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Neurogenesis in the R6/1 transgenic mouse model of Huntington's disease: effects of environmental enrichment

Stanley E. Lazic,[1] Helen E. Grote,[2] Colin Blakemore,[2] Anthony J. Hannan,[2,3] Anton van Dellen,[2] Wendy Phillips[1,4] and Roger A. Barker[1,4,5]
[1]Centre for Brain Repair, University of Cambridge, Cambridge CB2 2PY, UK
[2]University Laboratory of Physiology, University of Oxford, Oxford OX1 3PT, UK
[3]Howard Florey Institute, University of Melbourne, Vic. 3010, Australia
[4]Department of Neurology, Addenbrooke's Hospital, Cambridge CB2 2QQ, UK
[5]Edith Cowan University, Perth, Australia

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Abstract

Previous work has demonstrated that the transgenic R6/1 mouse model of Huntington's disease has decreased proliferation of neural precursor cells (NPCs) in the dentate gyrus of the hippocampus. This study therefore examined the survival and differentiation of NPCs in presymptomatic and symptomatic R6/1 mice and the effects of environmental enrichment on these variables. Here it is demonstrated that the survival of bromodeoxyuridine-positive ($BrdU^+$) NPCs in the dentate gyrus is decreased in the transgenic mice. In addition, the number of doublecortin-positive (DCX^+) cells is greatly reduced in these mice, as is the total number of new mature neurons, while the proportion of $BrdU^+$ cells differentiating into mature neurons was not significantly different between genotypes. Furthermore, the DCX^+ cells in the R6/1 mice had smaller and irregular-shaped somas, shorter neurites, and migrated a shorter distance into the granular cell layer compared with wild-type mice. Older symptomatic mice housed in an enriched environment had an increased number of $BrdU^+$ and DCX^+ cells as well as longer neurites and increased migration of DCX^+ cells. There was no significant difference between genotypes or environments in the number of $BrdU^+$ cells in the subventricular zone. These results suggest that decreased neurogenesis might be responsible, in part, for the hippocampal deficits observed in these mice and that environmental enrichment produces morphological changes in newborn granule neurons in both wild-type and R6/1 mice, which could underlie some of the beneficial effects of enrichment.

Introduction

Approximately 1 in 10 000 people suffer from Huntington's disease (HD), with onset commonly occurring in the fourth decade (Barker & Dunnett, 1999). The disease is caused by an unstable CAG repeat (which encodes the amino acid glutamine) in exon 1 of the *huntingtin* gene. Asymptomatic individuals have fewer than 36 CAG repeats and symptomatic individuals have 36 or more (reviewed in Ho *et al*., 2001). The most striking clinical feature of this disorder is the abnormal movements, but cognitive impairment and psychiatric disturbances also occur and patients die of a progressive motor disorder with dementia 15–20 years after disease onset.

The first reported transgenic mouse models of HD were the R6 lines developed by Mangiarini *et al*. (1996). These mice express exon 1 of the human *huntingtin* gene, with an expanded CAG repeat under control of the human huntingtin promoter. The R6/1 line have 112–120 CAG repeats and the transgene is expressed at 31% of endogenous levels while the R6/2 line have 139–148 CAG repeats that are expressed at 75% of endogenous levels (Mangiarini *et al*., 1996).

It is now widely accepted that neurogenesis occurs in the dentate gyrus (DG) of the hippocampus and in the subventricular zone/olfactory bulb system (SVZ/OB) in adult mammals. These newly created cells form functional connections with existing circuits (Carlen *et al*., 2002; van Praag *et al*., 2002; Belluzzi *et al*., 2003) and levels of neurogenesis correlate with performance on behavioural tasks (van Praag *et al*., 1999a; Shors *et al*., 2001; Kempermann & Gage, 2002a, b; Drapeau *et al*., 2003). Neurogenesis can be altered by a variety of endogenous and exogenous factors (Lazic & Barker, 2005) and can be increased by rearing animals in an enriched environment (Kempermann *et al*., 2002). Environmental enrichment can also delay the onset of symptoms in the R6 HD mice (van Dellen *et al*., 2000; Hockly *et al*., 2002; Lazic *et al*., 2004; Spires *et al*., 2004b), although it is not clear what role neurogenesis has in the development of symptoms in these mice. Previously, we have demonstrated that the proliferation of neural precursor cells (NPCs) in the DG is decreased in R6/1 mice (Lazic *et al*., 2004), whereas there is no change in the SVZ; similar results have been found in the R6/2 line by others (Gil *et al*., 2004, 2005; Phillips *et al*., 2005). These results are at odds with those obtained from human post-mortem tissue, which shows increased NPC proliferation in the SVZ (Curtis *et al*., 2003; Curtis, Penney *et al*., 2005a, b).

We therefore sought to establish if the survival and differentiation of NPCs in the DG and SVZ differed between R6/1 and wild-type control mice, and if the morphology of these newly formed neurons in the DG was similar between genotypes. In addition, the effect of environmental enrichment on the above variables was examined.

Correspondence: Dr S. E. Lazic, as above.
E-mail: stan.lazic@cantab.net

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Methods

Animals

Wild-type CBA ($n = 27$) and R6/1 transgenic mice ($n = 25$) from a CBA background were housed in the University Laboratory of Physiology, Oxford University. Animal work conformed to the UK Animals (Scientific Procedures) Act 1986 and was performed under appropriate Home Office project and personal licences. Mice were bred from a colony that originated from crossing male R6/1 mice (Mangiarini *et al*., 1996; The Jackson Laboratory, Bar Harbor, ME, USA) with female CBA mice. All mice were kept on a 12-h light/dark cycle, with food pellets and water freely available. Four to six mice of the same sex were housed per cage (dimensions 28 cm × 44 cm × 12 cm). Half of the mice (selected randomly) were given environmental enrichment in the home cage from the age of 4 weeks, whereas the other half were reared in a standard environment. The enriched mice had various plastic and cardboard objects in their cage that were changed every 2 days. At 4 weeks of age, tail tissue was taken from the mice for PCR genotyping, and a microchip (Labtrac, Uckfield, UK) for identification was inserted subcutaneously under general anaesthesia (to minimize pain and discomfort) induced with hypnorm (fentanyl citrate; Janssen Pharmaceutica, Berse, Belgium) and hypnovel (midazolam; Roche) in distilled water (1 : 1 : 2; 2.7 mL/kg). Both male and female mice were used in this experiment.

BrdU administration

The proliferation of endogenous neural precursor cells was detected using a 50 mg/kg intraperitoneal injection of bromodeoxyuridine (BrdU) dissolved in a 0.9% NaCl solution once a day for 10 days. Two cohorts of mice were used with an injection regime commencing at either 5 or 20 weeks. Mice were killed 4 weeks after the final injection, thus giving cohorts of mice aged 10 and 25 weeks at the time of analysis. These groups are hereafter referred to as the 10-week and 25-week mice, respectively. The 4-week period from final injection to histology was adopted to allow for the differentiation/maturation of new neurons.

Behavioural testing

Mice were placed on a rotarod (Ugo Basile, Comerio, VA, USA; model 7650), which accelerated from 4 to 40 r.p.m. in 3 min, and the length of time the mice remained on the rotarod was recorded. Mice were tested once at 5 weeks to acquaint them with the apparatus and to obtain baseline values. Mice were retested before they were killed at either 10 or 25 weeks. The purpose of the rotarod testing was to determine if the R6/1 mice had significantly reduced motor performance. It is known that levels of physical activity can influence neurogenesis (van Praag *et al*., 1999b), and a motor impairment may indicate that the R6/1 mice are getting less physical activity, which would then affect interpretation of the results.

Histology

Mice were killed using a 0.5-mL intraperitoneal injection of Euthatal (pentobarbitone sodium, 200 mg/mL; Merial, UK), which was followed by dislocation of the neck once the mice were unresponsive to stimuli. Mice were perfused transcardially by opening the thorax and inserting a cannula into the left ventricle. The right atrium was then cut and approximately 50 mL phosphate-buffered saline (PBS) solution was used to flush out the blood, followed by another 50 mL of 4% paraformaldehyde fixative. Following decapitation, the brains were removed and placed in 4% paraformaldehyde overnight and then transferred to a 30% sucrose solution until they sank. Brains were sectioned at 40-μm intervals in the coronal plane using a freezing stage microtome. Sections were then placed into 96-well plates containing a Tris-buffered saline (TBS) plus 0.05% sodium azide solution and were stored at 4 °C.

Immunohistochemistry

Staining was performed on free-floating sections. Incubation and washing solutions contained 0.2% Triton X-100 (Sigma) in TBS (Tx-TBS) unless otherwise noted. For BrdU staining, sections were treated with 2 M HCl for 30 min at room temperature to denature the DNA, followed by a wash with PBS (3 × 10 min). Sections were then quenched for 10 min using 10% H_2O_2/10% methanol in distilled water followed by three 10-min washes with Tx-TBS. A 1-h block with 3% normal donkey serum (NDS) was followed by incubation with the anti-BrdU primary antibody (1 : 1000; Santa Cruz Biotechnology, Santa Cruz, CA, USA) in a 1% NDS blocking solution at room temperature and left overnight. After washing (3 × 10 min with Tx-TBS), incubation with a biotin-conjugated donkey anti-sheep IgG secondary antibody (1 : 200; Sigma; plus 1% block) in Tx-TBS for 2 h was followed by another wash (3 × 10 min with Tx-TBS). Next, a streptavidin–biotin-conjugated complex (1 : 200; ABC Elite kit; Vector Laboratories, Burlingame, CA, USA) in a Tx-TBS solution was applied for a further 2 h. After another three 10-min washes with Tx-TBS, three drops (per 5 mL of Tx-TBS solution) of the Vector SG chromogen (Vector Laboratories) and three drops of H_2O_2 (per 5 mL of Tx-TBS solution) were used to visualize the antigen and left on for approximately 2–3 min. Sections were then washed three times with Tris non-buffered saline (TNS) for 10 min each. Sections were mounted on gelatinized glass slides and dehydrated using a series of ascending ethanol solutions (70, 95 and 100%) and xylene. Slides were then coverslipped using DPX. Staining with the other antibodies followed the same procedure but without the HCl step. Staining for doublecortin used a goat anti-DCX primary antibody (1 : 400; Santa Cruz Biotechnology) followed by a donkey anti-goat biotin secondary antibody (1 : 200; Sigma). Streptavadin–biotin treatment (ABC Elite kit) was followed by visualization with the Vector SG chromogen. Blocking solutions contained NDS. Staining for cleaved caspase-3 (CC-3) used a rabbit anti-CC-3 primary antibody (1 : 100; Cell Signaling Technology, Beverly, MA, USA) followed by a sheep anti-rabbit biotin secondary antibody (1 : 200; Chemicon). Streptavadin–biotin treatment (ABC Elite kit) was followed by visualization with the Vector SG chromogen. Blocking solutions contained NGS and equal amounts of bovine serum albumin (BSA; Sigma).

Fluorescence immunohistochemistry

Fluorescent double-labelling for BrdU and NeuN involved treatment with 2 M HCl for 30 min to denature the DNA followed by a wash with TBS (3 × 10 min). The mouse-on-mouse (MOM) blocking kit (Vector Laboratories) was used according to the manufacturer's instructions to reduce background staining. A 2-h block consisted of 5% NDS plus the MOM blocking solution (2 drops per 2.5 mL Tx-TBS). This was followed by application of the two primary antibodies at the same time – anti-BrdU (1 : 1000; Santa Cruz Biotechnology) and NeuN (1 : 200; Chemicon) – along with 3% NDS and the MOM diluent solution (80 μL/mL), and left overnight at

4 °C. After washing with Tx-TBS (4 × 15 min), donkey anti-sheep IgG FITC (1 : 200; Sigma) secondary antibody was added along with 3% NDS and left for 3 h in the dark. After another four 15-min washes, a goat anti-mouse Alexa 568 antibody (1 : 500; Invitrogen, Carlsbad, CA, USA) plus the MOM diluent solution was added and left for 3 h in the dark. Sections were washed (3 × 20 min) with Tx-TBS followed by two 10-min washes with TNS and coverslipped using Vectashield mounting medium (Vector Laboratories).

Confocal microscopy

Cells that stained for both BrdU and NeuN were examined using a Leica TCS-NT-UV confocal laser scanning system that excited fluorophores at 488 nm (Ar laser) and 543 nm (HeNe laser). In order to eliminate bleed-through from the green channel onto the red channel, the power of the argon laser was kept low (~3%) and any further bleed-through was eliminated by adjusting the gain and offset. Image stacks were acquired at 0.25-μm intervals (*z*-axis) with two scans per slice, which were averaged to improve the image quality. Images were collected under a 25× oil-immersion objective and analysed using Leica software (version 2.61) and edited in Photoshop 5.0. The total number of double-labelled cells was determined by imaging up to six regions of the DG per section (three on each side) and determining the number of double-labelled cells. As three sections per animal were examined in a 1 : 12 series, the number of cells counted was multiplied by 12, to give the total number of double-labelled cells in the DG. The percentage of cells that were double-labelled was calculated by dividing the number calculated above by the number of $BrdU^+$ cells as determined by the optical fractionator method (described below) and multiplying by 100. Owing to practical limitations, a random subset ($n = 19$) of the total number of mice were analysed for double labelling ($n = 11$ at 10 weeks, $n = 8$ at 25 weeks; $n = 12$ R6/1, $n = 7$ wild-type).

Stereology and quantification

All quantification was done with the experimenter blind to the genotype and the housing conditions of the mice. The Olympus CAST-Grid system (version 1.09; Denmark) was used for quantifying the number of $BrdU^+$ and DCX^+ cells using the $N_v \times V_{ref}$ method (Gundersen *et al*., 1988). Sections through the structure/region of interest (e.g. SVZ, DG) were sampled in a systematic random manner using a 1 : 12 series. The DG/hilus was defined from Bregma −1.22 mm to −2.80 mm, and the SVZ from Bregma 1.34 mm to −0.10 mm. For the SVZ, only the side adjacent to the striatum was quantified. On each section, the region of interest was outlined unilaterally under a 4× objective and the enclosed area was calculated by the CAST-Grid software. Sections within the highlighted area were then sampled at random by the software, and cell counts under the 100× (BrdU) or 20× (DCX) objective were used to determine the total number of immunoreactive cells within the sampled region (N_v). This was then repeated on the other side of the brain and the values combined to give the number of cells counted per unit of sampled volume. The volume was calculated by summing the values of all of the area measurements, multiplying by the thickness of the sections (40 μm) and then multiplying by 12 to account for the frequency of sections (V_{ref}). For analysis of BrdU, the size of the counting window was 3746 μm^2 and approximately 250 samples from three sections were examined. For analysis of BrdU in the SVZ, the size of the counting window was 2341 μm^2 and approximately 100 samples from three sections were examined. For analysis of DCX staining in the DG, the size of the counting window was 4844 μm^2 and approximately 50 samples from three sections were examined.

Morphological measurements of DCX^+ cells were performed using a Leica (Leitz DRMB) light microscope under a 20× objective, and images were acquired with a Nikon DXM 1200 digital camera. Lucia software (version 4.82; Laboratory Imaging, Czech Republic) was used for quantification of lengths and distances. Measurements were taken from five random areas from three different sections of each DG. The length of the longest neurite from each area was measured from mid cell body to the tip of the apical dendrite, and the resulting values averaged for each mouse. Similarly, to determine the migration of DCX^+ cells into the granular cell layer (GCL), measurements were taken from five random areas from three different sections of each DG and the distance travelled by the furthest cell from the subgranular zone (SGZ) from each area was measured.

Statistical analysis

Analysis was conducted with SPSS (version 11.5.0). Data were analysed with a two-way ANOVA, with genotype and environment as factors, and the Type I error rate (α) was set at 0.05. It can be assumed that all assumptions of the statistical tests (normality, homogeneity of variance, etc.) have been met unless otherwise indicated. Some dependent variables had unequal variances between groups and therefore these data were $\log_{10}$-transformed prior to analysis (Howell, 1992; Crawley, 2002); all graphs display original untransformed data. Data were analysed separately for the 10-week and 25-week groups, but were combined for some analyses to increase the sample size and thus power of the tests.

Results

BrdU immunohistochemistry

At 10 weeks, the R6/1 mice had 64% fewer $BrdU^+$ cells in the DG compared with wild-type mice (Fig. 1A; $F_{1,23} = 26.6$, $P < 0.001$). There were no significant differences between the enriched and non-enriched mice ($F_{1,23} = 0.1$, $P = 0.806$) and there was no interaction effect ($F_{1,23} = 0.4$, $P = 0.514$). The 25-week mice had much fewer $BrdU^+$ cells than the 10-week mice (Fig. 1B; note the different scale on the *y*-axis). In addition, the older R6/1 mice had 31% fewer $BrdU^+$ cells than wild-type mice, but this was not significant (Fig. 1B; $F_{1,21} = 0.07$, $P = 0.790$). The older enriched mice had almost double the number of $BrdU^+$ cells compared with older mice reared in standard conditions ($F_{1,21} = 9.1$, $P = 0.007$), irrespective of genotype, and there was no interaction effect ($F_{1,21} = 0.2$, $P = 0.644$).

In the SVZ, there were no significant differences between genotypes (Fig. 1C; $F_{1,23} = 2.2$, $P = 0.162$) or environments ($F_{1,23} = 3.0$, $P = 0.109$) in the 10-week group, and no interaction effect ($F_{1,23} = 0.3$, $P = 0.614$). In addition, there were no significant differences between genotypes (Fig. 1D; $F_{1,21} = 1.8$, $P = 0.200$) or environments ($F_{1,21} = 1.7$, $P = 0.206$) in the 25-week group, and no interaction effect ($F_{1,21} = 0.7$, $P = 0.416$).

The percentage of $BrdU^+$ cells in the DG that also expressed the mature neuronal marker NeuN was quantified using confocal microscopy (Fig. 2A and B). There was no significant difference between genotypes in the proportion of $BrdU^+$ cells that also expressed NeuN (Fig. 2C; $F_{1,15} = 0.003$, $P = 0.957$), nor was there any influence of environmental enrichment ($F_{1,15} = 0.6$, $P = 0.468$), and no interaction effect was found ($F_{1,15} = 0.1$, $P = 0.751$, both age groups combined). There was, however, a significant difference between

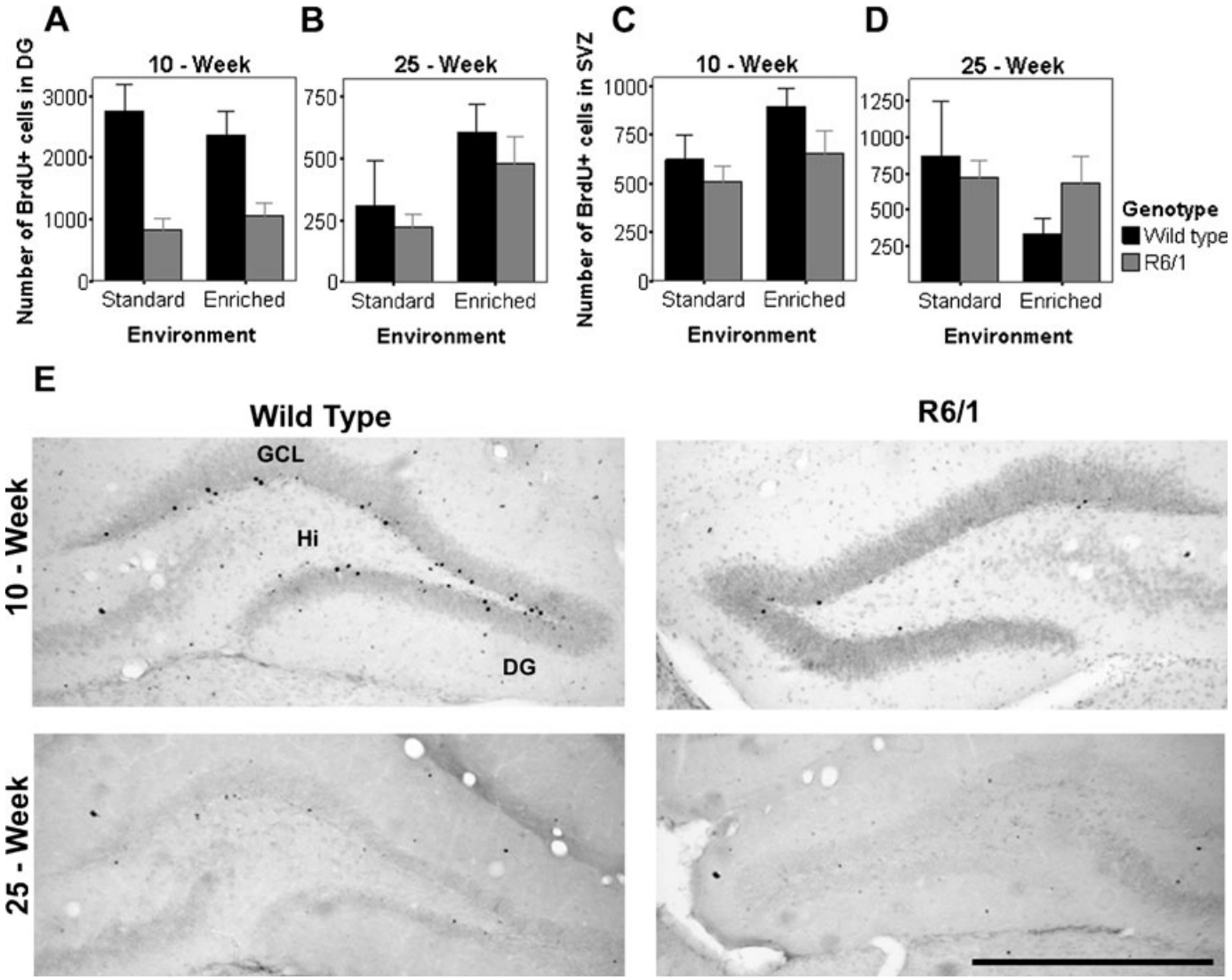

FIG. 1. BrdU staining in the hippocampus 4 weeks after BrdU injections. The number of $BrdU^+$ cells in the dentate gyrus is decreased in the R6/1 mice at 10 weeks (A; $P < 0.001$) and also at 25 weeks, but not significantly so at this later time point (B; $P = 0.790$). The 25-week mice reared in an enriched environment had significantly more $BrdU^+$ cells compared with non-enriched mice in the same age group ($P = 0.007$), irrespective of genotype. There were no differences in the number of $BrdU^+$ cells in the SVZ at 10 weeks (C) and 25 weeks (D) between genotypes or conditions. Representative histological pictures of BrdU immunostaining in the DG (E). DG, dentate gyrus; GCL, granular cell layer; Hi, Hilus. Scale bar, 0.5 mm.

genotypes in the total number of $BrdU^+/NeuN^+$ cells, with the R6/1 mice having 76% fewer double-labelled cells than wild-type mice (Fig. 2D; $F_{1,15} = 10.8$, $P = 0.005$, both age groups combined). There was no significant difference between environments ($F_{1,15} = 0.5$, $P = 0.490$) and no interaction effect ($F_{1,15} = 0.4$, $P = 0.516$) for the total number of $BrdU^+/NeuN^+$ cells.

Doublecortin immunohistochemistry

DCX is a microtubule-associated protein that is expressed in virtually all migrating neural precursors in the developing central nervous system (Gleeson *et al.*, 1998) and has been validated as a marker for neurogenesis in adult rodents (Brown *et al.*, 2003; Rao & Shetty, 2004; Couillard-Despres *et al.*, 2005). In the 10-week group, the number of DCX^+ cells was decreased in the R6/1 mice by 56% (Fig. 3A and I; $F_{1,23} = 85.7$, $P < 0.001$), and by 66% in the 25-week group (Fig. 3B; $F_{1,21} = 36.7$, $P < 0.001$), compared with age-matched wild-type controls. In the 10-week group, there were no significant differences between environments ($F_{1,23} = 0.9$, $P = 0.362$) and no interaction effect ($F_{1,23} = 0.4$, $P = 0.550$). However, in the 25-week group, the enriched mice had twice as many DCX^+ cells ($F_{1,21} = 15.6$, $P = 0.001$) as the non-enriched mice, irrespective of genotype. The genotype-by-environment interaction effect was not significant ($F_{1,21} = 0.9$, $P = 0.360$). In addition, at this time point, male mice had approximately 45% fewer DCX^+ cells than female mice ($F_{1,19} = 5.43$, $P = 0.031$). However, no significant differences between sexes were observed at 10 weeks, or at either time point for other variables.

The DCX^+ cells in the R6/1 mice appeared morphologically different compared with those found in the wild-type mice. The R6/1 DCX^+ cells had smaller and irregular shaped somas, fewer and smaller neurites, and appeared to cluster in the SGZ and did not migrate as far into the GCL (Fig. 3G and H). In view of this, the

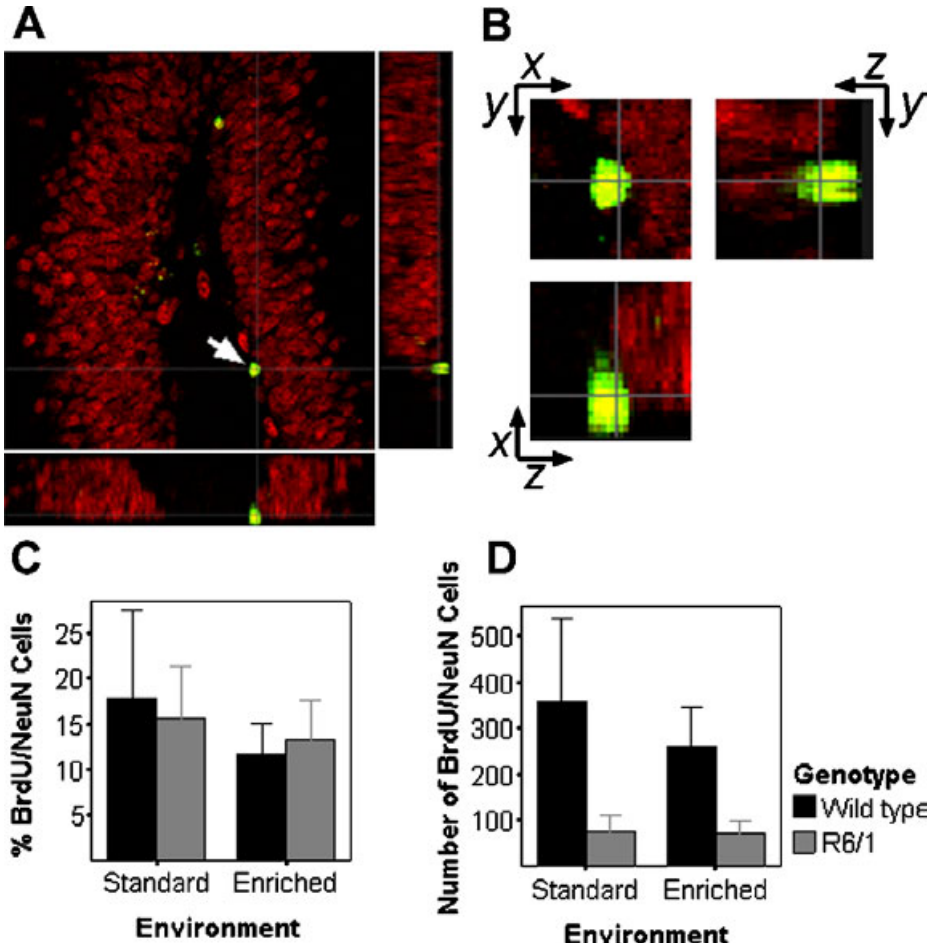

FIG. 2. Differentiation of neural precursor cells and total number of new neurons. BrdU and NeuN double-labelling (A and B) was used to determine the percentage of recently divided cells that differentiated into mature neurons (C) as well as the total number of new neurons (D) with the data for both age groups combined. There were no differences between genotypes or environments in the percentage of BrdU cells that also expressed NeuN. The total number of new neurons was 76% less in the R6/1 mice compared with the wild-type mice ($P = 0.005$), with no significant difference between environments.

length of the neurites and the distance that these cells migrated into the GCL was quantified. The R6/1 mice in the 10-week group (Fig. 3C; $F_{1,23} = 267.0$, $P < 0.001$) and 25-week group (Fig. 3D; $F_{1,21} = 51.1$, $P < 0.001$) had approximately 54% shorter neurites, compared with age-matched wild-type controls. In the 10-week group, there were no significant differences between environments ($F_{1,23} = 0.01$, $P = 0.915$) and no interaction effect ($F_{1,23} = 1.1$, $P = 0.304$). However, in the 25-week group, the enriched mice had significantly longer neurites ($F_{1,21} = 15.3$, $P = 0.001$) than the non-enriched mice, irrespective of genotype. The genotype-by-environment interaction effect was not significant ($F_{1,21} = 0.01$, $P = 0.941$).

In both age groups, the DCX^+ cells in the R6/1 mice migrated a shorter distance into the GCL from the SGZ (Fig. 3E and F; 10 weeks: $F_{1,23} = 65.2$, $P < 0.001$; 25 weeks: $F_{1,21} = 25.3$, $P < 0.001$). In the 10-week group, there were no significant differences between environments ($F_{1,23} = 2.9$, $P = 0.101$) and no interaction effect ($F_{1,23} = 2.6$, $P = 0.118$). However, in the 25-week group the DCX^+ cells in the enriched mice had migrated significantly further into the GCL ($F_{1,21} = 16.9$, $P = 0.001$) compared with the non-enriched mice. There was also a significant genotype-by-environment interaction effect ($F_{1,21} = 7.8$, $P = 0.011$). This occurred because although both wild-type and R6/1 mice had increased migration when reared in an enriched environment (compared with a standard environment), the R6/1 mice had a relatively greater increase than the wild-type mice.

Cleaved caspase-3 immunohistochemistry

Because differences between genotypes in the number of hippocampal $BrdU^+$ and DCX^+ cells has been demonstrated, it was of interest to determine if there were also different rates of cell death in the GCL and adjacent SGZ. Sections were stained for the apoptotic marker cleaved CC-3 (Fig. 4A and B), and because there were few labelled cells in the GCL/SGZ, the cells were counted directly (i.e. the optical fractionator method was not used). In addition, the data from both age groups were combined to increase the sample size, but there was still no significant difference between genotypes (Fig. 4C, $P = 0.926$) or environments (Fig. 4D, $P = 0.845$).

Behavioural testing

R6/1 mice develop a progressive motor disability and we determined if there were differences in performance on the rotarod test. At 10 weeks, there were no significant differences in rotarod performance between genotypes (Fig. 5A; $F_{1,23} = 2.3$, $P = 0.144$) or environments ($F_{1,23} = 0.3$, $P = 0.568$), and no interaction effect ($F_{1,23} = 0.2$,

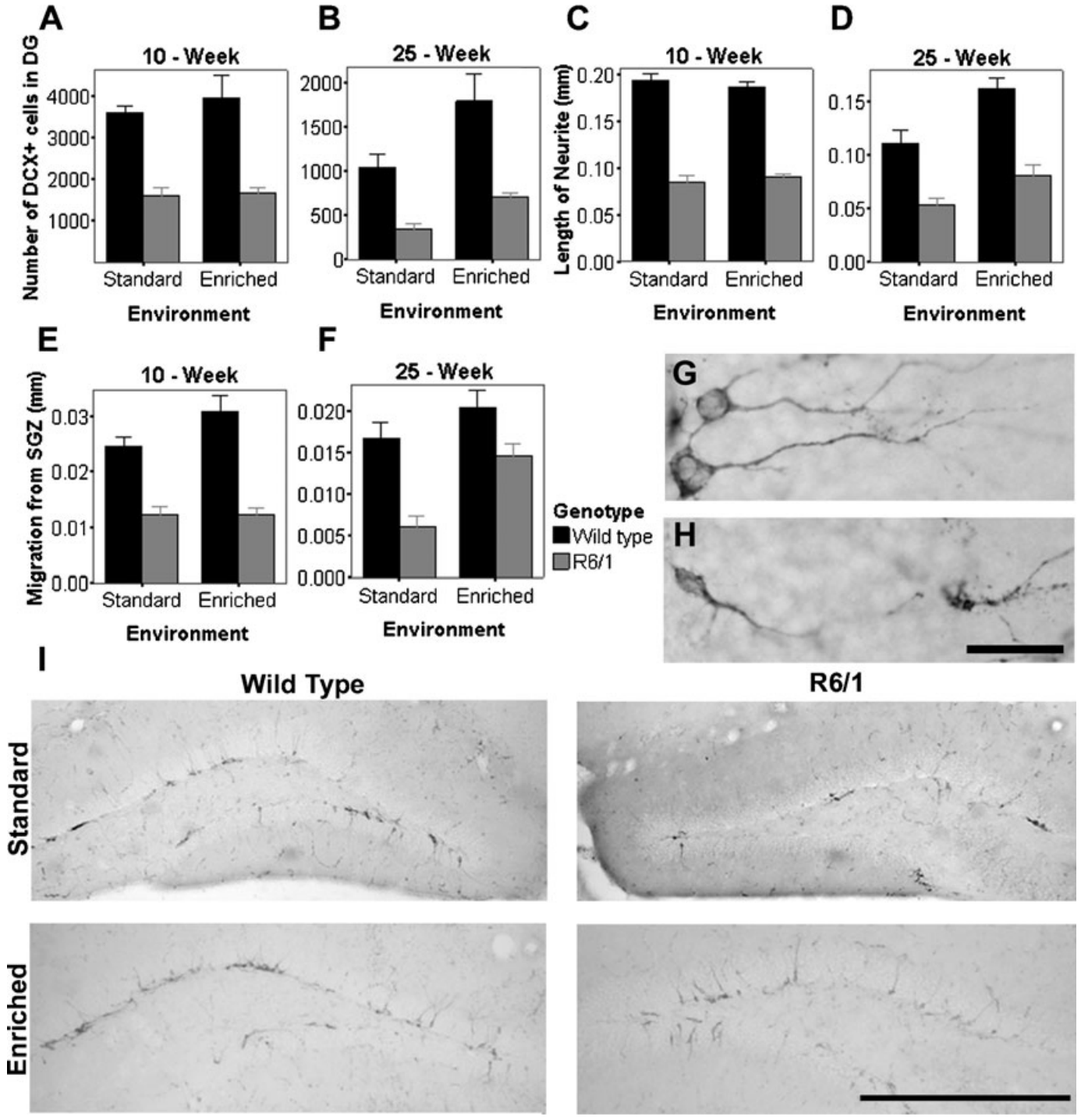

FIG. 3. DCX immunohistochemistry in the dentate gyrus. The number of DCX^+ cells is decreased in the R6/1 mice at 10 weeks (A; $P < 0.001$) and 25 weeks (B; $P < 0.001$), and at 25 weeks the enriched mice had a greater number of DCX^+ cells compared with non-enriched mice ($P = 0.001$). R6/1 mice also had shorter neurites at 10 weeks (C; $P < 0.001$) and 25 weeks (D; $P < 0.001$), and at 25 weeks the enriched mice had longer neurites compared with non-enriched mice ($P = 0.001$). The DCX^+ cells in the R6/1 mice migrated a shorter distance into the granular cell layer at 10 weeks (E; $P < 0.001$) and 25 weeks (F; $P < 0.001$), and at 25 weeks the DCX^+ cells of enriched mice migrated a greater distance compared with non-enriched mice ($P = 0.001$). Representative immunostained sections showing the morphology of DCX^+ cells in wild-type (G) and R6/1 (H) mice, and overall cell numbers in mice housed in a standard environment (I). Scale bars, 0.025 mm (G and H), 0.5 mm (I).

$P = 0.646$). In addition, there were no significant differences between genotypes (Fig. 5B; $F_{1,21} = 0.9$, $P = 0.344$) or environments ($F_{1,21} = 3.3$, $P = 0.087$) in the 25-week group, and no interaction effect ($F_{1,21} = 0.2$, $P = 0.653$); however, the R6/1 mice performed consistently worse than wild-type mice under all conditions.

Discussion

The number of $BrdU^+$ cells in the DG was decreased in both the older and the younger R6/1 mice compared with wild-type litter-mates, but this was only significant in the younger 10-week group. This indicates

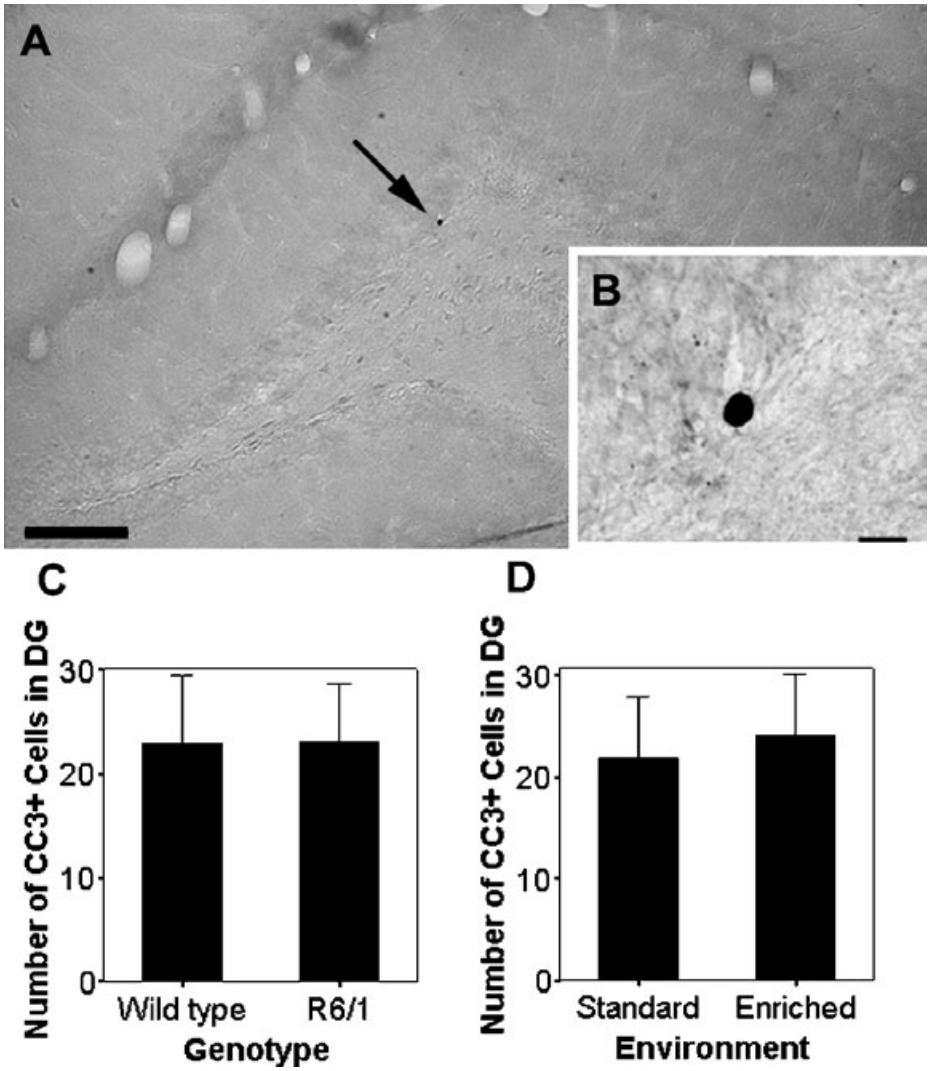

FIG. 4. Cleaved caspase-3 (CC-3) staining in the granular cell layer of the dentate gyrus under a 10× (A) and 63× (B) objective. There are no significant differences between genotypes (C; $P = 0.926$) or environments (D; $P = 0.845$), with the data for both age groups combined. Scale bars, 100 μm and 10 μm for A and B, respectively.

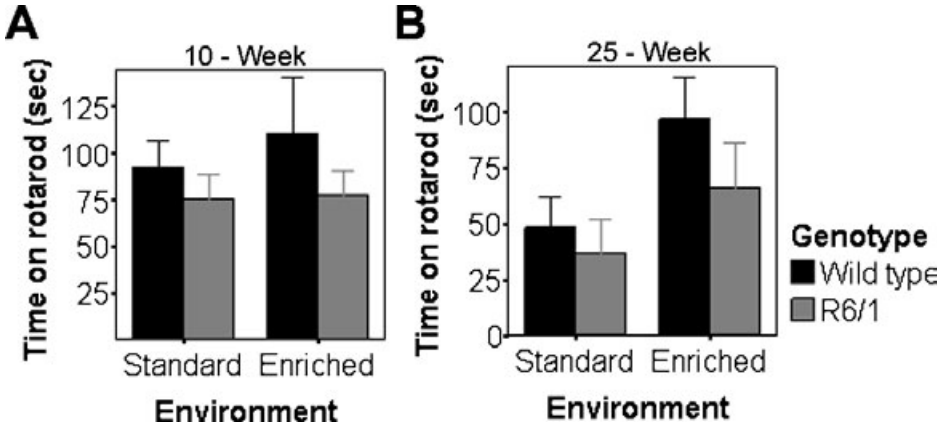

FIG. 5. Performance on the rotarod test. There was no significant difference between genotypes or environments at 10 weeks (A) or 25 weeks (B), although R6/1 mice consistently did less well than wild-type mice in all groups.

that the survival of NPCs is already compromised in the young transgenic mice, ahead of the development of overt neurological deficits. This is the exact same pattern that has been described in the R6/2 transgenic line, with a greater difference between genotypes in younger mice compared with older mice (i.e. a significant age-by-genotype interaction effect; Gil *et al*., 2005). Furthermore, in our

study, there was no effect of enrichment in the younger mice, which might reflect the limited time (6 weeks) that these mice were housed under such conditions. In contrast to the older mice, which had been enriched for 21 weeks, the environmentally enriched mice had significantly more $BrdU^+$ cells compared with non-enriched mice, irrespective of genotype. Whether the effect of enrichment in the older mice was due to these mice being more responsive to enrichment, or more likely, due to a much longer exposure to an enriched environment remains unresolved, but it does indicate that both transgenic and wild-type mice can respond to enrichment with an increase in the number of $BrdU^+$ cells.

The proportion of $BrdU^+$ cells that also expressed the mature neural marker NeuN did not differ between genotypes or environments, indicating that the differentiation of NPCs was not affected by transgenic status or housing conditions, but the total number of $BrdU^+/NeuN^+$ cells was 76% less in the transgenic mice. Similar results were also found in the R6/2 mice by Gil *et al*. (2005). Thus, there is evidence that both the proliferation (Lazic *et al*., 2004) and now survival of NPCs is reduced in the R6 transgenic mice, while the proportion that differentiate into mature neurons is the same as wild-type mice.

In this study, we also studied DCX as a marker of newly born neurons, and the results for the number of DCX^+ cells largely reflects the BrdU results. R6/1 mice had fewer DCX^+ cells in the DG at both 10 and 25 weeks, and in the older 25-week group, the mice housed in an enriched environment had a greater number of labelled cells. Again, these results are in good agreement with results from the R6/2 mice (Gil *et al*., 2005). The morphology of DCX-labelled cells was also investigated and it was discovered that the R6/1 mice had cells with shorter neurites, smaller and irregular shaped cell bodies, and migrated a shorter distance into the GCL, as we have also recently described in the R6/2 mice (Phillips *et al*., 2005). Environmental enrichment could rescue these deficits in the 25-week group, suggesting that these cells are still amenable to manipulation in the older, diseased rodent brain. It is not known if these differences in morphology persist in the more mature neurons of the GCL.

As the R6/1 mice have decreased neurogenesis, it was of interest to examine whether cell death was altered in the GCL, given that the induction of apoptosis in the GCL can increase proliferation (Pawlak *et al*., 2002). In addition, there seems to be a balance between proliferation and apoptosis in wild-type mice, with higher levels of proliferation being associated with higher levels of apoptosis and vice versa (Kim *et al*., 2001; Biebl *et al*., 2004; Heine *et al*., 2004). In the present experiment, however, there were no differences between genotypes or environments in the number of CC-3 immunopositive cells in the GCL/SGZ. Although the actual number of apoptotic cells is low, our findings are consistent with other studies which have also not found increased apoptotic (or necrotic) cell death in the striatum and other brain regions of the R6 compared with wild-type mice (Turmaine *et al*., 2000).

The reasons for a decrease in hippocampal NPC proliferation (Lazic *et al*., 2004) and/or survival are not known, but there are several possibilities. First, it could be due to the absence of a positive neurotrophic factor such as brain-derived neurotrophic factor (BDNF). BDNF is known both to increase proliferation in the hippocampus (Lee *et al*., 2002) and to be increased by environmental enrichment (Spires *et al*., 2004b). Previous work has demonstrated that BDNF protein and mRNA levels are decreased in the cortex and striatum of transgenic HD mice (Zuccato *et al*., 2001, 2005), and protein levels are decreased in the hippocampus of R6/1 mice (Spires *et al*., 2004b). However, in a study examining post-mortem human tissue, there was no evidence of decreased BDNF levels in the hippocampus (Ferrer *et al*., 2000). A second possibility relates to deficits in dopaminergic transmission in the R6 mice (Cha *et al*., 1998; Bibb *et al*., 2000; Yohrling *et al*., 2003). A third, more prosaic explanation is reduced motor activity in the R6/1 mice, given that physical activity is known to increase proliferation of hippocampal NPCs (van Praag *et al*., 1999a, b). However, the motor ability of the mice, as determined by rotarod testing, was similar between genotypes in both the 10-week and the 25-week groups (although worse in the transgenic mice), which suggests that the ability of mice to move about was not significantly compromised, although the daily activity of individual mice in their cages was not measured. Finally, a decreased number of $BrdU^+$ cells might be due to an increase in stress hormones, which can decrease neurogenesis (Lemaire *et al*., 2000; Wong & Herbert, 2004). There is evidence in humans that serum cortisol levels are higher in HD patients than controls (Heuser *et al*., 1991; Leblhuber *et al*., 1995), although it is not known if there are changes in these transgenic mouse models. Whatever the cause, it is likely that there is not a major deficit in the NPCs themselves, as Phillips *et al*. (2005) have demonstrated that NPCs from both wild-type and R6/2 mice have similar rates of proliferation *in vitro*.

Unlike the results in the DG, there was no difference in the number of $BrdU^+$ cells in the SVZ between R6/1 and wild-type mice. Gil *et al*. (2004, 2005) found similar histological results using R6/2 mice, but there appears to be an increase in proliferative cells in this region in the human brain at post-mortem (Curtis *et al*., 2003; Curtis *et al*., 2005a, b). These discrepant results may reflect differences in human vs. rodent neural precursor cells, neurogenic environments or organization of the SVZ (Sanai *et al*., 2004). Alternatively, the difference might be due to the large amount of cell loss that is generally found in the human HD striatum at post-mortem, which is not seen in the R6 mouse models (Turmaine *et al*., 2000), as cell loss or cell death has been shown to stimulate NPCs to increase their rate of proliferation/survival (Nait-Oumesmar *et al*., 1999; Magavi *et al*., 2000; Arvidsson *et al*., 2002). In particular, mice with striatal quinolinic acid lesions have increased proliferation of NPCs in the SVZ (Tattersfield *et al*., 2004; Collin *et al*., 2005), although such a response seems to be absent in the R6/2 mouse model of HD (Phillips *et al*., 2005). This suggests that even if there was cell loss in the striatum of the transgenic mice, the NPCs may not be able to respond normally.

The functional significance of these changes in neurogenesis remains speculative, but they may help explain some of the cognitive deficits, such as impaired spatial cognition, which is characteristic of these mice, although deficits in synaptic plasticity and changes in existing circuits also contribute to the behavioural deficits (Lione *et al*., 1999; Murphy *et al*., 2000; Spires *et al*., 2004a; Gibson *et al*., 2005). Furthermore, the beneficial effects of environmental enrichment on these mice (van Dellen *et al*., 2000; Hockly *et al*., 2002; Spires *et al*., 2004b) may be mediated, in part, by changes in neurogenesis. This is further supported by the finding that increasing neurogenesis with fluoxetine treatment improves the performance of R6/1 on a hippocampal-dependent memory task (Grote *et al*., 2005). In terms of patients with HD, it is possible that such abnormalities in neurogenesis may underlie some of the cognitive aspects of this disease as well as the depression, given that decreased neurogenesis is associated with depression (Santarelli *et al*., 2003). Depression can be the initial presenting feature of HD, can precede the onset of motor symptoms and cognitive symptoms by many years, and occurs in individuals who are not even aware of their risk (reviewed in Craufurd & Snowdon, 2002). This is consistent with the finding here that hippocampal neurogenesis is decreased early in the disease, before mice display motor abnormalities.

In summary, the R6/1 mouse model of HD has reduced hippocampal neurogenesis, and this is mainly the result of decreased proliferation and survival of NPCs. In addition, neural precursor cells in this region have an altered morphology and migrate a shorter distance into the GCL. Furthermore, these deficits can be partly restored by rearing the animals in an enriched environment. Because environmental enrichment is known to delay the onset of symptoms in these mice (van Dellen *et al*., 2000), it is possible that neurogenesis may be manipulated to improve performance in these mice. As such, therapies aiming to increase neurogenesis may prove efficacious for some symptoms of HD.

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Abbreviations

BDNF, brain-derived neurotrophic factor; CC-3, cleaved caspase-3; BrdU, bromodeoxyuridine; DCX, doublecortin; DG, dentate gyrus; GCL, granular cell layer; HD, Huntington's disease; OB, olfactory bulb; NPC, neural precursor cell; SGZ, subgranular zone; SVZ, subventricular zone.

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