Research report

Increased thirst and drinking in Huntington’s disease and the R6/2 mouse

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Abstract

While Huntington’s disease (HD) is a condition that primarily involves the basal ganglia, there is evidence to suggest that the hypothalamus is also affected. Because the osmoreceptors regulating thirst are situated in the circumventricular region of the hypothalamus, we were interested in whether altered thirst is a part of the HD phenotype. We used the LABORAS behavioural monitoring system and water consumption to show that drinking behaviour was abnormal in R6/2 mice. By 10 weeks of age, R6/2 mice spent significantly more time drinking and drank a greater volume than their wild-type (WT) littermates. The numbers of immunoreactive vasopressin neurons in the paraventricular nucleus (PVN) of the hypothalamus in R6/2 mice were significantly decreased from 8 weeks of age, suggesting that the change in drinking behaviour may be the result of hypothalamic dysfunction. We gave a xerostomia (dry mouth) questionnaire to HD patients and control subjects, and also measured their urine osmolality and serum vasopressin. The mean total xerostomia score was significantly higher in HD patients than in controls, indicating greater thirst in HD patients. Urine osmolality was unaffected in HD patients up to clinical stage III, and none of the patients had diabetes. However, serum vasopressin was increased, suggesting a dysregulation in the control of hypothalamic vasopressin release. A dry mouth can affect taste, mastication and swallowing, all of which may contribute to the significant weight loss seen in both HD patients and R6/2 mice, as can dehydration. We suggest that increased thirst may be an important and clinically relevant biomarker for the study of disease progression in HD.

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1. Introduction

Huntington’s disease (HD) is a fatal neurodegenerative disorder, characterised by a progressive decline in cognitive and motor function. While HD primarily affects the basal ganglia, there is evidence to suggest that the hypothalamus is also involved [14,15,22]. Functions under hypothalamic control already known to be affected in HD include sleep, metabolism and weight maintenance.

Sleep disturbances are characteristic of progressive neurodegenerative diseases including HD [10,23,34]. The hypothalamus, and in particular the suprachiasmatic nucleus (SCN), is thought to be the principal circadian oscillator, so it is possible that disrupted sleep in HD patients is caused by hypothalamic dysfunction. Evidence for this comes from the demonstration that in the R6/2 mouse model of HD, a disrupted circadian rhythm occurs alongside dysregulated expression of...
the circadian clock genes mPer2 and mBmal1 in the SCN [20].

Defects in metabolism have been observed in HD [13,25,33], and although these may be due in part to mitochondrial dysfunction [2], it has also been suggested that loss of neurons in the lateral tuberal nucleus of the hypothalamus is involved [13]. An inability to maintain body weight is a common clinical symptom in HD [6,9,13], and is one of the most obvious phenotypic signs in R6/2 mice [18,33]. The underlying cause of weight loss in HD is not clear. Patients have been reported to lose weight when eating a nutritionally adequate diet [19], and even when consuming a greater number of calories than control subjects [31], which suggests a greater expenditure of energy in HD patients. Although it has been suggested that this may be related to the chorea [25], there is also evidence from HD patients for an imbalance in production of orexin and ghrelin, both of which act on the hypothalamus to control feeding [24]. Loss of orexin has also been reported in the R6/2 mouse [22].

Although it is proposed that deficits in sleeping, metabolism and maintenance of body weight may be due at least in part to hypothalamic dysfunction, it is not known whether other functions mediated by the hypothalamus, such as thirst and fluid consumption, are also affected. Thirst commonly manifests as a dry mouth [4], which has implications for HD symptoms such as food dysphagia and weight loss. A chronically dry mouth and a reduction in saliva could affect the patient’s ability to chew and swallow, and may contribute to the high incidence of choking and coughing in HD patients [11,16,21]. This may also be a consideration in rodent models of HD, since laboratory animals are routinely fed dry food pellets. When mastication difficulties and solid food dysphagia were found in HD patients, they were significantly associated with weight loss [31]. In addition, a dry mouth will reduce the ability of HD patients to taste food properly, and hence may influence the type of food that they eat, the frequency of eating, and the enjoyment derived from eating.

The current study was designed to investigate whether thirst and the associated dry mouth are factors in HD patients and R6/2 mice. If thirst and dry mouth are present, they may be important clinically relevant biomarkers for disease progression. We administered a xerostomia (dry mouth) questionnaire to HD patients and controls, and measured serum vasopressin levels and urine osmolality. We also studied drinking behaviour in R6/2 mice, and measured numbers of immunoreactive vasopressin neurons in the paraventricular nucleus (PVN) of the hypothalamus.

2. Materials and methods

2.1. Animal study

2.1.1. Drinking behaviour

Mice from two different colonies were used in two separate studies. For the first experiment, mice were taken from a colony of R6/2 transgenic mice [18] established in the Department of Pharmacology, University of Cambridge, and maintained by backcrossing onto CBA × C57BL/6 F1 female mice. Twenty-four female mice (12 R6/2, 12 WT) were used, which were kept in mixed-genotype cages. CAGI repeat lengths of the mice were in the range 247–277, with a mean of 267 ± 2 repeats (S.E.M.). The mice were maintained on a 12 h light:12 h dark cycle, at a temperature of 21–23 °C and a humidity of 55 ± 10%. The mice had ad libitum access to water and standard dry laboratory food (RM3(E) rodent pellets, Special Diet Services, Witham, UK). Lowered drink spouts were used to improve access to the water bottles. In addition, once 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 mice. This feeding regime has been shown previously to be beneficial for long-term health of the animals [5]. For measurement of drinking behaviour, mice were monitored for 24 h in the LABORAS apparatus (Metris b.v., Hoofddorp, The Netherlands). The LABORAS apparatus measures locomotor activity, immobility, grooming, eating, drinking and climbing [26,27,32]. During the monitoring period mice were singly housed with ad libitum access to food and water. For each mouse, testing was carried out 3 times, at 7, 10 and 18 weeks of age. Immediately before being put into the LABORAS cages, all mice were tested for glycosuria using Diazistix ( Bayer plc, Newbury, Berks., UK). All components of this experiment were carried out in accordance with the UK Animals (Scientific Procedures) Act, 1986.

In the second experiment, R6/2 mice were purchased (Jackson Laboratories, Bar Harbor, ME, USA) and maintained in the Department of Experimental Medical Sciences, Lund. CAG repeat lengths of the mice were in the range 163–174, with a mean of 168 ± 1 repeats (S.E.M.). The mice were housed in groups and maintained on a 12 h light:12 h dark cycle, at a temperature of 21–23 °C and a humidity of 55 ± 10%. The mice had ad libitum access to water and food. The experimental procedures were approved by the Regional Ethical Committee in Lund, Sweden. Water consumption was measured in male R6/2 mice and their wild-type littermates (n = 8 per group). At 5 weeks of age, 1 week prior to the start of the experiment, the mice were housed singly with ad libitum access to water and food. To facilitate drinking the water bottles were equipped with long, non-leaking tips. Water consumption was measured twice per week by weighing the bottle and comparing this to the weight of the full bottle that had been put in the cage 3 or 4 days earlier. The weight of water that the mice had consumed during those days was divided by the density of water (0.99) and by the number of days (3 or 4) to express the values as millilitres water consumed per mouse per day. At 12 weeks of age the animals were anaesthetised with pentobarbital and perfused with saline and 4% paraformaldehyde.

2.1.2. Mouse urine osmolality and volume

Urine was collected from R6/2 mice in the Cambridge colony by singly housing the mice in clean cages for up to 30 min each. Once each mouse had urinated it was immediately removed and returned to its home cage. The urine was tested for glycosuria (Diazistix (Bayer plc, Newbury, UK)), collected into individual Eppendorfs and frozen for later analysis. Urine osmolality was measured by the freezing point depression technique, using an Advanced Instruments 3320 Osmometer (Advanced Instruments, Norwood, MA, USA) in the Department of Clinical Biochemistry, Addenbrooke’s Hospital, Cambridge.

2.1.3. Immunohistochemistry

Histological studies were performed in the University of Lund, Sweden. In addition to the 12-week-old mice from the Lund colony mentioned above, 4-week-old male and female R6/2 mice and 8-week-old female R6/2 mice from Lund were anaesthetised with pentobarbital and perfused with saline and 4% paraformaldehyde. Brains were post-fixed for 24 h in 4% paraformaldehyde and dehydrated in 20% sucrose/0.1 M phosphate buffered saline. All brains were cut into coronal sections (30 μm). Endogenous peroxidase of free floating sections was quenched with 3% hydrogen peroxide and the sections were incubated for 1 h at room temperature in 5% goat serum/0.3% Triton X-100.1 M phosphate buffered saline. The sections were incubated overnight with a primary antibody against vasopressin (diluted 1:5000; Chemicon International Inc., CA, USA). The following day they were incubated with the corresponding biotinylated secondary antibody (1:200) for 1 h, and bound antibody was visualised using the ABC system (Vectastain ABC kit, Vector Laboratories) with 3,3′-diaminobenzidine as chromogen. The sections were lightly counterstained with cresyl violet (ICN Biomedicals Inc., Aurora, OH, USA). All analyses were performed on blind-coded slides using an Olympus BX50 microscope (Visio-pharm Integrator System, version 2.8.5.0, Hørsholm, Denmark). As the total number of vasopressin-positive neurons in the PVN of the hypothalamus is low, the technique of systematic random sampling (i.e., stereological principles) is not applicable. Therefore, we decided to count all stained cells unilaterally...
in all sections. All cells in the PVN of the left hemisphere were counted and included regardless of their size or shape. The average cell soma diameter (i.e., the mean length of the long and short axis) of the cells was estimated from 20 randomly selected vasopressin-immunopositive neurons per animal. The total number of stained cells was then calculated using the Abercrombie formula [1].

2.2. Human study

2.2.1. Xerostomia questionnaire

The xerostomia questionnaire used for this part of the study was taken from Eisbruch et al. [7]. It has high test–retest correlations, high internal consistency, and sensitivity for changes in dryness. The questionnaire consists of eight questions that asked the subject to:

1. Rate your difficulty in talking due to dryness.
2. Rate your difficulty in chewing due to dryness.
3. Rate your difficulty in swallowing solid food due to dryness.
4. Rate the frequency of your sleeping problems due to dryness.
5. Rate your mouth or throat dryness when eating food.
6. Rate your mouth or throat dryness while not eating.
7. Rate the frequency of sipping liquids to aid swallowing food.
8. Rate the frequency of sipping liquids for oral comfort when not eating.

The questionnaire did not include questions related to taste perception or oral pain or discomfort. Subjects rated each symptom on an 11-point ordinal Likert scale from 0 to 10, with higher scores indicating greater dryness or discomfort due to dryness. The item scores were added together to form a total in which higher scores represented greater levels of xerostomia.

The xerostomia questionnaire was given to all HD patients attending the Cambridge Centre for Brain Repair HD clinic between February and August 2006 (n = 117). Of these, more than half were taking medications that had drying of the mouth as a possible side effect. For a full list of the medications potentially causing xerostomia, see Table 1. These patients were excluded from the analysis, giving a final group of n = 46. The control group was made up from the immediate family of the patient, or their caretaker (n = 96). For demographic data, see Table 2A.

All individuals completed the questionnaire independently.

2.2.2. Urine osmolality and serum vasopressin

Patients were recruited to the study from the HD clinic at the National Hospital for Neurology and Neurosurgery (NHNN), London, UK. All had a positive genetic diagnosis of HD. Patients were clinically staged ranging from presymptomatic gene carriers through clinical stages I–IV [29]. Healthy controls were recruited from non-consanguineous relatives and friends of patients attending the clinic and from healthy volunteers. For numbers of patients and controls, see Table 2B. All participants or their next of kin gave written informed consent prior to entering the study. The study was approved by the ION/NHNN ethical review board and the NHNN Research and Development Committee. Blood and urine were collected in sterile containers and frozen at −70 °C within 2 h of collection. Samples were collected between 2 and 5 p.m. to minimise any effect of diurnal variation.

Serum vasopressin was determined using a commercially available 125I RIA kit (Phoenix Pharmaceuticals, Belmont, CA, USA). Duplicate samples were assayed. Urine osmolality was determined by routine laboratory analysis, Clinical Chemistry, Lund University Hospital, Lund, Sweden.

2.3. Statistical analysis

Drinking and mouse urine osmolality data were analysed by analysis of variance (ANOVA) (Prism 4.0, GraphPad Inc.) with Bonferroni post hoc test. For non-parametric data, a Friedman test was used instead of ANOVA. The number of vasopressin-positive cells and their diameters in WT and R6/2 mice were analysed by a two-factor analysis of variance (ANOVA) followed by a Student’s t-test at the different timepoints. Xerostomia questionnaire data were analysed using Student’s t-test. Because the patients had a wide range of scores on the Unified Huntington’s Disease Rating Scale (UHDRS), a regression analysis was carried out to examine the relationship between UHDRS score and xerostomia questionnaire score.

Human vasopressin and urine osmolality data were analysed using a two-tailed unpaired t-test or two-factor analysis of variance (ANOVA), with Bonferroni’s multiple comparison test. A critical value for significance of p < 0.05 was used throughout the study.

3. Results

3.1. Animal study

The different colonies of R6/2 mice showed similar pathologies, but slightly different rates of progression. Fig. 1 shows timelines for the two colonies.
Table 2A
Demographic data from HD patients and control subjects (Cambridge cohort)

<table>
<thead>
<tr>
<th></th>
<th>All patients</th>
<th>Selected cohort (no xerostomia-inducing medication)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>57 F/44 M/16 unknown</td>
<td>27 F/20 M</td>
</tr>
<tr>
<td>Age (years)</td>
<td>51.4 ± 11.9 (F)/53.3 ± 12.2 (M)</td>
<td>50.2 ± 2.4 (F)/52.6 ± 2.2 (M)</td>
</tr>
<tr>
<td>IS (a.u.)</td>
<td>77.5 ± 1.6 (n = 117)</td>
<td>81.3 ± 2.7 (n = 47)</td>
</tr>
<tr>
<td>UDHRS motor (a.u.)</td>
<td>27.9 ± 2.0 (n = 117)</td>
<td>24.7 ± 3.3 (n = 47)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>69.5 ± 1.4 (n = 117)</td>
<td>66.0 ± 2.0 (n = 47)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.4 ± 0.6 (n = 117)</td>
<td>23.9 ± 0.6 (n = 47)</td>
</tr>
</tbody>
</table>

Table 2B
Demographic data from HD patients and control subjects (London cohort)

<table>
<thead>
<tr>
<th></th>
<th>Control 22 F/12 M</th>
<th>Pre-manifest 4 F/3 M</th>
<th>Clinical stage I/II (early) 10 F/7 M</th>
<th>Clinical stage III (moderate) 9 F/4 M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>22 F/12 M</td>
<td>4 F/3 M</td>
<td>10 F/7 M</td>
<td>9 F/4 M</td>
</tr>
<tr>
<td>Age (years)</td>
<td>42.0 ± 2.0 (n = 34)</td>
<td>35.0 ± 2.0 (n = 7)</td>
<td>47.0 ± 3.0 (n = 17)</td>
<td>53.0 ± 3.0 (n = 13)</td>
</tr>
</tbody>
</table>

Abbreviations: F, female; M, male; a.u., arbitrary units; BMI, body mass index; IS, independence score; UHDRS, unified Huntington’s disease rating scale.

3.1.1. Drinking behaviour

We used the LABORAS system to examine frequency of drinking behaviour of R6/2 mice at 7, 10 and 18 weeks. In the Cambridge colony, onset of phenotype (stereotyped hindlimb grooming) occurs at around 12 weeks of age. The mice cease to gain weight from this point, with loss of body weight occurring at around 18–20 weeks. At 7 weeks, there was no difference in drinking between genotypes ($F_{(1,44)} = 1.49, p > 0.05$; Figs. 2A and B, 3A). However, by 10 weeks of age R6/2 mice were spending significantly more time drinking than their WT littermates ($F_{(1,44)} = 141.8, p < 0.0001$; Figs. 2C and D, 3A). There was a clear difference between genotypes in both the light and dark phases of the circadian cycle ($p < 0.001$ in both cases, Fig. 3A). This pattern persisted to 18 weeks of age, by which time the R6/2 mice were clearly symptomatic ($F_{(1,44)} = 240.3, p < 0.0001$; Figs. 2E and F, 3A). Differences between genotypes were significant during both light and dark phases ($p < 0.001$ in both cases, Fig. 3A).

Because the R6/2 mice showed such a marked increase in time spent drinking, we wondered whether they were suffering from a generalised hyperactivity. LABORAS collects a number of different parameters simultaneously, including drinking, eating, locomotor activity, climbing and grooming. We examined locomotor activity from the LABORAS data. At 7 weeks there was no main effect of genotype ($F_{(1,44)} = 1.724, p > 0.05$; Fig. 3B), although post hoc analysis revealed a decrease in loco-
Fig. 2. Activity plots of drinking behaviour during 24 h. Each data point is total time spent drinking per minute, averaged across the groups. Data in Figures A, C, and E come from wild-type (WT) mice, those in B, D, F are from R6/2 mice. Mice were tested at seven weeks (A and B), 10 weeks (C and D) and 18 weeks (E and F) of age.

Motor activity in R6/2 mice during the light phase ($p < 0.05$). By 10 weeks there was a significant difference between genotypes, with the R6/2 mice being less active ($F_{(1,44)} = 5.695$, $p < 0.05$; Fig. 3B). Again, this difference was due to hypoactivity in R6/2 mice during the light phase ($p < 0.01$). However, by 18 weeks there was no difference between genotypes, although this was due to a reduction in daytime activity in the WT mice rather than a change in R6/2 mice ($F_{(1,44)} = 0.001$, $p > 0.05$; Fig. 3B). Therefore, the increase in drinking in R6/2 mice does not seem to be due to a non-specific increase in activity. Nor was it due to the presence of diabetes. Measurements of glycosuria in the R6/2 mice revealed that at 7 weeks of age, none of the mice were diabetic. In humans, diabetes is considered to be present with a glycosuria level greater than 18 mmol/L. At 10 weeks 1 out of 12 R6/2 mice had a glycosuria reading of 14 mmol/L, while all others were 5.5 mmol/L or lower. By 18 weeks, all R6/2 mice had readings between 14 and >111 mmol/L, so at this age diabetes may have contributed to the increase in drinking.

The results obtained from the Cambridge mice were confirmed in the Lund cohort of mice, where the amount of water
Fig. 3. Drinking behaviour in R6/2 mice. The average time spent drinking was elevated in the Cambridge cohort of R6/2 mice from 10 weeks of age, with significant increases in both the light and dark phases of the circadian cycle (A). This increase in drinking behaviour was not the result of a generalised hyperactivity in the R6/2 mice (B). Total water consumption in R6/2 mice from the Lund colony was significantly increased compared to WT littermates from 10.5 weeks of age (C). Data are means ± S.E.M. *p < 0.05, **p < 0.01, ***p < 0.001.

consumed in a 24 h period was shown to be significantly greater in R6/2 mice (F(1,152) = 43.69, p < 0.0001; Fig. 3C), with significant differences at 10.5 weeks (p < 0.05), 11.5 weeks and 12 weeks (both p < 0.001) (Fig. 3C). None of the mice were diabetic at this age. These results were very similar to the data from the Cambridge mice, although the CAG repeat lengths and age at onset of hindlimb clasping (5 weeks in the Lund mice, compared to 12 weeks in the Cambridge colony) were different.

3.1.2. Mouse urine osmolality and volume

Urine osmolality in the Cambridge cohort of R6/2 mice was not different to that of WT littermates at 6 and 12 weeks of age (Fig. 4A). By 18 weeks, urine osmolality of R6/2 mice had significantly increased relative to that measured at either 6 or 12 weeks (F(2,27) = 6.2, p < 0.01). The urine osmolality of 18-week-old R6/2 mice was significantly higher than that of 6-, 12- or 18-week-old WT mice (F(1,27) = 9.72, p < 0.01).

The volume of urine produced by the mice during the 30 min collection period did not differ by age or genotype (Fig. 4B). Two mice in the 18 weeks R6/2 group did not produce enough urine for an accurate measure of osmolality to be possible.

3.1.3. Immunohistochemistry

The number of vasopressin-positive neurons in the PVN of the hypothalamus was measured unilaterally at 4, 8 and 12 weeks in the Lund mice. The cell number was significantly decreased in R6/2 mice compared to WT littermates (two-factor ANOVA: genotype F(1,36) = 19.166, p < 0.0005; age F(2,36) = 0.230, p = 0.796; age × genotype, F(2,36) = 1.493, p = 0.241). There was a significant difference between genotypes at 8 (p < 0.05) and 12 (p < 0.01) weeks of age (Fig. 5A). Atrophy...
of the vasopressin-immunopositive cells, as assessed by measuring cell diameter, declined progressively in R6/2 mice compared to WT mice (two-factor ANOVA; genotype \( F_{(1,35)} = 24.581, p < 0.0005 \); age, \( F_{(2,35)} = 0.744, p = 0.484 \); age \( \times \) genotype \( F_{(2,35)} = 6.292, p = 0.005 \)), and was significantly different at 12 weeks \( (p < 0.001, \text{Fig. 5B}) \). Micrographs of the PVN from WT (Fig. 5C) and R6/2 mice (Fig. 5D) clearly show reduced staining intensity and decreased cell volumes in R6/2 mice.

3.2. Human study

3.2.1. Xerostomia questionnaire

The mean total xerostomia score was significantly higher in patients (patients 20.8 ± 2.5 arbitrary units (a.u.), control subjects 6.3 ± 1.4 a.u., \( p < 0.01 \). Data are means ± S.E.M.). The answers to all eight questions in the xerostomia questionnaire were analysed separately. All were significantly different between patients and control subjects (see Fig. 6A). For the question “Rate the frequency of your sleeping problems due to dryness”, \( p < 0.05 \). For all other questions, \( p < 0.001 \).

There was a significant correlation between UHDRS score and xerostomia score \( (R^2 = 0.16, p < 0.01; \text{Fig. 6B}) \).

3.2.2. Human urine osmolality

No significant differences in urine osmolality were found between controls and HD patients \( (F_{(3,67)} = 0.242, p > 0.05, \text{Fig. 6C}) \).

3.2.3. Serum vasopressin

There was a significant main effect of genotype on serum vasopressin levels \( (F_{(3,67)} = 6.044, p < 0.001; \text{Fig. 6C}) \). Post hoc analysis showed that levels were significantly elevated in moderate (stage III) patients compared to age- and sex-matched control subjects \( (p < 0.01, \text{Fig. 6C}) \).

4. Discussion

The aim of this study was to investigate thirst and drinking in R6/2 mice and HD patients. We found that water consumption was significantly elevated in R6/2 mice from the Lund colony from 10.5 weeks of age, and in the Cambridge colony from 10 weeks of age. The time of onset of altered drinking behaviour in the two colonies was thus very similar, despite the large difference in CAG repeat lengths (mean 267 in the Cambridge colony, 168 in the Lund colony). When this study was conducted, the classical disease signs of R6/2 mice (hindlimb grooming, excessive face washing, weight loss) did not appear in the Cambridge colony until at least 12 weeks of age. At 10 weeks of age the Cambridge R6/2 mice appeared overtly normal and healthy, and had normal levels of locomotor activity, suggesting that the increase in drinking is not the result of a generalised hyperactivity. Therefore, an increase in drinking behaviour seems to provide an early marker for the disease in R6/2 mice. The increase in drinking at 10 weeks is unlikely to be related to diabetes. Although one R6/2 mouse from the Cambridge colony group had slightly elevated...
glycosuria, the other 11 had urinary glucose levels in the normal range. In the Lund colony, increases in blood glucose levels and the presence of diabetes have not been recorded below 12 weeks of age [3]. By 18 weeks, most of the Cambridge R6/2 mice were diabetic, so this is likely to have contributed to their increased drinking at this age. Urine osmolality was also elevated in 18-week R6/2 mice, although we found no difference in the volume of urine produced by the R6/2 mice with increasing age. The presence of increased drinking at 10 weeks of age, before the onset of increased urine osmolality or glycosuria, is therefore, likely to reflect hypothalamic rather than pancreatic dysfunction, although we cannot completely rule out the possibility that diabetes affects the drinking behaviour of R6/2 mice. It should also be noted that we measured micturition during a 30 min period only. It would clearly be interesting to measure urine production in these mice over extended periods.

Although the Cambridge R6/2 mice spent more time engaged in drinking behaviour than their WT littermates, and Lund mice ‘drank’ significantly more water, it is not known whether this reflects the amount of water actually ingested. It is possible that a neuromuscular disability such as we have reported previously [28] may lead to difficulty in swallowing. A reduced ability to swallow water may then lead to an increase in drinking behaviour in an attempt to swallow the required amount. However, there was no evidence of the mice having difficulties in swallowing. They did not have unusually wet mouths or fur, nor was there spillage below the water bottles. Furthermore, data from the Lund mice showed that the number of vasopressin-positive neurons in the PVN of R6/2 mice was decreased from 8 weeks, suggesting that the cause of the change in drinking behaviour is cellular, rather than neuromuscular. A similar decrease in vasopressin mRNA has been shown in R6/2 mice at 8 weeks by others, although no behavioural data were presented in that study [12].

We think that the changes in vasopressin immunoreactivity are due to transcriptional dysregulation rather than neurodegeneration. We have reported that the total number of cresyl violet-positive cells in the hypothalamus is not reduced in R6/2 mice compared to wild-type littermates [33], although the vasopressin-immunopositive cells are significantly atrophied at 12 weeks of age. This suggests that the loss of immunoreactivity is not caused by neuronal cell loss in the hypothalamus of R6/2 mice, although we appreciate that the number of vasopressin-secreting cells is low, and it is, therefore, difficult to exclude the possibility that some may have died (loss of such a small population of cells would not be detected by the quantification of cresyl violet-positive cells in the entire hypothalamus). Counting cells in the PVN may increase the chance of detecting loss of a small population of cells, but the lack of natural boundaries around this structure makes this option very difficult. If there is a loss of vasopressin-secreting cells then there may be a concurrent reduction in the level of serum vasopressin. It would, therefore, be interesting to measure circulating vasopressin levels as the phenotype develops. Nevertheless, transcriptional dysregulation in R6/2 mice is widely recognised [17,30], and on balance, our evidence suggests that loss of vasopressin expression is a part of a repertoire of general hypothalamic dysfunctions that includes gene dysregulation. The progressive loss of several different hypothalamic peptides (cocaine- and amphetamine-regulated transcript (CART), melanin-concentrating hormone (MCH), pro-opiomelanocortin (POMC) and orexin) in the R6/2 mouse [22,33] supports the theory of a widespread hypothalamic dysfunction. The loss of these peptides is not limited to a specific subregion of the hypothalamus, but occurs in at least the lateral hypothalamus and PVN as well as arcuate nucleus.

In contrast to our findings in R6/2 mice, HD patients had elevated levels of serum vasopressin, although there was no change in plasma osmolality. The increase in serum vasopressin may be caused by dehydration, since severely dehydrated rats have been shown to have elevated serum vasopressin [8]. However, in the rats this occurred in combination with increased plasma osmolality. It is unlikely that HD patients would be as badly dehydrated (the rats in the Gottlieb study were water-deprived for 48 h), so
the effects on plasma osmolality in patients may be less dramatic. If dehydration were the trigger, the fact that plasma osmolality is normal suggests that patients up to clinical stage III were still able to maintain water balance by increased vasopressin release. An alternative explanation for the human data is that there is a decrease in V2 receptors in the kidney. Under normal circumstances, increased plasma vasopressin leads to decreased urine flow and increased urine osmolality [35]. The finding in HD patients that an increase in serum vasopressin is not accompanied by a change in urine osmolality suggests an insensitivity to levels of circulating vasopressin. It would be interesting to measure V2 receptor numbers in post-mortem HD kidney.

Having found a dysfunction in drinking behaviour in R6/2 mice, we were interested to find out whether HD patients suffer from a similar problem. By using the xerostomia questionnaire, we found that HD patients were significantly more likely to report problems related to being thirsty and having a dry mouth than were control subjects. These included difficulties in talking, chewing, swallowing and sleeping due to a dry mouth. Although other symptoms of HD (such as neuromuscular dysfunction) may contribute to these problems, it is important to note that the patients themselves feel that xerostomia is a contributing factor. We also found a positive correlation between UHDRS score and xerostomia rating, suggesting that the perception of the patients is that problems associated with a dry mouth increased with disease progression. Our findings support earlier work showing that HD patients have a high incidence of choking and coughing [11,16,21]. Difficulties in chewing and swallowing may also contribute to the significant weight loss seen in both HD patients and R6/2 mice. We did not measure saliva production in HD patients, although it would be interesting to do so, since it may be difficult to distinguish between the dry mouth which is a sensation of thirst, and a dry mouth caused by a deficit in saliva production.

Xerostomia may be an important and clinically relevant biomarker for the study of disease progression in both HD patients and R6/2 mice. However, in the course of administering the xerostomia questionnaire we found that a large number of patients were taking medications that can in themselves cause a dry mouth (e.g., diazepam and paroxetine, see Table 2). As these drugs are likely to exacerbate the xerostomia, it would be helpful for physicians to avoid prescribing compounds that are associated with this side effect. It is just as important to note that xerostomia is comparatively simple to treat in HD patients. Although other symptoms of HD (such as neuromuscular dysfunction) may contribute to these problems, it is important to note that the patients themselves feel that xerostomia is a contributing factor.

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We have demonstrated an increase in drinking and a reduction in vasopressin-positive neurons in the PVN of R6/2 mice, and xerostomia and elevated levels of serum vasopressin in moderate-stage HD patients. These findings contribute to the body of evidence pointing to hypothalamic dysfunction in HD.

Conflict of interest

The authors declare no conflict of interest.

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