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Supplemental Information

ESC-Derived BDNF-Overexpressing Neural Progenitors Differentially Promote Recovery in Huntington's Disease Models by Enhanced Striatal Differentiation

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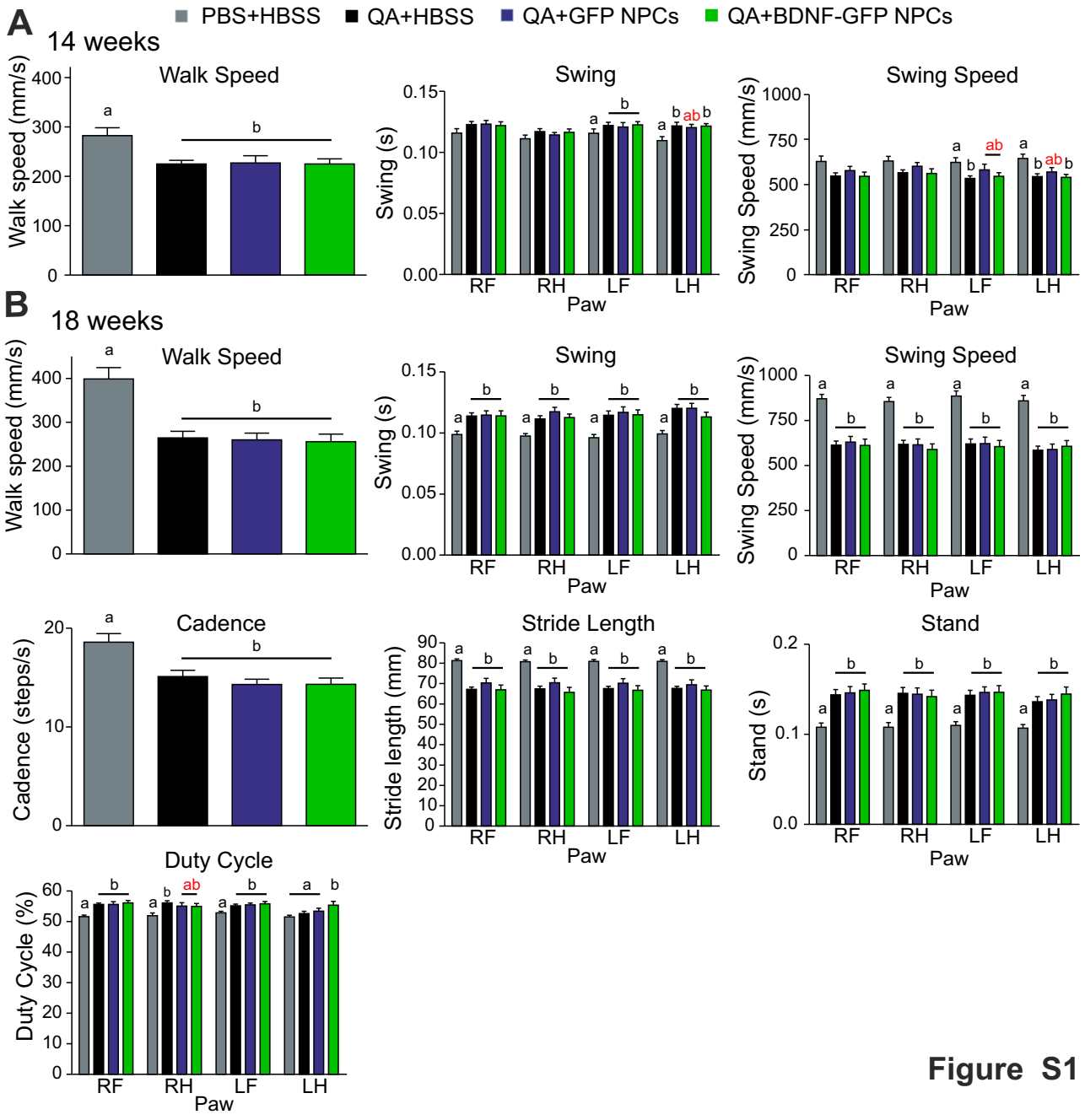
A
PBS+HBSS
QA+HBSS
QA+GFP NPCs
QA+BDNF-GFP NPCs
14 weeks
Walk Speed
Walk speed (mm/s)
Swing
Swing (s)
Swing Speed
Swing Speed (mm/s)
RF
RH
LF
LH
Paw
B
18 weeks
Walk Speed
Swing
Swing Speed
Cadence
Cadence (steps/s)
Stride Length
Stride length (mm)
Stand
Stand (s)
Duty Cycle
Duty Cycle (%)

Figure S1

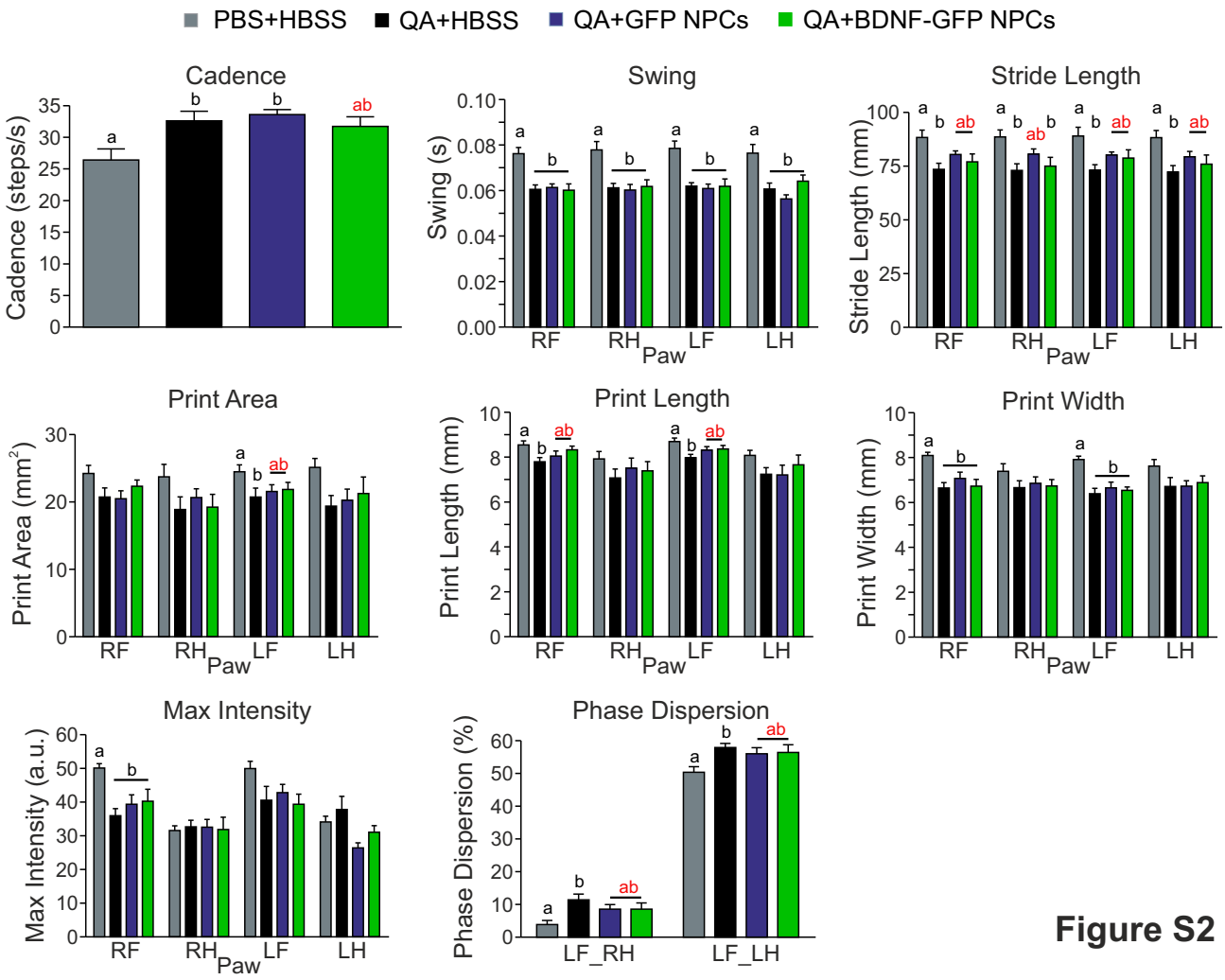
PBS+HBSS
QA+HBSS
QA+GFP NPCs
QA+BDNF-GFP NPCs
Cadence
Cadence (steps/s)
Swing
Swing (s)
Stride Length
Stride Length (mm)
Print Area
Print Area (mm²)
Print Length
Print Length (mm)
Print Width
Print Width (mm)
Max Intensity
Max Intensity (a.u.)
Phase Dispersion
Phase Dispersion (%)
RF
RH
LF
LH
Paw
LF_RH
LF_LH

Figure S2

Supplemental Figure Legends

Figure S1, related to Figure 3. Affected CatWalk parameters of QA lesioned mice. Detailed graphs for the table in Figure 3E. An influence of NPCs can be seen at 14 weeks of age (A) and 18 weeks of age (B). One-way ANOVA with post-hoc Tukey's test between groups for each paw (n=10-12). Data represent mean ± SEM. Values of bars with a different letter (a or b) were significantly different ($p<0.05$) from each other. Values of bars with the same letter (a or b) were not significantly different from each other, hence values of bars marked with "ab" (marked in red) are not significantly different from either a or b, thereby indicating a partial/incomplete rescue of the phenotype. RF= right forepaw, RH= right hindpaw, LF= left forepaw, LH= left hindpaw.

Figure S2, related to Figure 5. Affected CatWalk parameters in the N171-82Q mouse model. Detailed graphs for the table in Figure 5E. Transplanted NPCs had a lasting effect on transgenic compared to wildtype mice. One-way ANOVA with post-hoc Tukey's test between groups for each paw (n=10-12). Data represent mean ± SEM. Values of bars with a different letter (a or b) were significantly different ($p<0.05$) to each other. Values of bars with the same letter (a or b) were not significantly different from each other, hence values of bars marked with "ab" (marked in red) are not significantly different from either a or b, which indicates a partial/incomplete rescue of the phenotype. RF= right forepaw, RH= right hindpaw, LF= left forepaw, LH= left hindpaw.

QA Mouse Model	parameters significant different (p≤0.05)	Paw	PBS+HBSS	QA+HBSS	p vs PBS+HBSS	QA+GFP NPCs	p vs PBS+HBSS	QA+BDNF-GFP NPCs	p vs PBS+HBSS
14 weeks	Walk speed [mm/s]		282.4 ± 16.17	225.3 ± 7.35	p=0.009	227.1 ± 14.89	p=0.018	225.3 ± 10.28	p=0.013
	Swing [s]	LH	0.1095 ± 0.003203	0.1215 ± 0.003048	p=0.018	0.1201 ± 0.00271	p=0.057	0.1214 ± 0.00201	p=0.027
	Swing speed [mm/s]	LF	623.7 ± 25.98	534.7 ± 14.03	p=0.038	582.00 ± 31.69	p=0.109	546.59 ± 19.6	p=0.596
		LH	645.7 ± 24.21	544.4 ± 16.00	p=0.005	570.00 ± 24.96	p=0.067	540.22 ± 17.16	p=0.005
18 weeks	Walk speed [mm/s]		399.0 ± 25.82	264.6 ± 15.08	p=0.0001	259.9 ± 15.65	p=0.0001	255.9 ± 17.36	p=0.0001
	Swing [s]	RF	0.09871 ± 0.0027	0.1138 ± 0.0026	p=0.009	0.1146 ± 0.0034	p=0.005	0.1140 ± 0.0041	p=0.008
		RH	0.09744 ± 0.0019	0.1114 ± 0.0026	p=0.004	0.1179 ± 0.0037	p=0.0001	0.1125 ± 0.0029	p=0.002
		LF	0.09600 ± 0.0027	0.1144 ± 0.0035	p=0.004	0.1169 ± 0.004	p=0.001	0.1148 ± 0.0039	p=0.003
		LH	0.09924 ± 0.0028	0.1201 ± 0.0032	p=0.001	0.1202 ± 0.0040	p=0.0001	0.1131 ± 0.0039	p=0.003
	Swing speed [mm/s]	RF	871.0 ± 24.65	614.0 ± 22.63	p=0.0001	630.4 ± 32.02	p=0.0001	611.8 ± 34.87	p=0.0001
		RH	855.2 ± 23.76	618.3 ± 21.71	p=0.0001	615.7 ± 32.49	p=0.0001	589.2 ± 31.28	p=0.0001
		LF	885.8 ± 28.56	620.6 ± 27.23	p=0.0001	621.9 ± 35.88	p=0.0001	605.7 ± 34.01	p=0.0001
		LH	859.6 ± 30.24	585.7 ± 22.36	p=0.0001	589.4 ± 30.07	p=0.0001	607.3 ± 31.56	p=0.0001
	Cadence [steps/s]		18.6 ± 0.86	15.11 ± 0.62	p=0.005	14.31 ± 0.52	p=0.0001	14.32 ± 0.63	p=0.0001
	Stride length [mm]	RF	81.28 ± 0.7961	67.13 ± 1.185	p=0.0001	70.30 ± 2.267	p=0.0001	66.94 ± 2.310	p=0.0001
		RH	80.73 ± 0.8576	67.37 ± 1.380	p=0.0001	70.39 ± 2.268	p=0.001	65.65 ± 2.505	p=0.0001
		LF	80.91 ± 0.8731	67.55 ± 1.093	p=0.0001	70.18 ± 2.230	p=0.0001	66.72 ± 2.214	p=0.0001
		LH	80.87 ± 0.8301	67.60 ± 1.086	p=0.0001	69.43 ± 2.380	p=0.0001	66.77 ± 2.073	p=0.0001
	Stand [s]	RF	0.1079 ± 0.0047	0.1440 ± 0.0057	p=0.001	0.1461 ± 0.0069	p=0.0001	0.1489 ± 0.0070	p=0.0001
		RH	0.1079 ± 0.0052	0.1456 ± 0.0064	p=0.001	0.1445 ± 0.0070	p=0.001	0.1418 ± 0.0072	p=0.003
		LF	0.1099 ± 0.0042	0.1434 ± 0.0056	p=0.001	0.1467 ± 0.0061	p=0.0001	0.1467 ± 0.0074	p=0.0001
		LH	0.1069 ± 0.0039	0.1363 ± 0.0056	p=0.005	0.1382 ± 0.0062	p=0.003	0.1447 ± 0.0079	p=0.0001
	Duty cycle [%]	RF	51.60 ± 0.5119	55.60 ± 0.4700	p=0.001	55.63 ± 0.92	p=0.001	56.14 0.79	p=0.0001
		RH	51.91 ± 0.8960	56.13 ± 0.6862	p=0.012	55.05 ± 3.54	p=0.088	54.97 ± 3.05	p=0.100
		LF	52.87 ± 0.5117	55.19 ± 0.5481	p=0.049	55.46 ± 0.62	p=0.022	55.82 ± 0.78	p=0.008
		LH	51.51 ± 0.5758	52.65 ± 0.7506	p=0.794	53.43 ± 0.94	p=0.415	55.37 ± 1.25	p=0.017

Table S1, related to Figure 3 and S1. Affected CatWalk parameters of QA lesioned mice. Data represent mean ± SEM. One-way ANOVA with post-hoc Tukey's test between groups for each paw (n=10-12). This table shows exemplarily p-values for all QA-lesioned mice (vehicle and transplanted) compared to non-lesioned mice at 14 weeks of age (4 weeks after transplantation) and 18 weeks of age (8 weeks after transplantation). Values labeled in red are not significantly different to non-lesioned mice, representing the red letter "ab" in Figure S1. This indicates a partial rescue of the phenotype.

Mouse Model	parameters significant different (p≤0.05)	Paw	WT	TG	p vs WT	TG+GFP NPCs	p vs WT	TG+BDNF-GFP NPCs	p vs WT
R6/2	Stride length [mm]	RF	79.95 ± 1.08	71.21 ± 2.53	p=0.015	75.24 ± 1.24	p=0.348	72.38 ± 2.37	p=0.049
		LF	80.46 ± 1.22	71.18 ± 2.59	p=0.014	74.74 ± 1.36	p=0.231	72.97 ± 2.54	p=0.071
		LH	80.86 ± 1.29	70.99 ± 2.57	p=0.006	75.15 ± 1.11	p=0.209	72.62 ± 2.43	p=0.032
	Lateral support [%]		0.55 ± 0.36	5.67 ± 1.94	p=0.041	5.66 ± 0.63	p=0.048	5.43 ± 1.47	p=0.064
N171-82Q	Cadence [steps/s]		26.41 ± 1.75	32.59 ± 1.50	p=0.031	33.59 ± 0.79	p=0.018	31.70 ± 1.53	p=0.069
	Swing [s]	RF	0.0761 ± 0.0027	0.0605 ± 0.0019	p=0.0001	0.0613 ± 0.0015	p=0.002	0.0601 ± 0.0028	p=0.0001
		RH	0.0778 ± 0.0037	0.0611 ± 0.0019	p=0.002	0.0602 ± 0.0024	p=0.002	0.0617 ± 0.0029	p=0.002
		LF	0.0785 ± 0.0032	0.0619 ± 0.0015	p=0.001	0.0609 ± 0.0019	p=0.001	0.0618 ± 0.0032	p=0.0001
		LH	0.0764 ± 0.0039	0.0606 ± 0.0025	p=0.004	0.0562 ± 0.0018	p=0.001	0.0641 ± 0.0027	p=0.026
	Stride length [mm]	RF	88.37 ± 3.39	73.54 ± 2.82	p=0.01	80.46 ± 1.68	p=0.359	77.02 ± 3.71	p=0.058
		RH	88.61 ± 3.25	73.02 ± 3.07	p=0.011	80.64 ± 2.38	p=0.399	75.03 ± 4.05	p=0.025
		LF	89.07 ± 4.06	73.14 ± 2.59	p=0.011	80.24 ± 1.36	p=0.328	78.79 ± 3.84	p=0.139
		LH	88.33 ± 3.29	72.29 ± 2.99	p=0.01	79.34 ± 2.52	p=0.316	75.92 ± 4.25	p=0.054
	Print area [mm²]	LF	24.85 ± 0.98	20.74 ± 1.32	p=0.047	22.16 ± 1.04	p=0.359	22.26 ± 1.01	p=0.304
	Print length [mm]	RF	8.54 ± 0.18	7.79 ± 0.19	p=0.028	8.04 ± 0.22	p=0.286	8.32 ± 0.17	p=0.816
		LF	8.69 ± 0.15	7.97 ± 0.15	p=0.009	8.31 ± 0.15	p=0.358	8.36 ± 0.15	p=0.399
	Print width [mm]	RF	8.09 ± 0.14	6.64 ± 0.24	p=0.001	7.07 ± 0.28	p=0.031	6.73 ± 0.29	p=0.001
		LF	7.91 ± 0.15	6.39 ± 0.24	p=0.0001	6.65 ± 0.26	p=0.001	6.54 ± 0.15	p=0.0001
	Max intensity [a.u.]	RF	50.14 ± 1.29	35.95 ± 2.11	p=0.001	39.38 ± 2.79	p=0.033	40.27 ± 3.55	p=0.030
	Phase dispersion [%]	LF_RH	3.89 ± 1.24	11.43 ± 1.72	p=0.009	8.56 ±1.39	p=0.222	8.57 ± 1.92	p=0.151
		LF_LH	50.39 ± 1.67	57.90 ± 1.28	p=0.029	56.00 ± 1.87	p=0.194	56.44 ± 2.34	p=0.090

Table S2, related to Figures 4, 5, and S2. Affected CatWalk parameters in the R6/2 and N171-82Q mouse model. Data represent mean ± SEM. One-way ANOVA with post-hoc Tukey's test between groups for each paw (n=10-12). This table shows exemplarily p-values for all transgenic mice (vehicle and transplanted) compared to wildtype mice. Values labeled in red are not significantly different to wildtype, representing the red letter "ab" in Figure S2. This indicates a partial rescue of the phenotype.

Supplemental Experimental Procedures

Generation and Differentiation of ESCs

Recombinant ESCs had been obtained by knock-in targeting strategy into the Rosa26 locus. The targeting vector contained the coding sequence of mouse pre-pro-BDNF-GFP (fused to the long 3'UTR of the BDNF gene) or the GFP coding sequence respectively. The ubiquitous CAG promoter (cytomegalovirus enhancer element and the chicken b-actin promoter) was placed upstream of the coding sequence. Furthermore, a floxed-stop cassette was introduced as a transcriptional blocker, which included a neomycin resistance gene. Neomycin resistant target clones obtained after homologous recombination were later transfected with the Cre-Recombinase harboring-vector pCrepac. Then, BDNF-GFP and GFP expressing ESCs were selected with puromycin and clonally expanded. A detailed description of how cells were generated and analyzed has been published by Leschik et al., 2013.
ESCs were maintained on feeder cells (mouse embryonic fibroblasts) and cultured in the presence of leukemia inhibitory factor (LIF). For differentiation, cells were cultured for two passages on gelatin (stage 1) and then differentiated according to the protocol of Bernreuther et al. (2006). This protocol includes embryoid body (EB) formation of ES cells (stage 2), selection of Nestin-positive cells from plated EBs (stage 3) and expansion of Nestin-positive cells (stage 4). Embryoid body formation was induced by plating 2.5 x 10^4 cells/cm² on non-adherent bacterial plastic dishes (Starlab) in ES culture medium without LIF. Embryoid bodies kept in suspension culture for 4 days were plated onto a tissue culture surface (Starlab). On the next day, the medium was switched to ITSFn medium and Nestin-positive cells were selected for 10 days with a medium change every other day. Cells maintained in ITSFn medium were dissociated and replated at a density of 1.5 x 10^5 cells/cm^2 on precoated poly-o/laminin dishes. The expansion medium containing bFGF was changed every 2 days. See a list of ESC culture and differentiation reagents below. After 6 days expansion of Nestin-positive cells, cells were enriched for polysialylated-neural cell adhesion molecule (PSA-NCAM) positive cells and depleted for stage-specific embryonic antigen 1 (SSEA-1) positive cells by MACS (Miltenyi Biotec, Bergisch Gladbach, Germany) as described previously (Butenschön et al., 2016). SSEA-1^-/PSA-$NCAM^+$ sorted cells were plated onto poly-ornithin/laminin coated dishes at a density of 2.0 x 10^5 cells/cm^2 in DMEM/F12/Glutamax supplemented with B27 and differentiated for 3 days by the omission of bFGF.

List of ESC culture and differentiation reagents

Medium	Components	Final concentration	Company	Catalog number
ESC culture medium	DMEM	1x	Sigma	D5671
	FBS	10%	PAA	A15-101
	Non-essential AA	0.1 mM	Invitrogen	11140035
	Sodium Pyruvate	1 mM	Sigma	S8636
	2-Mercaptoethanol	0.1 mM	Sigma	M7522
	L-Glutamine	2 mM	Sigma	G7513
	Pen/Strep	100 U/ml	Sigma	P0781
	LIF	recombinant LIF from supernatant of transfected HEK cells (kind gift from Ari Weissmann)		
ITSFn medium	DMEM/F12 with Glutamax	1x	Invitrogen	10565-018
	Fibronectin	5 µg/ml	Sigma	F2006
	Insulin	5 µg/ml	Sigma	I4011
	Transferrin	50 µg/ml	Sigma	T8158
	Selenium Chloride	30 nM	Sigma	323527
	L-Glutamine	2 mM	Sigma	G7513
	Pen/Strep	100 U/ml	Sigma	P0781
Expansion medium	DMEM/F12 with Glutamax	1x	Invitrogen	10565-018
	bFGF	10 ng/ml	Sigma	F0291
	B27	1x	Invitrogen	17504-044
	L-Glutamine	2 mM	Sigma	G7513
	Pen/Strep	100 U/ml	Sigma	P0781
Gelatin		0.1%	Sigma	1393
Poly-L-ornithine hydrobromide (poly-O)		10 mg/ml	Sigma	P3655
Laminin		5 µl/ml	Sigma	L2020

Animals

Animals were single housed in a temperature- and humidity-controlled room with a 12 h/12 h light / dark cycle (lights on 5 am - 5 pm) and had access to food and water ad libitum. All experiments were carried out in accordance with the European Community's Council Directive of 22 September 2010 (2010/63EU) and were approved by the local animal care committee (Landesuntersuchungsamt Koblenz, permit number G 12-1-097). This study was performed on male C57BL/6J mice (Harlan Laboratories), lesioned with quinolinic acid (Sigma Aldrich), male R6/2 mice (Jackson's laboratories, mouse strain stock number: 002810) and wild-type littermates maintained on the F1 hybrid B6CBA strain and N171-82Q mice (Jackson's laboratories, mouse strain stock number: 003627) and wild-type littermates maintained on the F1 hybrid B6C3H strain. Animals were subjected to genotyping to confirm the presence of the transgene with the mutated huntingtin. CAG repeat sizes were determined from genomic DNA by Laragen Inc. (Culver City, CA). The N171-82Q mice used in this study had a mean CAG repeat length of 84.00 ± 0.06 and the R6/2 mice used in this study a mean CAG repeat length of 167.40 ± 0.91. There was no difference in CAG repeats between transgenic groups. For randomization littermates were split among groups. Experimenters were blind to the genotype of mice and to the treatment.

Quinolinic acid lesion and cell transplantation

Mice were anaesthetized with isoflurane anesthesia and received a subcutaneous injection of buprenorphine (0.05 mg/kg) after the surgery. Mice that were lesioned before the cell transplantation received an additional injection of midazolam (50 mg/kg), to reduce quinolinic acid-induced seizures after waking up from surgery. One week before transplantation, the right striatum (0.0 mm AP, −2.0 mm ML, −3.0 mm DV from bregma) of C57BL6/J mice was lesioned by stereotactic injection of 1 µl 60 nmol quinolinic acid (Sigma, P63204). ESCs, differentiated for 3 days, were trypsinized and resuspended at a concentration of 100,000 cells/µl in HBSS without Ca^{2+} and Mg^{2+} (Gibco, 14175-046). Before transplantation, viability (>95%) of NPCs was validated by Trypan blue exclusion. 1 µl of the cell suspension was injected either in the right striatum of quinolinic acid lesioned mice (same coordinates as for lesioning) or bilaterally in transgenic mice (0.0 mm AP, ±2.0 mm ML, −3.0 DV from bregma) with a flow rate of 500 nl/min with a 26G beveled NanoFil needle. The injection needle was left in place for additional 5 min and then slowly removed. Sham-injected animals received an injection of 1 µl HBSS instead of the cell suspension.

Behavioral assays

All behavioral tests were performed during the light phase of the cycle, starting at 8 am. QA-lesioned and transgenic (R6/2 or N171-82Q) animals had been randomized into treatment groups before transplantation.

Body Weight

Body weight was measured once per week to observe any possible weight loss because of tumor formation of transplanted cells.

Rotation Test

To assess the functional lesion of the right striatum, mice were injected intraperitoneally with apomorphine (Sigma) at a concentration of 2 mg/kg body weight and immediately placed in an acrylic cylinder (20 cm diameter, 25 cm tall) in an Open Field box. The behavior of the mouse was video recorded for 45 min. 5 min after the injection, rotations were counted for 30 min with EthoVision. Net rotations were calculated: number of ipsilateral rotations − number of contralateral rotations.

Rotarod

The rotarod apparatus (Ugo Basile) was used to measure motor coordination and balance. Mice were placed on the rotarod at an accelerating speed ranging from 4 to 40 rpm over 5 min. Mice received three trials per day with a rest period of at least 1 hour in between trials for three consecutive days. On the third day (testing day) the maximum latency to fall off the rotarod for each mouse was recorded. The testing day was repeated every 2 weeks.

Quantitative gait analysis using the CatWalk system

CatWalk XT 9 (Noldus, The Netherlands) was used to assess gait and locomotion. Mice traversed a green illuminated glass plate, the reflected light from the paws that touch the glass was captured with a high-speed video camera and the illuminated paw prints were recorded. The recorded section was 9 mm long and automatic detection settings were applied. The intensity threshold was set to 0.25, the camera gain was set to 37.5. The testing was performed in the dark. Animals were placed on the glass plate and allowed to explore the walkway freely for 3 min. Then the runs were acquired, while the animal was running back and forth voluntarily. The maximum variation was set to 20% and the three fastest trials were used for subsequent analysis.

The following parameters were analyzed (RF= right forepaw, RH= right hindpaw, LF= left forepaw, LH= left hindpaw):

Temporal parameters: walk speed (distance of the runway divided by the time needed to cross), cadence (number of steps per second), stance duration (average time in seconds that the paw is in contact with the glass plate),

swing duration (average time in seconds that the paw is not in contact with the glass plate) and swing speed. Individual paw statistics: maximum contact area, maximum intensity, print area, print width, print length. Comparative paw statistics: stride length (distance between successive placements of the same paw), duty cycle (percentage of time the paw accounts for the total step cycle of the paw), base of support (average distance between front paws or hind paws).

Open Field

Mice were placed in the center of a white box (40 x 40 x 40 cm) illuminated at 90 lux and their behavior was video recorded for 10 min and tracked using EthoVision (Noldus). The distance moved was analyzed.

Grip Strength

To measure forelimb grip strength, a mouse was suspended by the tail and lowered towards the apparatus (Ugo Basile) until it grasped a handle with both front paws. The mouse was pulled back until it released its grip from the handle. All mice were tested for five consecutive trials, the peak pull-force (g) was recorded and the mean pull-force of all five trials was calculated.

Hindlimb Clasping

A marker for disease progression of neurodegeneration in R6/2 mice is hindlimb clasping. Mice were taken by the tail and lifted up in the air for 30 sec (Lee et al., 2009). The foot-clasping time was scored as the following: no clasping equals a score of 0, 0 to 5 sec a score of 1, 5 to 10 sec a score of 2 and more than 10 sec a score of 3.

Immunofluorescent staining

For immunocytochemistry, cells were fixed 15 min with 4% paraformaldehyde (PFA) and washed with PBS. Coverslips were rinsed for 5 min in 0.2% PBS-TX and blocked with 4% goat serum for 20 min. Cells were incubated with primary antibodies in 4% goat serum overnight at 4°C. The second day, coverslips were washed three times 5 min with PBS and incubated with the secondary antibody for 2 h at room temperature. Afterwards, cells were washed, counterstained with DAPI and mounted on (SuperFrostPlus, Menzel, Braunschweig, Germany) slides with Mowiol (Sigma Aldrich).

The following primary antibodies were used: mouse anti-Nestin (1:200), mouse anti-PSA-NCAM (1:100), mouse anti-MAP2 (1:200), mouse anti-OLIG2 (1:1000, all Merck Milipore), rabbit anti-KI67 (1:500), guinea pig anti-DCX (1:500), rabbit anti-GFAP (1:1000) and rabbit anti-FOXG1 (1:500), rabbit anti-BDNF (1:100, all Abcam, Cambridge, UK). Prior to immunostaining with anti-BDNF antibody, antigen retrieval at 98°C in Sodium citrate buffer (10 mM Sodium citrate, 0.05% Tween 20, pH 6.0) was performed. For PSA-NCAM staining, all buffers were used without TX.

For immunohistochemistry, mice were perfused transcardially with PBS followed by 4% PFA, brains were removed, post-fixed overnight in 4% PFA and treated with 30% sucrose for 48 h. Then brains were sectioned 30 µm thick in coronal plane and stored at 4°C in cryoprotection solution until use. Sections were blocked in PBS containing 5% normal donkey serum (NDS), 2.5% BSA and 0.3% TX for 90 min and incubated with the respective primary antibody in PBS containing 1% NDS, 0.1% BSA and 0.3% TX overnight: goat anti-DARPP32 (1:50, Santa Cruz Biotechnologies Inc., Santa Cruz, CA, USA) or guinea pig anti-DCX (1:500, Abcam). For double staining with BrdU, sections were washed 5 min with 0.2% TBS-TX and incubated in 1 N HCl for 1 h at 37 °C to denature DNA, followed by 3 x 5 min washes with TBS. Brain slices were then blocked in TBS with 1% NDS, 0.1% BSA and 0.3% TX for 90 min and incubated with the primary antibodies in blocking buffer overnight at 4°C. On the next day, appropriate secondary antibodies were applied for 2 h. To visualize cell nuclei in non-BrdU treated sections, brain slices were incubated with DAPI for 5 min, washed with PBS and mounted with Mowiol onto slides. The following primary antibodies were used: rat anti-BrdU (1:500), rabbit anti-FOXP1 (1:500), rabbit anti-GFAP (1:1000), rabbit anti-S100B (1:500, all Abcam), rabbit polyclonal anti-MAP2, 1:200 (Merck Milipore), and rabbit anti-ASPA (1:1000, kind gift from Matthias Klugmann, Sydney, Australia).

The respective secondary antibodies were applied: goat anti-mouse IgM or donkey anti-mouse, goat anti-rabbit, goat anti-guinea pig, donkey anti-goat or goat anti-rat AlexaFluor546 and goat anti-mouse, anti-rabbit or anti-rat AlexaFluor647 (all 1:1000; Invitrogen). See a list of all used antibodies below.

List of antibodies

Antibody	Species	Dilution	Company	Catalog number
Nestin	mouse	1:200	Merck Milipore	MAB353
PSA-NCAM	mouse	1:100	Merck Milipore	MAB5324
MAP2	mouse	1:200	Merck Milipore	MAB3418
OLIG2	mouse	1:1000	Merck Milipore	MABN50
KI67	rabbit	1:500	Abcam	ab15580
DCX	guinea pig	1:500	Abcam	ab2253
GFAP	rabbit	1:1000	Abcam	ab7260
FOXG1	rabbit	1:500	Abcam	ab18259
DARPP32	goat	1:50	Santa Cruz	sc-8483
BrdU	rat	1:500	Abcam	ab6326
FOXP1	rabbit	1:500	Abcam	ab16645
S100B	rabbit	1:500	Abcam	ab41548
ASPA	rabbit	1:1000	kind gift from Matthias Klugmann	
anti-mouse IgM AlexaFluor546	goat	1:1000	Invitrogen	A-21045
anti-mouse IgG AlexaFluor546	donkey	1:1000	Invitrogen	A-10036
anti-rabbit IgG AlexaFluor546	goat	1:1000	Invitrogen	A-11010
anti-guinea pig IgG AlexaFluor546	goat	1:1000	Invitrogen	A-11074
anti-goat IgG AlexaFluor546	donkey	1:1000	Invitrogen	A-11056
anti-rat IgG AlexaFluor546	goat	1:1000	Invitrogen	A-11081
anti-mouse IgG AlexaFluor647	goat	1:1000	Invitrogen	A-21235
anti-rabbit IgG AlexaFluor647	goat	1:1000	Invitrogen	A-21244
anti-rat IgG AlexaFluor647	goat	1:1000	Invitrogen	A-21247
Microbeads			**Company**	**Catalog number**
anti-SSEA-1 Micro Beads			Miltenyi	130-094-530
anti-PSA-NCAM-APC Micro Bead Kit			Miltenyi	130-097-859

Microscopic analysis of histology

Slides were observed under a Leica DM5500 (Leica Camera, Wetzlar, Germany) fluorescence microscope or a Zeiss Axiovert LSM 710 (Carl Zeiss, Oberkochen, Germany) laser scanning confocal microscope. For laser scanning confocal microscopy, z-stacks with optical sections of 1 µm of the graft were recorded.
Lesion volume and cell survival were assessed based on the Cavalieri principle of stereology. Lesion size was determined by staining every 10^{th} brain section of the entire striatum of QA-lesioned animals for DARPP32, a medium spiny neuron marker. The lesion area was outlined on digitized images and measured by ImageJ, National Institutes of Health, Bethesda, MD, USA. The lesion volume was calculated by multiplying the distance between sections (300 µm), number of sections and the measured area. Transplanted cells were quantified by staining marked cells with a BrdU antibody. Positive cells were counted in every 6^{th} section (180 µm apart) and calculated for the whole graft. For each parameter four animals were analyzed.
To dissect the differentiation pattern of transplanted cells, BrdU positive cells and BrdU cells positive for the respective cell fate marker were counted in 2 sections of each graft in eight animals per group, analyzing at least 1000 cells per marker and animal.
For evaluation of endogenous precursor proliferation, every 10^{th} brain section was immunostained with DCX and DAPI and positive cells in the ipsilateral dorsolateral subventricular zone (SVZ) were counted.

Statistical Analysis

Data are represented as the mean ± standard error of the mean (SEM). Statistical analysis was done using GraphPad Prism 4 (GraphPad Software Inc, La Jolla, CA, USA) and IBM SPSS Statistics 22v software (IBM Corporation, Armonk, NY, USA). For analysis of behavior over time, analysis of variance (ANOVA) with repeated measures and consecutive Tukey's post hoc tests (for significant group differences) or simple effects

analysis with Sidak correction (for significant group x time interaction) was conducted. The factors were groups x time. Motor behavior at distinct time points was analyzed with one-way ANOVA with post-hoc Tukey's test. For analysis of immunostaining of cells *in-vitro*, two-way ANOVA followed by Bonferroni's corrected multiple comparisons was applied. When comparing only two groups in immunohistology (BDNF-GFP NPCs and GFP NPCs), the two-tailed unpaired Student's t-test was applied. For lesion volume (three groups) and endogenous precursor proliferation (four groups), one-way ANOVA with post-hoc Tukey's or Dunnett's test was used. Differences were assumed to be significant if $p<0.05$.