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Direct Comparison of Rat- and Human-Derived Ganglionic Eminence Tissue Grafts on Motor Function

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Huntington's disease (HD) is a debilitating, genetically inherited neurodegenerative disorder that results in early loss of medium spiny neurons from the striatum and subsequent degeneration of cortical and other subcortical brain regions. Behavioral changes manifest as a range of motor, cognitive, and neuropsychiatric impairments. It has been established that replacement of the degenerated medium spiny neurons with rat-derived fetal whole ganglionic eminence (rWGE) tissue can alleviate motor and cognitive deficits in preclinical rodent models of HD. However, clinical application of this cell replacement therapy requires the use of human-derived (hWGE), not rWGE, tissue. Despite this, little is currently known about the functional efficacy of hWGE. The aim of this study was to directly compare the ability of the gold standard rWGE grafts, against the clinically relevant hWGE grafts, on a range of behavioral tests of motor function. Lister hooded rats either remained as unoperated controls or received unilateral excitotoxic lesions of the lateral neostriatum. Subsets of lesioned rats then received transplants of either rWGE or hWGE primary fetal tissue into the lateral striatum. All rats were tested postlesion and postgraft on the following tests of motor function: staircase test, apomorphine-induced rotation, cylinder test, adjusting steps test, and vibrissae-evoked touch test. At 21 weeks postgraft, brain tissue was taken for histological analysis. The results revealed comparable improvements in apomorphine-induced rotational bias and the vibrissae test, despite larger graft volumes in the hWGE cohort. hWGE grafts, but not rWGE grafts, stabilized behavioral performance on the adjusting steps test. These results have implications for clinical application of cell replacement therapies, as well as providing a foundation for the development of stem cell-derived cell therapy products.

Key words: Huntington's disease (HD); Motor deficits; Cell transplantation; Ganglionic eminence

INTRODUCTION

Huntington's disease (HD) is a neurodegenerative disease caused by an autosomal dominant gene mutation on chromosome 4, which results in an aberrant expansion of a polyglutamine tract within the huntingtin protein. Medium spiny neurons of the striatum are particularly susceptible to the toxic impact of the intracellular aggregated protein inclusions early in the course of disease manifestation, although neuronal cell loss eventually occurs throughout cortical and subcortical brain regions. The most overt symptoms of the disease include motor impairments, such as chorea and rigidity, but cognitive and neuropsychiatric symptoms also manifest early in the course of disease onset. No disease-modifying interventions are available, and current treatment options are restricted to pharmaceuticals that modulate only some specific symptoms.

Cell replacement therapies aim to repair the nervous system by reintroducing the cells that degenerate and thereby restore efferent and afferent projections between cortical and basal ganglia nuclei. To achieve this, the region from which the striatum originates in the developing brain, the ganglionic eminence, is dissected from the fetal brain, dissociated as a cell suspension, and transplanted into the adult host brain, where it matures into the projection neurons and interneurons that comprise the striatum.

Proof of principle of the efficacy of this intervention has been demonstrated in preclinical rat lesion models of HD. In these studies, the whole ganglionic eminence (WGE) was harvested from rat embryos at day

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E14–15 and transplanted into the degenerated striatum of the adult rodent host. Long-term behavioral assessment revealed improvements on tasks of fine motor function (13,25,37) and tests of cognitive performance (11). Importantly, however, these proof-of-concept studies have, almost exclusively, been conducted with rat-derived WGE (rWGE) grafts. As a result, little is known regarding the ability of human-derived fetal striatal (hWGE) grafts to alleviate functional deficits in preclinical models of HD. To date, studies reporting the intrastriatal grafting of hWGE tissue into rodent hosts have predominately described morphological and immunohistochemical data (17,18,29), with much less comprehensive analysis of functionality (28,32).

Despite this, the safety and efficacy of transplanting hWGE tissue has been investigated in early phase clinical trials, in which HD patients received either cell suspension or diced tissue transplants. The results obtained from follow-up assessments administered up to 6 years postgraft have confirmed the safety of the procedure and the survival of metabolically active tissue transplants (14,15,20,31). Interestingly, in one safety study, three out of five grafted patients demonstrated long-term stabilization of performance on motor and cognitive tasks relative to a nongrafted cohort of patients who demonstrated a progressive deterioration of these functions. Moreover, signals were evident on MRI and PET scans that suggest surviving fetal tissue grafts and some evidence of circuitry reconstruction were present (1–3,16). Thus, the hWGE grafts appear to at least partially alleviate symptoms in at least some patients, although the underlying cortical and subcortical neurodegeneration associated with the disease appears not to have been halted, and the observed functional improvements declined over 5–7 years.

Given how little is known regarding the ability of hWGE grafts to improve functional deficits, it is necessary to establish the relationship between the "gold standard" rWGE grafts and hWGE grafts. The aim of this study was to determine whether hWGE grafts might be capable of improving motor function in a manner comparable to rWGE grafts. Moreover, the tests are undertaken in a model involving relatively pure striatal cell loss in the lesioned rats, without the additional extensive cortical degeneration that can compromise interpretation of the clinical studies. Based on the longer maturation times necessary for human-derived cells (18), it was hypothesized that the improvement in motor function observed in rats receiving hWGE grafts would be delayed relative to rWGE grafts, which typically yield significant enhancement of performance from 10 to 12 weeks posttransplant. Thus, in the present study, rats grafted with either rWGE or hWGE were tested up to 21 weeks postgraft on a battery of five tests of motor function.

MATERIALS AND METHODS

Animals

Forty-eight female Lister hooded rats (Harlan, Shardlow, UK) were housed in groups of four per cage and maintained on a 14:10-h light/dark cycle. Rats had ad lib access to food and water, except during staircase testing when rats were maintained at ~90% of their free-feeding weights by providing a set daily portion of laboratory chow. All experiments were conducted in compliance with the UK Animals (Scientific Procedures) Act 1986 under Home Office License No. 30/3036 and with the approval of the local Cardiff University Ethics Review Committee.

Experimental Design

Rats ($n = 48$) were pretrained on the staircase task for 3 weeks before a subset ($n = 39$) of rats received unilateral quinolinic acid (Sigma-Aldrich, Dorset, UK) lesions to the striatum. Two weeks postlesion, all rats were tested on the staircase task for 5 days, and baseline functional impairments were also measured on the adjusting steps test, vibrissae test, cylinder test, and apomorphine (Sigma-Aldrich)-induced rotation test (see below). Lesioned rats were divided into three equal groups based on performance on the staircase and apomorphine-induced rotation tests. Rats then either remained as lesion-only controls ($n = 9$) or received rWGE grafts ($n = 14$) or hWGE grafts ($n = 16$). Commencing 4 weeks postlesion, daily cyclosporine (Sandoz Pharmaceutical, Surrey, UK) injections were initiated for all rats. Rats receiving rWGE or hWGE grafts underwent transplant surgery the following day. Testing on the staircase task commenced 10 weeks postgraft and continued weekly up to 20 weeks postgraft. Apomorphine-induced rotations were undertaken at 15, 18, and 21 weeks postgraft. Performance on the stepping, vibrissae, and cylinder tests was assessed at 20 weeks postgraft. At 21 weeks postgraft, rats were perfused, and tissue was taken for immunohistochemical analyses.

Surgical Procedures

Lesions. Rats were anesthetized with isoflourane (2–4% with carrier gases oxygen and nitrous oxide; TEVA UK Ltd., Eastbourne, UK) in a stereotaxic frame. The lateral striatum was targeted with 4 × 0.25-µl injections of 0.12 M quinolinic acid using the following stereotaxic coordinates: 1) AP −0.4, ML −3.7 (from bregma), DV −5.0/−4.0 (from dura) and 2) AP +1.2, ML −2.9, and DV −5.0/−4.0. All rats received Metacam (Boehringer Ingelheim, Ingelheim am Rhein, Germany) for pain relief, and diazepam (Hameln Pharmaceuticals, Gloucester, UK) was administered subcutaneously immediately after the surgical session.

Transplantation of rWGE. The uterine horn was removed from terminally anesthetized time-mated Lister hooded rats by Cesarean section at embryonic day 14 (E14) and placed into a sterile dish of Dulbecco's modified Eagle medium (DMEM; Life Technologies/ThermoFisher, Carlsbad, CA, USA). The brain was removed from each embryo, and WGEs were subsequently dissected out bilaterally into fresh DMEM. For cell preparation, WGEs were washed twice in DMEM/F12 (Life Technologies) then incubated at 37°C for 20 min in TrypLE Express (Life Technologies) containing 20 U/ml dornase alfa (Pulmozyme; Roche, Hertfordshire, UK). Tissue was dissociated by trituration in 200 µl of DMEM/F12 to obtain a quasi-single-cell suspension. Cell viability was assessed by Trypan blue exclusion (Life Technologies) in a hemocytometer and revealed that ~96% of all cells were viable.

Transplantation of hWGE. Human fetal tissue was collected from medical terminations of pregnancy with full donor consent, through the SWIFT human fetal tissue bank (http://www.biobankswales.org.uk/swift-research-tissue-bank), under UK Human Tissue Authority research license (No. 12457) held by Cardiff University and with ethical approval of the project from the Bro Taf local research ethics committee. Gestational age was estimated through ultrasound scan prior to the procedure in combination with measurement of fetal regions (12). The human WGE tissue was harvested from three fetuses with a mean (and standard deviation) crown–rump length of 33.6 ± 4.6 mm, which corresponds to approximately 10 weeks of gestation (8 weeks postconception). Embryos were not pooled; instead, each rat received tissue from one of the three donors. Tissue was incubated at 37°C for 20 min in TrypLE Express containing 20 U/ml dornase alfa. Tissue was dissociated by trituration in 200 µl of DMEM/D to obtain a quasi-single-cell suspension. The viability for each sample was estimated as a mean of 85.3 ± 4.9%.

For both the rWGE and hWGE grafts, each rat received a 2-µl suspension containing approximately 500,000 cells in total. Cells were implanted at a rate of 1 µl/min and distributed over two stereotaxic depths (−4.5, −3.7) at the site AP: +0.4, ML: −3.2 mm.

Behavioral Testing

All behavioral testing was conducted blind to the treatment of the rats.

Apomorphine-Induced Rotations. A bank of 16 automated rotometer bowls (Rotorat; Med Associates, St. Albans City, VT, USA) recorded the frequency of clockwise and anticlockwise rotations. Apomorphine-induced rotations were recorded for 60 min after subcutaneous injection of 1.0 mg/kg apomorphine hydrochloride hemihydrate (Sigma-Aldrich). Rotation scores were expressed as the net (ipsilateral minus contralateral) rotations over the test session. Rotational bias was tested postlesion and at 15, 18, and 21 weeks postgraft.

Cylinder Test. Forelimb asymmetry was measured using the cylinder test (34). Rats were placed in a clear Perspex cylinder, in front of a three-sided mirror (allowing simultaneous all round observation of rat behavior), and monitored for up to 5 min while they explored the restricted environment until they achieved approximately 30 paw touches. Behavior was captured via a video recorder, and the number of times the rat touched the side walls with either the ipsilateral or contralateral paw was counted from video images. Cylinder data were collected on two occasions: at 2 weeks postlesion and at 20 weeks posttransplantation. Data are expressed as the number of contralateral touches as a percentage out of the total number of touches made using both paws.

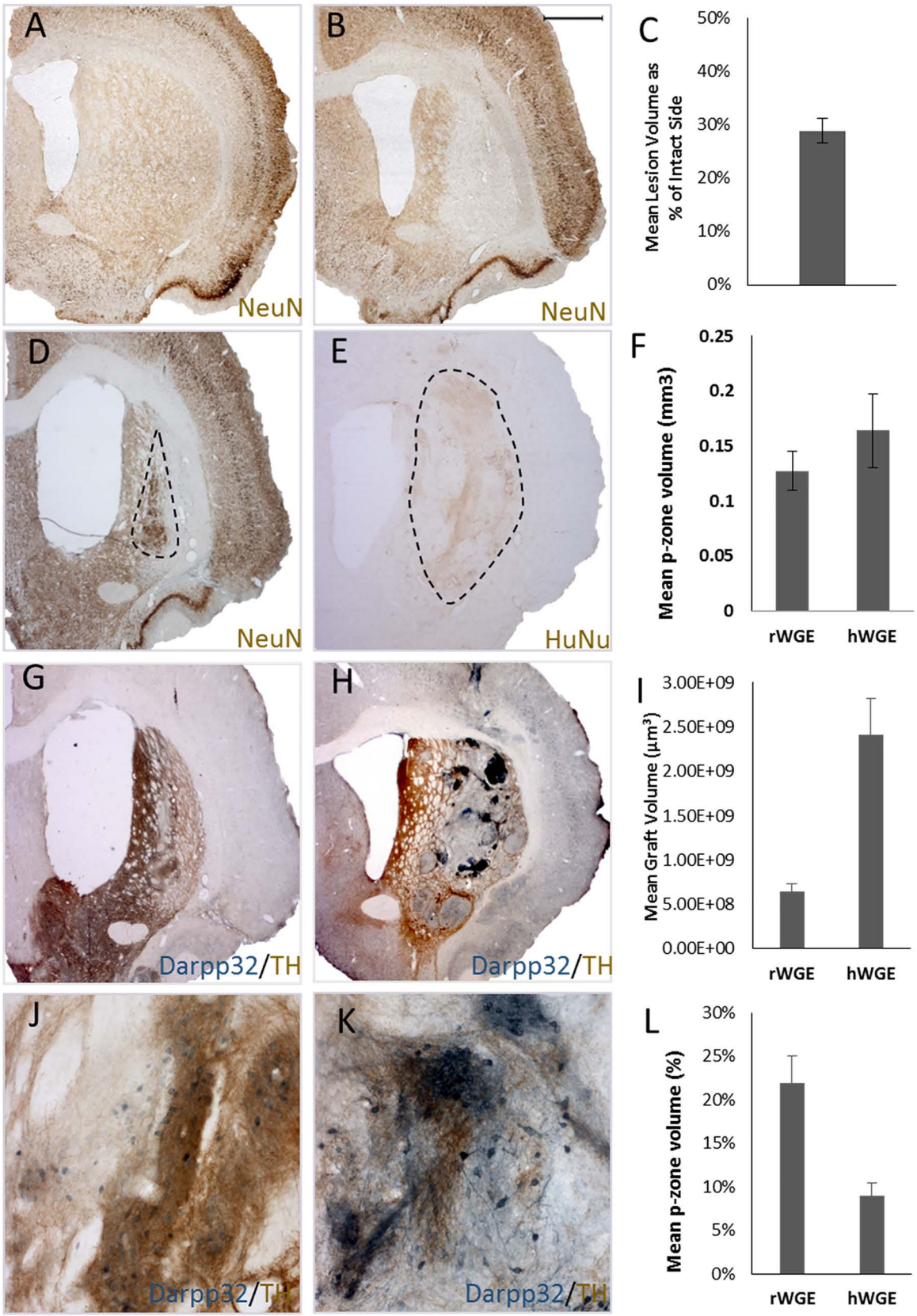
Staircase Task. Lateralized paw dexterity was measured in the staircase test (24). Rats were food restricted to approximately 90% of their free-feeding body weight for testing. The seven steps on either side of the central plinth were each baited with two sugar pellets (45 mg). All rats were pretrained on the reaching task over 15 days prior to surgery and then tested for lesion effects over 5 days at 2 weeks postlesion. Finally, rats were tested for 15 min each day for 50 days from 10 to 20 weeks postgraft. The number of successful retrievals was recorded separately for the ipsilateral and contralateral sides. Data are presented as the mean number of successful retrievals the rat made with its contralateral and ipsilateral paws over the final 20 days of testing (16–20 weeks posttransplantation).

Vibrissae-Evoked Forepaw Placing Test. In this task of spontaneous sensorimotor function (34,35), rats were lifted upward toward the edge of a table so that only the vibrissae would touch the table edge. An unoperated control rat will reflexively place its free forepaw onto the table surface as soon as the vibrissae contact the table surface. This test was performed 10 times per side during each experimental day.

Adjusting Steps Test. Forelimb akinesia was assessed using the adjusting steps test (27). This involved restraining one forelimb and counting the number of adjusting steps made by the unrestrained forelimb when a rat was moved sideways along a table surface for 100 cm in either a medial or lateral direction with respect to the unrestrained paw. Stepping data were collected postlesion and at 21 weeks postgraft. The data presented are the number of adjusting steps made with the contralateral paw in forward and backward trajectories.

Immunohistochemical Techniques

Perfusion. Rats were terminally anesthetized with sodium pentobarbital (Euthatal; Merial, Woking, UK) and sacrificed by transcardial perfusion with 0.01 M phosphate buffer followed by 1.5% paraformaldehyde (pH 7.4;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L
Mean p-zone volume (%)
30%
25%
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10%
5%
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rWGE
hWGE

Sigma-Aldrich). Brains were postfixed for 24 h in 1.5% PFA before being transferred to 25% sucrose solution. Tissue was sectioned on a freezing sledge microtome at 40 μm thickness in a 1:12 series.

Bright-Field and Fluorescent Immunohistochemistry. Free-floating sections were blocked in 3% normal serum, then incubated in primary antibody with 1% serum overnight at room temperature. Tissue was incubated in secondary antibody (1:200) with 1% serum for 3 h, then immersed in an avidin-biotinylated enzyme complex (Vector Laboratories, Peterborough, UK) for 2 h, then stained with either DAB or Vector SG (Vector Labs).

The primary antibodies used were DARPP-32 (human specific, 1:1,000; Abcam, Cambridge, UK); DARPP-32 (1:30,000; kind gift from Cornell University), tyrosine hydroxylase (TH, 1:1,000; Millipore, Watford, UK), β-tubulin (1:500; Sigma-Aldrich, Poole, UK), NeuN (1:1,000; Millipore), and HuNu (1:1,000; Millipore).

Statistics

Statistical analyses were conducted using SPSS (v20; IBM, Armonk, NY, USA). Using analysis of variance (ANOVA) tests, the between-subjects factor of Group and within-subjects factor of Time were used. Graphical data are represented as means and standard error of the means (SEM). Newman–Keuls method was used for post hoc analyses. Results were deemed to be statistically significant with a value of $p < 0.05$.

RESULTS

Histological Analyses

Histological analysis of the brain tissue revealed large lateral lesions that comprised approximately 30% of the right hemisphere (Fig. 1A–C) and resulted in an increased ventricular volume. In the graft groups, 11/14 rWGE and 10/13 hWGE grafts survived to 21 weeks post-transplantation. rWGE grafts (Fig. 1D) were smaller on average than hWGE grafts [$F(1, 19) = 19.25$, $p < 0.001$] (Fig. 1E, I). DARPP-32 expression was observed in all grafts (Fig. 1G, H, and J–L), distributed with a patchy staining ["P-zones" (19)]. The mean P-zone volume was similar between rWGE and hWGE grafts overall ($F < 1$, $p =$ n.s.), but the proportion of P-zone (as a percentage of graft volume) was greater in rWGE than in hWGE grafts [$F(1, 19) = 5.45$, $p < 0.05$] (Fig. 1F and L). Considerable innervation of dopaminergic fibers was evident in both rWGE (Fig. 1G and J) and hWGE grafts (Fig. 1H and K). Thus, similar rates of survival and TH innervation were observed in both graft groups, although differences in graft volume and DARPP-32 expression were evident.

Behavioral Analyses

Apomorphine-Induced Rotations. Rotational bias data were analyzed by ANOVA with Time (Baseline, 15 weeks, 18 weeks, and 21 weeks postgraft) and Group as factors (Fig. 2A). Unlike lesioned and grafted rats, control rats did not rotate at any of the time points. At the postlesion baseline phase, there was no difference between the rotational bias of Lesion, rWGE, and hWGE grafted rats [Time × Group: $F(9, 99) = 6.39$, $p < 0.001$; Effect of Group at Baseline: $F(3, 33) = 28.41$]. However, at 15, 18, and 21 weeks postgraft, rWGE and hWGE rats were rotating significantly less than Lesion rats [Effect of Group at weeks 15–21: minimum $F(3, 33) = 47.49$; Lesion vs. rWGE and hWGE rats, all $p \leq 0.01$]. At all time points, rWGE and hWGE grafted rats performed comparably (rWGE vs. hWGE, n.s.) and control rats differed significantly to all other groups [Group: $F(3, 33) = 76.13$, all $p < 0.001$]. Thus, rWGE and hWGE induced a comparable reduction in apomorphine-induced rotational bias.

Cylinder. Analysis of the percentage of contralateral paw use in the cylinder test revealed no effect of Group, nor any differences between the postlesion and postgraft time points [maximum: $F(3, 33) = 2.34$, n.s.] (Fig. 2B).

Staircase. Data from the staircase test were analyzed separately for the ipsilateral and contralateral sides, with Group and Time (postlesion vs. postgraft) as factors (Fig. 2C). On the ipsilateral side, Lesion rats performed significantly worse than Control rats, but grafted rats did not differ from either the Lesion or Control groups [Group: $F(3, 33) = 3.30$, $p < 0.05$; Control vs. Lesion: $p < 0.05$]. On the contralateral side, Control rats performed significantly better than all other groups, but no differences were evident between the lesion and grafted rats [Group: $F(3, 33) = 20.66$, $p < 0.001$; Control vs. Lesion, rWGE, hWGE: all $p < 0.001$].

Performance improved relative to baseline for Control, rWGE, and hWGE rats, on both the ipsilateral and contralateral paws [minimum $t(9) = 2.28$, $p < 0.05$] (Fig. 2D), except for Lesion rats, which did not meet conventional levels of significance [$t(6) = 2.07$, $p = 0.075$, and 2.15, $p = 0.084$, for the ipsilateral and contralateral sides, respectively].

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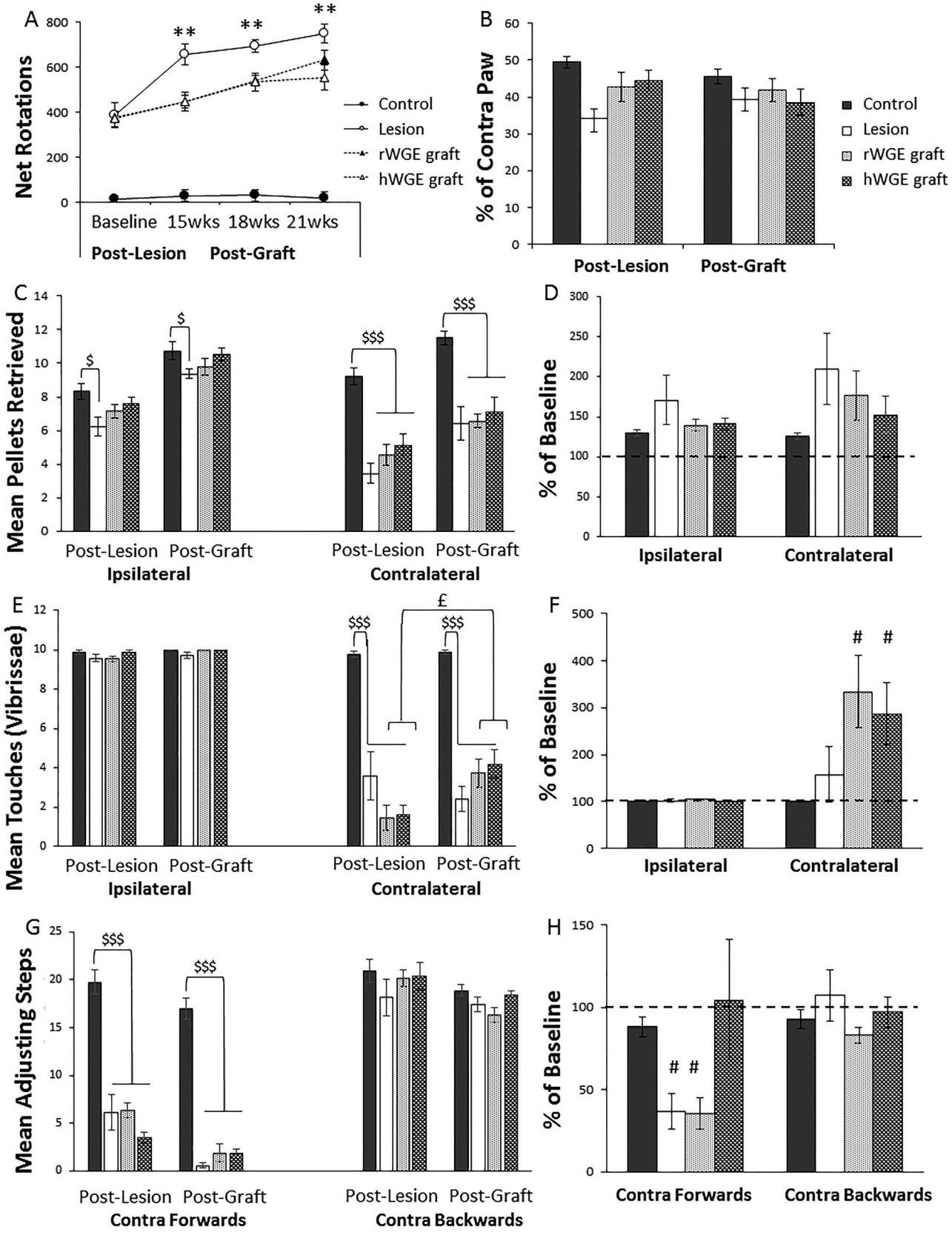
Figure 1. Immunohistochemical analysis of rat- and human-derived WGE grafts at 21 weeks. Representative images of Control (A) and Lesion (B) brain tissue, stained with NeuN. Representative images of a rWGE graft (D) stained with NeuN and a hWGE graft (E) stained with HuNu. DARPP-32 staining (blue) and TH staining (brown) in a rWGE graft (G) and a hWGE graft (H). Higher magnification of TH innervation in a rWGE (J) and a hWGE (K) graft. Mean percentage of lesion area, relative to the intact tissue (C) and mean P-zone area in mm^3 for rWGE and hWGE grafts (F). Mean graft area (I) and the mean P-zone volume, as a percentage of total graft volume (L) for rWGE and hWGE grafts. Scale bar: 1,000 μm. Error bars: standard error of the mean (SEM).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Vibrissae Test. Data from each side were analyzed with Time (postlesion, postgraft) and Group as factors. On the ipsilateral side, Lesion rats performed significantly worse than Control rats [Group: $F(3, 33) = 4.00$, $p < 0.05$; Control vs. Lesion, $p < 0.05$] (Fig. 2E), but no differences were observed in the grafted rats. On the contralateral side, Control rats performed significantly better than all other groups [Group: $F(3, 33) = 37.38$, $p < 0.001$; Control vs. Lesion, rWGE, hWGE: all $p < 0.001$]. Interestingly, however, only rWGE and hWGE grafted rats demonstrated improved performance at the postgraft time point relative to their postlesion performance [Group × Time: $F(3, 33) = 3.02$, $p < 0.05$; Effect of Time for rWGE and hWGE, minimum $F(1, 33) = 6.33$, $p < 0.05$].

To explore this further, we asked to what extent postgraft performance had changed relative to postlesion baseline performance. The results reveal no change in performance on the ipsilateral side (all groups compared 100%, n.s.) (Fig. 2F). On the contralateral side, however, the performance of rats grafted with both rWGE and hWGE significantly improved relative to their postlesion baseline [$t(10) = 3.02$ and $t(9) = 2.82$, for rWGE and hWGE, respectively, both $p < 0.05$; n.s. for Control and Lesion].

Adjusting Steps Test. Data from the ipsilateral and contralateral paws were analyzed with Time (postlesion, postgraft) and Group as factors. No effects of lesion or graft were evident in the forward or backward adjusting steps made using the ipsilateral paw (data not shown). Analysis of the forward steps on the contralateral side revealed a significant effect of the lesion on performance, but no overt recovery was evident from the rWGE or hWGE grafts [Group: $F(3, 33) = 77.33$, $p < 0.001$; Control vs. Lesion, rWGE, hWGE, all $p < 0.001$] (Fig. 2G). Overall, there was also a reduction in the number of adjusting steps during the postgraft test phase, relative to the postlesion phase [Time: $F(1, 33) = 36.26$, $p < 0.001$].

No effect of striatal lesion was evident on the backward steps using the contralateral paw, nor any effect of the grafts [Group: $F(3, 33) = 1.13$, n.s.].

To investigate further the change in performance over time, we compared postgraft performance to the postlesion baseline. Results revealed a significant reduction in the number of forward adjusting steps for Lesion and rWGE graft groups, which was not evident in the Control or hWGE-grafted rats [$t(6) = -5.27$ and $t(10) = -6.61$, both $p < 0.01$, Lesion and rWGE, respectively; Control and hWGE, n.s.].

Correlations. Interestingly, no significant correlations were evident between total P-zone or total graft volume, and performance on any of the behavioral tasks at week 20 (data not shown). However, an expected correlation was found between P-zone volume and graft volume ($r = 1.000$, $n = 21$, $p < 0.000$).

DISCUSSION

The aim of this study was to compare directly the functional efficacy of rWGE and hWGE grafts on tasks of motor function to determine whether clinically relevant hWGE grafts alleviated motor impairments to the same extent as the gold standard rWGE grafts. The data reveal subtle but comparable improvements in behavioral performance in rats grafted with rWGE and hWGE primary tissue, despite significant differences in the total volume of surviving graft tissue. The implications of these results are discussed in greater detail below.

Immunohistochemical Analysis of rWGE and hWGE Grafts

Although postmortem immunohistochemical examination verified the survival of intrastriatal grafts in each cohort up to 21 weeks, differences in the volume of surviving graft tissue was evident. rWGE grafts were of a modest size, surviving within the degenerated lateral neostriatum of the host brain, but not demonstrating complete replacement of all the lesioned tissue. In contrast, hWGE grafts were of significantly larger volume, filling the lesioned area. Both rWGE and hWGE grafts presented with DARPP-32-positive P-zones, which indicates replacement of the appropriate GABAergic medium spiny neurons within the degenerated brain, although the proportions of P-zones, relative to the total graft volume, were greater in the rWGE grafts. Moreover, clear indication of circuitry reconstruction was evident in the innervation of tyrosine hydroxylase-positive projections from the substantia nigra onto the grafted rat and human fetal neurons.

Impact of rWGE and hWGE Grafts on Behavioral Performance

Apomorphine-Induced Rotational Bias. The apomorphine-induced rotational bias test is used as a simple and robust measure to reveal the loss of GABAergic medium spiny neurons in the striatum and the effect of any

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Figure 2. Behavioral impact of rWGE and hWGE grafts. Raw data showing performance of Control, Lesion, rWGE, and hWGE cohorts on apomorphine-induced rotational bias (A), cylinder test (B), staircase test (C), vibrissae-evoked forepaw placing test (E), and adjusting steps test (G). Data are also represented as the percentage change, relative to the baseline (postlesion) phase for staircase (D), vibrissae-evoked forepaw placing test (F), and adjusting steps test (H). $^{**}p \leq 0.01$ for Lesion versus rWGE and hWGE comparisons. $\$p \leq 0.05$, $\$\$\$p \leq 0.001$ for Control versus any experimental groups. $£p \leq 0.05$ for effect of time (postlesion vs. postgraft comparisons). $\#p \leq 0.05$ for comparisons to baseline performance (100%).

restorative intervention on effective striatal reconstruction. Previous studies have reported mixed results, with evidence of recovery after the grafting of E15 WGE but only in animals that received targeted behavioral training (10); E14 from the lateral, but not whole or medial, GE (26,37); and E13, but not E14 or E15, WGE grafts (33). To date, the only behavioral recovery reported using human-derived GE grafts has utilized apomorphine-induced rotations to assess functionality (28,32). In the present study, recovery was induced in rats grafted with E14 rWGE and hWGE, at all (15–21 weeks) postgraft time points. The magnitude of recovery was also comparable between rWGE and hWGE grafts, despite differences in graft volume. Interestingly, apomorphine-induced rotations have also been effective in revealing the functional efficacy of human stem cell-derived neuronal cells in rodent models of HD (6,36).

Of note is the increase in rotations observed across all lesioned groups at the postgraft stage relative to the postlesion stage. This increase could be explained by an interaction between cyclosporine and apomorphine activity. Since cyclosporine inhibits CYP3A4 and P-glycoprotein, and apomorphine is a substrate of these, the net result is increased plasma levels of apomorphine when administered alongside cyclosporine, leading to the increased rotational bias effect.

Cylinder Test. The cylinder test was utilized in the present study based on previous evidence that rWGE graft-mediated improvements have been observed using this task with E15 and E13 WGE transplants (8,33). In this study, however, the behavioral impact of the lesion was not sufficient to allow investigation of any impact of the rWGE or hWGE graft interventions.

Staircase Test. It is well established that rWGE grafts are capable of alleviating gross motor impairments (9,10) and improving fine motor control (22). The most frequently reported improvements are observed on the staircase test, although it is well established that extensive training and relearning to use the graft are essential prerequisites to observing graft-mediated recovery. It has been reported that grafts derived from both E14 and E15 WGE are capable of improving skilled paw-reaching ability in the staircase test (8,9,10,13) and that the extent of recovery depends upon the proportion of DARPP-32-positive P-zones present (25). However, recovery on this task has not always been consistent. Where a lack of graft-mediated recovery has been observed in paw reaching, the inclusion of an environmental enrichment manipulation has been necessary to improve performance (7). In other circumstances, the E14 medial GE has been found to induce recovery more effectively than the E14 WGE or lateral GE (37). Occasionally, no graft-mediated recovery is evident on this task (38). The lack of improvement observed in our data set may relate to unidentified differences in graft composition or integration. The effect of daily cyclosporine on recovery of performance is not well understood and should be considered, although despite the immunosuppression regimen, all rats did continue to respond vigorously in the task each day. Alternatively, it may be explained by the reduced volume of rWGE grafts, although despite their greater volume, hWGE grafts also failed to improve paw-reaching performance.

Vibrissae-Evoked Forepaw Placing Test. The vibrissae-evoked forepaw placing test is a test of spontaneous sensorimotor function that is used fairly infrequently in the context of HD but which has previously revealed the functional efficacy of viable grafted cells. For example, Klein and colleagues (22) demonstrated recovery of motor function using the vibrissae test after transplantation of E15 WGE grafts into a rat model of HD. Our study further supports these results by revealing improvement in spontaneous reaching after vibrissae stimulation both in rats grafted with rWGE and hWGE tissue.

Adjusting Steps Test. Previous studies have reported improved performance on the adjusting steps test after transplantation of E15 WGE (8), although a lack of recovery has also been reported using the same tissue source (22). Similar to Klein et al. (24), we observed a decrease in stepping behavior over time in lesioned rats. Interestingly, however, while rats grafted with rWGE also demonstrated a decrease in adjusting steps between the postlesion and postgraft test phases, rats grafted with hWGE maintained a stable level of performance between the testing time points. Although this stabilization of behavior does not amount to improved performance per se, it is noteworthy that data gathered from clinical trials with patients with HD has often revealed a stabilization of behavior in grafted patients, relative to the deterioration observed in ungrafted control patients (4,20,31). That is, patients' performance on a task is maintained and does not demonstrate the natural decline observed in ungrafted patients. Thus, these preclinical results may parallel those observed clinically.

hWGE in Preclinical Studies

Few preclinical studies have investigated the functional efficacy of human-derived GE grafts. Several studies report the cellular profiles of hWGE grafts, demonstrating medium spiny neuron maturation and noting that the percentage of P-zone formation is less in hWGE than in rWGE grafts (17,28). Human striatal grafts have also been shown to form long efferent and afferent connections with the host brain (5,21,28,39). However, behaviorally, only two papers report recovery of function posttransplantation of hWGE. Pundt et al. (28) report a

reduction in apomorphine-induced rotation starting from 50 days posttransplant after intrastriatal transplantation of WGE from a fetus measuring 110 mm, which is equivalent to 14 weeks gestation. Similarly, human fetal tissue of 7–9 weeks postconception was found to reduce apomorphine-induced rotational bias as early as 1 month postgraft (32). Thus, the data presented here represent the first comprehensive investigation of the functional efficacy of hWGE grafts. Our data support the previous literature on hWGE grafts and apomorphine rotations and extend our knowledge by demonstrating effects on vibrissae-evoked touching and adjusting steps.

Although not directly tested here, the review of the preclinical literature raises questions as to the optimal cells necessary for behavioral recovery. The WGE, which consists of medial GE containing interneurons and lateral GE, is the most frequently investigated source of tissue. However, it is not clear to what extent the medial GE is necessary to support the medium spiny neurons arising from the WGE. Conflicting results have been obtained, for example, using the staircase test in which the proportion of DARPP32-positive cells has been reported to correlate with behavioral recovery (25), while others observe relatively more recovery using medial GE than lateral GE or WGE (37). It will be critical to address such questions as the field progresses toward the effective development of ES-derived medium spiny neurons for transplantation.

hWGE in Clinical Studies

Most clinical studies conducted thus far have endeavored to test the safety and feasibility of grafting hWGE tissue into patients and to establish the optimal parameters for clinical application of this therapeutic intervention. Where assessment of the functional efficacy of the grafts has been undertaken, interesting results have so far been obtained. For example, Hauser et al. (20) reported a transient and mild stabilization of motor scores on the UHDRS at 12 months posttransplant using LGE harvested from fetuses of 8–9 weeks postconception. Patients in the mild-moderate stages of disease grafted with tissue from fetuses of 8–12 weeks postconception also demonstrated a slowed progression of the disease in the early stages, despite no long-term improvement (up to 10 years) in the grafted group relative to a control, ungrafted cohort (4,31). The authors suggest that too few cells were grafted to provide sufficient clinical benefit.

Importantly, however, Bachoud-Levi and colleagues (1,2) observed improvements in motor and cognitive functions up to 2 years postgraft, followed by stabilization for a further 4 years on tasks of motor function and for 6 years on cognitive tasks in a subset of grafted patients. Similarly, it has been shown that that one out of two patients showed increased striatal D2 receptor binding on the PET scan and demonstrated sustained clinical improvement up to 5 years postgraft (30).

rWGE Versus hWGE Grafts

Previous studies utilizing hWGE have only reported functional efficacy in vivo using the apomorphine rotation task, while an abundance of studies have reported improvements in a range of motor tests, as well as enhanced cognitive function, after grafting of rWGE. Interestingly, our data suggest comparable improvements on the apomorphine rotation test and vibrissae task in both graft groups, but only hWGE stabilized performance on the adjusting steps test.

Fundamental differences exist in the development of the striatum of the rat and the human, which will undoubtedly impact upon the rates of maturation and integration of these ganglionic eminence grafts. For example, the proportion of P-zone volume in rWGE grafts has been found to be greater than that observed in hWGE grafts (18), and the maturation of hWGE grafts is slower than rWGE (17,18,23). Thus, the optimal stage for utilizing hWGE for functional recovery still remains to be definitively determined and the effect of differing maturation profiles on functional efficacy is not yet well understood.

CONCLUSION

The data presented here confirm the ability of rWGE grafts to reduce rotational bias and improve sensorimotor function in the vibrissae task, but in contrast, no improvements in paw reaching were observed on the staircase task, despite clear effects of the lesion on performance for all groups. Interestingly, we observed similar recovery in rats grafted with hWGE, with the additional benefit of the hWGE grafts maintaining performance on the adjusting steps test. These data are, to our knowledge, the first direct comparison of rWGE and hWGE grafts on motor function, as well as the first comprehensive, preclinical investigation into the functional efficacy of hWGE. While further investigation into the ability of these cells to improve cognitive functions is warranted, the results presented here support the continued evaluation of this cell source (alongside other pluripotent stem cells) for clinical translation to develop novel cell therapies that can stabilize or alleviate motor impairments in people with HD.

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