Imbalance of the oxytocin-vasopressin system contributes to the neuropsychiatric phenotype in the BACHD mouse model of Huntington disease

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\textbf{ABSTRACT}

Neuropsychiatric disturbances with altered social cognition, depression and anxiety are among the most debilitating early features in the fatal neurodegenerative disorder Huntington disease (HD) which is caused by an expanded CAG repeat in the huntingtin gene. The underlying neurobiological mechanisms are not known. Neuropathological analyses of postmortem human HD hypothalamic tissue have demonstrated loss of the neuropeptides oxytocin and vasopressin. The dynamic interplay between these neuropeptides is crucial for modulating emotional and social behavior but its role in HD is unclear. In the present study, we have investigated the effect of expressing the mutant huntingtin gene on the development of behavioral changes using the transgenic BACHD mouse model at different ages. We show for the first time that BACHD mice exhibit deficits in social behavior with parallel aberrations in the balance of the oxytocin-vasopressin system. Importantly, our data also show that restoration of the interplay within the system with an acute dose of intranasal oxytocin immediately prior to behavioral testing can rescue the depressive-like phenotype but not anxiety-like behavior in this transgenic model. These findings demonstrate that imbalances in the oxytocin-vasopressin interplay contribute to the neuropsychiatric component of HD and suggest that interventions aimed at restoring the blunted levels of oxytocin may confer therapeutic benefits for this disease.

\textbf{1. Introduction}

Huntington’s disease (HD) is a fatal hereditary neurodegenerative disorder caused by a CAG trinucleotide repeat expansion within the first exon of the \textit{huntingtin} (\textit{HTT}) gene (The Huntington’s Disease Collaborative Research Group, 1993). It is inherited in an autosomal dominant fashion with full penetrance. A clinical diagnosis typically occurs in mid-life based on the presence of typical motor signs including chorea in combination with a positive gene test. Thereafter, the disease then progresses over the next 20 years leading to severe disability and inevitably premature death. Non-motor features including cognitive dysfunction, psychiatric symptoms, disturbance of the circadian rhythm, and sleep disorder often manifest in the prodromal stage and precede characteristic motor symptomology by many years (Ross et al., 2014). Despite the monogenetic etiology, no effective therapies are currently available to prevent, delay or reverse the progression of HD even though several clinical trials are currently underway (Rodrigues et al., 2019). Significant research efforts to date have targeted the clearance of mutant HTT with several approaches including genome editing, antisense oligonucleotides and RNA interference (Tabrizi et al., 2019). These efforts have focused mainly on neuropathology in the striatum of the basal ganglia and the cerebral cortex along with their respectively-associated motor and cognitive changes.

The underlying neurobiological substrate for the early non-motor features in HD with disturbances of sleep and emotion is not fully understood but may be due to neuropathology in the hypothalamus, a region that controls these functions. Indeed, pathological changes have been found in the hypothalamus using both PET and MRI analyses of HD patients as well as neuropathological examination of post-mortem tissue from HD cases (Baldo et al., 2019; Bartlett et al., 2019; Gabery et al., 2010; Kassubek et al., 2004; Kremer et al., 1990; Petersen et al., 2005; Politis et al., 2008; Soneson et al., 2010; van Wamelen et al., 2013). Inactivation of mutant HTT in the transgenic BACHD mouse model has led to the amelioration of non-motor features including...
depressive-like behavior, further supporting a causal link between hypothalamic pathology and non-motor features in HD (Hult Lundh et al., 2013; Hult et al., 2011).

The neuropsychiatric features of HD include altered social cognition, apathy, depression, anxiety as well as irritability and aggression (Anderson et al., 2018; Kempnich et al., 2018). Oxytocin and arginine vasopressin are nonapeptideic hormones synthesized in the paraventricular nucleus and supraoptic nucleus of the hypothalamus. The oxytocin and vasopressin neuropeptide systems are of particular interest given the dynamic interplay of both neuropeptides in regulating emotional behavior and stress circuits (Neumann and Landgraf, 2012), apart from their classical functions of regulating parturition, lactation and osmolality. While both oxytocin and arginine vasopressin are released centrally and peripherally to regulate physiological function, affective behaviors are controlled by the central release of these neuropeptides. The combined anxiolytic effects of oxytocin and the anxiogenic effects of vasopressin are thought to be pertinent to modulate the neural response that governs affective behavior (Baribeau and Anagnostou, 2015). In HD, it is possible that the balance between oxytocin and vasopressin levels becomes dysregulated, which impacts upon the hypothalamic and limbic circuitries and in turn alters the vulnerability to develop non-motor symptoms. Changes in oxytocin and vasopressin expression on the mRNA and protein level have in fact been reported in experimental models as well as in post-mortem HD cases which have been neuropathologically graded 2–4, indicating severe striatal pathology and disease (Gabery et al., 2010; Hult et al., 2011; Kotiljarova et al., 2005; Soylu-Kucharz et al., 2016; Wood et al., 2008; Yamanaka et al., 2010). Furthermore, these changes have also been reported in post-mortem hypothalamic tissue from an HD gene carrier with only non-motor symptoms and that was neuropathologically graded with the lowest grade of 0 due to the absence of striatal pathology (Gabery et al., 2015). These data suggest that imbalances in the oxytocin and vasopressin systems may underpin the pathophysiology of early HD. Several studies have indicated the therapeutic use of oxytocin and vasopressin in affective and autism-spectrum disorders (Neumann and Landgraf, 2012). In the present study, we tested the hypothesis that neuropsychiatric behaviors related to the oxytocin-vasopressin system are altered in HD and that delivery of oxytocin would have a beneficial effect using the transgenic BACHD mouse model of the disease.

2. Materials and methods

The complete experimental procedures can be found online as supplementary material (see Supplementary data Appendix 1: Supplementary Material and Methods).

2.1. Animals

Experiments were undertaken in separate groups of neonatal (postnatal day 0 (P0), P5 and P10) as well as adult BACHD mutant mice with 97 mixed CAA/CAG repeats (Gray et al., 2008) (age 2, 4, 6, 12, 15 and 18 months old) and wild-type (WT) littermates as control. Both male and female mice were used in this study. All experimental procedures were approved by the Regional Ethical Committee in Lund, Sweden (Permit number: M360–12, M65–13 and 15,499/2017).

2.2. Isolation-induced ultrasonic vocalizations

Neonatal pups were individually isolated in a sound attenuating chamber and ultrasonic calls were recorded with an Avisoft UltraSoundGate 116Hb recording interface (Model CM16-CMPA, Avisoft Bioacoustics, Germany). USV calls were recorded for 5 min at a sampling rate of 250,000 Hz in 16 bit format. Sound recordings were analyzed using the Avisoft SASLab Pro software (Version 5.02.07, Avisoft Bioacoustics). Spectrogram analysis was performed using an automatic threshold-based algorithm. Various other spectrum-based parameters including peak frequency, peak amplitude and frequency modulation and temporal parameters such as call duration, total calling time, duration of intervals between subsequent calls and the total number of calls emitted were also determined automatically.

2.3. Behavioral procedures

Adult mice underwent a battery of behavioral tests for total locomotor activity, anxiety-like behaviors, depressive-like behaviors, social behavior, aggression, digging, burrowing and nesting. All behavioral tests were performed between zeitgeber time (ZT) 3 and ZT9 according to procedures described previously (Baldo et al., 2014; Hult Lundh et al., 2013; Yu-Taeger et al., 2012) and analyzed by experimenters blinded to the genotype and groupings of the mice.

2.4. Intranasal oxytocin treatment

Mice were picked up by the scruff of the neck and exposed in a supine position to immobilize them for the intranasal instillation. Oxytocin (OXT) or 0.9 % physiological saline (vehicle) was administered as a droplet over both nares, bilaterally on the rhinarium using a micropipette (Syntocinon, 0.032 IU/g mouse, Sigma-Tau Pharmaceuticals, Pomezia, Italy). This dose corresponded to a supraphysiologic dose equivalent to 40 IU/50 kg body weight human and showed no adverse effects when administered acutely (Ditzen et al., 2009; Lee et al., 2018; MacDonald et al., 2011). Mice were treated with either OXT or vehicle and returned to the home cage for 5 min before being tested on the elevated plus maze (EPM) or Porso/forced swim test (FST).

2.5. Radioimmunoassay

The hypothalamus, amygdala, pituitary and plasma were collected from a separate cohort of mice at 2, 6, 12 and 15 months of age between ZT11 and ZT13. All samples from female mice were collected in the diestrus phase of the estrous cycle, monitored by vaginal cytology. Plasma, brain and pituitary concentrations of oxytocin and arginine vasopressin were determined in duplicates by radioimmunoassay (RIA, Phoenix Pharmaceuticals, California, USA). The sensitivity of the assay was 6.7 pg/mL for the oxytocin assay and 29.7 pg/mL for the arginine vasopressin assay. For the oxytocin assays, the intra- and inter-assay coefficient of variance was 3.72 % and 3.65 % respectively, while the arginine vasopressin assays had an intra- and inter-assay coefficient of variance of 3.90 % and 7.17 % respectively.

2.6. Statistical analyses

All data are presented as group means ± SEM in the bar graphs and overlayed with dot plots of individual data points. Statistical analyses were performed using the SPSS statistical package (Version 23, IBM Inc., Chicago, IL, USA). The data were analyzed using a two or three-way ANOVA followed by a post-hoc unpaired Student’s t-test when appropriate to determine differences between genotype, sex and timepoint. Data which showed a clear sex difference were presented separately while those without a sex difference were presented together, collapsed across sex. Differences were considered to be significant for p values < 0.05. Full statistical results can be found in Supplementary data Appendix 2: Supplementary Statistical Results.

3. Results

3.1. Early postnatal development of anxiety-like behaviors

Isolation-induced USV emission is a consistent, robust phenomenon that has been considered to be an early measure of communicative function and greatly regarded to be closely linked to a predisposition to
Development of postnatal psychiatric HD-related phenotypic behavior in BACHD mice.

A. Bar graph show the number of isolation-induced ultrasonic vocalizations (USVs) emitted from P0, P5 and P10 BACHD and WT mouse pups. BACHD pups evoked significantly less calls than WT littermates at P10 (three-way ANOVA, effect of genotype $F_{(1,211)} = 0.57$, $p = 0.44$, effect of sex $F_{(1,211)} = 0.51$, $p = 0.47$, effect of timepoint $F_{(2,211)} = 132.67$, $p < 0.0001$, interaction between genotype*timepoint $F_{(2,211)} = 5.67$, $p < 0.001$; post-hoc Student's unpaired t-test $p < 0.05$ for P10). B. Representative sonographs depict types of isolation-induced USVs recorded in 150 ms. Calls can be classified as simple or multi-component calls. C-D. Bar graphs show the quantification of simple (C) and multi-component calls (D) between BACHD and WT mouse pups at P0, P5 and P10. BACHD mice displayed a trend towards reduction in the number of simple calls evoked at P10 (three-way ANOVA, effect of genotype $F_{(1,211)} = 0.001$, $p = 0.97$, effect of sex $F_{(1,211)} = 1.65$, $p = 0.20$, effect of timepoint $F_{(2,211)} = 95.91$, $p < 0.0001$, post-hoc Student’s unpaired t-test $p = 0.055$ for P10). BACHD mice show increased number of complexed multi-component calls at P0 and a trend to increase at P5 (three-way ANOVA, effect of genotype $F_{(1,211)} = 1.85$, $p = 0.17$, effect of sex $F_{(1,211)} = 0.0001$, $p = 0.995$, effect of timepoint $F_{(2,211)} = 125.00$, $p < 0.0001$; interaction between genotype*timepoint $F_{(2,211)} = 8.15$, $p < 0.0001$; interaction between genotype*timepoint*sex $F_{(2,211)} = 5.07$, $p < 0.01$; post-hoc Student’s unpaired t-test, P0 $p = 0.055$, P5 $p = 0.065$ for FS). Multi-component calls were significantly reduced at P10 ($p < 0.05$). E. Total general locomotor activity between BACHD and WT littermates at 4, 12 and 18 months of age remained unchanged. F. Measurement of anxiety-like behaviors with the elevated plus maze (EPM). BACHD mice spent significantly less time on the open arms compared to WT mice at 4 and 12 months of age (three-way ANOVA, effect of genotype $F_{(1,88)} = 21.06$, $p < 0.0001$, effect of sex $F_{(1,88)} = 0.17$, $p = 0.67$, effect of timepoint $F_{(2,88)} = 1.9$, $p = 0.03$; post-hoc Student’s unpaired t-test, 4 months $p < 0.0001$, 12 months $p < 0.01$). G. Adult BACHD mice display typical depressive-like behaviors at 4 and 12 months of age as indicated by the Porsolt forced swim test (FST) with BACHD mice spending a significantly increased time immobile than WT mice (three-way ANOVA, effect of genotype $F_{(1,96)} = 16.32$, $p < 0.0001$, effect of sex $F_{(1,96)} = 0.57$, $p = 0.45$, effect of timepoint $F_{(2,96)} = 2.72$, $p = 0.05$; post-hoc Student’s unpaired t-test, 4 months $p < 0.0001$, 12 months $p < 0.01$). Data presented as mean ± SEM, ***$p < 0.001$, **$p < 0.01$ and *$p < 0.05$ represents a genotype difference, three-way ANOVA with post-hoc Student’s t-test. The number of animals used in each group is indicated in parenthesis.
Fig. 2. BACHD mice display impaired social interaction behavior.

A-D. Assessment of social affiliation. Bar graphs depict the percentage preference for the cup enclosed with the stranger mouse over empty cup (A), the percentage of time spent sniffing around the vicinity of the empty cup (B) and around the cup enclosed with a stranger mouse (C, D). BACHD mice display deficits in social affiliation at 6 months of age (percentage preference: three-way ANOVA, effect of genotype $F_{(1,79)} = 2.31, p = 0.13$, effect of sex $F_{(1,79)} = 2.86, p = 0.09$, effect of timepoint $F_{(2,79)} = 5.50, p < 0.01$, interaction between sex*timepoint $F_{(2,79)} = 3.15, p < 0.05$; post-hoc Student's unpaired t-test, $p < 0.05$). Female BACHD mice show differences in time spent sniffing around the perimeter of the cup enclosed with a stranger mouse at 6 months of age (three-way ANOVA, effect of genotype $F_{(1,79)} = 3.05, p = 0.08$, effect of sex $F_{(1,79)} = 10.30, p < 0.01$, effect of timepoint $F_{(2,79)} = 18.12, p < 0.0001$, interaction between genotype*timepoint $F_{(2,79)} = 4.07, p < 0.05$, sex*timepoint $F_{(2,79)} = 4.39, p < 0.01$, post-hoc Student’s t-test, females 6 months $p < 0.01$).

E-G. Assessment of social novelty. Bar graphs show the preference for the cup enclosed with the novel mouse over the cup with the familiar mouse (E) as well as the percentage time spent sniffing the cup enclosing the novel mouse (three-way ANOVA, effect of genotype $F_{(1,78)} = 14.63, p < 0.0001$, effect of sex $F_{(1,78)} = 0.47, p = 0.49$, effect of timepoint $F_{(2,78)} = 2.00, p = 0.14$; post-hoc Student’s t-test, 6 months $p < 0.0001$, 12 months $p < 0.05$).

H-I. Assessment of aggressive behaviors in BACHD mice. Bar graphs show the duration (A) and frequency of aggressive attacks (B) in BACHD and WT mice at 2, 6 and 12 months of age with the resident-intruder test. No differences in the duration and frequency of attacks were detected between BACHD and WT mice. Data presented as mean ± SEM, ***$p<0.001$, **$p<0.01$ and *$p<0.05$ represents a genotype difference, three-way ANOVA with post-hoc Student’s t-test. The number of animals used in each group is indicated in parenthesis.
frequency steps and subsequently further subdivided into simple calls, the call repertoire. The calls were then separated into five different categories based on frequency modulation and sound complexity. The number of calls emitted between WT and BACHD mice at P0 and P5 was significantly different. Interestingly, BACHD mice show alterations in the number of complex, multi-component calls with a significant increase at P0 (Fig. 1D; p < 0.05) and a trend to increase at P5 (Fig. 1D; p = 0.065). The number of multi-component calls was significantly reduced at P10 (Fig. 1D; p < 0.05). The isolation-induced USVs were not different across the sexes. In addition to the total number of calls, we empirically tested other temporal parameters such as the total calling time, average call duration, duration of intervals as well as spectrum-based parameters such as peak amplitude, peak frequency and frequency modulation (Supplementary data Appendix 3: Table S1).

As noted previously, BACHD mice display anxiety- and depressive-like behaviors already at 2 months of age as assessed using the EPM and FST, respectively (Hult Lundh et al., 2013). We were now interested in examining whether these early non-motor behaviors persisted across different ages. No differences in general locomotor activity as measured by the open field test was detected between BACHD and WT mice across all age points (Fig. 1E). We found that BACHD mice exhibited anxiety-like behaviors as observed by the significant decrease in the percentage time spent on the open arms of the EPM at 4 and 12 months of age (Fig. 1F; 4 months p < 0.0001, 12 months p < 0.01). However, this response was not present in the 18 months group. Similarly, BACHD mice displayed depressive-like behaviors evidenced by the increased time spent being immobile in the FST compared to age-matched WT littermates at 4 and 12 but not 18 months of age (Fig. 1G; 4 months p < 0.0001, 12 months p < 0.01).

3.2. Normal innate rodent behavior

Classical innate rodent behaviors such as nesting, digging and burrowing were assessed as an indication of wellbeing in BACHD and WT mice at 2, 6 and 12 months of age. Nesting behavior was scored and a sex difference was detected (p < 0.001). In females, further post-hoc analyses revealed that the nesting score was significantly different between BACHD and WT mice at 6 but not at 2 and 12 months of age (Supplementary data Appendix 4: Fig. S1A; p < 0.05). In males, nesting behavior was not different at all ages tested (Supplementary data Appendix 4: Fig. S1B). Digging behavior, measured with the marble burying test, also showed a sex difference (p < 0.01). However, further post-hoc analyses revealed no differences between genotype at all ages tested in females (Supplementary data Appendix 4: Fig. S1C). In males, there was a trend towards a decreased number of marbles buried in BACHD mice at 12 months of age but not at 2 and 6 months of age (Supplementary data Appendix 4: Fig. S1D; p = 0.06). Similarly, the ability to burrow did not differ between BACHD and WT mice at 2 and 6 months of age. At 12 months of age, BACHD mice show a trend towards reduction in the burrowing behavior compared to WT mice (Supplementary data Appendix 4: Fig. S1E; p = 0.07).

3.3. Impairments in social behavior

The three-chamber test for social interaction revealed altered social affiliation and social novelty in BACHD mice. In the first test for social affiliation, the preference for contact with the cup enclosed with a stranger mouse compared to the empty cup and the percentage time spent sniffing around each cup were evaluated. All mice displayed increased preference for the stranger mouse compared to the empty cup, regardless of genotype. BACHD mice at 6 months of age had a trend for increased preference for the stranger mouse compared to the empty cup (Fig. 2A; p = 0.081). Both genotypes spent a similar amount of time sniffing around the empty cup (Fig. 3B). The trend for increased preference for the stranger mouse compared to the empty cup was not present at 12 months of age (Fig. 2B; p = 0.24). In the second test for social novelty, the preference for the stranger mouse compared to the cup enclosed with a familiar mouse was evaluated. There was no difference in the preference for the stranger mouse compared to the familiar mouse at 6 months of age (Fig. 2C; p = 0.39). However, at 12 months of age, BACHD mice showed a significant decrease in the preference for the stranger mouse compared to the familiar mouse (Fig. 2C; p < 0.01). Similarly, there was no difference in the preference for the stranger mouse compared to the familiar mouse at 6 months of age (Fig. 2D; p = 0.18) but a trend for decreased preference for the stranger mouse compared to the familiar mouse at 12 months of age (Fig. 2D; p = 0.07).
preference for the stranger mouse at 6 months of age is reflected in the time spent sniffing around the perimeter of the cup enclosed with a stranger mouse where a clear sex difference was detected with only the females showing these changes (Fig. 2C; p < 0.01). No changes between genotype were detected at all time points in males (Fig. 2D). In the second test for social novelty, preference for the cup enclosed with an unfamiliar novel mouse compared to the cup enclosed with a familiar stranger (mouse from first session) and the corresponding time spent sniffing each cup were measured. In general, both genotypes had an increased preference for the novel mouse compared to the familiar mouse at 6 and 12 months of age. BACHD mice showed an increased preference for the novel mouse compared to the familiar mouse at both 6 and 12 months of age compared to WT mice (Fig. 2E; 6 months p < 0.01, 12 months p < 0.05). Again, this is reflected in the time spent sniffing around the perimeter of the cup enclosed with the novel mouse (Fig. 2F; 6 months p < 0.0001, 12 months p < 0.01). Consequently, there is a reduced time spent sniffing around the cup with the familiar mouse at 12 months of age but this decreased preference was not detected at 6 months of age (Fig. 2G; p < 0.05).

Next, we assessed aggressive behavior in the BACHD model using the resident-intruder test given that irritability and aggression are part of the behavioral manifestations in HD. However, the resident-intruder test showed no differences in the duration and frequency of aggressive behavior between BACHD and WT littermates at all time points (Fig. 2H and I).

3.4. Intranasal oxytocin treatment ameliorates depressive-like behavior but does not rescue anxiety-like behavior

Dysfunction of the oxytocin system has been reported in HD and given the importance of oxytocin in the regulation of emotional behaviors, we set out to determine whether oxytocin treatment could restore the neuropsychiatric behaviors in BACHD mice. We treated BACHD and WT mice with either OXT or vehicle and measured depressive- and anxiety-like behaviors with the FST and EPM, respectively. Interestingly, acute OXT treatment just prior to the behavioral task ameliorates the depressive-like phenotype in BACHD mice (Fig. 3A; p < 0.01). The increased immobility as observed in the vehicle treated BACHD group, classic of a depressive-like behavior, was completely abolished with OXT treatment (p < 0.01). In terms of anxiety-like behaviors, we found that vehicle-treated BACHD mice at 12 months of age displayed an anxiety-like phenotype but OXT treatment failed to improve the anxiety-like behaviors (Fig. 3B).

3.5. Oxytocin and arginine vasopressin neuropeptide levels are altered in BACHD mice

Given the effects of intranasal OXT on behavior and the involvement of the oxytocin and vasopressin system in emotional regulation, we wanted to understand whether these systems were also affected in BACHD mice. To assess oxytocin and arginine vasopressin levels, we measured levels with RIA in a controlled fashion by timed-collection of samples and controlling for fluctuations in the estrous cycle. Measurements of oxytocin revealed reduced circulating oxytocin levels in the plasma at 6 and 15 months of age (Fig. 4A; 6 months p < 0.001, 15 months p < 0.05). Oxytocin levels in the hypothalamus of 12 months old BACHD mice were significantly elevated compared to age-matched WT mice (Fig. 4B; p < 0.01). No differences were detected at other time points. We also measured oxytocin levels in the amygdala, a brain region in the limbic system that is essential for the processing of emotions and receives innervations by oxytocin neuron projections. We found no alterations in oxytocin levels in the amygdala (Fig. 4C). Pituitary levels of oxytocin showed a clear sex difference whereby female BACHD mice at 6 months of age exhibited significantly less oxytocin levels compared to WT mice, but not at other time points (Fig. 4D; p < 0.01). No change in oxytocin levels in the pituitary was detected in males at all time points measured (Fig. 4E).

Measurements of arginine vasopressin revealed elevated circulating levels in the plasma of BACHD mice at 2, 6 and 12 months of age (Fig. 5A; 2 months p < 0.001, 6 months p < 0.05 and 12 months p < 0.05). Vasopressin levels in the hypothalamus, amygdala and pituitary did not differ between genotype and sex (Fig. 5B, C, D).

4. Discussion

Non-motor symptoms and signs are among the most difficult features in HD and accumulating studies point to the importance of the hypothalamus in mediating these features. More effective treatments targeting the neuropsychiatric disturbances in HD are needed (Eddy et al., 2016; Mason and Barker, 2016; Moulton et al., 2014). In post-mortem tissue from HD patients, the number of neurons expressing oxytocin is reduced already before the manifestation of motor symptoms and the development of striatal pathology (Gabery et al., 2015). In this study, we addressed the neuromodulatory role of oxytocin and arginine vasopressin in HD and also explored the potential for therapeutic efficacy of oxytocin in mitigating the neuropsychiatric behaviors. Here, we show for the first time that BACHD mice exhibit alterations in social behavior and in the oxytocin-vasopressin system. Finally, we provide first evidence for a positive effect of intranasal oxytocin administration on the depressive-like phenotype in BACHD mice.

Social behavior is in part governed by the central effects of oxytocin. Oxytocin null mutant mice display deficits in social memory and social recognition (Choleris et al., 2003; Ferguson et al., 2000; Modir and Young, 2012) and an increased propensity to anxiety and stress responses (Amico et al., 2004; Mantella et al., 2003). In the same manner, deletion of the oxytocin receptor in mice impairs the ability to develop social memory and increases the level of aggression (Takayanagi et al., 2005). A key player in this molecular cascade is CD38, a transmembrane receptor and leukaemia cell marker that regulates the axonal transport of oxytocin and arginine vasopressin secretion. It is likely that disturbance in the oxytocin signaling cascade alters the interplay between the oxytocin-vasopressin systems which in turn results in impaired social behavior (Neumann and Landgraf, 2012). This is in agreement with the R6/2 mouse model where mice also display deficits in social behavior combined with reduced oxytocin protein and mRNA levels (Wood et al., 2008; Wood and Morton, 2015; Yamanaka et al., 2010). Surprisingly, the increased vasopressin secretion in the plasma did not parallel aggressive-like behaviors using the resident-intruder test. A possible explanation for this could simply be that the resident needs to be socially isolated for a longer period of time for offensive conflicts to be triggered or that the levels of arginine vasopressin being secreted need to be even more elevated to result in aggression. The precise signaling mechanisms leading to the disrupted oxytocin-vasopressin system in HD and the expression of these specific neuropeptide transporters across disease progression are not known.

Our results also show alterations in the levels of oxytocin and arginine vasopressin secreted in the plasma in BACHD mice. These changes in the peripheral circulation are likely to be consequent of central changes at the level of the hypothalamic/hypophyseal regulation and/or due to defects in the feedback mechanism, resulting in problems with either the production or the release of these neuropeptides. Surprisingly, no neuronal loss of oxytocin and vasopressin has been reported in BACHD mice to date (Hult Lundh et al., 2013; Soylu-Kucharz et al., 2016), suggesting an intact neuropeptide production mechanism. Although the neuropeptide production in the hypothalamus remained unaltered in this study, our findings implicate the possibility that mutant HTT could have rearranged the synaptic
Fig. 4. Oxytocin levels in the circulation and associated brain regions. A–E. Bar graphs show oxytocin levels in the plasma (A), hypothalamus (B), amygdala (C) and pituitary (D, E) of WT and BACHD mice at 2, 6, 12 and 15 months of age as measured with radioimmunoassay. Reduced circulating oxytocin levels was detected in the plasma at 6 and 15 months of age (three-way ANOVA, effect of genotype $F_{(1,97)} = 25.32$, $p < 0.0001$, effect of sex $F_{(1,97)} = 1.54$, $p = 0.21$, effect of timepoint $F_{(3,97)} = 17.69$, $p < 0.001$; post-hoc Student’s unpaired $t$-test 6 months $p < 0.001$, 15 months $p < 0.05$). Oxytocin levels in the hypothalamus were significantly elevated at 12 months old BACHD mice compared to age-matched WT mice (three-way ANOVA, effect of genotype $F_{(1,94)} = 7.62$, $p < 0.01$, effect of sex $F_{(1,94)} = 2.47$, $p = 0.11$, effect of timepoint $F_{(3,94)} = 43.54$, $p < 0.0001$; post-hoc Student’s unpaired $t$-test, $p < 0.01$). No differences were detected in the levels of oxytocin in the amygdala between BACHD and WT mice. Pituitary levels of oxytocin showed a clear sex difference with only female BACHD mice at 6 months of age exhibiting less oxytocin levels compared to WT (three-way ANOVA, effect of genotype $F_{(1,94)} = 8.19$, $p < 0.01$, effect of sex $F_{(1,94)} = 13.69$, $p < 0.0001$, effect of timepoint $F_{(3,94)} = 61.23$, $p < 0.0001$, post-hoc Student’s unpaired $t$-test, 6 months $p < 0.01$). Data presented as mean ± SEM, **$p < 0.01$ and *$p < 0.05$ represents a genotype difference, three-way ANOVA with post-hoc Student’s $t$-test. The number of animals used in each group is indicated in parenthesis.
environment such that the feedback mechanism is now no longer sensitive enough to react to changes in hormonal milieu. It is also possible that half-life of oxytocin/arginine vasopressin is specifically altered and/or that behavior-regulatory-brain areas and cells, responsive to oxytocin/arginine vasopressin, such as prefrontal interneurons or medium spiny neurons in the striatum/nucleus accumbens are differentially affected by the associated HD neurodegenerative processes (Nakajima et al., 2014; Nardou et al., 2019). This may be reflected in the compromised secretion of these neuropeptides and their distinct and time/age/disease process dependent alterations.

In the present study, we show that the abnormalities in the oxytocin-vasopressin system are paralleled by early neurodevelopmental changes, as reflected in the isolation-induced USVs. In agreement with a previous study, BACHD neonatal mice exhibit reduced total number of USVs at P10 (Siebzehnrubl et al., 2018). Interestingly, when the sound repertoire was further categorized into simple and multi-component calls, BACHD pups evoked more complex calls compared to WT littermates during the initial days with significantly reduced complex calls in the second week of life. Although the precise implication of each call type has not yet been deciphered, it is likely that the distinct spectral and temporal features of each USV call category have functional relevance on behavior. The increased vocalization of multi-component calls emitted in the first week could be indicative of an increased need for maternal care-giving since these calls are communicative signals to their mother signifying the need for immediate retrieval back to the nest. Comparatively, a weaker vocalization subsequently at P10 indicates a reduced sensitivity or responsiveness to maternal isolation, suggesting the possibility that social bonding behavior may already be aberrant from an early developmental stage. The increase and decrease in vocalization is thought to be related to the balance in the oxytocin-vasopressin system. Neonatal mice deficient for CD38 and the oxytocin receptor emit reduced USVs (Higashida et al., 2011; Takayanagi et al., 2005). In addition, vasopressin 1b receptor null mice have reduced isolation-induced USVs at P9 (Scattoni et al., 2008). The results of the current study support the notion that the oxytocin-vasopressin balance system is tightly regulated but vulnerable and any subtle disruption to this balance during early development might manifest in emotional deficits during later life (Dolen et al., 2013; Nardou et al., 2019).

The rationale for treatment of the different psychiatric symptoms in HD is mainly based on the practice in general psychiatry and expert-based recommendations for HD with sparse scientific evidence (Anderson et al., 2018; Eddy et al., 2016). Pharmacological treatments for apathy and altered social cognitions do not exist. In this study, we tested whether intranasal oxytocin intervention could confer an alternative treatment for the HD neuropsychiatric component. It is conceivable that exogenous restoration of the oxytocin-vasopressin balance...
can improve behavior. Our findings show that depressive-like behaviors may be ameliorated after an acute administration of oxytocin in adult BACHD mice but not anxiety-like behaviors. A recent study in early HD patients observed that intranasal oxytocin treatment selectively modulates the processing of disgust (Labuschagne et al., 2018), indicating the possibility that oxytocin could be used to alleviate emotional and social cognitive deficits in HD. However, an important caution to the treatment data we present here is that the extent to which an acute supraphysiological dose remains beneficial is not known. It is important to note that oxytocin was administered immediately prior to the behavior test but given its short half-life, it would be interesting to determine whether chronic oxytocin administration or treatment with an oxytocin receptor agonist would achieve similar beneficial effects. In light of these results, it would also be tempting to speculate whether treatment during early development can help prevent or delay the onset of psychiatric HD behavior in adulthood.

There has also been some debate with regards to whether exogenous oxytocin passes through the blood-brain-barrier, how much exogenous oxytocin enters the brain via the intranasal administration route and which brain areas are targeted. Neumann and colleagues reported increased levels of oxytocin in the amygdala and hippocampus as well as increased oxytocin levels in the CSF and periphery following nasal administration in mice (Neumann et al., 2013). Within the HD field, peptides like tagged neuropeptide Y (NPy) have also been administered intranasally to mouse models of HD (R6/2 model) and shown to be able to target brain regions that are behaviorally relevant (Fatoba et al., 2018). It is also likely that the supraphysiological dose used in this study would raise peripheral levels of oxytocin from which some would cross the blood-brain-barrier to exert positive feedback effects on the brain, thereby increasing brain levels of oxytocin. Anatomically, the hypothalamus is situated in the immediate proximity to circumventricular organs such as the vascular organ of lamina terminals, median eminence and pituitary gland which has no blood-brain-barrier and in direct contact with the blood and CSF. Hence, given the anatomical location of the hypothalamus, it is very likely that the increases in CSF oxytocin levels following nasal administration had reached relevant brain regions for psychiatric behaviors (Quintana et al., 2018; Veening and Olivier, 2013). Although we did not pharmacologically modulate the vasopressin system in this study, there is an ongoing randomized Phase I/II clinical trial for safety and tolerability of SRX246, a highly selective vasopressin 1a receptor antagonist to treat neuropsychiatric symptoms in HD (NCT02507284, Azevan Pharmaceuticals) (Fabio et al., 2013; Rodrigues et al., 2019).

In conclusion, our study reports for the first time that BACHD mice exhibit alterations in social behavior with parallel changes in the balance of the oxytocin-vasopressin system. We also provide evidence for a positive effect of intranasal oxytocin administration on the depressive-like phenotype in BACHD mice. Taken together, our study raises the possibility that interventions aimed at restoring the function of the oxytocin-vasopressin system may confer therapeutic benefits to psychiatric HD behavior.

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Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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RYC, SvH and AP conceived and designed experiments. RYC and ST performed experiments and analyzed the data. RYC and AP wrote the manuscript. We thank Björn Anzelius, Anna Hansen and Anneli Josefsson at Lund University for their valuable technical assistance. We also thank Susann Ullén for her valuable statistical input to the study.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.psyneuen.2020.104773.

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