Hypothalamic expression of mutant huntingtin contributes to the development of depressive-like behavior in the BAC transgenic mouse model of Huntington’s disease

Sofia Hult Lundh1, Nathalie Nilsson1, Rana Soylu1, Deniz Kirik2 and Åsa Petersén1,*

1Translational Neuroendocrine Research Unit and 2Brain Repair and Imaging in Neural Systems (BRAINS) Unit, Department of Experimental Medical Science, Lund University, Lund SE-22184, Sweden

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Psychiatric symptoms such as depression and anxiety are important clinical features of Huntington’s disease (HD). However, the underlying neurobiological substrate for the psychiatric features is not fully understood. In order to explore the biological origin of depression and anxiety in HD, we used a mouse model that expresses the human full-length mutant huntingtin, the BACHD mouse. We found that the BACHD mice displayed depressive- and anxiety-like features as early as at 2 months of age as assessed using the Porsolt forced swim test (FST), the sucrose preference test and the elevated plus maze (EPM). BACHD mice subjected to chronic treatment with the anti-depressant sertraline were not different to vehicle-treated BACHD mice in the FST and EPM. The behavioral manifestations occurred in the absence of reduced hippocampal cell proliferation/neurogenesis or upregulation of the hypothalamic–pituitary–adrenal axis. However, alterations in anxiety- and depression-regulating genes were present in the hypothalamus of BACHD mice including reduced mRNA expression of neuropeptide Y, tachykinin receptor 3 and vesicular monoamine transporter type 2 as well as increased expression of cocaine and amphetamine regulated transcript. Interestingly, the orexin neuronal population in the hypothalamus was increased and showed cellular atrophy in old BACHD mice. Furthermore, inactivation of mutant huntingtin in a subset of the hypothalamic neurons prevented the development of the depressive features. Taken together, our data demonstrate that the BACHD mouse recapitulates clinical HD with early psychiatric aspects and point to the role of hypothalamic dysfunction in the development of depression and anxiety in the disease.

INTRODUCTION

Psychiatric symptoms are early and important features of the hereditary and fatal Huntington’s disease (HD). HD is caused by an expansion of a CAG repeat in the huntingtin (HTT) gene, which leads to an expanded polyglutamine tract in the ubiquitously expressed HTT protein (1). Wild-type HTT has important regulatory functions in cells including gene expression and synaptic function (2). The CAG expansion in the gene is thought to give rise to a toxic gain-of-function for the mutant HTT resulting in the neuropathology in the brain, while loss of the normal function of the protein might also contribute to the phenotype (3). The disease is characterized by progressive neuronal dysfunction and ultimately neuronal loss most prominently in the basal ganglia and cerebral cortex (4,5). These changes have been linked to the classic motor symptoms with involuntary movements as well as the cognitive symptoms with frontal dysfunction. The presence of the involuntary movements typical for HD together with the confirmation of the mutant gene forms the clinical diagnosis (6). The diagnosis is usually made in midlife leading to death within 20 years due to the progressive nature of the disease and lack of any disease-modifying treatments today.

Despite the fact that HD has traditionally been seen as movement disorder, the early psychiatric symptoms often precede the motor dysfunction and cause significant distress to the affected individuals and their families (7–9). The psychiatric symptoms

*To whom correspondence should be addressed at: Translational Neuroendocrine Research Unit, BMC D11, 221 84 Lund, Sweden. Tel: +46 462221686; Fax: +46 462223436; Email: asa.petersen@med.lu.se

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include anxiety, irritability, aggression, obsessive-compulsive behavior and depression, the latter being one of the most common symptoms (8). Although part of the psychiatric symptoms may arise from the difficulty of living in a family with HD, the observation that unaffected siblings have a lower prevalence of psychiatric symptoms than HD gene carriers argues for the existence of a neurobiological component (10–13). In agreement with this view, several mouse models expressing fragments of or the full-length mutant HTT gene develop depressive-like and/or anxiety-like features (14–22). Interestingly, in the R6/1 mouse model, which expresses a short fragment of mutant HTT, only female HD mice display depressive-like behavior which is associated with changes in serotonin receptor expression (15,17,23). No sex-specific differences in the depressive-like behavior have been found in one of the full-length mutant HTT models, the YAC128 mouse, where specific cleavage of mutant HTT by caspase 6 has been shown to be important in mediating the depressive-like behavior (16,18). However, the neuropathological processes leading to the psychiatric symptoms in HD are not fully known. In fact, the cause of depressive-like disorder in general is not fully understood but is under heavy investigation. Given that around 40% of patients with depression do not respond to current medical treatment options, there is a major unmet need for better therapies (24). Prevailing hypotheses for the neurobiological basis of depression include reduced adult hippocampal neurogenesis, alterations in the hypothalamic-pituitary-adrenal (HPA) axis and changes in different neuropeptide systems in the hypothalamus (24). Interestingly, early hypothalamic changes are present in HD and neuropathological studies of the hypothalamus have revealed alterations in neuropeptides regulating both emotion and metabolism in clinical HD (25–28). The affected neuropeptide populations express orexin (hypocretin), oxytocin, vasopressin and cocaine and amphetamine regulated transcript (CART) (25,26,29,30).

In order to further the understanding of the neurobiological origin of depression and anxiety in HD, we have used a mouse model that expresses full-length mutant HTT with 97Q engineered by a bacterial artificial chromosome (BAC) approach (BACHD mouse) (31). Given the presence of hypothalamic pathology in HD and the proposed role of hypothalamic circuits in emotion control, we hypothesized that expression of mutant HTT in the hypothalamus would be involved in causing the psychiatric features of the BACHD mice. We found that specific gene expression changes of anxiety and depression-regulating factors were indeed present in the hypothalamus of BACHD mice, and that inactivation of mutant HTT in a subset of hypothalamic neurons prevented the development of depressive-like features. Hence, this study supports the idea that expression of mutant HTT induces a neuropathology that contributes to the development of psychiatric symptoms in HD and points to the role of hypothalamic dysfunction in this process.

RESULTS

Development of depressive- and anxiety-like behavior in the BACHD mice

The BACHD colony was bred by mating male BACHD mice with female wild-type mice in order to ensure that all pups were bred by and cared for by a healthy mouse mother and that any potential behavioral change in the offspring would be of genetic origin. The BACHD mouse model has previously been reported to display depressive-like behavior at 12 months of age (16). Here, we show that BACHD mice display depressive-like behavior both at 2 and 6 months of age when assessed using the Porsolt forced swim test (FST) as well as using the sucrose preference test (SPT) (Fig. 1A and B). These tests are both well established to assess depressive-like behavior in mice (32–36). The data in the two tests were statistically analyzed using a three-factor ANOVA with genotype, age and sex as factors. For the FST, the analyses revealed a significant effect of genotype ($F_{(1,67)} = 10.66, P = 0.02$), but no significant effect of sex or age. However, as there was a significant interaction between genotype and sex ($F_{(1,67)} = 7.47, P = 0.008$), we analyzed the data from males and females separately and found that there was only a significant difference in the FST between male BACHD and wild-type mice (Student’s unpaired $t$-test, $P = 0.007$), but not in females ($P = 0.656$). In the SPT, the analyses revealed a significant effect of genotype ($F_{(1,41)} = 38.38, P < 0.001$), but no significant effect of sex or age. In this test of depressive-like behavior, there was no significant interaction between genotype and sex.

Furthermore, we found that the BACHD mice also displayed anxiety-like behavior at 2 and 6 months of age (Fig. 1C). Anxiety was assessed using the elevated plus maze (EPM), a well-established test to measure anxiety in rodents (37,38). A three-factor ANOVA with genotype, age and sex as factors revealed a significant effect of genotype ($F_{(1,66)} = 25.23, P < 0.001$), a significant effect of age ($F_{(1,66)} = 4.41, P = 0.04$) but no effect of sex.

In order to exclude that the findings of depressive- and anxiety-like behavior in BACHD mice were due to motor dysfunction and/or reduced swimming capacity, we measured general motor activity in the open field (OF) test as well as swim speed in a swim ability test (SAT) at both 2 and 6 months of age (Fig. 1D and E) (18). The results from these tests were similar for wild-type and BACHD mice, indicating that the BACHD mice did not display any reduced general motor activity or swimming activity which could account for the results in the FST or EPM.

No alterations in cell proliferation or neurogenesis in the hippocampus

Reduced cell proliferation and/or neurogenesis in the dentate gyrus (DG) of the hippocampus have been implicated in depression, although current research indicates that it may rather have a role as a target for antidepressant actions than be involved in the development of the disease (39–41). Interestingly, reduced cell proliferation in the DG has been found in several mouse models of HD (42–52). We assessed the number of newborn cells and their neuronal differentiation in the DG of 3-month-old BACHD mice (where a depressive-like phenotype is apparent) and wild-type littermates in order to see whether this was a feature also of the BACHD mice. The mice were subjected to two daily intraperitoneal (i.p.) injections with bromodeoxyuridine (BrdU) for 3 days. Twelve hours after the last injections, the animals were perfused and serial sections of the brains were subjected to immunohistochemistry (IHC) targeting...
BrdU, doublecortin (DCX) and NeuroD1. BrdU is a thymidine analogue and can therefore incorporate into the DNA of cells undergoing cell division (53). DCX and NeuroD1 are markers commonly used to assess neuronal differentiation in the adult hippocampus (54). We found no significant differences in the number of BrdU, DCX or NeuroD1 positive cells in the DG of the hippocampus between BACHD and wild-type mice nor any sex differences (Fig. 2A–I). There appeared to be a trend for a reduction of DCX positive cells in BACHD mice compared with wild-type mice (effect of genotype in the ANOVA analysis, P = 0.095).

Neuroendocrine factors in the BACHD mice

Another prevailing hypothesis for depression is alterations in the HPA axis. Changes in this neuroendocrine system have been found in both HD patients and in some HD animal models (24,29,55–57). The HPA axis constitutes of corticotropin-releasing hormone (CRH) released from the hypothalamus, which induces release of adrenocorticotropic hormone (ACTH) from the pituitary, which subsequentially leads to cortisol (corticosterone in rats and mice) release from the adrenal cortex. Similar to CRH, vasopressin stimulates release of ACTH from the pituitary, and it has been suggested that vasopressin might be responsible for the hyperactivity of the HPA axis sometimes found in depression (58). We therefore measured circulating levels of corticosterone and vasopressin in BACHD and wild-type mice but could not detect any differences between the groups (Fig. 3A and B). Hypothalamic pathology has been found in postmortem tissue from HD patients with reductions in the number of neurons expressing the emotion-regulating neuropeptides orexin, oxytocin and vasopressin (25,26). We have previously reported that there was no general loss of cells in the hypothalamus in BACHD mice at 4 months of age (59), so here we decided to assess the number of orexin, oxytocin and vasopressin neurons using stereology in 18-month-old female and male BACHD mice in order to study whether these neuronal populations would at all be affected in this mouse model. Interestingly, we found a significant increase in the number of orexin neurons in both female and male BACHD mice compared with their wild-type littermates together with a significant reduction in the cross-sectional area of these neurons (Fig. 3C). However, there were no differences in the number of oxytocin and vasopressin immunopositive neurons between the groups (Fig. 3D and E). These results show that several of the critical emotion-controlling neuronal populations are maintained in BACHD mice, but the changes in orexin neurons suggest the presence of dysfunction in this region.

Inactivation of mutant HTT in the hypothalamus in BACHD mice prevents the development of the depressive-like phenotype

Based on the fact that hypothalamic changes are present in clinical HD and that this region is part of the emotion control of the brain, we hypothesized that expression of mutant HTT in this region may be involved in causing the depressive- and anxiety-like behavior in BACHD mice. We therefore took advantage of the fact that BACHD mice are constructed with LoxP sites around exon1 of the mutant HTT gene, allowing the transgene to be inactivated in the presence of the enzyme cre-recombinase (cre) (31). Using recombinant adeno-associated viral vectors of serotype 5 expressing cre (rAAV-cre) under the neuronal specific synapsin promoter, we have previously shown that inactivation of mutant HTT in a subset of hypothalamic neurons could prevent the development of the metabolic phenotype in...
BACHD mice (59). Here, we injected the same vectors (or a vehicle solution as control) bilaterally into the hypothalamus of another group of female BACHD and wild-type mice at 2 months of age and assessed their behavior at 4 months of age. For the analyses of data obtained in the FST, a two-factor ANOVA revealed a significant effect of both genotype ($F(1,35) = 20.73, P < 0.001$) and treatment ($F(1,35) = 4.79, P = 0.035$). Further analyses of all groups using a one-factor ANOVA showed a significant effect of genotype ($F(3,35) = 8.56, P < 0.001$), and Tukey’s post hoc tests revealed a significant difference between BACHD-vehicle and all other groups, but that BACHD-cre was only significantly different to BACHD-vehicle and not to the wild-type groups (see Supplementary Statistical Results). Hence, inactivation of mutant HTT occurred specifically in the hypothalamus (Fig. 4A). A two-factor analysis of the data obtained in the EPM revealed a significant effect of genotype ($F(1,35) = 6.78, P = 0.013$) but no effect of the treatment ($F(1,35) = 0.045, P = 0.834$), i.e. inactivation of mutant HTT in the hypothalamus did not have any effect on the anxiety-like behavior in the mice (Fig. 4B).

Correct targeting of the hypothalamus in all mice was confirmed by IHC for cre (Fig. 4C). Stereological analysis of cre-immunopositive neurons in the hypothalamus revealed that around 60 229 ± 10 334 cells per side of the hypothalamus expressed cre in the BACHD and 37 000 ± 11 790 cells in wild-type mice. This corresponded to around 25 ± 4 and 14 ± 5%, respectively, of the total number of neurons in this region, as we estimated there to be around 242 400 ± 14 690 neurons in the BACHD and 256 133 ± 5174 cells in the wild-type mouse hypothalamus in this study. These numbers are in agreement with our earlier observations in non-injected animals (59). Hence, the stereological analysis showed that cre-expression did not cause neuronal loss. Furthermore, PCR analysis confirmed that cre excision of mutant HTT occurred specifically in the hypothalamus in BACHD mice but not in other brain regions such as the striatum, hippocampus or cerebral cortex (Fig. 4D). To confirm that, in line with our previous study (59), inactivation of mutant HTT in the hypothalamus in this experiment would prevent the body weight gain in BACHD mice, we also assessed body weight in the mice. Whereas vehicle-injected BACHD mice gained 40 ± 6% ($n = 8$) of body weight from 2 to 4 months of age, the cre-injected BACHD mice only gained 25 ± 3% ($n = 12$) of body weight, which was significantly different from the vehicle-injected BACHD mice ($P = 0.026$) but not different to cre- or vehicle-injected wild-type mice [14 ± 2% ($n = 9$) and 16 ± 3% ($n = 10$), respectively, see also Supplementary Statistical Results].

As the orexin cell population was found to be increased and showed cellular atrophy in 18-month-old BACHD mice, we decided to assess the effect of rAAV-cre on the orexin system in this group of 4-month-old mice. The total numbers of orexin neurons were assessed bilaterally, but the data are expressed as the average mean ± SEM per unilateral side to allow for comparisons to the data in 18-month-old mice. The numbers of orexin neurons in the unilateral hypothalamus of BACHD with rAAV-cre (2153 ± 71, $n = 12$) and vehicle (2145 ± 56, $n = 8$) were not statistically different to the numbers in wild-type mice with rAAV-cre (2063 ± 77, $n = 10$) and vehicle (2091 ± 46, $n = 9$). Interestingly, the cross-sectional area of orexin neurons in BACHD treated with rAAV-cre (167 ± 3 μm², $n = 12$) was significantly larger than in vehicle-treated BACHD mice (155 ± 2, $n = 8$; $P = 0.028$, Tukey’s post hoc test), and the cell size in BACHD treated with vehicle showed a trend to be reduced compared with wild-type mice treated.
Specific alterations of genes encoding for emotion-regulating factors in the hypothalamus of BACHD mice

Transcriptional dysregulation has been a consistent finding in both postmortem tissue from HD patients and several animal models of the disease (60). We hypothesized that mutant HTT in the hypothalamus of BACHD may affect expression of critical emotion controlling genes in this region. We therefore performed quantitative real-time PCR (qRT-PCR) analysis of a selection of 27 candidate genes involved in emotion control in the hypothalamus (Table 1). Hypothalami were dissected from 4-month-old BACHD mice with or without inactivation of mutant HTT in the hypothalamus after rAAV-cre injections at 2 months of age or from wild-type littermates. We found that the expression of four genes were significantly downregulated and that one gene was significantly upregulated in BACHD mice compared with wild-type littermates (Table 1). The downregulated genes included orexin receptor 2 (HCRTR2), neuropeptide Y (NPY), vesicular monoamine transporter type 2 (VMAT2) and tachykinin receptor 3 (TACR3) and rAAV-cre (165 + 4, n = 10; P = 0.124, Tukey’s post hoc test). These data indicate that inactivation of mutant HTT in the hypothalamus at this early stage has a positive effect on the orexin system. See also Supplementary Statistical Results.
Dopamine and serotonin system into the hypothalamus in 2-month-old female mice reduced the development of depressive-like behavior (Table 1). The mRNA for CART was significantly upregulated in BACHD mice (Table 1). No changes in gene expression in the hypothalamus of BACHD mice were significantly corrected after injections of rAAV-cre, but this may be due to the fact that we only targeted around 25% of the neurons in the hypothalamus in the previous experiment. Taken together, the data suggest

![Figure 4](https://academic.oup.com/hmg/article-abstract/22/17/3485/572159)

**Figure 4.** Inactivation of mutant HTT in the hypothalamus in young mice reduced the development of depressive- but not anxiety-like behavior. rAAV-cre injection into the hypothalamus in 2-month-old female mice reduced the development of depressive-like behavior (A) at 4 months but had no effect on the development of anxiety-like behavior (B) in the BACHD mice (n = 8–12/treatment and genotype). (C) Representative photomicrograph of cre-immunopositive cells 2 months after injections of rAAC-cre in the hypothalamus demonstrating correct targeting. Scale bar represents 250 μm. (D) PCR analysis confirmed the successful excision of mutant HTT in the hypothalamus, but not in the striatum, hippocampus or cerebral cortex, after hypothalamic expression of cre in BACHD mice. All data are expressed as means ± SEM, significant differences from BACHD vehicle mice: *Indicates a significant difference in Tukey’s post hoc test compared with WT + cre as well as BACHD + cre (P < 0.05). †Indicates significant effect of genotype in the ANOVA analyses (P < 0.05) (see also Supplementary Statistical Results).

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All data are expressed as the mean fold change ± SEM compared with the WT group. The gene expression levels were assessed in 4-month-old mice, where the BACHD/Cre-hyp group had been bilaterally injected with rAAV-Cre at 2 months of age (n = 7/group). Statistical analyses were performed using one-way ANOVA followed by Tukey’s post hoc test. CRH, corticotropin releasing hormone; Sim1, single-minded 1; Bm2, Brain2; NPY, neuropeptide Y; CART, cocaine and amphetamine regulated transcript; MCH, melanin-concentrating hormone; GHRH, growth hormone releasing hormone; TRH, thyrotropin-releasing hormone; VMAT2, vesicular monoamine transporter 2; AADC, aromatic l-amino acid decarboxylase; Sirt1, Sirtuin 1; BDNF, brain-derived neurotrophic factor; TACR3, tachykinin receptor 3.
early transcriptional dysregulation of specific emotion controlling genes in the hypothalamus of BACHD mice.

No effect of sertraline treatment on the psychiatric phenotype in BACHD mice

Finally, we were interested in investigating whether the psychiatric phenotype in BACHD mice would respond to chronic treatment with sertraline, a selective serotonin reuptake inhibitor (SSRI) commonly used for treatment of depression and anxiety. There is no evidence for SSRI treatment to have an effect in clinical HD, but it is often prescribed to treat the depressive-like symptoms or anxiety according to the standard clinical guidelines (9). We chose the dose of 20 mg/kg of sertraline which has previously been shown to ameliorate the depressive-like phenotype in R6/1 mice (23). A cohort of 2-month-old female and male BACHD and wild-type mice was treated with sertraline or vehicle i.p. daily for 4 weeks and was then tested using the FST and EPM at 3 months of age (Fig. 5A and B). The data were analyzed using a three-factor ANOVA with genotype, treatment and sex as factors. For the FST, we found a significant effect of genotype ($F_{(1,49)} = 12.13$, $P = 0.001$), but no significant effect of treatment or sex. Further analyses using Tukey’s post hoc test revealed that BACHD mice treated with vehicle were significantly different from both wild-type mice treated with sertraline ($P = 0.026$) and vehicle ($P = 0.002$) but not to BACHD mice treated with sertraline ($P = 0.443$). Interestingly, BACHD mice treated with sertraline was not statistically significant different to the wild-type mice treated with sertraline ($P = 0.511$) or vehicle ($P = 0.144$). Hence, the depressive-like behavior in BACHD was reproduced in this cohort, but there was no statistically significant difference between BACHD mice treated with sertraline compared with vehicle. We therefore performed a post hoc power calculation based on the above data to find out how many animals would be required to prove efficacy of sertraline and found that 53 BACHD mice treated with sertraline and 61 BACHD mice treated with vehicle would be required to, with the probability of 80% (power 0.8), find a true difference between the two BACHD groups. For the EPM, we found a significant effect of genotype ($F_{(1,50)} = 20.74$, $P < 0.001$), but no significant effect of treatment or sex. Taken together, these data indicate that the depressive- and anxiety-like behaviors in BACHD mice were not fully responsive to SSRI treatment.

DISCUSSION

Depression and anxiety are common early features of the clinical manifestation in HD. In this study, we show that depressive- and anxiety-like behaviors are present at an early time in the BACHD mouse which recapitulates clinical HD. Dysregulation of hypothalamic circuits has been suggested to play a role in the development of depression and anxiety disorders (24). The underlying neurobiological mechanisms of the psychiatric symptoms in HD are not known. Hypothalamic changes have been found in early stages of clinical HD using MR and PET imaging studies, and postmortem studies have demonstrated specific changes in emotion-regulating neuronal populations in the hypothalamus in HD (25–28,61,62). We therefore hypothesized that disrupted neurocircuits in the hypothalamus induced by expression of mutant HTT would be involved in the psychiatric features in HD. Using rAAV vectors expressing cre, we demonstrated that the development of depressive-like features can be prevented by inactivation of mutant HTT in part of the hypothalamus in BACHD mice, indicating that hypothalamic dysfunction can be important for the depressive-like features in BACHD mice.

In order to investigate what hypothalamic circuitries would be affected in the BACHD mice, we compared the expression of key emotion controlling genes in the hypothalamus from 4-month-old BACHD mice compared with wild-type mice, as well as BACHD mice injected with rAAV-cre. Our analyses demonstrated that four genes were significantly downregulated and that one gene was significantly upregulated in BACHD mice compared with wild-type littermates. Four of these changes were also detected in the group of BACHD mice injected with rAAV-cre, which confirms the robustness of the findings although it fails to reveal the direct underlying mechanism of mutant HTT-induced depressive-like behavior. In fact, as the expression levels of these genes are not corrected by the inactivation of mutant HTT in the hypothalamus, they are unlikely to be involved in the depressive-like behavior in the BACHD mice. Interestingly, we found that the mRNA level of HCRTR2 was reduced by 31% in BACHD mice compared with wild-type mice. For the rAAV-cre injected group, the expression of HCRTR2 did not significantly differ from either BACHD or wild-type mice. This suggests a partial effect of hypothalamic mutant HTT inactivation at the level of HCRTR2. The HCRTR2 gene encodes the orexin receptor 2, one of the two receptors for orexin. Orexin is a neuropeptide involved in the regulation of emotion, metabolism and sleep, and a reduction in the number of orexin immunopositive cells has been found in both postmortem human HD tissue as well as in several HD animal models (25,26,28,63). BACHD mice,
on the contrary, display increased numbers of orexin neurons at a late stage, which may be a sign of dysfunctional orexin circuitry and could be related to the reduced HCRTR2 levels. Interestingly, the Flinders Sensitive Line (a rat model of depression) has also increased number of orexin immunopositive neurons in the hypothalamus and knock-out mice for HCRTR2 display depressive-like but no anxiety-like behavior (64,65). Furthermore, BACHD mice showed a trend for cellular atrophy of the orexin population at 4 months of age, which became significant at 18 months of age. Importantly, inactivation of mutant HTT in BACHD mice resulted in significantly larger cellular area of the orexin neurons compared with vehicle-treated BACHD mice, indicating a direct link between mutant HTT expression and pathological effects on the orexin system.

Expression of NPY mRNA was reduced by around 35% in BACHD mice compared with wild-type mice. Reduced NPY signaling in the central nervous system has been suggested to play a role in both anxiety and depression (66,67). Stereological analyses of the number of NPY-expressing neurons in the HD hypothalamus as a whole did not reveal any changes, but later analysis focusing on the infundibular nucleus has indeed detected a 30% loss of NPY neurons (25,68). Moreover, in the Flinders Sensitive Line rat, treatment with electroconvulsive stimulation has been shown to increase NPY levels in the hypothalamus parallel to reducing immobility in the FST, further suggesting a role for impaired NPY signaling in the hypothalamus for depression (69).

Our analyses also revealed that the expression of TACR3 mRNA was reduced by 40% in BACHD mice compared with wild-type littermates. Tachykinins are a group of widely distributed neuropeptides such as substance P, neurokinin A (NKA) and neurokinin B (NKB) that function as excitatory neurotransmitters. The TACR3, also known as the NK3 receptor, preferentially binds neurokinin B (tachykinin 3) (70). A substantial number of clinical studies have been conducted with NK3 receptor antagonists for mainly schizophrenia, but also for major depression and anxiety, but the studies so far have yielded disappointing results. Interestingly, mutations in the TACR3 genes lead to hypogonadotropic hypogonadism in patients and the expression of the receptors in the hypothalamus plays an important role in integrating metabolic signals at the time of puberty and in reproduction (71,72).

mRNA of VMAT2 was downregulated in BACHD mice by 20% compared with wild-type mice. Mice with reductions in VMAT2 in the central nervous system display depressive- and anxiety-like behavior (73,74), but it is not known what the effect of a VMAT2 reduction specifically in the hypothalamus would be. Finally, the mRNA for CART was significantly upregulated in BACHD mice by 22% compared with wild-type mice. This is particularly interesting as CART has been found to be increased in cerebrospinal fluid and postmortem hypothalamic tissue from HD patients and is thought to mediate anxiety (25,30,75,76).

A sex-specific depressive-like phenotype has been consistently found in the R6/1 line where female mice display early depressive-like behavior (15,17,23). Sex differences for the depressive-like behavior in BACHD mice or for the other full-length mutant HTT mouse model YAC128 have not been reported (16,18). In this study, we found no effect of sex in the SPT or FST, but a significant interaction was detected between genotype and sex in the FST as shown in Figure 1, where only BACHD males were significantly different to their wild-type littermates. However, in the other FST experiments (Figs 4A and 5A), BACHD females significantly differed from wild-type female mice, which suggests that there are no major sex differences in the depressive-like behavior in BACHD mice. Furthermore, we found no effect of sex on anxiety-like behavior in the BACHD mice. However, BACHD male mice have previously been shown to display anxiety-like behavior already at 4 weeks of age in the light–dark choice test, whereas female BACHD first displayed anxiety at 12 weeks (14). We did not perform any behavioral analyses before 2 months of age in the present study, but it would be interesting to explore what the earliest time would be to detect behavioral changes in BACHD mice.

Reduced cell proliferation in the DG has been found in several HD mouse models (42–52). These mice also display hippocampal-dependent learning and memory deficits at the time of altered cell proliferation or neurogenesis in the DG (77–80). In the present study, we found no changes in cell proliferation determined by BrdU-labeling, or in the number of NeuroD1 or DCX positive cells in the DG of BACHD mice at 3 months of age of either sex. Male BACHD mice have been found to display hippocampal-dependent cognitive deficits at 6 months of age (81), but it is not yet known whether these are present in female BACHD mice or at the age of 3 months when cell proliferation in the DG was examined in this study. Interestingly, the YAC128 mice that develop reduced neurogenesis later in life do not exhibit any deficits in hippocampal neurogenesis when they first manifest depressive-like behavior (50). Hence, it is possible that altered hippocampal neurogenesis is primarily linked to hippocampal-dependent cognitive deficits rather than depressive-like behavior at least in full-length HD mouse models (52). Furthermore, in order to fully determine whether neurogenesis is affected in the BACHD mice, further experiments would be required to investigate the fate of the newborn BrdU-labeled cells using different immunohistochemical markers.

Treatment with sertraline did not significantly affect the depressive- or anxiety-like behaviors as assessed in the FST and EPM in BACHD mice. The dose and treatment length was similar to what has previously been shown to be effective in female R6/1 mice (23). Interestingly, BACHD mice treated with sertraline were no longer statistically different to the wild-type groups as assessed in the FST, although they were also not different to the vehicle-treated BACHD mice. It is therefore possible that sertraline had a partial effect on the BACHD mice that might reach a statistically significance with a much larger cohort of mice. YAC128 mice display a depressive-like behavior on the BACHD mice that might reach a statistically significance with a much larger cohort of mice.

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sertraline using tests that assess other behavioral domains in BACHD mice.

In conclusion, our study shows that the BACHD mouse display an early depressive- and anxiety-like phenotype which recapitulates the psychiatric aspects of clinical HD. The data point to interesting gene expression changes in the hypothalamus with relevance for emotional control and encourage further studies using the BACHD model to dissect out what neuro-circuitries underlie the psychiatric aspects of HD.

MATERIALS AND METHODS

Animals

The transgenic BACHD mouse expresses full-length mutant HTT containing 97 polyglutamine repeats (31). BACHD breeding males on the FVB/N strain were obtained from the Jackson Laboratories and crossed with wild-type female mice on the FVB/N strain. The genotype of the offspring was determined from tail or ear samples using PCR as described previously (31). All mice were housed in groups with ad libitum access to normal chow diet and water, and were maintained at a 12 h light/dark cycle. The behavioral tests were performed during the light phase of the circadian rhythm. The numbers of animals included in the different analyses are presented in Supplementary Material, Table S1. All experimental procedures were approved by the Regional Ethical Committee in Lund, Sweden.

Behavioral analyses

Depressive-like behavior, in terms of behavioral despair, was assessed in the FST (33,35). The mice were placed in a 17 cm wide and 18 cm high glass cylinder filled with 10–12 cm of 25°C water. The mice were filmed for 6 min with a digital video camera and the total time spent immobile during the last 4 min of the test was estimated manually after the test. The criterion for immobility were that no swimming movements were made and only movements to keep the head above the water surface was included [according to the criteria defined by Porsolt (35)]. The observer was blinded to the identity of each mouse.

For 2- and 6-month-old mice, depressive-like behavior was further assessed with the SPT (34,36). The SPT measures anhedonia, defined as a reduction in the perceived rewarding property of an event, which is a hallmark of depression. The mouse is given the choice between drinking from a bottle with either regular water or a bottle with sucrose containing water, and reduced sucrose consumption is interpreted as impaired perception of reward, i.e. anhedonia (34,36). On the first day of the test, two water bottles with non-dripping bottle caps were introduced. One bottle was placed on the right side (where the water bottle normally is placed) and one on the left side of the cage, and the mice were given 6 days to accommodate to the two bottles. After the accommodation period, the water in the left bottle was replaced by a 1% sucrose solution and the preference for sucrose was measured daily for 3 days. Neither water nor sucrose solution was changed during the 3-day test period. The water bottle measurements were carried out on an electronic scale at the same time point each day. Preference for sucrose (intake of sucrose solution divided by total fluid intake) is presented as an average over the three test days. Most mice were housed in pairs based on genotype and sex; however, some mice needed to be single caged due to aggressive behavior.

Anxiety-like behavior was assessed in the EPM using the Ethovision 3.1 Software system (Noldus Information Technology) (37,38). The EPM was elevated 50 cm above its base and consisted of four 30 cm long and 6 cm wide arms. Thirty centimeter high walls enclosed two of the opposing arms. The mice were placed in the center of the maze and their behavior was recorded for 5 min. The percentage time spent on the open arms was assessed and calculated with the software. For the analyses presented in Figure 1, the same group of mice was tested in the FST, SPT and EPM at 2 and 6 months of age.

Two separate groups of mice were assessed at 2 and 6 months of age for general activity and swimming capacity in the OF test and SAT, respectively. The OF test was performed using the PAS-Open field system (San Diego Instruments). Mice were placed in the center of the activity box (40.6 × 40.6 × 38.1 cm) and general activity, in terms of infrared beam crossings (16 × 16 beams), was recorded during 4 h. The data are presented as horizontal ambulatory beam crossings.

The SAT protocol was modified from Pouladi et al. (18). The linear swimming test chamber (65 × 12 cm) was filled with 7 cm 25°C water. A 7 cm high escape platform (8 × 12 cm) was placed at one end of the chamber. Mice were placed at the other end of the chamber and were trained to reach the platform to escape the water in the shortest time possible. All mice performed ten trials, 5 min apart. The first three trials were considered test trials and not recorded. If a mouse could not find the platform during 1 min, it was slowly guided to the platform. The time it took for the mice to reach the platform was recorded manually during the last seven trials. The average swim speed (cm/s) was calculated for the two fastest trials.

Serum analyses

Serum levels of corticosterone were assessed in duplicates with Corticosterone Enzyme Immunoassay kit (Assay Designs) according to the manufacturer’s instructions. Serum levels of vasopressin were assessed with Vasopressin RIA kit (Phoenix Pharmaceuticals, Inc.) in duplicates according to the manufacturer’s instruction.

BrdU injections

To study changes in cell proliferation in young BACHD mice, 3-month-old male and female mice were injected with BrdU (n = 6/genotype/sex). BrdU (Sigma Aldrich) was dissolved in PBS at 50°C and injected i.p. at 200 mg/kg every 12th hour for three consecutive days (six injections/animal in total). The mice were perfused with paraformaldehyde (PFA) 12 h after the last BrdU injection.

Immunohistochemistry

Mice were perfused with 4% PFA for neuropathological analyses. The brains were postfixed in PFA for 6 h and then cryoprotected in 25% sucrose. Brains were cut in coronal sections in series of six at a thickness of 35 μm using a freezing microtome.
Sections were stored in antifreeze solution at −20°C until further processing for IHC with primary antibodies targeting 5-bromo-2′-deoxyuridine (BrDU, 1:100, made in rat, Serotec), Neurogenetic differentiation 1 (NeuroD1, 1:1000, made in goat, Santa Cruz Biotechnology), DCX (1:200, made in goat, Santa Cruz Biotechnology), Cre recombinase (1:20 000, made in rabbit, Covance, antibody pre-incubated with other mouse tissue before applying to sections), orexin A (1:4000, made in rabbit, Phoenix Pharmaceuticals, Inc.), oxytocin (1:2000, made in rabbit, Phoenix Pharmaceuticals, Inc.) and arginine-vasopressin (AVP, 1:10 000, made in rabbit, Chemicon). Briefly, sections were incubated with the primary antibodies overnight at room temperature followed by 1 h incubation at room temperature with the appropriate biotinylated secondary antibody. Bound antibodies were visualized by incubation in an avidin–biotin–peroxidase solution (Vectastain ABC Standard Kit, Vector Laboratories) at room temperature for 1 h followed by a 3,3′-diaminobenzidine reaction. Sections processed for BrDU IHC were denaturated in 1 M HCl at 65°C for 10 min and then at room temperature for 20 min before incubation with primary antibody.

**Stereological analyses**

The numbers of immunopositive cells in the hypothalamus and the DG were assessed using design-based stereology with an unbiased counting frame (83). A Nikon Eclipse 80i light microscope connected to the NewCast Module in VIS software (Visiopharm A/S) was used. The brains were assessed unilaterally besides for the cre-recombinase estimation, the orexin population in 4-month-old mice and the analysis of cresyl violet stained sections, where both sides of the hypothalamus were included. The hypothalamic area included in the analyses ranged from bregma =−0.58 to =−2.18 mm. For the orexin and oxytocin analyses, the regions of interest (ROIs) were delineated to include all peptide positive cells, resulting in 4–6 sections/animal and 9–11 sections/animal, respectively. The ROIs for the AVP quantification were limited to the paraventricular nucleus resulting in two to three sections with AVP positive cells. For the cre recombinase and cresyl violet quantification, all the sections between above-mentioned bregma levels were included. Delineation of hypothalamic and the definition of neurons were performed as described elsewhere (59). Delineation of the DG of the hippocampus started from bregma =−0.98 mm and continued until bregma =−3.88 resulting in 13–15 analyzed sections per animal (84). All quantification (using 60x Plan-Apo oil objective) was carried out on blind-coded slides and fraction settings were adjusted so at least 200 cells were counted per brain. For the AVP and oxytocin, a fraction of 100% was used since a number of 200 cells could not be achieved. The optical fractionator method was then used to estimate the total number of objects (85).

**Sertraline treatment**

To test the response of BACHD mice to SSRI, a chronic treatment of the commonly used the SSRI sertraline was performed in 2-month-old male and female mice (n = 6–8/genotype/sex). Sertraline (Sigma Aldrich) was dissolved in saline and injected i.p. at 20 mg/kg daily for 4 weeks (23). Fresh solutions of sertraline were prepared daily and injected at the same time point each day. Control animals received vehicle injections. In the last week of the sertraline treatment, depressive- and anxiety-like behavior was assessed in the FST and EPM, respectively.

**rAAV vector production and stereotactic surgery**

To determine the effect of targeted deletion of mutant HTT in the hypothalamus on depressive- and anxiety-like behavior and the orexin neuronal population as well as on hypothalamic gene transcription, 2-month-old female BACHD and wild-type mice were injected with recombinant AAV vectors of serotype 5 expressing cre-recombinase (rAAV5-Cre). The vectors were produced as previously described (59). Two separate groups of mice were injected, one for behavioral and neuropathological analyses and one for transcriptional analyses. The production titer for rAAV5-Cre for the first group (behavioral and neuropathological analyses) was determined to 1.0 E13 gc/ml (batch 1) and for the second group (transcriptional analyses) to 6.3 E13 gc/ml (batch 2). Stereotactic hypothalamic injections were performed using a 5 μl syringe (Hamilton) fitted with pulled glass pipette tips (outer diameter 80 μm) at the following stereotactic coordinates: 0.7 mm posterior to the bregma, 0.55 mm lateral to the bregma and 5.2 mm ventral to the dura. For the second group of mice used for transcriptional analyses, the anterior–posterior coordinate was adjusted to 0.6 mm posterior to the bregma. The rAAV5-Cre was injected bilaterally (0.5 μl/side) at a 1:1 dilution for batch 1 and at a 1:3 dilution for batch 2 in order to inject a similar concentration in both groups. The speed of injection was 0.05 μl per 15 s, followed by a 5 min wait before the capillary was slowly retracted.

**PCR for hypothalamic cre-excision validation after rAAV-cre injections**

The successful deletion of mutant HTT exon 1 in hypothalamus in BACHD mice injected with rAAV5-cre was validated in genomic DNA from dissected fresh frozen brain regions 2 months post-injection. DNA was extracted from the tissues with the DNeasy Blood and Tissue kit (Qiagen) and PCR was performed on a P2x2 Thermal Cycler (Thermo Electron Corporation). The PCR products were run on a 1% agarose gel with SYBR® Safe DNA gel stain (Invitrogen). The primers were designed to detect the loxP flanked HTT exon 1 of the BACHD mice. Forward primer 5'-ATTCAATTGCCCGCTGGTCTGA-3' and reverse primer 5'-AGCCCTCTTCCCTCTCACTAGAAGAGG-3'. The PCR products from BACHD mice were around 1050 bp for the non-excised HTT exon 1 and around 600 bp for the cre-excised HTT exon 1.

**Analysis of hypothalamic mRNA expression**

The mRNA expression of different transcripts associated with emotional control was assessed in hypothalami from 4-month-old female BACHD and wild-type mice (n = 7/genotype) as well as in a group of BACHD mice injected with rAAV5-cre in hypothalamus at 2 months of age (n = 7). Mice were decapitated and the hypothalamus was dissected on a cold plate and then immediately frozen on dry ice until further storage at −80°C. mRNA expression analysis for all genotypes
was carried out using qRT-PCR. Total RNA was isolated by using RNeasy Lipid Tissue Kit (Qiagen) with an on-column DNase digestion (RNase-free DNase set, Qiagen) and cDNA was generated using random primers and SuperScript III Reverse Transcriptase (Invitrogen) according to the manufacturer’s instructions. qRT-PCR was performed on a LightCycler 480 (Roche) in a two-step cycling protocol using SYBR Green I Master (Roche). Calculations were performed with the ΔΔCT method. Once normalized to the expression of the three housekeeping genes β-actin, hypoxanthine–guanine phosphoribosyltransferase and glyceraldehyde 3-phosphate dehydrogenase, the averaged expression value was estimated. All values are presented as ratios to the average wild-type value. The primer sequences used for the gene expressions analyses are found in Supplementary Material, Table S2.

Statistical analyses
Data are presented as mean ± SEM. Statistical analyses were performed using PASW 19 statistical package (SPSS Inc.). The data were analyzed with full factorial ANOVAs followed by appropriate post hoc tests. Significant statistical difference is considered at P < 0.05. Power analysis was performed with the PS: Power and Sample Size Calculation version 3.0, 2009 program (http://biostat.mc.vanderbilt.edu/wiki/Main/PowerSampleSize). Detailed information of the statistical analyses is presented in Supplementary Statistical Results.

SUPPLEMENTARY MATERIAL
Supplementary Material is available at HMG online.

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Conflict of Interest statement. None declared.

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