Dopaminergic manipulations and its effects on neurogenesis and motor function in a transgenic mouse model of Huntington's disease

M.L. Choi a,1, F. Begeti a,b,1, J.H. Oh c, S.Y. Lee c, G.C. O’Keeffe a, C.D. Clelland a, P. Tyers a, Z.H. Cho a, Y.B. Kim a, R.A. Barker a,d,1

a Department of Clinical Neurosciences, John van Geest Centre for Brain Repair, University of Cambridge, Cambridge CB2 0PY, UK
b School of Clinical Medicine, University of Cambridge, Addenbrooke’s Hospital, Cambridge CB2 0SP, UK
c Neuroscience Research Institute, Gachon University, Incheon 405-760, Republic of Korea
d Department of Neurology, Addenbrooke’s Hospital, Cambridge CB2 0QQ, UK

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A B S T R A C T

Huntington’s disease (HD) is an inherited neurodegenerative disorder that is classically defined by a triad of movement and cognitive and psychiatric abnormalities with a well-established pathology that affects the dopaminergic systems of the brain. This has classically been described in terms of an early loss of dopamine D2 receptors (D2R), although interestingly the most treatments most effectively used to treat patients with HD block these same receptors. We therefore sought to examine the dopaminergic system in HD not only in terms of striatal function but also at extrastriatal sites especially the hippocampus, given that transgenic (Tg) mice also exhibit deficits in hippocampal-dependent cognitive tests and a reduction in adult hippocampal neurogenesis. We showed that there was an early reduction of D2R in both the striatum and dentate gyrus (DG) of the hippocampus in the R6/1 transgenic HD mouse ahead of any overt motor signs and before striatal neuronal loss. Despite downregulation of D2Rs in these sites, further reduction of the dopaminergic input to these sites by either medial forebrain bundle lesions or receptor blockade using sulpiride was able to improve both deficits in motor performance and adult hippocampal neurogenesis. In contrast, a reduction in dopaminergic innervation of the neurogenic niches resulted in impaired neurogenesis in healthy WT mice. This study therefore provides evidence that D2R blockade improves hippocampal and striatal deficits in HD mice although the underlying mechanism for this is unclear, and suggests that agents working within this network may have greater effects than previously thought.

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Introduction

Huntington’s disease (HD) is an autosomal dominant neurodegenerative disorder that is clinically defined by a movement disorder (typically chorea) together with cognitive and psychiatric disturbances. As in other neurodegenerative disorders, the functional abnormalities in HD have been proposed to result from a compromise of neural circuitry associated with cellular dysfunction or death (Ross and Tabrizi, 2011). The neuronal loss in HD is extensive, and in particular, many areas of the CNS including the hippocampus are affected by the disease process even in the earliest stages (Ross and Tabrizi, 2011). Indeed, HD transgenic (Tg) mouse models exhibit a range of dopaminergic-dependent impairments, one of the best described being a reduction in adult neurogenesis in the dentate gyrus (DG) (Gil et al., 2005; Lazic et al., 2004; Phillips et al., 2005). During this process, endogenous neural precursor cells give rise to immature neurons which eventually mature and functionally integrate into the circuitry (Ming and Song, 2011), a process which is critical to some aspects of cognition (Clelland et al., 2009; Sahay et al., 2011).

Dopaminergic disturbances are also a common feature of the disease particularly a progressive reduction in striatal and extrastriatal D2 receptor (D2R) binding which begins before clinical manifestations and correlates with disease progression and frontostriatal cognitive impairment (Bäckman and Farde, 2001; Ginovart et al., 1997; Pavese et al., 2003, 2010; Ramos et al., 2013; van Oostrom et al., 2009). In line with human HD studies, there is evidence of an early reduction in striatal D2R expression in HD Tg mice (Benn et al., 2010; Cha et al., 1998; Cummings et al., 2006; Glass et al., 2004) although there have been limited approaches to demonstrate this in vivo using PET imaging as has been done in human HD patients. Furthermore, whether similar

Abbreviations: 6-OHDA, 6-hydroxydopamine; D1R, dopamine D1 receptor; D2R, dopamine D2 receptor; DCX, doublecortin; DG, dentate gyrus; HD, Huntington’s disease; L-DOPA, 3,4-dihydroxy-L-phenylalanine; micro-PET, micro-positron emission tomography; MPB, medial forebrain bundle; SNZ, subventricular zone; TH, tyrosine hydroxylase; VTA, ventral tegmental area.

⁎ Corresponding author at: Department of Clinical Neurosciences, John van Geest Centre for Brain Repair, University of Cambridge, Cambridge CB2 0PY, UK.
E-mail address: tab46@cam.ac.uk (R.A. Barker).
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1 M. L. Choi and F. Begeti are the first authors.
changes occur in the dentate gyrus is unknown. Such questions have significant clinical interest as the majority of drugs used to effectively treat this disease block these receptors and/or deplete presynaptic dopamine (Mason and Barker, 2009; Priller et al., 2008).

We therefore sought to examine the dopaminergic system in HD not only in terms of striatal function but also at extrastriatal sites, especially the hippocampus. We used the R6/1 HD Tg mouse model which expresses exon 1 of the human huntingtin (Htt) gene carrying 110–120 CAG repeats (Mangiarnini et al., 1996). This line of HD mice has been well studied in terms of both the dopaminergic system and adult neurogenesis and also has a relatively slow evolution of progressive motor signs which makes them amenable especially to studies looking at the premanifest motor phase of the illness (Lazic et al., 2004; Ortiz et al., 2011; Petersen et al., 2002; Walker et al., 2011). We now demonstrate that in these HD Tg mice, using in vivo micro-PET imaging and mRNA expression, there is a significant reduction of D2R expression in the striatum and DG respectively which begins ahead of overt disease manifestations and neuronal loss in agreement with previous studies. Despite this reduction of D2R, further reduction of the dopaminergic input from a partial bilateral medial forebrain bundle (MFB) lesion paradoxically improved motor behavior and partially ameliorated adult hippocampal neurogenesis in the R6/1 mouse model of HD while having opposite effects in wild-type (WT) mice. Using the D2R antagonist which is commonly used to treat chorea and behavioral symptoms in HD, we observed a partial increase in hippocampal neurogenesis while there was no improvement in motor impairment. These findings have important clinical implications especially given that dopaminergic drugs are widely used in the treatment of patients with HD.

**Materials and method**

**Animals**

C57BL/6 wild type (WT) mice were purchased from Harlan Laboratories (UK). R6/1 Tg mice were purchased from the Jackson Laboratory (USA) and the colony was maintained by backcrossing to C57BL/6 females purchased from Harlan Laboratories (UK). After the mice were weaned, tissue from ear biopsies was sent to Largen Inc. (USA) for genotyping. All animals were kept in a temperature and humidity-controlled (22 °C) room on a 12-hour light/dark cycle. The mice were separately housed in single-sex cages of 3 males purchased from Harlan Laboratories (UK). After the mice were weaned, tissue from ear biopsies was sent to Largen Inc. (USA) for genotyping. All animals were kept in a temperature and humidity-controlled (22 °C) room on a 12-hour light/dark cycle. The mice were separately housed in single-sex cages of 3–4 mice per cage. Water and food was made freely available in the home cage. All experiments were performed using only female mice in the John van Geest Centre for Brain Repair, University of Cambridge, UK and Neuroscience Research Institute, Gachon University, Republic of Korea in strict accordance with appropriate Home Office project and personal licenses. Only female mice were used because of the need to avoid problems of fighting and sex hormone influences on any behavioral tests. The protocols were approved by the ethical committees of the University of Cambridge (UK) and the University of Gachon (Republic of Korea).

**Micro-PET imaging**

For the micro-PET study, animals were shipped via an air courier to NRI, Gachon University of Medicine & Science, Republic of Korea and allowed to recover for 2 weeks prior to scanning. R6/1 (n = 4) and WT littermate (n = 4) mice were scanned twice, at 12–13 weeks and at 21–23 weeks of age. Anesthesia was induced and maintained with passive oxygen/isoflurane at 1.5 l/min. Each mouse was positioned prone on a bed with its brain centered in the gantry and its head fixed by a nose bar. A PET scan was performed using a Focus 120 MicroPET system (Concorde Microsystems, Knoxville, TN) with 1.18 mm (radial), 1.13 mm (tangential) and 1.45 mm full width at half maximum (FWHM) resolution at the center (Kim et al., 2007). Dynamic scans were performed for 90 min immediately after a [C] raclopride injection (9.25–12.95 MBq, 200 μl) via the tail vein. The acquired data was reconstructed using a 2D-filtered back-projection algorithm (microPET ManagerTM, Siemens Medical Solutions, Knoxville, USA). Regions of interest (ROIs) for the striatum were drawn on the reconstructed PET images using a mouse brain atlas (Paxinos and Franklin, 2001). Regional radioactivity was determined for each dynamic frame in the ROIs. Tracer binding was calculated on the basis of the binding potential (BP) derived from a Logan plot graphical analysis technique (Logan et al., 1996).

**mRNA expression**

The DG was micro-dissected according to a previously described protocol (Hagihara et al., 2009) and samples were stored at −80 °C until use. RNA was extracted using an RNeasy Mini Kit (Qiagen, 74106) according to the manufacturer instructions and eluted in 30 μl of RNase-free water, the concentration of which was determined using a Nanodrop 2000c spectrophotometer (Thermo Scientific) and stored at −80 °C. cDNA was reverse transcribed using a Transcriptor First Strand cDNA Synthesis Kit (Roche) with anchored-oligo(dt) primers according to the manufacturer instructions. Quantitative Polymerase Chain Reactions (qPCR) were subsequently undertaken using Solaris Mouse qPCR Gene Expression Assays for D2D. ATP5b was chosen as the reference gene due to its stable level of expression in HD mouse models when compared to other commonly used housekeeping genes (Benn et al., 2008). All qPCR reactions were performed in triplicates. Each dissection contained 3–5 R6/1 mice and 3–5 WT littermates and results were repeated with three independent dissections at different ages: 8, 12 and 16 weeks.

**Dopamine lesion surgery**

12–13 week old WT (n = 30 from Harlan Laboratories) mice or R6/1 (n = 22) mice and their WT littermates (n = 19) received bilateral injections of 6-OHDA (Sigma, UK) into the medial forebrain bundle (MFB). A stock of 6-OHDA was prepared in 0.01% ascorbic acid/saline and stored at −20 °C. The working doses were diluted daily and then carried on ice to the operating room. Mouse surgery was performed under isoflurane anesthesia using a mouse stereotaxic frame. The stereotaxic coordinates for MFB lesions were A/P: −1.1 (from bregma), M/L: ±1.1, and D/V: −4.7 (from the dura surface). Drugs were injected bilaterally at the rate of 0.5 μl/min through a Hamilton syringe (Hamilton Company, Switzerland) and the needle was left in situ for 5 min following delivery of the toxin to allow for its diffusion. For sham surgery, the identical volume of saline was injected using the same protocols for the 6-OHDA injections.

**Drug administration**

Quinpirole and S–(-) sulpiride were purchased from Tocris (UK) and dissolved in saline with ethanol (quinpirole: to 100 mM and S–(-) sulpiride: to 10 mM, all of which were soluble in saline) using a warm bath (30 °C). Stacks of a constant volume were stored in a −20 °C freezer until use. 3,4-Dihydroxy-L-phenylalanine (l-DOPA, Sigma UK) was dissolved in saline using a warm bath on the day of administration (6 mg/kg) and was stabilized by co-injection with benserazide hydrochloride (12 mg/kg, Sigma UK). Stock drugs were dissolved in saline to obtain final working concentrations (quinpirole: 5 ml/kg, sulpiride: 10 ml/kg). Either drug or saline was injected intraperitoneally (i.p.) at 10 ml/kg volume in R6/1 (n = 27) and their WT littermate mice (n = 24).

**Immunohistochemistry and cell quantification**

Animals were perfused with 4% paraformaldehyde (PFA) and brains were post fixed overnight and then cryoprotected in 30% sucrose.Brains
were cut into 40 μm free-floating coronal sections which were stored in 30% glycerol anti-freezing solution until use. The following immunohistochemistry was undertaken:

- Adult neurogenesis: a one in twelve series of sections was stained with doublecortin (DCX) (goat anti-DCX, 1:250, Santa Cruz Biotechnology) and visualized with a fluorescent secondary antibody (Alexa donkey anti-goat, 1:250, Invitrogen). DCX positive cells were counted throughout the SVZ of the LV and the rostrocaudal extent of the granular cell layer (GCL) and SGZ within the DG. All sections were double stained with neuronal nuclei (NeuN).

- Striatal neuronal population: a one in twelve series of sections was stained with NeuN (mouse anti-NeuN, 1:500, Millipore) and visualized with fluorescent secondary antibodies (Alexa donkey anti-mouse 647, 1:250, Invitrogen). NeuN positive cells were counted in both the dorsal and the ventral striatum.

- Dopaminergic innervation: a one in six series of sections was stained with tyrosine hydroxylase (TH, rabbit anti-TH, 1:4000, Millipore) visualized by biotinylated secondary antibody (anti-rabbit, 1:200, Invitrogen) and the diaminobenzidine system (Vectastain ABC kit, Vector Laboratories) in three areas, the substantia nigra (SN), VTA and striatum. The number of TH positive cells was counted in the SN and VTA and the intensity of TH fibers was measured in the striatum using images of the whole striatum captured by the Olympus Stereo Investigator. The degree of TH fiber reduction by a 6-OHDA lesion was obtained by comparing it to that recorded in the control group. The degree of TH immunoreactivity in the SVZ and SGZ was insufficiently intense to be quantified.

After staining, cells were counted using either a 20× or 40× objective on a Leica fluorescence microscope or Olympus stereo system and numbers counted were multiplied by either 12 or 6 depending on the fraction of sections stained to get an estimate of the total number of cells in the regions of study. Representative fluorescent images were obtained using a Leica confocal microscope.

**Results**

**D2Rs are down-regulated early in the disease course of R6/1 mice in the striatum and DG**

We used [11C]raclopride PET imaging to examine D2R changes in premanifest and manifest R6/1 mice compared to WT littermates. Mice were scanned twice, between 11–13 weeks and 21–23 weeks of age, and the whole striatum was used as the region of interest. Striatal D2R binding was significantly decreased in the 11–13 week old R6/1 mice compared to their WT littermates ($P = 0.006$). This was also the case in the 21–23 week old mice ($P = 0.009$) although there was no further reduction in D2R binding at this time (Fig. 1B), despite the animals having developed motor features (Mangiarini et al., 1996). Histological analysis showed no significant difference in the number of mature NeuN+ neurons in the striatum of 11–13 week R6/1 mice indicating that the reduction in D2R binding was not due to neuronal loss (Fig. 1D). Manifest 21–23 week old R6/1 mice had a reduction in the number of neurons in the striatum ($P = 0.0009$, Fig. 1D), despite there being no further reduction in D2R binding. Since the current resolution of PET is too low to quantify D2R in the DG, we micro-dissected this region in R6/1 mice aged 8, 12 and 16 weeks old, in order to measure mRNA expression. We observed a reduction in D2R mRNA at all age points studied ($P = 0.0009$, Fig. 1C). Again the loss of D2R expression was not progressive in R6/1 mice.

**Partial 6-OHDA lesions of the MFB lead to a decrease in adult neurogenesis in both the SVZ and SGZ of WT mice**

We next investigated the role of dopamine in WT mice by reducing dopaminergic input to both constitutive neurogenic sites via selective 6-hydroxydopamine (6-OHDA) partial bilateral lesions of the MFB (Borta and Höglinger, 2007). Since complete bilateral MFB lesions have been associated with high morbidity and mortality (von Bohlen und Halbach, 2011), we first determined the optimal dose of 6-OHDA and found that doses greater than 2 μg/μl were associated with a significant mortality rate in WT mice (data not presented). A dose of 1.5 μg/μl of 6-OHDA was therefore used and resulted in lesions with a 21% and 18% loss of dopaminergic cells in the SN ($P = 0.011$) and VTA ($P = 0.017$), respectively, and a 33% loss of dopaminergic fibers in the striatum ($P = 0.0009$) 4 weeks after the lesion (Fig A1).

We then examined the effect of this loss of a dopaminergic input followed by dopamine replacement on adult neurogenesis. Two weeks after the MFB lesions, animals received either saline or L-DOPA (6 mg/kg) stabilized by benserazide hydrochloride (12 mg/kg) daily for 7 days. All mice were perfused 2 weeks after the last drug administration in order to quantify adult neurogenesis through counting the number of doublecortin (DCX) positive immature neurons in the SGZ and SVZ (see Fig. 2A for experimental timeline). We found that partial bilateral MFB lesions led to a significant decrease in the number of DCX positive cells in the SGZ ($P = 0.0009$) and SVZ ($P = 0.0009$), which was rescued by L-DOPA treatment ($P = 0.021$) at both sites (Fig. 2C and D). This suggests that the dopaminergic input to the SVZ and SGZ is essential in maintaining normal levels of adult neurogenesis in mice.

**6-OHDA lesions of the MFB ameliorate motor impairments and increased adult hippocampal neurogenesis in the R6/1 mouse model of HD**

We then investigated the effects of similar dopaminergic lesions in the R6/1 mouse model. We found that R6/1 mice exhibited greater sensitivity to 6-OHDA than WT littermate mice in that doses greater than 1 μg/μl were more likely to be fatal (data not presented). As a result, we used a dose of 0.5 μg/μl 6-OHDA which resulted in lesions with a 16.5% ($P = 0.039$) and 12.2% ($P = 0.028$) reduction in midbrain dopaminergic cells in the SN and VTA respectively and a 6.3% reduction
in dopaminergic fibers to the striatum in both WT and R6/1 mice (Fig. A2).

This smaller dose of 0.5 μg/μl of 6-OHDA had no effect on rotarod performance (Fig. 3B) and did not lead to a significant reduction in the number of DCX-positive cells in the DG or the SVZ in WT mice (Fig. 3E and F). In contrast, this lesion strikingly improved rotarod performance (P = 0.0009, Fig. 3E) and led to a significant increase in the number of DCX-positive cells in the SGZ in R6/1 mice (P = 0.007, Fig. 3E) indicating that reducing the dopaminergic input had a beneficial effect on motor performance and adult hippocampal neurogenesis. No effect was observed in the SVZ (Fig. 3F), a site at which adult neurogenesis is not reduced in these mice (Grote et al., 2005; Lazic et al., 2006).

**Deleterious effects of dopamine in R6/1 mice are mediated through D2Rs**

Since the majority of drugs used in the treatment of HD patients block D2Rs (Mason and Barker, 2009), we subsequently explored whether the deleterious effects of dopamine in HD are mediated via these receptors. We used the D2R antagonist sulpiride which, although commonly used to treat chorea and behavioral features in HD patients,
has yet to be investigated in a mouse model of HD and compared its
effects with L-DOPA and the D2R agonist quinpirole. Naïve R6/1 and WT
littermate mice were treated with these drugs for 7 days and then
were perfused 2 weeks after the last injection in order to study the
effects of these drug treatments on adult neurogenesis (see Fig. 4Af or
experimental timeline). Neither D2R agonist nor L-DOPA treatment had
any effect in R6/1 mice (Figs. 4B and C) while treatment with the D2R
antagonist sulpiride showed a partial increase of the number of DCX
positive cells in the SGZ of R6/1 mice (P = 0.044, Fig. 4D) indicating
that blockade of D2R improves adult hippocampal neurogenesis in
these mice. However, sulpiride treatment had no effect on motor
performance (Fig. 4G) nor did L-DOPA or quinpirole treatment (Figs. 4E
and F). In WT littermates, 5 mg/kg quinpirole did enhance hippocampal
neurogenesis but no effect was observed with either 6 mg/kg L-DOPA or
10 mg/kg sulpiride.

Discussion

In this study we have demonstrated a seemingly “paradoxical role”
for dopamine in HD when compared with the normal brain. Although
D2Rs were downregulated early in the course of HD in both the striatum
and the DG of the R6/1 mice, a reduction of dopaminergic input via MFB
lesions or sulpiride treatment was beneficial, unlike that observed in
WT mice. Given that dopamine blocking/depleting drugs are widely
used in the management of HD, our observations may have wide
reaching implications in the treatment of these patients.

The normal role of dopamine in adult hippocampal neurogenesis

It is widely accepted that dopamine controls the proliferation of
neural precursor cells (NPCs) in the SVZ and that this is mediated
through D2Rs leading to the paracrine release of epidermal growth
factor (EGF) (O’Keeffe et al., 2009) and ciliary neurotrophic factor
(CNTF) (Yang et al., 2008). Although there is substantially less evidence
regarding the role of dopamine on the SGZ, there are some studies
implying that dopamine may play a similar role at this site. Firstly, the
dopaminergic terminals contact proliferating cells in the SGZ (Hoglinger et al., 2014;
Hajszan, 2007) and the dopaminergic terminals contact
proliferating cells in the SGZ (Hoglinger et al., 2004). Secondly, Yang
and colleagues have demonstrated that there is an increase in the num-
ber of proliferating cells in both the SVZ and SGZ of WT mice when
D2Rs are pharmacologically stimulated along with up-regulation of
CNTF (Yang et al., 2008). Additionally, dopamine and its receptors are
significantly involved in synaptic transmission, long-term potentiation
(LTP) and memory function in the hippocampus all of which may
involve adult neurogenesis (Blackwell et al., 2008; Crook and Housman,
2012; Mu et al., 2011; Rossato et al., 2009).
In this study, we have provided additional evidence for the role of dopamine in maintaining adult hippocampal neurogenesis by demonstrating that lesioning the dopaminergic input to the DG in WT mice leads to a decrease in normal adult hippocampal neurogenesis which is rescued by administration of L-DOPA, in line with a previous MPTP study (Hoglinger et al., 2004). In contrast, studies that have tried to block dopamine receptors have been less clear cut—for example, haloperidol has been shown to decrease (Backhouse et al., 1982; Wakade et al., 2002), increase (Dawirs et al., 1998; Kippin et al., 2005) or have no effect (Malberg et al., 2000) on hippocampal neurogenesis. Similar contradictory results have also been seen with regard to neurogenesis in the SVZ (Backhouse et al., 1982; Wakade et al., 2002). However, in addition to dopamine receptor blockade, haloperidol has additional pharmacological properties on the glutamatergic, noradrenergic and serotoninergic systems which are all known to influence neurogenesis (Grote and Hannan, 2007), which complicates the interpretation of such studies.

The primary roles of the CNS dopaminergic system are in controlling motor function, aspects of cognition and motivational behaviors as well as endocrine functions (Wise, 2004). In this study, we have shown that inhibiting the dopaminergic system in R6 transgenic mice improved motor performance as assessed using a rotarod test, a test which seems to not be sensitive to motivational aspects of behavior (Brooks et al., 2012; Jones and Roberts, 1968). It is though still possible that this improvement related to a non-motor effect of the dopaminergic lesions, such an an anti-depressive effect. The reason for this is that the female R6 line mice have been reported to show early depression-like phenotypes (Pang et al., 2009) and that the dopaminergic system has also been implicated in the beneficial effects of some antidepressant drugs in these same mice (Renoir et al., 2012). Indeed, an improved rotarod performance was reported in the N171-82Q mouse model of HD when the Tg mice were treated with the SSRI antidepressant sertraline (Duan et al., 2008).

The "paradoxical" role of dopamine in HD

D2R downregulation is one of the earliest findings in HD patients and has also been described in several mouse models. In this study, we confirmed that D2Rs are downregulated in the striatum but have also now shown for the first time that D2Rs are downregulated in the DG in a HD mouse model from 8 weeks of age, in a non-progressive fashion.

Despite this reduction in D2R, the majority of drugs currently used to treat HD block D2Rs and/or deplete presynaptic dopamine (Mason and...
indicating that a reduction of the dopaminergic input appears to be of benefit. However, despite the widespread use of such drugs in clinical practice, very few studies have examined the effects of these agents in HD mouse models and ours is the first study to investigate the effects of the D2R antagonist sulpiride in such a model and show a beneficial effect on both motor function and adult hippocampal neurogenesis. Similar beneficial effects of dopamine reduction in vivo in transgenic HD mouse models have previously been described with lesions of the substantia nigra (Stack et al., 2007) or the administration of tetrabenazine (Tang et al., 2007), whereas increased dopamine levels, via administration of L-DOPA, have deleterious effects (Hickey et al., 2002). Although the mechanism by which dopamine blockade is beneficial is unknown, and something that is currently is under investigation, there is some evidence to suggest that dopaminergic abnormalities lead to excitotoxic cell death in HD. One such piece of evidence is that in striatal lesions produced by 3-nitropropionic acid (3-NP), which were used as an early model of HD, the neurotoxic effects were attenuated by reducing the dopaminergic input to the striatum using dopaminergic lesions (Jakel and Maragos, 2000; McLaughlin et al., 1998; Reynolds et al., 1998). Furthermore while there is no difference in neuronal survival in striatal cultures of WT and R6 mouse models, HD striatal neurons are more susceptible to dopamine-induced stress with increased cell death (Charvin et al., 2005; Petersén et al., 2001). One reason for this may be that dopamine is sufficient to directly damage neurons via oxidative stress and the production of free radicals which leads to impaired autophagy (Petersén et al., 2001). However, other studies have found that dopamine does not directly cause cell death but may work synergistically with glutamate causing excitotoxicity (Tang et al., 2007). This may be important since both the striatum and the DG are key sites of glutamatergic and dopaminergic input (Leranth and Hajszan, 2007) hence explaining the motor impairments and reduction in neurogenesis observed in a variety of mouse models of HD.

The vulnerability of striatal neurons may also be directly linked to reduced D2R levels given that overactivation of dopaminergic signaling such as elevated dopamine release promotes apoptosis/regeneration of striatal neurons (Cyr et al., 2003; Stephens and Yamamoto, 1996). D2R is a main modulator of medium spiny neurons (MSNs), the main output neuron in the striatum (Lalchandani et al., 2013), and elevated dopamine levels have been reported in both human and animal HD models (Garrett and Soares-da-Silva, 1992; Jahanshahi et al., 2010). Thus in HD there may be abnormal over-activation of the dopaminergic networks which target susceptible neuronal populations such as the striatal MSNs, with secondary downregulation and loss of D2Rs.

In our study, we observe a partial increase of neurogenesis in the DG after treatment with the D2R antagonist sulpiride which suggests that at least some of the detrimental actions of dopamine in the DG are mediated by D2R. However, we did not examine a role for D1R which
has also been shown to be abnormal in HD (Andrews et al., 1999; Ginovart et al., 1997; Turjanski et al., 1995). Indeed, there have been emerging studies suggesting a role for D1R in HD. For example, mice in which striatal D1Rs are genetically ablated have some of the neuro-pathological features of HD including reduced body weight, impaired locomotor and striatal atrophy (Kim et al., 2014). Mutant huntingtin has also been reported to promote striatal cell death through D1R (Paoletti et al., 2008), and a D1R agonist has been reported to rescue impaired long-term potentiation in a mouse model of HD (Dallerac et al., 2011). We therefore cannot exclude the possibility that D1Rs are also involved in the beneficial effects of dopaminergic lesions in R6/1 mice and further studies on this are needed.

**Implications for the treatment of human HD**

Despite the recent advances in better understanding the pathogenesis of HD, there have been very few breakthroughs in terms of medical treatments. At present this disease is incurable and treatment focuses on symptom management mainly based on anecdotal observation (Mason and Barker, 2009). Tetrabenazine, the only licensed drug for use in HD, is a vesicular monoamine transporter blocker which depletes the monoamine transmitters throughout the central nervous system including dopamine and this has been shown to reduce chorea in HD (Huntington Study Group, 2006). In addition to tetrabenazine, other dopaminergic drugs such as sulpiride are also commonly used off label to treat chorea and mood fluctuations in HD. This is the first study to investigate the effects of sulpiride in a mouse model of HD, and we found that it led to an increase in adult hippocampal neurogenesis, a process which we have previously shown to be important in rodent cognition (Clelland et al., 2009). This gives rise to the possibility that these drugs could have more than just symptomatic benefits on motor aspects of the disorder. Nevertheless, further studies are needed to establish whether these agents have significant cognitive benefits in patients.

**Conclusions**

Overall our findings reveal an unexpected role for dopamine in HD mice, and that despite a decrease in D2Rs in the striatum and DG, further reducing this dopaminergic input is beneficial and opposite to that observed in WT mice. These findings may have significant clinical relevance given that currently dopamine blockers and D2R antagonists are used as symptomatic treatments of chorea and mood fluctuations in human HD. It is therefore imperative that we better understand how dopamine regulates adult neurogenesis and neuronal function in the HD brain given that manipulation of this system may have consequences on disease progression and expression.

Supplementary data to this article can be found online at [http://dx.doi.org/10.1016/j.nbd.2014.02.004](http://dx.doi.org/10.1016/j.nbd.2014.02.004).

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