Delaying the onset of Huntington's in mice

This unremitting disease develops later in animals stimulated by their environment.

Huntington's disease is an inherited (autosomal dominant) disorder in which there is progressive neurodegeneration, affecting the corpus striatum and cerebral cortex of the brain, and for which there is no known cure. Transgenic mice have been created[1,2] that develop a neurodegenerative syndrome that closely models the human disease. Here we show that exposure of these mice to a stimulating, enriched environment from an early age helps to prevent the loss of cerebral volume and delays the onset of motor disorders.

We randomly allocated 30 male Huntington's disease (HD) R6/1 mice to either a normal or a stimulating environment. Sixteen of these mice were positive for the HD transgene, in which exon 1 of the human *huntingtin* gene[1] is expressed with an expanded CAG repeat[2] encoding an extended polyglutamine tract. All mice were housed in groups in large standard cages. Routine care included provision of normal feed and bedding, whereas the cages of the 'environmentally enriched' groups also contained cardboard, paper and plastic objects, which were changed every two days, from the age of 4 weeks.

We characterized the response of the mice to their new environment by testing each mouse once, at 19–22 weeks of age, in an experimental cage, where it had the option of approaching or avoiding such stimuli. The cage was identical to the home cages except that only one half contained 'enrichment' objects. The mouse was put in the middle of the cage and the time spent in each half of the cage was measured over 3 min. Mice from the enriched groups spent much of their time exploring the objects (75% for wild-type and 70% for HD mice).

Environmental enrichment was not associated with any effect on body mass or spontaneous motor activity. HD mice were on average 16% lighter at 20 weeks than wild-type littermates (29.6 g versus 35.3 g; $P<0.01$), but enrichment had no significant effect on body weight in either group. Blood and urine glucose levels were normal in all mice.

To define the onset of disease, motor coordination was tested every week in a 'turning task', by placing each mouse at the end of a suspended, horizontal wooden rod. This test reveals deficits in motor coordination while minimizing the influence of memory and muscular strength. Strikingly, only one of the environmentally enriched group of HD mice (14%) had developed this behavioural sign at the end of testing at 22 weeks (Fig. 1a; $P<0.000001$).

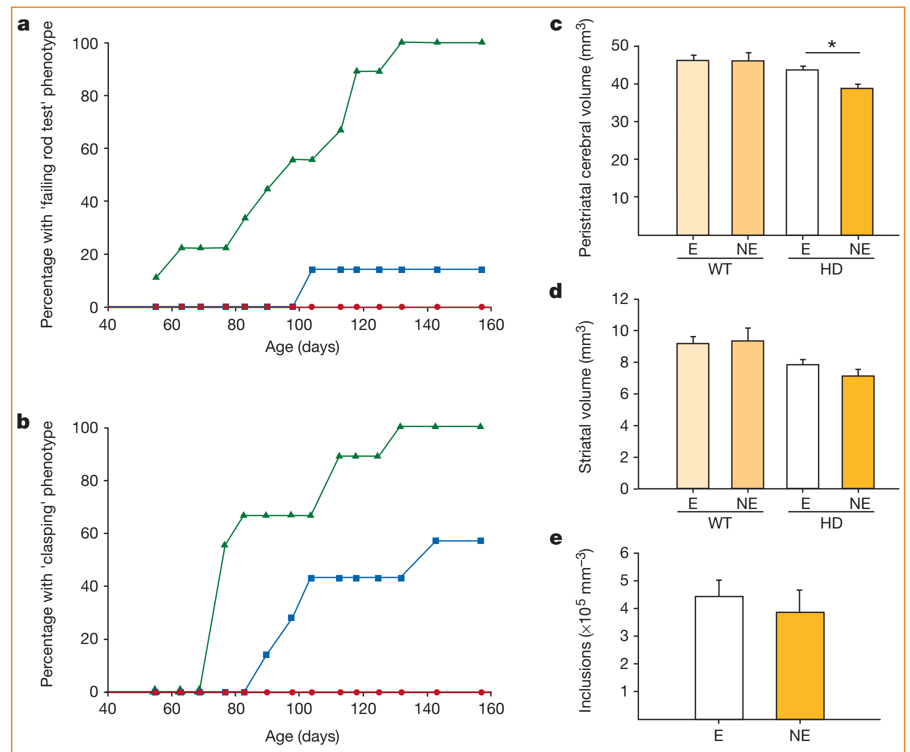

Figure 1 Exposure to a stimulating environment delays the onset of the disease phenotype in R6/1 HD mice. **a,** The cumulative percentage of mice consistently failing a test of motor coordination is plotted as a function of age. A failure is defined as consistent falling or inability to turn around on a suspended horizontal rod. The enriched environment markedly delayed the appearance of this behavioural sign in HD mice ($P<0.000001$; χ^2 test). **b,** This environment also delayed the appearance of sustained rear-paw clasping, another indication of HD phenotype in R6/1 mice. The cumulative percentage of mice reaching the onset of this sign is plotted as a function of age. Onset was highly significantly delayed in the enriched environment ($P<0.000001$; χ^2 test). Squares, HD mice in enriched environment; triangles, HD mice given routine care; circles, wild-type (WT) mice (enriched (E) and non-enriched (NE) groups pools: no failures). **c,** Effect of environmental enrichment on the volume of the corpus striatum and the surrounding portion of the entire forebrain in HD mice. The outlines of the whole brain and of the striatum on both sides were digitized for a series of coronal sections running from the caudal to the rostral pole of the striatum. The 'peristriatal cerebral volume' was calculated separately for E and NE groups of WT and HD mice. Analysis of variance (ANOVA) indicates a significant effect of enrichment ($F[1,21]=4.63$, $P=0.043$), a highly significant effect of genotype ($F[1,21]=16.17$, $P=0.0006$) and an enrichment × genotype interaction that did not reach significance ($F[1,21]=4.18$, $P=0.054$). Asterisk, $P<0.01$, Student's *t*-test. **d**, ANOVA of differences between the groups in the volume of the striatum (both sides of the brain combined) indicates no effect of enrichment ($F[1,21]=0.394$, $P=0.54$), a significant effect of genotype ($F[1,21]=18.89$, $P=0.0003$) and no enrichment × genotype interaction ($F[1,21]=1.00$, $P=0.33$). **e**, Exposure to an enriched environment does not retard the appearance of ubiquitinated neuronal inclusions in the corpus striatum of R6/1 HD mice. The densities of ubiquitinated inclusions (means + s.e.m.) were estimated from counts through the entire striatum of E and NE HD mice. They did not differ significantly ($P=0.55$, Student's *t*-test).

Another early sign of disease in HD mice is clasping of the rear paws when briefly suspended by the tail[2]. This sign is presumably independent of muscle strength and acquired motor skills. Again, the appearance of this sign was significantly delayed in the enriched group compared with the normally reared HD animals (Fig. 1b; $P<0.000001$).

A late component of the disease phenotype in HD mice is the onset of seizures[2]. Two of the non-enriched group had seizures, at 14 and 19 weeks old respectively, but none of the enriched mice had them.

As disease was advancing in the control HD mice, we examined their brains at 22 weeks of age by quantitative histology using an automated stereological analysis system (Neurolucida).

The combined volume of the striatum, 'striatal volume', on both sides of the brain was calculated, as was the volume of the entire coronal block of cerebrum between the rostral and the caudal poles of the striatum, which includes the part of the cerebral cortex with the densest projections to the striatum. This 'peristriatal cerebral volume' was 13% larger in the environmentally enriched HD mice than in the non-enriched HD group (Fig. 1c; $P<0.05$), whereas enrichment did not affect the peristriatal cerebral volume of wild-type mice. The striatum was slightly larger in enriched than

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in non-enriched HD mice, but this difference was not significant (Fig. 1d; $P=0.33$). These results suggest that environmental enrichment delays the degenerative loss of cerebral volume in HD mice and that the corticostriatal pathway plays a role in the pathogenesis of HD.

HD mice develop neuronal inclusions that are immunopositive for ubiquitin[3]. There was no significant difference in the overall density of inclusions[3] in the striatum between enriched and non-enriched HD mice (Fig. 1e). This shows that enrichment does not prevent the formation of inclusions, despite its effects on the appearance of symptoms. The TUNEL assay revealed no occurrence of apoptotic cell death.

The remarkable effect of environmental enrichment in delaying the onset of neurological signs in HD mice could be explained by increased sensory input and/or motor activity having a direct influence on the striatum, for example by altering the synaptic number and morphology of medium spiny neurons[4,5]. Alternatively, the effect could be mediated through the cortical input. Environmental enrichment might, for instance, counter the reduction in activity in parts of the cerebral cortex that occurs in Huntington's disease[6].

Deficits in a form of synaptic plasticity — long-term potentiation (LTP) — have been found in hippocampal slices from HD mice[7,8]. Environmental enrichment in rats increases the strength of specific hippocampal synapses, regulates the induction of LTP, increases the binding of glutamate to the AMPA receptor[9], and protects against excitotoxicity[10]. Environmental enrichment may therefore overcome deficiencies of synaptic plasticity, particularly at intracortical and corticostriatal[4,5] synapses, and help to improve the defects in HD mice.

Monozygotic twins with Huntington's disease and identical CAG-repeat lengths can display distinctly different clinical symptoms and behavioural abilities, implying that the disease is affected by environmental factors[11]. Our findings suggest that occupational therapy based on the principles of environmental enrichment might delay the onset of Huntington's disease in humans as well.

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1. Huntington's Disease Collaborative Research Group *Cell* **72,** 971–983 (1993).
2. Mangiarini, L. *et al. Cell* **87,** 493–506 (1996).
3. Davies, S. W. *et al. Cell* **90,** 537–548 (1997).
4. Comery, T. A., Shah, R. & Greenough, W. T. *Neurobiol. Learn. Mem.* **63,** 217–219 (1995).
5. Comery, T. A., Stamoudis, C. X., Irwin, S. A. & Greenough, W. T. *Neurobiol. Learn. Mem.* **66,** 93–96 (1996).
6. Deckel, A. W., Cohen, D. & Duckrow, R. *Neurology* **51,** 1576–1583 (1998).
7. Murphy, K. P. S. J. *et al. J. Neurosci.* (in the press).
8. Hodgson, J. G. *et al. Neuron* **23,** 181–192 (1999).
9. Foster, T. C., Gagne, J. & Massicotte, G. *Brain Res.* **736,** 243–250 (1996).
10. Young, D. H., Lawlor, P. A., Leone, P., Dragunow, M. & During, M. J. *Nature Med.* **5,** 448–453 (1999).
11. Georgiou, N. *et al. Mov. Disord.* **14,** 320–325 (1999).

Biogeochemistry

Hexadecane decay by methanogenesis

The potential for the biological conversion of long-chain saturated hydrocarbons to methane under anaerobic conditions has been demonstrated by using an enrichment culture of bacteria to degrade pure-phase hexadecane[1]. The formation of methane in hydrocarbon-rich subsurface zones could be explained if a similar conversion of long-chain alkanes to methane were to take place in subsurface environments. If this process could be stimulated in the subsurface, it could be used to enhance hydrocarbon recovery from petroleum reserves[1,2]. Parkes[2], however, questions the environmental significance of the enrichment-culture results[1] on the grounds that alkane conversion to methane is very slow and because sulphate-reducing and methanogenic bacteria might both be necessary for even this slow process to occur, restricting the conversion to specialized, unusual zones in sediments. Here we show that, on the contrary, subsurface bacteria can adapt to convert hexadecane to methane rapidly and in the absence of sulphate-reducing bacteria.

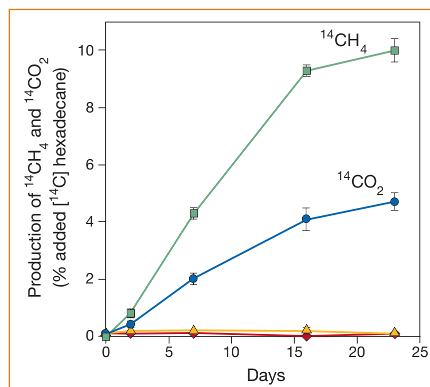

Figure 1 Metabolism of [^{14}C]hexadecane in contaminated aquifer sediments collected from a subsurface crude-oil spill site near Bemidji, Minnesota. Methanogenic oil-containing sediments readily produced $^{14}CH_4$ (squares) and $^{14}CO_2$ (circles), whereas there was no metabolism of hexadecane in oil-free, anaerobic sediments ($^{14}CH_4$, diamonds; $^{14}CO_2$,triangles) collected further downgradient within the contaminant plume. Results are the means of triplicate incubations; error bars show the standard error of the means.

The study of microbial metabolism in deep petroleum reservoirs is problematic owing to the technical difficulty of sampling sediments from these environments. Shallow petroleum-contaminated subsurface environments provide a likely analogue for petroleum reservoirs because microbial processes found in shallow subsurface environments are commonly found in deeper subsurface environments as well[3].

We sampled oil-bearing sediments from an aquifer located in Bemidji, Minnesota, that had been contaminated with crude oil. The sediments were incubated under strict anaerobic conditions at 20 °C as previously described[4]. The sediments were depleted of nitrate (to less than 5 μM) and sulphate (to less than 10 μM) and more than 95% of the HCl(0.5 M)-extractable iron was in the Fe(II) state. These sediments actively produced methane over time. When [2-^{14}C]acetate was injected into the sediments, $^{14}CH_4$ and $^{14}CO_2$ were produced in a ratio of 4:1, which is typical in sulphate-depleted sediments where methane production is the electron-accepting process. The concentration of hydrogen was 5–8 nM, characteristic of sulphate-depleted, methanogenic sediments[3].

When we injected [^{14}C]hexadecane into the sediments, it was converted to $^{14}CH_4$ without a lag (Fig. 1). This demonstrated that the microorganisms were already adapted for conversion of hexadecane to methane and suggests that hexadecane was being degraded *in situ*. In contrast, sediments that were slightly downgradient from the oil zone, and which were also anaerobic but not exposed to alkanes, did not degrade hexadecane. The ratio of $^{14}CH_4$ to $^{14}CO_2$ produced from [^{14}C]hexadecane in the oil-containing sediments was consistent with the ratio expected[1] for hexadecane conversion under methanogenic conditions.

Parkes' reservations[2], together with reports that alkanes are not significantly converted to methane in actual anaerobic sediments[5,6], have raised questions about how well the results from the enrichment culture experiment[1] can be extrapolated to typically sulphate-depleted, methanogenic subsurface environments. Our results show that subsurface microbial communities can adapt to rapidly convert alkanes to methane in the complete absence of sulphate reduction. The conversion of alkanes to methane is likely to be significant in many petroleum-containing anaerobic environments, including petroleum reservoirs.

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1. Zengler, K., Richnow, H. H., Rosselo-Mora, R., Michaelis, W. & Widdel, F. *Nature* **401,** 266–269 (1999).
2. Parkes, J. *Nature* **401,** 217–218 (1999).
3. Lovley, D. R. & Chapelle, F. H. *Rev. Geophys.* **33,** 365–381 (1995).

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