 months in WT-VEH and WT-CNS10 mice, this was not evident in CNS10-transplanted ASO mice (Fig. 1E). This may suggest early age-related decline in hippocampal function, resulting in compensatory function in the cortex which enhances NOR memory [35, 36]. Collectively, these results suggest that CNS10-hNPCs provided sustained cognitive improvement in both cognitive domains over time, even despite this age-related trend.



**Figure 2.** Human neural progenitor cells (NPCs) engraft and migrate throughout the striatum. CNS10-hNPCs were injected into a dorsal and a ventral site within the medial striatum. Cells engraft well at 1 month (mos) and migrate throughout the striatum by 3-month post-transplantation (A). By 3 mos, more hNPCs have populated the injection site (line of cells), although no other clustering of cells is observed. At this time point hNPCs remain largely within the corpus callosum and lateral ventricle anatomical boundaries of the striatum. Cells survive and increase significantly in number from 1 to 3 mos independent of genotype both near the injection site (square) (B; wild-type [WT]-hNPC<sub>1mos</sub> = 57.5, ASO-hNPC<sub>1mos</sub> = 79.7, WT-hNPC<sub>3mos</sub> = 103.1, ASO-hNPC<sub>3mos</sub> = 116.1 cells/2 mm<sup>2</sup>) and at more dispersed locations (circle) (C; WT-hNPC<sub>1mos</sub> = 56.2, ASO-hNPC<sub>1mos</sub> = 60.3, WT-hNPC<sub>3mos</sub> = 84.8, ASO-hNPC<sub>3mos</sub> = 84.5 cells per 2mm<sup>2</sup>). Scalebar = 2 mm. *n* = 6–8 per group. All values are means ± SEM. Abbreviations: hNPC, human neural progenitor cells; mos, month; WT, wild-type.

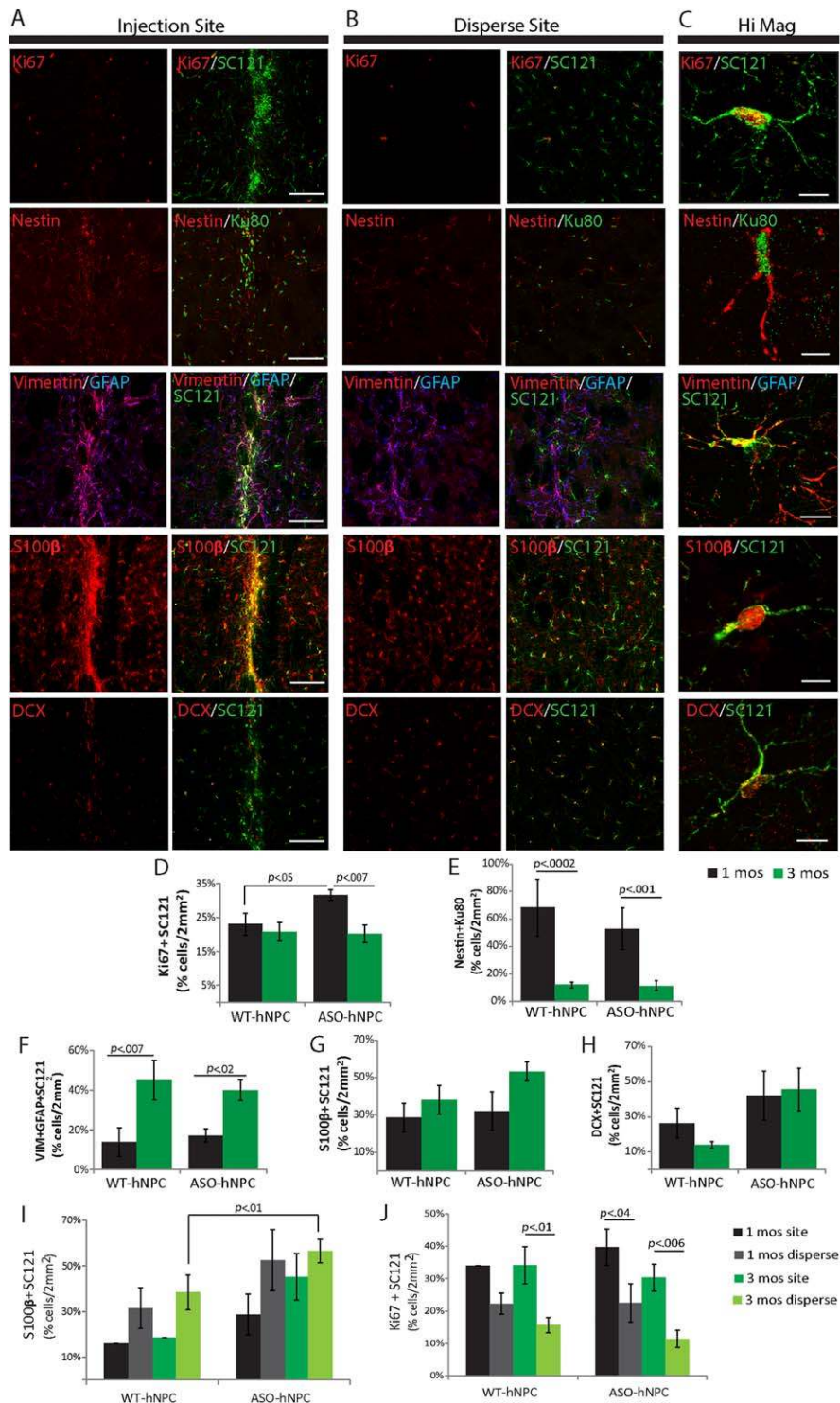
### hNPCs Show Robust Engraftment and Glial Differentiation

Whereas improved behavioral function in ASO mice was maintained at similar levels from 1 to 3 months following transplantation, CNS10-hNPC engraftment and migration increased considerably over time (Fig. 2A–2C;  $F_{(1, 25)} = 5.4$ ,  $p < .03$ ). Importantly, CNS10-hNPCs showed robust engraftment independent of genotype, suggesting that h- $\alpha$ -syn overexpression does not impair survival of transplanted human cells. Relevant to this result, h- $\alpha$ -syn aggregates were not observed within transplanted CNS10-hNPCs at either 1 or 3 months (data not shown). The duration-dependent increase in hNPC number occurred both at the injection site (Fig. 2B;  $F_{(1, 14)} = 15.8$ ;  $p < .001$ ) and in the disperse regions to which CNS10-hNPCs migrated within the striatum (Fig. 2C;  $F_{(1, 14)} = 10.3$ ,  $p < .005$ ). While CNS10-hNPCs increased in number, their overall proliferative capacity as assessed by Ki67 colabeling remained low at both 1 and 3 months in WT-CNS10 mice, and was reduced in ASO-CNS10 mice by 3 months (Fig. 3A–3D;  $F_{(1, 25)} = 3.5$ ;  $p < .03$ ). This result critically indicates decreased proliferation and thereby reduced tumorigenicity. Supporting this conclusion, no clustering of transplanted hNPCs was observed in either WT or ASO mice. Finally, CNS10-hNPCs that remained at the injection site expressed a more proliferative potential compared to the minimally proliferative phenotype of the disperse populations, which one might expect if the proximity of transplanted cells to one another affects proliferation rates (Fig. 3J).

The progenitor state of CNS10-hNPCs as assessed by nestin immunolabeling declined dramatically from 1 to 3 months post-transplantation independent of genotype (Fig. 3A, 3B, 3E;

$F_{(1, 25)} = 78.6$ ;  $p < .0001$ ), indicating a shift from progenitor state toward fate commitment. Given that CNS10-hNPC have been shown to preferentially differentiate toward an astrocytic fate in vivo [22, 25], we anticipated largely astrocytic differentiation profiles at both 1-month and 3 month post-transplantation. Immunohistochemical analysis confirmed that by 3-month post-transplantation, differentiating CNS10-hNPCs largely expressed the astrocytic intermediate filaments vimentin and glial fibrillary acidic protein (GFAP) (Fig. 3A, 3B, 3F). Indeed, vimentin+ and GFAP+ human cells increased approximately fourfold from 1 to 3 months post-transplantation ( $F_{(1, 25)} = 15.1$ ;  $p < .0009$ ) as transplanted cells matured into astrocytes in both genotypes. Furthermore, expression of the astrocyte-associated calcium binding protein, S100 $\beta$  indicated early astrocytic maturation of hNPCs in WT and ASO mice (Fig. 3A, 3B, 3G). Site-specific analysis revealed a trend toward increased S100 $\beta$  hNPCs at disperse sites compared to injection sites in both WT and ASO mice (Fig. 3A, 3B, 3I). Finally, an influence of genotype was observed as S100 $\beta$ + hNPCs were significantly greater in number at 3-month disperse sites in ASO compared to WT mice (Fig. 3A, 3B, 3I;  $F_{(1, 14)} = 4.5$ ;  $p < .04$ ). This suggests that host h- $\alpha$ -syn overexpression may encourage glial differentiation in hNPCs that have migrated away from the injection site.

Doublecortin (DCX) labeling for early neuronal fated CNS10-hNPCs revealed a nonsignificant trend toward increased neuronal potential in ASO-CNS10 mice versus WT-CNS10 mice at both time points (Fig. 3A, 3B, 3H), though no CNS10-hNPCs expressed the mature neuronal marker NeuN (data not shown). This elevated expression of DCX in ASO-CNS10 hNPCs is intriguing given reports that astrocytic fate in vitro decreases following overexpression of



**Figure 3.** Human neural progenitor cells (NPCs) differentiate predominantly toward a glial fate independent of host genotype. Confocal micrographs demonstrate largely glial differentiation of human cells (green, SC121 cytoplasm or Ku80+ nuclei) near the injection site (A) and at dispersed locations (B). Counts of Ki67 revealed that ~20% of human cells remained mitotic at 3 months (mos), although ASO mice exhibited a significant reduction in Ki67 CNS10-hNPCs between 1-mos and 3-mos time points (C; 1 mos black, 3 mos green). Nestin-labeled hNPCs decreased significantly over time, independent of genotype (D). Whereas Vimentin/GFAP (E) and S100β colabeling of human cells increased over time (F), no significant changes in Doublecortin expression between 1 and 3 mos (G;  $p = .08$ ). Further analysis of site-specific differentiation showed that S100β-labeled CNS10-hNPCs trended toward increased expression at disperse locations at both 1-mos and 3-mos time points, and expression is significantly greater in ASO compared to wild-type (WT) mice at 3-mos disperse locations (H). In contrast, Ki67-labeled CNS10-hNPCs are significantly decreased at disperse locations compared to the injection site at 3 mos in WT and both time points in ASO mice (I). Scale bar = 100  $\mu$ m, inset scale bar = 10  $\mu$ m.  $n = 6-8$  per group. All values are means  $\pm$  SEM, expressed as percent of within-group total CNS10-hNPCs. Abbreviations: hNPC, human neural progenitor cells; mos, month; WT, wild-type.

wild-type  $\alpha$ -syn in CNS10-hNPCs [37]. In the current report, h- $\alpha$ -syn aggregates were not observed within transplanted CNS10-hNPCs (data not shown), however extrinsic cues from host h- $\alpha$ -syn may have led to increased early neuronal differentiation of transplanted CNS10-hNPCs, but did not hinder overall astrocytic differentiation of CNS10-hNPCs in ASO-CNS10 mice.

Finally, we assessed the host glial response to CNS10-hNPC transplantation in ASO and WT mice by quantifying the total endogenous glial response to vehicle or CNS10-hNPC. Host vimentin+/GFAP+ cells were evident in larger numbers in the CNS10-hNPC-transplanted striatum compared to vehicle-injected mice, independent of genotype (Supporting Information Fig. 3A, 3B;  $F_{(1, 25)} = 55.6$ ;  $p < .0001$ ). Although no adaptive immune response to CNS10-hNPCs was possible in ASO and WT mice due to the homozygous Rag2/Il2r $\gamma$  knockout, resident microglial cells remain capable of mounting an innate response. Microglial recruitment as well as changes in morphology were therefore assessed at sites proximal to the injection site using confocal microscopy and IMARIS software analysis. Interestingly, no changes in total microglial number, process length, or process branching were observed post-transplantation between ASO and WT mice (Supporting Information Figs. 4A, 4B, 5A, 5B). This suggests that microglia played a minimal role in modulating the host response to CNS10-hNPC transplantation in Rag-ASO versus Rag-WT mice.

### Striatal Dopaminergic and Glutamatergic Regulation is Restored by hNPC Transplantation

ASO mice have previously been shown to exhibit impairments in both dopaminergic and glutamatergic systems that correlate with motor and cognitive dysfunction [10]. We therefore examined several protein markers associated with these key striatal neurotransmitters ( $n = 4$ ). Dopamine regulation was assessed both by the rate-limiting enzyme in DA synthesis, tyrosine hydroxylase (TH), and its phospho-epitope (pSer31) that is associated with increased TH activity. The ratio of striatal pSer31/TH protein was then calculated, revealing a significant decrease in ASO-VEH mice that was rescued by CNS10-hNPC transplantation at 1-month and 3-month time points (Fig. 4A, 4B). This analysis demonstrated a significant interaction between genotype, treatment, and time point in the striatal pSer31/TH protein ratio ( $F_{(1, 24)} = 6.6$ ,  $p < .02$ ), indicating that the main effects of CNS10-hNPC transplantation were greater in ASO mice compared to WT mice, and were further enhanced at 3-month post-transplantation compared to 1 month.

We next examined expression of glutamate transporters in the striatum in order to determine whether  $\alpha$ -syn or CNS10-hNPCs influence corticostriatal and hippocampal-striatal glutamatergic projections. Significant main effects of CNS10-hNPCs were observed on expression of the glial glutamate reuptake transporter, GLT-1, as transplantation was able to rescue expression at both 1-month and 3-month post-transplantation (Fig. 4A, 4C, Supporting Information Fig. 5C;  $F_{(1, 24)} = 7.3$ ,  $p < .01$ ). Corroborating the specific role of glial GLT-1 in CNS10-hNPC-mediated effects on glutamate regulation, the neuron-specific transporter EAAC1 was not altered by either genotype or treatment (Fig. 4A, 4D, Supporting Information Fig. 5D).

Because we and others have reported that brain-derived neurotrophic factor (BDNF) signaling plays an important role in the multiple homeostatic effects of murine NSC transplantation, we examined the neurotrophin BDNF (Fig. 4A, 4E, Supporting Information Fig. 5E). Western blot quantification showed that mature BDNF expression was not altered by genotype or CNS10-hNPC

treatment at this age, as was the case for the other neurotrophins, glial cell line-derived neurotrophic factor (GDNF) and nerve growth factor (NGF; data not shown). In addition, we have routinely observed by ELISA that the CNS10-hNPCs do not secrete GDNF in culture [38]. This suggests that increased expression of these mature neurotrophins is not required for CNS10-hNPC-mediated behavioral improvement.

Although neurotrophin signaling was unaffected, pSer31/TH and GLT-1 expression in individual ASO mice correlated with changes in motor and cognitive behaviors due to h- $\alpha$ -syn and CNS10-hNPC transplantation (Fig. 4F). For example, pSer31/TH correlated with both cognitive ( $R^2 = 0.52$ ,  $F_{(1, 13)} = 13.9$ ,  $p < .003$ ) and motor ( $R^2 = 0.43$ ,  $F_{(1, 13)} = 9.9$ ,  $p < .008$ ) performance. In contrast, GLT-1 expression correlated with cognitive performance ( $R^2 = 0.41$ ,  $F_{(1, 13)} = 9$ ,  $p < .01$ ), but not with motor function ( $R^2 = 0.13$ ,  $F_{(1, 13)} = 1.9$ ,  $p = .2$ ). These associations indicate a CNS10-hNPC-dependent recovery of dopamine and glutamate neurotransmitter regulation that is associated with the observed improvements in behavior.

### hNPC Transplantation Does Not Impact $\alpha$ -Synuclein Inclusions or Insoluble Protein

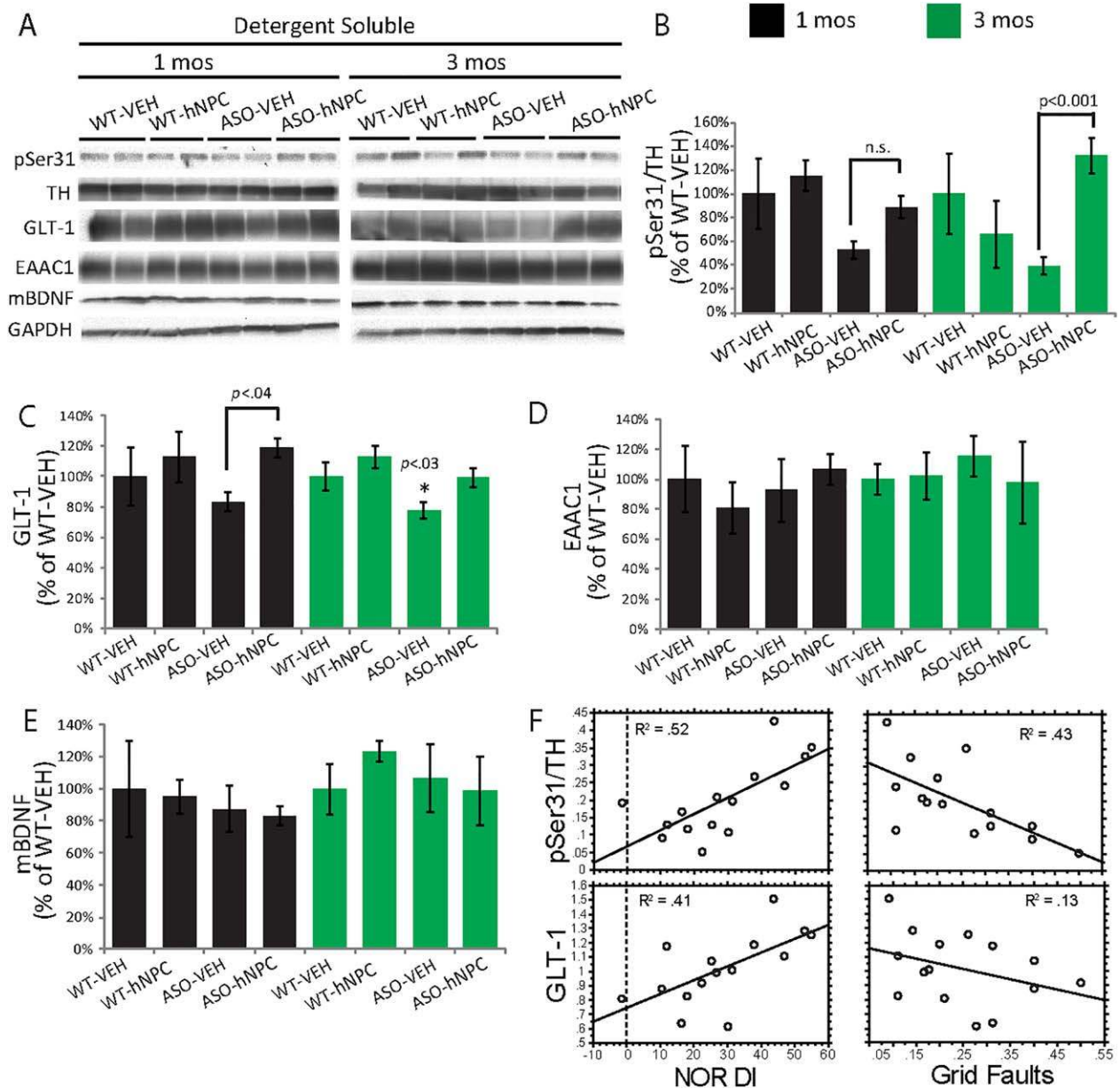
We next examined the role of  $\alpha$ -syn pathology on the ability of CNS10-hNPC transplantation to modify behavior and neurotransmitter homeostasis using several approaches. We began by assessing whether insoluble forms of the protein might be affected by CNS10-hNPC transplantation. Quantification of h- $\alpha$ -syn inclusion bodies (Syn211) revealed that total inclusion number was not altered by CNS10-hNPCs, even by 3-month post-transplantation ( $n = 5-6$ ) (Fig. 5A, 5B). The absence of an effect on inclusion number, however, does not preclude potential influence on additional insoluble h- $\alpha$ -syn conformers that could affect behavior. Therefore, striatal samples extracted by formic acid treatment were examined by Western blot ( $n = 4$ ). A main effect of genotype was observed in insoluble h- $\alpha$ -syn ( $F_{(1, 24)} = 43.3$ ,  $p < .0001$ ). However, there was no impact of CNS10-hNPC transplantation on insoluble monomeric h- $\alpha$ -syn or total human/murine (Fig. 5C, 5D).

Although multimeric forms of h- $\alpha$ -syn (Syn211) were not observed in the insoluble fraction (Fig. 5C, 5D), Western blots showed clear expression of a dimer (28 kDa) and a smear of high molecular weight (HMW) species in total  $\alpha$ -syn, the specificity of which was verified by their absence in  $\alpha$ -syn knockout samples (Fig. 5C, 5E, 5F). A detected band at 12 kDa may represent a truncated form of monomeric  $\alpha$ -syn [39]. Intriguingly, while expression of monomeric, dimeric and HMW conformers was significantly elevated in ASO mice at the 1-month time point, only the HMW species was significantly higher at 3 months, suggesting an effect of age on expression of this particular species (Fig. 5F;  $F_{(1, 24)} = 5.3$ ,  $p < .02$ ).

### hNPC Transplantation Alters Soluble Monomeric and Oligomeric $\alpha$ -Synuclein Species

The absence of CNS10-hNPC-induced changes in insoluble  $\alpha$ -syn does not preclude alterations to the soluble protein. Therefore, we next assessed detergent soluble human- $\alpha$ -syn monomers (Fig. 6A, Supporting Information Fig. 5G;  $n = 4$ ). As expected, a significant main effect of genotype confirmed that h- $\alpha$ -syn (Syn211) was expressed only in ASO mice ( $F_{(1, 24)} = 107.6$ ,  $p < .0001$ ). However, we also observed a significant interaction between treatment and time point, indicating the increased ability of CNS10-



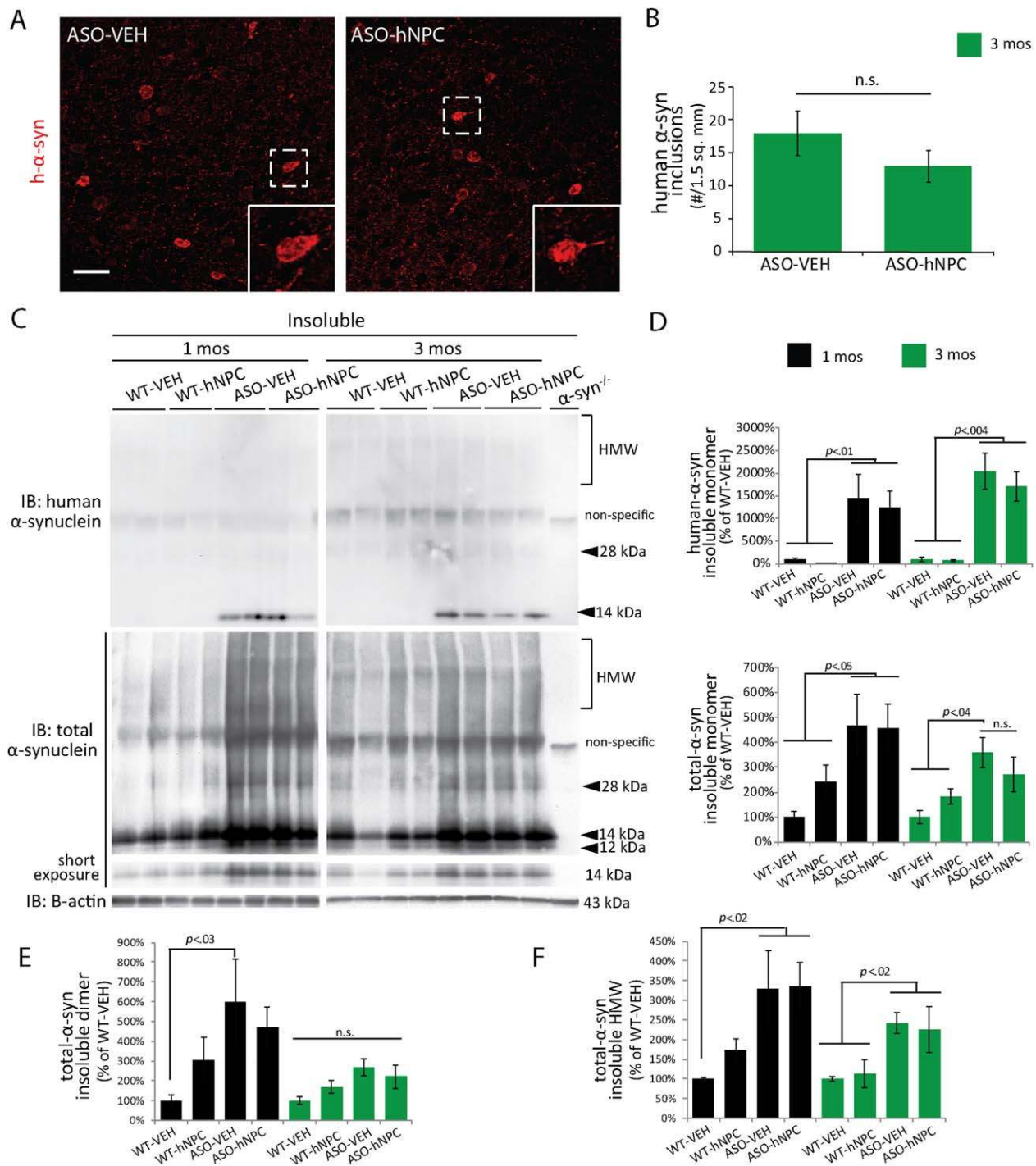


**Figure 4.** Human neural progenitor cells (NPC) transplantation enhances dopaminergic and glutamatergic markers. Western blots of detergent soluble striatal fractions were normalized to GAPDH (A). The ratio of phosphorylated serine 31 tyrosine hydroxylase (pSer31) to total tyrosine hydroxylase (TH) protein was modestly increased by CNS10-hNPCs at 1 month (mos) (black) in ASO mice, and significantly elevated by CNS10-hNPCs after 3 mos (B; green). The glial glutamate transporter GLT-1 was also significantly increased by CNS10-hNPCs at both 1-mos and 3-mos post-transplantation in ASO mice (C). Distinctly, the neuronal glutamate transporter EAAC1 was unaltered by genotype or treatment (D). Mature BDNF was not altered by genotype or CNS10-hNPC transplantation (E). The most significant correlations to behavioral outcome in ASO mice were pSer31/TH and GLT-1, with pSer31/TH showing association with both cognitive (novel object recognition, discrimination index (DI)) and motor (beam traversal) tasks whereas GLT-1 was only significantly correlated with cognitive performance (F).  $n = 4$  per group. All values are means  $\pm$  SEM, expressed as percent of within-time point wild-type-VEH group. \* $p$  values are significantly different from all other within-time point groups. Abbreviations: hNPC, human neural progenitor cells; mos, month; WT, wild-type.

hNPCs to reduce soluble h- $\alpha$ -syn over time ( $F_{(1, 24)} = 5.8, p < .02$ ). While monomeric h- $\alpha$ -syn (Syn211) was not reduced by CNS10-hNPCs at 1 month in ASO-CNS10 mice, CNS10-hNPCs did significantly reduce h- $\alpha$ -syn monomers by 3 months in the soluble fraction (Fig. 6A, 6B). The ASO transgenic model expresses both human and mouse  $\alpha$ -syn, therefore we examined whether either h- $\alpha$ -syn or CNS10-hNPCs impacted total levels of human and murine  $\alpha$ -syn. As would be expected, total levels of soluble  $\alpha$ -syn

were elevated in ASO mice (Fig. 6A, 6C, Supporting Information Fig. 5H;  $F_{(1, 24)} = 10.36, p < .004$ ). However, CNS10-hNPC transplantation had no impact on total  $\alpha$ -syn levels, suggesting that CNS10-hNPC-induced changes in  $\alpha$ -syn are specific to the overexpressed human form of this protein.

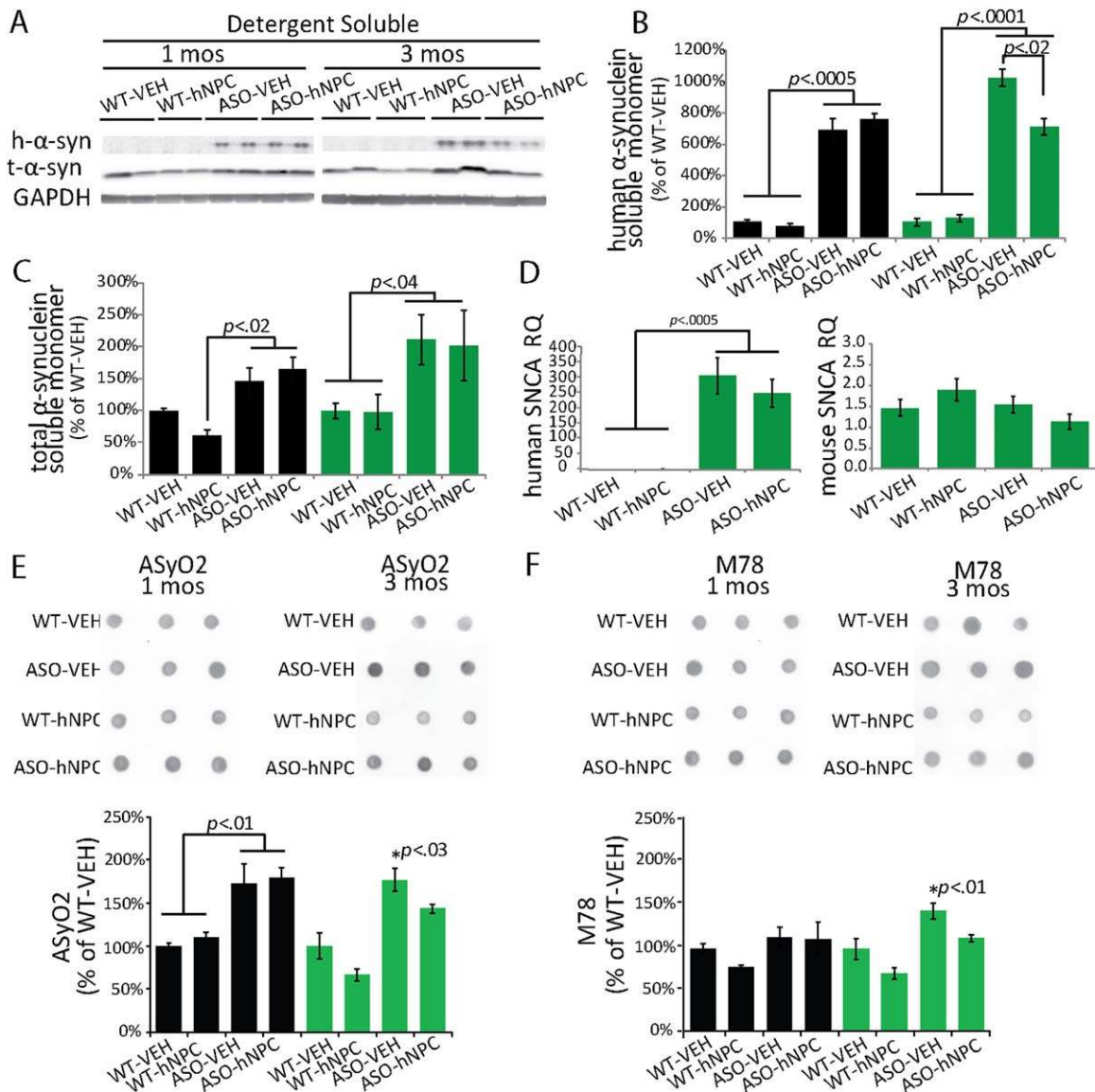
Next, to confirm that changes in monomeric  $\alpha$ -syn were being driven at the level of protein accumulation rather than transgene expression, we conducted quantitative real-time PCR of both



**Figure 5.**  $\alpha$ -Synuclein inclusions and insoluble protein are unaltered by human neural progenitor cells (hNPCs). Quantification of confocal micrographs of h- $\alpha$ -syn (Syn211)-labeled inclusions reveal no difference between VEH and CNS10-hNPC treated ASO mice at 3 mos ( $n = 5$ –6 per group; Scale bar = 20  $\mu$ m) (A, B). Immunoblots likewise indicate no effect of CNS10-hNPCs on insoluble human- $\alpha$ -syn or total  $\alpha$ -syn at 1-month (mos) (black) or 3-mos (green) post-transplantation ( $n = 4$  per group) (C, D). Dimer (E) and High Molecular Weight (F)  $\alpha$ -syn species were exclusively observed in total  $\alpha$ -syn immunoblots, although CNS10-hNPC transplantation once again had no effect on these insoluble conformers. All values are means  $\pm$  SEM, expressed as percent of within-time point wild-type-VEH group. Abbreviations: hNPC, human neural progenitor cells; mos, month; WT, wild-type.

human and mouse  $\alpha$ -syn. As predicted, h- $\alpha$ -syn transgene expression was unchanged between ASO-VEH and ASO-CNS10 groups at 3 months and undetectable in WT-VEH and WT-CNS10 groups, verifying that CNS10-hNPC driven changes occur at the protein

level (Fig. 6D). Further, mouse  $\alpha$ -syn was also not affected by genotype or treatment between groups, supporting the explanation that total  $\alpha$ -syn changes were likely driven at the protein level (Fig. 6D).



**Figure 6.** Soluble human  $\alpha$ -synuclein monomers and oligomers are reduced by human neural progenitor cells (hNPC) transplantation. Western blot analysis of detergent soluble h- $\alpha$ -syn monomers (**A**) are decreased by CNS10-hNPC transplantation only after 3 months (mos) in ASO mice (**B**), whereas total human and mouse- $\alpha$ -syn (t- $\alpha$ -syn) monomers are unaltered by CNS10-hNPCs ( $n = 4$  per group) (**C**). Quantitative real-time PCR shows h- $\alpha$ -syn transgene expression only in ASO mice and unaltered by CNS10-hNPCs, while mouse- $\alpha$ -syn mRNA is present at similar levels in all four groups ( $n = 4$  per group) (**D**). Dot blots probed with the oligomer-specific antibody ASyO2 show significant CNS10-hNPC-induced decreases in soluble oligomers at both 1-mos (black) and 3-mos (green) time points in ASO mice (**E**), while the oligomer-specific M78 antibody reveals significant CNS10-hNPC-induced reduction only after 3 mos ( $n = 4-7$  per group) (**F**). All values are means  $\pm$  SEM, expressed as percent of within-time point wild-type-VEH group. \* $p$  values are significantly different from all other within-time point groups. Abbreviations: hNPC, human neural progenitor cells; mos, month; WT, wild-type.

Finally, we sought to address whether this change in monomeric h- $\alpha$ -syn was impacting larger soluble  $\alpha$ -syn oligomers. We therefore assayed total  $\alpha$ -syn oligomers by dot blot using an oligomer-specific antibody, ASyO2 ( $n = 4-7$ ) (Agriser, Sweden, <http://www.agrisera.com/>) [40]. Staining with this antibody showed significant main effects of genotype ( $F_{(1, 32)} = 53.8$ ,  $p < .0001$ ) and time point ( $F_{(1, 32)} = 14.9$ ,  $p < .0005$ ), and an interaction of time point and treatment ( $F_{(1, 32)} = 5.4$ ,  $p < .02$ ) indicating that CNS10-hNPCs have a more substantial impact on oligomer expression over time. Total ASyO2  $\alpha$ -syn oligomers in ASO-VEH mice were elevated compared to their respective WT groups at both 1-month and 3-month time points (Fig. 6E).

However, CNS10-hNPC transplantation significantly reduced oligomeric  $\alpha$ -syn to WT by 3-month post-transplantation (Fig. 6E), suggesting that CNS10-hNPCs can reduce both monomeric h- $\alpha$ -syn and total oligomeric  $\alpha$ -syn. Importantly, this pattern was confirmed using another oligomer-specific antibody (mOC 78, generously provided by Dr. Charles Glabe (UCI), Fig. 6F), which detects fibrillar oligomeric conformations of several pathological proteins, with no crossover to monomers [41]. In contrast to ASyO2, M78 showed main effects of genotype ( $F_{(1, 32)} = 6.3$ ,  $p < .02$ ), and an interaction of genotype and treatment ( $F_{(1, 32)} = 4.1$ ,  $p < .05$ ), where oligomers increased in ASO-VEH groups at both time points but only significantly at the 3-month time point (Fig. 6F). A

significant reduction of  $\alpha$ -syn oligomers by M78 was only observed after 3-month transplantation in ASO-CNS10 mice (Fig. 6F). The modest differences in signal detection between ASyO2 and M78 antibodies in the 1-month groups demonstrates the importance of corroborating total oligomer expression across multiple antibodies. Together, these results suggest that CNS10-hNPC-induced reductions in soluble human  $\alpha$ -syn monomers and oligomers play a critical role in the observed improvements in neurotransmission and behavior.

## DISCUSSION

Dementia with Lewy Bodies (DLB) is one of many proteinopathies in which the aberrant behavior of presynaptic  $\alpha$ -syn leads to cellular stress and neurodegeneration. In DLB, this pathology manifests in cortical and midbrain regions, which converge on the striatum through disruption of both nigrostriatal dopamine and corticostriatal glutamate signaling. Dysregulation of these neurotransmitter systems then leads to impairment of the motor and cognitive functions to which they contribute.

The potential of NSC transplantation to alleviate motor and cognitive impairments associated with  $\alpha$ -syn accumulation has been largely unexplored. However, our group recently found that allogeneic mouse NSCs can improve these deficits in immune-competent DLB mice [10]. To determine the translational potential of this approach using human-derived NPCs, we created a novel xenotransplantation-compatible model of DLB. The Rag-ASO model provides a unique platform to assess effects of human cell transplantation independently of the known effects of immunosuppressive drugs like cyclosporine and FK506 on  $\alpha$ -syn pathology [27, 28]. Effects of Rag2/il2r $\alpha$  knockdown on  $\alpha$ -syn have been heretofore undescribed, however we demonstrate that the absence of T-, B-, and NK-cells in the CNS does not impact inclusion number or soluble  $\alpha$ -syn protein levels in Rag-ASO mice up to 1 year in age. In contrast, our group recently reported that amyloid- $\beta$  pathology is significantly altered in a Rag2/il2r $\alpha$  null model of Alzheimer's disease [42], suggesting intriguing differences between models of neurodegeneration and the potential impact of the adaptive immune system on disease progression.

One caveat of our studies in the Rag-ASO model is that we did not assess hNPC transplantation in the context of immunosuppression which would typically be required in human patients. However, CNS10-hNPC line has a long history of successful transplantation in multiple models of neurodegeneration, including Huntington's disease (HD), Parkinson's disease (PD), retinal degeneration, and amyotrophic lateral sclerosis (ALS) using cyclosporine immunosuppression [19, 20, 43–45, 50]. Thus, we would anticipate that the need for immunosuppression in human trials (NCT02943850) would be unlikely to slow clinical translation of this approach. The cells show robust survival, extensive migration, and differentiation toward a glial phenotype [22, 25]. Critically, the cells do not display tumor formation or other adverse events in animals including rodents, primates, and pigs [19, 22, 46, 50]. The CNS10-hNPCs have recently been manufactured under cGMP [21], making them now ready for clinical application.

In this current study, the data reveal that preferentially gliogenic CNS10-hNPCs can dramatically improve both motor and cognitive deficits in  $\alpha$ -syn transgenic mice. Importantly, these benefits appear to be achieved by restoring dopaminergic and glutamatergic signaling and reducing soluble  $\alpha$ -syn monomers and

oligomers. Although we hypothesized that CNS10-hNPC transplantation might partially rescue behavioral deficits, motor and cognitive analyses instead yielded robust or nearly complete recovery. This is likely due to the multiple homeostatic mechanisms influenced by CNS10-hNPC transplantation, and the extensive migration of human cells throughout the striatum. CNS10-hNPCs engrafted and demonstrated impressive survival and migration by 3-month post-transplantation, likely due in part to their expression of the chemokine receptor CXCR4 which has been shown by several groups to mediate NPC migration within the brain [47–49]. CNS10-hNPCs that remained near the injection site expressed a more proliferative potential compared to the minimally proliferative phenotype of the disperse populations. After 140 days *in vivo*, Ki-67 expressing CNS10-hNPCs have been shown to diminish to 0.3% [50], suggesting a finite proliferation period that extends just beyond the scope of this study. Therefore, longer-term assessments will be required to fully confirm that these cells migrate and differentiate and do not form masses at later time points in the Rag-ASO model. However, it is important to point out that we observed no examples of proliferating cell clusters that would indicate tumor formation in any of the transplanted mice. Concomitantly, CNS10-hNPCs that had migrated away from the injection site expressed a more glial phenotype. This is as one might expect as cells decrease in proliferative potential as they differentiate. Of note, maturation of glial hNPCs was observed while maturation of early neuronal DCX-positive hNPCs was not. This could reflect that human progenitors require a longer time course for maturation compared to murine progenitors. Alternatively, such a preference could occur due to phenotypic fluidity under transplantation conditions [51], cues promoting astrocytic fate over neuronal in the host milieu [52], or the absence of species-appropriate cues promoting neuronal fate. This largely glial differentiation and enhanced expression of glial glutamate transporter GLT-1 in striatal tissue suggests that CNS10-hNPCs may aid glutamate regulation through a glial-dependent mechanism. It is unlikely that CNS10-hNPCs achieve changes in glutamate regulation through neuronal transport as evidenced by absence of change in EAAC1. This mechanism is also supported by reports that cortical  $\alpha$ -syn can impair cognitive performance through disrupting striatal glutamate transmission [53]. Cognitive performance correlated well with both glutamate and dopamine markers, substantiating the involvement of both neurotransmitter systems in object and spatial recognition [54, 55]. Motor recovery, in contrast, was clearly most strongly associated with dopamine regulation.

Based on our findings in ASO mice with allogeneic mouse NSC transplantation [10], we did not anticipate an impact of CNS10-hNPCs on  $\alpha$ -syn expression. However, our analyses revealed an unexpected decrease of soluble  $\alpha$ -syn monomers and oligomers in response to 3-month CNS10-hNPC transplantation. These results were contrasted by an absence of effects on insoluble  $\alpha$ -syn protein and Lewy body-like aggregates. The finding that total (human and mouse)  $\alpha$ -syn levels were not changed due to CNS10-hNPCs suggests that the human protein overexpressed in ASO mice is specifically targeted by CNS10-hNPCs. Although there are several hypotheses as to the toxic species of the intrinsically disordered  $\alpha$ -syn protein, debate continues regarding which of its many conformers is particularly disruptive to cellular function [5, 56–59]. One contention is that the cytosolic Lewy bodies themselves lead to inflammation and cell death by obstructing essential cellular processes. Another proposes that the toxic species are small soluble oligomers—wild-type or mutated—which react with

membranes to perturb vesicle trafficking, mitochondrial function, and protein turnover. While we find that soluble monomeric and oligomeric  $\alpha$ -syn is reduced by CNS10-hNPC transplantation, this does not suggest that these conformers are innately toxic. Rather, it is likely that an overabundance of these particular  $\alpha$ -syn conformers can have toxic effects. This hypothesis is supported by our observation of substantial  $\alpha$ -syn oligomers in WT mice. Another argument proposes that the toxicity of  $\alpha$ -syn is determined less by accumulation of a particular conformer, and more by the high extracellular concentrations of  $\alpha$ -syn achieved in disease phenotypes that can stimulate inflammation and overwhelm protein degradation and clearance mechanisms [7]. Although this proposition is beyond the scope of the studies reported here, characterization of the mechanisms by which hNPCs lead to reduced soluble  $\alpha$ -syn in ASO mice warrants further investigation. The results we report herein suggest that both soluble monomeric and oligomeric  $\alpha$ -syn contribute to the disruption of dopamine and glutamate neurotransmission that results in drastic cognitive and motor deficits in this model, and that CNS10-hNPC transplantation reduces these while having no effect on insoluble conformers.

Our finding that soluble human- $\alpha$ -syn monomers and oligomers, but not insoluble species are exquisitely altered by CNS10-hNPC transplantation strongly suggests that these changes are most relevant to CNS10-hNPC mediated behavioral recovery. Complementary to this is a recent report suggesting that multiple types of missense mutations in  $\alpha$ -syn can promote a shift from likely native tetramers to monomers [60, 61]. Excess soluble monomers are then free to assemble into  $\beta$ -sheet rich oligomeric intermediates which may participate in cytotoxicity [62]. Total murine and human soluble monomers were clearly not altered by CNS10-hNPC transplantation, and mouse specific mRNA was unaffected by either human- $\alpha$ -syn overexpression or CNS10-hNPCs. Therefore, it is likely that soluble mouse syn—specifically soluble monomers—continue to carry out native functions [63], while the human- $\alpha$ -syn contributes to disease phenotypes in the Rag-ASO mouse model.

## CONCLUSION

The absence of a transplantation effect on Lewy body-like inclusions and insoluble oligomeric species augments the evidence we report in soluble  $\alpha$ -syn protein. The role of Lewy bodies in neurodegeneration is also debated, as their occurrence is not likely sufficient to be a major cause of degeneration [64–66]. It has even been proposed that aggregation of  $\alpha$ -syn into Lewy bodies is an adaptive response to primary insults such as inflammation due to small soluble  $\alpha$ -syn oligomers and an attempt to sequester toxic levels of soluble species [67, 68]. Given these findings, further attention is warranted toward the shift in specific oligomeric

species in models of DLB and the potential use of hNPC transplantation as a mechanism to alleviate cellular stress and improve both dopamine and glutamate neurotransmission and cognitive and motor function. Further safety profiling of CNS10-hNPCs over longer time points of 6 months to 1 year, as well as optimizing survival and functional integration into the host microenvironment will be required to progress this approach toward clinical testing. In addition, testing of CNS10-hNPCs in other models of synucleinopathy including viral  $\alpha$ -synuclein models of Parkinson's disease [69] will no doubt be highly informative and could support additional clinical targets. In summary, the results demonstrate that transplantation of clinically relevant CNS10-hNPCs could offer a promising new therapeutic approach to treat both the motor and cognitive components, along with the biochemical perturbations, of synucleinopathies.

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## AUTHOR CONTRIBUTIONS

N.R.S.G.: conception and design, collection and/or assembly of data, analysis and interpretation, manuscript writing; S.E.M.: collection and/or assembly of data, data analysis and interpretation; J.O.: collection and/or assembly of data, data analysis and interpretation; B.S.: provision of study material; H.D.: data analysis and interpretation; L.M.T.: conception and design; J.S.S.: conception and design; C.N.S.: conception and design. M.B.J.: principal investigator, conception and design, manuscript writing, financial support; All authors: final approval of manuscript.

## DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

The authors indicated no potential conflicts of interest.

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