“Brain training” improves cognitive performance and survival in a transgenic mouse model of Huntington’s disease

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ABSTRACT

Environmental enrichment (EE) has been shown to improve neurological function and cognitive performance in animal models of Alzheimer’s disease (AD), Parkinson’s disease (PD), and Huntington’s disease (HD). We have shown recently that even when they are already living in an enriched environment, additional EE had beneficial effects in R6/2 mice. Here we examined the effects of three different enrichment paradigms on cognitive dysfunction in R6/2 mice in a longitudinal study. The EE consisted of either enforced physical exercise on the Rotarod (predominantly motor stimulation), training in a novel type of maze, the ‘noughts and crosses’ (OX) maze (mainly cognitive stimulation), or access to a playground, that gave the mice the opportunity for increased, self-motivated activity using running wheels and other toys in a social context (mixed EE). We designed the OX maze to test spatial memory in the R6/2 mouse while minimizing motor demands. Control mice remained in their home cages during the training period. Mice were given enrichment between 6 and 8 weeks of age, followed by cognitive (Lashley maze) and motor testing (Rotarod) between 8 and 10 weeks. Mice were then given a further period of enrichment between 10 and 12 weeks, and their behavior was re-tested between 12 and 14 weeks of age. We also collected body weights and age at death from all mice.

The OX maze was as sensitive for detecting learning deficits in the R6/2 mice as other types of mazes (such as the Morris water maze). Interestingly, providing cognitive stimulation via training in the OX maze produced significant improvements in subsequent cognitive performance by male, but not female, R6/2 mice in the Lashley maze task. OX maze training also significantly improved loss of body weight and survival in male R6/2 mice. These effects became apparent after as little as 2 weeks of training in the OX maze. These data suggest that there is a cognitive reserve that may be exploited in neurodegenerative disease. While brain training was not beneficial for all mice, it produced no deleterious effects, and so warrants further study in rodent models of HD.

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Introduction

HD is a genetic neurodegenerative disorder caused by an expanded CAG repeat in the coding region of the HTT gene (The Huntington’s Disease Collaborative Research Group, 1993). It is characterized by progressive striatal and cortical neurodegeneration (Reddy et al., 1999), although by the end stages of the disease many subcortical regions are also involved. Patients with HD develop progressive motor, cognitive and psychological symptoms that invariably lead to death within 15–25 years after the first onset of symptoms.

In the absence of an effective treatment for HD, increased environmental stimulation of HD patients has the potential to improve the symptoms and slow disease progression. It has already been demonstrated that an active lifestyle, involving enhanced social, physical and mental components, protects against dementia and Alzheimer’s disease in human patients (Fratiglioni et al., 2004; Baker et al., 2010; Lee et al., 2010). Similar beneficial effects of physical, social and cognitive stimulation might be seen in human HD patients (Sullivan et al., 2001; Zinzi et al., 2007).

The laboratory animal correlate of an enhanced lifestyle in humans is known as environmental enrichment (EE). This has been shown to have beneficial effects in animal models of Alzheimer’s disease (Mirochnic et al., 2009; Herring et al., 2010), Parkinson’s disease (Faherty et al., 2005; Jadavji et al., 2006), and on the progression of motor symptoms and the survival of the R6/1 and R6/2 mouse models of HD (Carter et al., 2000; van Dellen et al., 2000; Hockly et al., 2002). In these studies, environmental enrichment was provided primarily through different forms of enhanced housing (van Dellen et al., 2000; Hockly et al., 2002), although Carter et al. also demonstrated that improvements even to the feeding regime and regular behavioral testing significantly enhanced the life expectancy of R6/2 mice (Carter et al., 2010).
et al., 2000). We have also shown that R6/2 mice exposed to extended periods of time in a ‘playground’ (where they had access to a large cage containing toys, ladders and running wheels), were not only more active than R6/2 mice kept in their home cages, but also survived for longer, although there was little consistent effect of playground enrichment on cognitive function (Wood et al., 2010).

EE in the laboratory generally involves placing animals in a more ‘stimulating’ physical environment, such as improved home cage housing or playgrounds. However, EE can also be provided by increasing the level of cognitive stimulation. There is increasing evidence that maintaining a high level of mental stimulation can protect against dementia and other brain disorders (for reviews, see Valenzuela et al., 2008; Nithianantharajah and Hannan, 2009). This has led to the development of the concepts of ‘brain reserve’ and ‘cognitive reserve’ to explain these data. These theories may have relevance to HD, since preservation of cognitive function is a major goal in the search for effective therapies for this disease.

We were interested in testing the long-term effects of cognitive stimulation in R6/2 mice in the context of building a cognitive reserve. However, designing a suitable paradigm was problematic. R6/2 mice develop parallel motor and cognitive deficits (Carter et al., 1999; Lione et al., 1999), and also have retinal degeneration and dystrophy that restrict the use of visual learning tasks (Helmingler et al., 2002; Petrasch-Parwez et al., 2004). Young R6/2 mice are capable of carrying out traditional cognitive tasks such as the Morris water maze (Lione et al., 1999; Wood et al., 2007, 2010), but their deteriorating physical condition inevitably leads to reduced swim speed (Lione et al., 1999; Wood et al., 2007, 2010), and the retinal degeneration may affect discrimination of distal spatial cues. In order to minimize these potential confounds, we designed a cognitive test (the ‘noughts and crosses’ (OX) maze) that required minimal locomotion from the mice, and relied on proximal, rather than distal, visual cues. This test is described here for the first time.

We gave different types of EE to groups of R6/2 and wildtype (WT) mice. The EE consisted of either enforced physical exercise on the Rotarod (predominantly motor stimulation), training in the OX maze (mainly cognitive stimulation), or access to a playground (mixed EE). Control mice remained in their home cages during the training period. Following enrichment, we tested the mice for motor performance on the Rotarod, and for cognitive function using a standard task, the Lashley maze. We also collected body weights and age at death from all mice.

Materials and methods

Ethics statement

All components of this study were carried out in accordance with the UK Animals (Scientific Procedures) Act, 1986, and with the approval of the University of Cambridge Licence Review Committee.

Animals

Mice were taken from a colony of R6/2 transgenic mice (Mangiarini et al., 1996) established in the Department of Pharmacology, University of Cambridge, and maintained by backcrossing onto CBA × C57BL6 F1 female mice. Genotyping and CAG repeat length measurement were carried out by Laragen (Los Angeles, CA, USA). CAG repeat lengths were 182 ± 2 (mean ± SEM) as determined by GeneMapper. Note that the CAG repeat number measured by GeneMapper differs from that measured by sequencing. To convert the CAG repeat numbers determined by the GeneMapper technique to the CAG repeat number determined by the sequencing technique (which more closely represents the true CAG repeat number) the following formula needs to be applied: $\text{SEQ CAG no. (true CAG no.)} = 1.0427 \times \text{GM CAG no.} + 1.1695$ (personal communication, Dr J. Li, Laragen). For example, a GeneMapper repeat number of 182 CAG repeats would be calculated as 191 true CAG repeats.

Mice were group-housed in single sex, single genotype groups of 10. Clean cages were provided twice weekly, with grade 8/10-corncob bedding, and finely shredded paper for nesting. Mice were maintained on a 12 hour light:12 hour dark cycle, at a temperature of 21–23 °C and a humidity of 55 ± 10%. The mice had ad libitum access to water (using water bottles with elongated spouts) and dry laboratory food (RM3(E) rodent pellets, Special Diet Services, Witham, UK). EE was supplied to the home cages of all of the mice in the form of larger than usual quantities of bedding and nesting materials. In addition, twice a day, a mash was prepared by soaking 100 g dry food in 230 ml water until the pellets were soft and fully expanded. The mash was placed on the cage floor, improving access to food and water for the R6/2 transgenic mice. This feeding regime has been shown previously to be beneficial (Carter et al., 2000).

We used 64 male and 64 female mice for the study, half of which were hemizygotic transgenic (R6/2); the other half were WT control litter mates. All mice were born within a period of 7 days. Following genotyping, mice were assigned to one of 4 experimental groups. Each group contained 8 R6/2 and 8 WT mice of each sex. Each group of mice lived in a single-sex, mixed-genotype, home cage. Mice from individual litters were dispersed among the groups. The age of onset of overt symptoms in R6/2 mice with this CAG repeat length is approximately eight weeks, with the severity of symptoms progressing rapidly thereafter (Carter et al., 1999; Lione et al., 1999). In the current study, experimental testing began at 6 weeks of age, when R6/2 mice are phenotypically indistinguishable from their WT litter mates. Testing was carried out six days a week (Monday to Saturday). All mice were weighed daily until the end of the experiment at 13.5 weeks of age. Before the appearance of the distinctive phenotype, experimenters were blind to the genotype of individual animals; after the phenotypic changes became pronounced (from eight weeks onwards), it was impossible to conduct the experiment blind.

The experiment was divided into two sessions, that started when the mice were 6 and 10 weeks of age (Fig. 1). Each session comprised a training period of 14 days, followed by a testing period of 12 days in the Lashley maze plus one session on the Rotarod. During training, mice were exposed to one of four conditions: Control, Rotarod, OX maze, or Playground (for details, see below).

Training regime

Rotarod group

The Rotarod apparatus (Accelerating Model, Ugo Basile, Biological Research Apparatus, Varese, Italy) was used to provide motor stimulation. Mice were given two sessions daily of 5 minutes each at 15 rpm. As the phenotype became pronounced, R6/2 mice were unable to stay on the rod for five consecutive minutes at a time, even though they were still capable of locomotion. If they jumped or fell off the rod, they were replaced until they had accumulated five minutes per session.

Playground group

The playground apparatus consisted of a clear Perspex box (60 × 60 × 60 cm), containing 5 out of a selection of 10 ‘toys’, including running wheels, a nest box, ladders, climbing platforms, horizontal and vertical tubes (Fig. 2). Every day, 2/5 items were replaced, to maintain a stimulating environment. Mice were placed in the box in groups of four or five for one hour every day, after which they were returned to their home cage. After each use, the box and apparatus were cleaned with 1% acetic acid.
OX maze group

The OX maze was designed to test visual discrimination, learning and memory (Fig. 3). The test apparatus consisted of a square plastic-coated plywood box (60 cm × 60 cm × 30 cm high). The floor was marked with dark blue grid lines, producing twenty-five 12 cm × 12 cm squares, to allow for accurate positioning of each block. The maze consists of six white Perspex blocks (10 cm × 10 cm × 5 cm high). The blocks had a circular hole (2 cm diameter × 2 cm deep) drilled in each side, with each hole being located in the middle of one of four symbols (O, X, =, ⌘) which were drawn on with a permanent black marker (Fig. 3A). The orientation of the symbols in relation to each other was identical on each block. The blocks were positioned precisely using the grid lines according to particular maze layouts depending on orientation of the blocks (e.g., Fig. 3A). A fan was placed over the maze to circulate the air within the maze and to minimize the effects of olfactory cues on the acquisition of the task. Blank screens were arranged around the maze to minimize extra-maze spatial cues.

The reward comprised small pellets of dough made from flour, sugar and sunflower oil. A pellet of dough (20 mg in weight) was located in one of the four symbol-denoted holes in each block. The rewarded symbol was the same for each mouse (see below). Mice were placed in the center of the maze and allowed to explore the maze and blocks for 10 min. The number of correct and incorrect nose pokes into each hole of every block was recorded. After each 10-minute trial, the maze and blocks were cleaned with 1% acetic acid. Each mouse had one trial per day, with the opportunity to obtain six rewards. During the course of the experiment, the maze layout was altered randomly every day. The reward was located in the O position during acquisition in session one, and for four days of retention testing in session two. The X location was then used for the remaining 10 days of session 2 to assess reversal learning. The holes marked with = and ⌘ were not rewarded during this experiment.

Control group

Control mice remained in their home cage throughout the training period, except when they were removed for weighing.

Testing regime

Mice were trained under the various experimental conditions for 14 days between 6 and 8 weeks of age. They were then tested in the Lashley III maze (Nasello and Ramirez, 1978; Arendash et al., 1995) for 11 days between 8 and 10 weeks of age, and on the Rotarod (Carter et al., 1999) for 1 day. The mice were then returned to their training

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Fig. 1. Experimental timeline.

Fig. 2. Playground showing a range of toys used for enrichment.

Fig. 3. Noughts and crosses maze. Typical layout and dimensions of the maze (A). A mouse exploring the maze (B). For photographic purposes, clear walls are shown. During the experiment, the walls were opaque to reduce distal cues.
regime for 14 days between 10 and 12 weeks of age, and were re-tested at 12–14 weeks of age (Fig. 1).

Lashley III maze

The Lashley III maze is a complex task that can be solved by learning the correct sequence of right–left responses, or extra-maze cues, or both (Denenberg et al., 1991). The maze (40 cm long × 30 cm wide × 15 cm high) was constructed of white opaque plastic (Fig. 4). In this study, we filled the maze with water at room temperature to provide an extra incentive for the mice to escape. The mice were placed in the maze at point A, and had to swim through to the goal box at N, which contained an escape platform (Fig. 4). If they failed to find the goal box within 120 s, they were gently guided through the maze to the goal box, and allowed to climb onto the platform. A 60 W lamp was positioned above the goal box to illuminate the box and to provide an extra-maze cue.

Rotarod

The Rotarod was used for testing motor co-ordination and strength. Mice received two trials (maximum of 60 s each) at eight speeds (5, 8, 15, 20, 24, 31, 33 and 44 rpm). The mean latency to fall from the Rotarod (for the two trials at each speed) was recorded and used in the subsequent analysis.

Body weight

All mice were weighed daily. Because it was possible that consumption of the dough might have an effect on the OX group, an equal quantity of dough was fed individually to the mice in the other three groups at the end of training each day.

Survival

Age of death was recorded for all R6/2 mice. Mice were killed at end stage, i.e. if they were moribund, lacked a righting reflex, failed to rouse for their mash, or did not respond to gentle stimulation.

Statistical analysis

Group comparisons were made using ANOVA with repeated measures on one within subject factor (age/day/block of trials/Rotarod speed) as appropriate to the particular test. Differences between pairs of groups were evaluated using Dunnett’s test. For non-parametric data, a Friedman test with post-hoc Dunn’s multiple comparison test was used instead of ANOVA. Survival data were analyzed using a log rank test.

Statistical analyses were performed using StatSoft Statistica 19.0 (StatSoft Inc., Tulsa, USA) or Prism 5 (GraphPad Software Inc., San Diego, USA).

Results

The main effects of training in the various paradigms on cognitive and motor testing are shown in Table 1.

OX maze

We found that between the ages of 6–8 weeks, R6/2 mice were capable of solving the OX maze task, as shown by the increasing number of correct responses over time (Fig. 5). There was no difference in the rate of learning between WT and R6/2 mice of either sex (males, F(1, 182) = 0.005, p > 0.05; females, F(1, 182) = 3.847, p = 0.05). Following the first Lashley maze and Rotarod testing session between 8 and 10 weeks of age, the mice were returned to the noughts and crosses maze and given four days of retention testing (Fig. 5). Again, there was no difference between genotypes (males, F(1, 140) = 53.81, p = 0.0001; females, F(1, 140) = 26.15, p = 0.0001).

Rotarod

Rotarod testing revealed the expected deficits in R6/2 mice, with the transgenic mice impaired at staying on the rod, especially at higher speeds. By 9 weeks of age this deficit was already apparent in both male (Figs. 6A, E) and female (Figs. 6B, F) R6/2 mice. At 9 weeks,

Table 1

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↓: increase relative to Control group.
↑: decrease relative to Control group.
•: no change relative to Control group.

Fig. 4. Lashley III maze. The maze was filled to a depth of 15 cm with water at room temperature. A is the start position and N is the goal box, which contained an escape platform. The dashed line indicates the optimal route.
there was an effect of training group on Rotarod performance in male WT mice, with the OX group showing poorer performance than the Playground, Control and Rotarod groups (main effect F(3, 21) = 5.682, p<0.01; OX vs. Playground, p<0.001; OX vs. Control and Rotarod, both p<0.01; Fig. 6A). At 13 weeks, there was still a significant difference between OX and Playground groups, but the other differences seen at 9 weeks had disappeared (main effect F(3, 21) = 4.578, p<0.05; OX vs. Playground, p<0.05; Fig. 6C).

By contrast, in 9 week old female WT mice, the OX group showed superior performance to mice in the Control and Rotarod groups (main effect F(3, 21) = 6.475, p<0.01; OX vs. Control, p<0.001; OX vs. Rotarod, p<0.05; Fig. 6A). At 13 weeks of age, the effect of training group was no longer present in female WT mice (F(3, 21) = 4.264, p<0.05; Fig. 6D).

With the male R6/2 mice, the Playground group showed superior performance to the Control group at 9 weeks of age (main effect F(3, 21) = 5.367, p<0.01; Playground vs. Control, p<0.01; Fig. 6E). By 13 weeks, this effect was no longer present (F(3, 21) = 2.958, p=0.06; Fig. 6G).

The female R6/2 mice showed a beneficial effect of prior Rotarod training on Rotarod performance. At 9 weeks, the Rotarod group performed better than the Control and OX groups (main effect F(3, 21) = 8.749, p<0.001; Rotarod vs. Control, p<0.01; Rotarod vs. OX, p<0.05; Fig. 6G). By 13 weeks, all of the female R6/2 enriched groups out-performed the Control group, demonstrating a generally beneficial response to all kinds of EE on Rotarod performance in female R6/2 mice (main effect F(3, 21) = 5.910, p<0.01; Rotarod vs. Control, p<0.01; Playground and OX vs. Control, both p<0.05; Fig. 6H). It was surprising that there was no training effect in the other groups of mice (all males and female WT mice), but the finding of a clear beneficial effect in female R6/2 mice suggests exercise as a possible therapy in these mice.

**Lashley III maze**

Performance of mice in the Lashley maze was assessed by comparing the time taken to complete the task between day 1 and all subsequent days. At 8–10 weeks, WT mice from all groups and both sexes showed significant improvements in the time taken to complete the task from day 6 onwards (Table 2A, Figs. 7A, B, C, D). Male R6/2 mice from the OX group also showed improved performance from day 6 onwards (Table 2A, Fig. 7F). By contrast, male R6/2 mice in the Control, Rotarod and Playground groups showed no evidence of learning (Table 2A, Figs. 7E, F). Female R6/2 mice in the Control group displayed reductions in time to completion of the task from day 7 onwards (Table 2A, Fig. 7H), but those in the other groups showed no consistent evidence of learning (Table 2A, Figs. 7G, H).

During the second Lashley maze testing session (12–14 weeks of age), WT mice remembered how to solve the maze (Table 2B, Figs. 7A, B, C, D). R6/2 mice of both sexes showed marked impairments in solving the maze, showing no evidence of improving their performance in the task (Table 2B, Figs. 7E, F, C, H). The exceptions were mice from the male OX group, which showed improvements in performance from days 18–20 (Table 2B, Fig. 7F).

**Weights**

Body weights of all mice were recorded daily until the end of the experiment at 13.5 weeks. In the male WT mice, the Control group was the heaviest over the course of the experiment (Friedman statistic 48.39, p<0.001; Fig. 8A). The Playground group was also lighter than the OX group (p<0.05, Fig. 8A). Similar results were found with the female R6/2 mice, where the Control group was heavier than the other three groups (Friedman statistic 48.23, p<0.01; Fig. 8D). It is likely that the reduced body weights of enriched male WT and female R6/2 mice are a consequence of the difference in energy expended by EE mice during enrichment. Mice in the female WT OX group were heavier than all other groups (Friedman statistic 63.83, p<0.01; Fig. 8B). In addition, the female WT Rotarod group was heavier than the Control group (p<0.01, Fig. 8B). Male R6/2 mice from the OX group were also heavier than those from all other groups (Friedman statistic 40.12, p<0.001; Fig. 8C).

Together, these data demonstrate that giving mice the opportunity for increased exercise reduces their body weights relative to mice that stay in their home cage, suggesting that control mice were more sedentary than EE mice, and gained weight faster. However, exposure to the OX maze had positive effects on the weight of female WT and male R6/2 mice, especially from the second session (10 weeks of age) onwards, when they increased weight compared to all other groups. This effect was not due to consumption of the reward pellets since all mice in each group were fed an equivalent number of pellets at the end of each training day.

**Survival**

None of the WT mice died. In male R6/2 mice, the OX group mice lived longer than either Rotarod (p<0.01) or Playground (p<0.05) mice (Fig. 9A). Median ages at death for male mice were: Control, 112±3 days; OX maze, 117±5 days; Rotarod, 107±2 days; and Playground, 104±4 days. Exposure to the different types of enrichment had no effect on survival in female R6/2 mice (Fig. 9B). Median ages at death for female mice were: Control, 123±3 days; OX maze, 119±4 days;
Rotarod, 124 ± 2 days; and Playground, 118 ± 3 days. There were expected sex differences, with female R6/2 mice living longer than their male littermates in the Control (p < 0.05, Appendix Fig. 1A), Rotarod (p < 0.0001, Appendix Fig. 1B) and Playground (p < 0.01, Appendix Fig. 1C) groups. Interestingly, there was no sex difference in age at death in the OX group (Appendix Fig. 1D), suggesting again that training in the OX maze produced beneficial effects in male R6/2 mice.

Discussion

We reported recently that providing EE to R6/2 mice in the form of access to a playground (which gave the mice the opportunity for greater voluntary physical exercise as well as an opportunity for exploration and social interaction) produces a variety of outcomes, depending upon sex and genotype (Wood et al., 2010). In the current
study we explored this further, by providing mice with EE that was predominantly physical exercise (Rotarod), mixed motor/exploratory/social stimulation (Playground) or which provided cognitive stimulation through a novel (OX) maze that we designed to avoid the potential confounds of deteriorating motor strength and visual acuity in R6/2 mice. As in our previous study (Wood et al., 2010), we found that EE produces complex results. However, we also discovered that it was possible to build a cognitive reserve in R6/2 mice, through the use of EE in the form of the OX maze.

R6/2 mice can learn traditional water-based maze tasks such as the Morris water maze, and such tasks are valuable because they provide a measure of motor and cognitive function, with exposure to water providing a motivation to complete the task that is absent in ‘dry’ mazes (Lione et al., 1999; Wood et al., 2007, 2010). However, we were concerned that the stress of exposure to aversive tasks (Engelmann et al., 2006; Harrison et al., 2009), the requirement to swim by R6/2 mice that already have a motor deficit (Lione et al., 1999; Wood et al., 2007, 2010), and the possibility that R6/2 mice have deteriorating vision (Helmlinger et al., 2002; Petrasch-Parwez et al., 2004) could all have confounding effects on behavioral testing results of R6/2 mice in the water maze. We designed our novel OX maze as a spatial test that involved little movement and used proximal visual cues. Young (6–8 weeks) R6/2 mice quickly learned the acquisition task, and performed well on the retention task after a 2 week break, suggesting that their vision was adequate to distinguish the proximal cues. However, R6/2 mice were severely impaired in reversal learning, a finding that mirrors our results in the two-choice swim tank (Lione et al., 1999). These data suggest strongly that the OX maze can detect the same deficits as the two-choice swim tank, but without the stress of immersion in water. Our OX maze utilizes a highly palatable food reward that the R6/2 mice will work for without the need for food deprivation, thus the OX maze has the further advantage of not requiring food deprivation, as is necessary for cognitive tasks carried out in operant conditioning chambers. Since R6/2 mice develop a metabolic deficit and eat more as the phenotype develops (van der Burg et al., 2008; Wood et al., 2008), tasks that involve food deprivation should be avoided with these mice.

We were interested in trying to develop a cognitive reserve in R6/2 mice, and specifically, whether previous training in a motor task, a mixed task or a cognitive task could influence the decline in learning and memory found in R6/2 mice (Lione et al., 1999). There was no

### Table 2
Lashley III maze testing data.

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- No difference, *p<0.05, **p<0.01, ***p<0.001.
Fig. 7. Lashley III maze results. Time to navigate the maze to the escape platform in the Lashley maze. Mice were tested first between 8 and 9.5 weeks, and a second time between 12 and 13.5 weeks. WT mice learned and remembered the position of the platform (male WT mice, A, B; female WT mice C, D). R6/2 mice showed significant impairments in solving the task (male R6/2 mice, E, F; female R6/2 mice, G, H) with the exception of the male OX group (F). Data are means ± SEM.
effect of EE on Lashley maze performance in WT mice, probably due to a ceiling effect. However, male R6/2 mice from theOX group showed significant reductions in escape latencies up to 14 weeks of age, suggesting that they had retained greater cognitive capacities than R6/2 mice in the other training groups. Mice from theOX group increased (WT females) or maintained (R6/2 males) body weight relative to the other groups, demonstrating that in these mice, training in the OX maze had beneficial effects. This was not due simply to calories obtained from the reward pellets, since all mice in each group were fed equivalent numbers of pellets at the end of each training day. This beneficial effect in male R6/2 mice was reflected in the survival data, which showed longer survival of the R6/2 male OX group mice. Interestingly, although we found the expected sex differences in survival in the Control, Rotarod and Playground groups (where the female R6/2 mice lived significantly longer than their male R6/2 littermates), this difference was not present between the OX groups. This finding reinforces the beneficial effects that exposure to theOX maze had on cognitive function in male R6/2 mice.

One of the aims of our study was to investigate the use of environmental enrichment to prevent the deterioration of the behavior of R6/2 mice. Nevertheless, given that disease progression in these mice is so rapid, there is the possibility that some aspects of the enrichment could have negative effects. For example, subjecting the mice to a reversal task may induce ‘confusion’ and/or stress that might have adversely affected subsequent Lashley maze and Rotarod testing. However, since a major aim of this study was to develop a behavioral task that was less stressful than the traditional water-based tasks, we wanted to conduct a thorough comparison, including a reversal session. We found that in the OX maze task, although the R6/2 mice showed impaired reversal learning, they also showed clear evidence of learning the reversal, by performing above the level of chance. We chose to test the mice in the Lashley maze and Rotarod because these tests are very different to the OX maze. We wanted to minimize the effects (either positive or negative) that would arise from ‘familiarity’ with either a type of test, or a particular piece of testing apparatus. In future studies, it would be interesting to see whether repeated training in the OX maze (without testing reversal) would produce better effects when the mice are subsequently tested in the Lashley maze.

Other enrichment effects varied markedly. Male WT and female R6/2 mice that were exposed to the Rotarod had lower body weight than Control mice, but this did not affect age at death, suggesting that the effect was not detrimental to the animals’ general health. We expected that mice that had previously been trained on the Rotarod would show superior performance when tested on the same apparatus, and this was the case, for R6/2 female but not male mice. There was no effect of Rotarod training on cognitive ability when tested in the Lashley maze. It has been reported that exercise in humans improves cognitive performance (Kramer et al., 1999; Kramer et al., 2005), but we did not find this to be the case with our mice. It is possible that 5 min daily on the Rotarod was just not enough time to exert a beneficial effect. It may also be the case that, since this exercise was enforced rather than voluntary, it induced stress in the animals that might have masked any beneficial effect. As with the Rotarod, we expected that the playground would be a stimulating
environment for the mice. However, although it improved Rotarod performance in 9 week old male R6/2 and 13 week old female R6/2 mice, it had no major beneficial effects on either cognition or body weights. Furthermore, male R6/2 mice exposed to the playground died earlier than those in the other groups. This was unexpected, since we have shown recently that access to a playground improved survival in R6/2 mice (Wood et al., 2010). In the previous study, mice had access to the playground for 6 h a day, compared to 1 h per day in the current experiment. It may be that competition for the toys in the playground during the shorter period raises stress levels in the mice. These results reinforce the point that even very similar forms of EE can produce widely differing effects.

The focus of our study was the behavioral rather than the molecular or biochemical consequences of enrichment, so we can only speculate on the possible mechanisms underlying the beneficial effects we found. There are a great many systems through which enrichment may potentially improve function, behavior and lifespan, including increased levels of neurotrophins such as nerve growth factor and brain-derived neurotrophic factor (Ickes et al., 2000; Zhu et al., 2006), which in turn may promote neurogenesis (Kempermann et al., 1997; van Praag et al., 1999). In addition to neurogenesis, enrichment has been reported to increase dendritic growth (Leggio et al., 2005) and synaptic plasticity (Duffy et al., 2001). The dopaminergic (Segovia et al., 2008a), cholinergic (Segovia et al., 2008b), GABAergic (Segovia et al., 2006) and glutamatergic (Segovia et al., 2006; Nichols et al., 2007) neurotransmitter systems have all been implicated in the beneficial effects of environmental enrichment. Thus it seems likely that the beneficial results of enrichment arise from a widespread effect on a large number of systems.

In summary, we have studied the effects of various types of enrichment in R6/2 mice, including training the mice in a novel type of maze that does not require aversive stimuli or food restriction. In common with our previous work (Wood et al., 2010), we found clear sex differences in response to the various kinds of enrichment, reinforcing the idea that potential therapies must be tested in mice of both sexes before conclusions can be drawn as to global efficacy. We also found that exposure to the OX maze produced significant improvements in cognitive performance and survival in male mice, effects that were not seen in mice given access to a playground or exercised on a Rotarod. Our data suggest that 'brain training', and the subsequent development of a cognitive reserve, may be beneficial in HD.

Supplementary materials related to this article can be found online at doi:10.1016/j.nbd.2011.02.005.

Acknowledgment

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Fig. 9. R6/2 mouse survival data. Male mice from the OX group lived longer than those from the Rotarod and Playground groups. Playground mice died sooner than those from the Control group (A). Training condition had no effect on survival in female R6/2 mice (B).

References


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