Early postnatal behavioral, cellular and molecular changes in models of Huntington disease are reversible by HDAC inhibition

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Abstract

Huntington disease (HD) is an autosomal dominant neurodegenerative disorder caused by expanded CAG repeats in the *huntingtin* gene (*HTT*). Though mutant HTT is expressed during embryonic development and throughout life, clinical HD usually manifests later in adulthood. A number of studies document neurodevelopmental changes associated with mutant HTT, but whether these are reversible under therapy remains unclear. Here, we identify very early behavioral, molecular and cellular changes in pre-weaning transgenic HD rats and mice. Reduced ultrasonic vocalization, loss of pre-pulse inhibition and increased risk taking are accompanied by disturbances of dopaminergic regulation *in vivo,* reduced neuronal differentiation capacity in subventricular zone stem/progenitor cells, and impaired neuronal and oligodendrocyte differentiation of mouse embryo-derived neural stem cells *in vitro*. Interventional treatment of this early phenotype with the histone deacetylase inhibitor (HDACi) LBH589 led to significant improvement of behavioral changes and markers of dopaminergic neurotransmission, and complete reversal of aberrant neuronal differentiation *in vitro* and *in vivo*. Our data support that neurodevelopmental changes contribute to the prodromal phase of HD and that early, pre-symptomatic intervention using HDACi may represent a promising novel treatment approach for HD.

Significance Statement

In Huntington disease (HD) gene-carriers, the disease-causing mutant Huntingtin (mHTT) is already present during early developmental stages but surprisingly, HD patients develop clinical symptoms only many years later. While a developmental role of

HTT has been described, so far new therapeutic approaches targeting those early neurodevelopmental processes are lacking. Here, we show that behavioral, cellular, and molecular changes associated with mHTT in the postnatal period of genetic animal models of HD can be reverted using low dose histone-deacetylation-inhibitor treatment. Our findings support a neurodevelopmental basis for HD, and provide proof-of-concept that pre-HD-symptoms, including aberrant neuronal differentiation, are reversible by early therapeutic intervention *in vivo*.

/body

Introduction

Pathological CAG repeat expansions in the human *HTT* gene on chromosome 4p16.3 cause autosomal dominant Huntington disease (HD), which is clinically characterized by the triad of chorea, cognitive dysfunction, and psychiatric symptoms (1, 2). After clinical onset the disease inevitably progresses, leading to severe disability and premature death with no effective disease-modifying treatments available.

The CNS pathology in advanced stages of HD is characterized by a loss of striatal medium spiny neurons (3) and cortical structures (4), as well as the presence of HTT- and ubiquitin-positive cytoplasmic and nuclear protein aggregates in neurons (5-7). The lengths of pathological CAG repeat expansions, which extend the polyglutamine stretch within HTT, correlate with earlier disease onset and greater severity (1, 8, 9). Over the past decades, the field has shifted from considering HD solely as a late onset neurodegenerative disease: Instead HD is more recognized as a developmental disorder with various effects of mutant HTT (mHTT) on early neurodevelopment (10, 11).

HTT is expressed in preimplantation stages of the embryo (12, 13) and loss of HTT expression in mice is embryonic lethal (14-16), underlining its developmental importance. Several studies document mHTT-dependent changes during development, both *in vivo* and in stem cell-derived culture paradigms (11, 17-21). Specifically, mHTT affects striatal development in the Hdh-Q111 embryo (22), and conditional mHTT expression during development is sufficient to generate HD-like symptoms (23, 24). In

animal models, early behavioral, cellular and metabolic abnormalities are discernible in four week-old tgHD rats and R6/2 mice, and changes in myelination were found in 14-day-old Hdh-Q250 mice (25-28). These studies indicate that HD pathology includes a neurodevelopmental phase that is present long before the onset of HD-like neurodegeneration, and which not only contributes to the classical disease symptoms, but is also likely to shape prodromal stages of HD.

In contrast to the later stages of symptomatic HD, prodromal manifestations are much less clearly defined. Previous studies indicate that emotional dysfunction and minor motor signs may precede the characteristic symptomatology (29, 30). Longitudinal studies of HD carriers aim to define the spectrum of early psychiatric symptoms in HD (29, 31). Additionally, differences in cranial volume and growth have been observed in young, pre-symptomatic gene carriers (32). Thus, the clinical and molecular delineation of early stages of HD is a central issue in the implementation of future targeted treatment strategies and the identification of new biomarkers.

Here, we provide evidence that neuronal differentiation changes continue postnatally in two mouse and rat HD animal models, and that molecular, cellular and behavioral alterations may be reversible through early therapeutic intervention with histone deacetylase inhibitors (HDACi). HDACi are promising compounds that are neuroprotective in HD mice (33), alleviate motor symptoms in the R6/2 model (34, 35), and were in clinical trials for HD (36).

A previous study showed that mHTT affects neural development pathways in HD-derived iPSCs (21), and we extend these findings to the systems level, revealing cellular

and behavioral correlates of neurodevelopmental gene expression pathways. Importantly, these mHTT-induced changes are reversible by low-dose therapy with the histone deacetylase inhibitor LBH589 (Panobinostat), which restores neural differentiation phenotypes *in vitro* and *in vivo*.

Results

Transgenic rodent HD models display a postnatal behavioral phenotype with decreased anxiety

We began by investigating if the presence of mHTT in the developing CNS causes measurable changes in early postnatal HD animals. Therefore, we employed the earliest quantifiable behavioral assays and tested transgenic tgHD rat (51 CAG repeats, adult onset) and BACHD mouse (97 repeats, juvenile onset) pups for emotional behavior (ultrasonic vocalization, USV) at postnatal day (P) 10, sensorimotor gating (prepulse inhibition, PPI) at P17 and risk taking behavior (novel cage test, NCT (37)) at P21. USV analysis revealed a decreased number and shorter duration of ultrasonic calls from both tgHD rat and BACHD transgenic pups separated from their dam compared to wild type (WT) littermates (Fig. 1a,b), indicating changes in perception (reduced awareness), emotionality (reduced anxiety) and/or executive functions (reduced pattern to vocalize as an indicator of emotional and/or motor functions). We employed PPI to test for a reduction in sensorimotor gating, and observed a loss of PPI in tgHD rats, but not in BACHD mice (Fig. 1c,d). In a test for anxiety and risk taking behavior, transgenic pups

from both models showed greater mobility and remained in the center area of a new cage for significantly longer durations, suggesting reduced anxiety and increased risk-taking behavior (Fig. 1e,f). These combined tests indicate decreased anxiety levels in early postnatal HD rodents. Fig. 1g and h summarize the behavioral phenotypes of the tgHD rat model and the BACHD mouse model, respectively.

Dopaminergic and glutamatergic imbalance in postnatal tgHD rat striatum

We then analyzed gene expression in the striatum of homozygous tgHD P10 rat pups and age-matched WT littermates using Affymetrix arrays in order to identify molecular changes that may be the underlying cause for the early behavioral symptoms. This revealed a high number of differentially regulated genes (Fig. 2a, Dataset S1), and using the Ingenuity pathway analysis software to classify genes according to function, we identified 17 aberrantly regulated candidate genes associated with "behavior" (Fig. 2b,c). qRT-PCR validation confirmed aberrant regulation of the 7 candidates angiotensinogen (*Agt)*, ATPase, Ca^{2+} transport (*Atp2A2)*, Forkhead box G1B (*FoxG1B),* hypocretin (orexin) receptor (*Hcrtr2),* potassium channel, member 1 (*Kcnc1),* solute carrier family 18, 2 (vesicular) (*Slc6A3),* and tyrosine hydroxylase (*Th)* (Fig. 2b). Since the two most upregulated of these 7 candidates were related to dopaminergic signaling (Fig. 2c), we analyzed striatal dopaminergic circuits in more detail, and further quantified the mRNA levels of DARPP-32, the dopamine receptor D1A and protein kinase A (PKA) by qRT-PCR. In contrast to the observed upregulation of Slc6A3 and Th, we found that all three genes were significantly downregulated in the striatum of tgHD P10 pups (Fig. 2d).

Western Blot analyses of total protein extracts from striatal tissue at P10 confirmed significantly reduced protein levels of DARPP-32 (Fig. 2e). Of note, there was a trend towards increased phosphorylation at Thr-75 and -34 in DARPP-32.

These studies were complemented by autoradiography for striatal expression of D1A, D2, NMDA, 5-HT2, adenosine A1 and adenosine A2A receptors in P10 rat pups. We found decreased levels of dopaminergic receptor D1A (Fig. 2f) and NMDA receptor density (Fig. 2f and Fig. S1a). Further Western Blot analyses of NMDA receptor subunits revealed downregulation of NR1, NR2B and NR2C (Fig. S1b). Immunohistochemical analysis of P10 tgHD striata confirmed reduced expression of DARPP32 and TH (Fig. 1G).

As mRNA expression changes do not always translate into altered protein levels, and because other regulatory mechanisms may also affect protein synthesis, we performed proteomic analysis using 2D-electrophoresis and subsequent MALDI-TOF of P10 pup striata as well. In the R6/2 model, large-scale alterations of protein levels precede the onset of classical symptoms (38). Similarly, we found a total of 156 proteins to be significantly altered in pre-HD tgHD rat brains. These could be classified into 6 KEGG pathways, confirming the observed alterations in metabolic pathways (38), and further identifying changes in axon sprouting and neurodegenerative pathways (Dataset S2). These studies revealed an additional downregulated regulatory subunit of protein phosphatase 1 (PPP1R7) (39, 40). Taken together, these data reveal imbalances in dopaminergic and glutamatergic pathways in the early postnatal period of tgHD rats sufficient to explain the behavioral phenotype.

Altered neuronal and oligodendroglial differentiation capacity in HD

Olfactory dysfunction is among the earliest discernible symptoms in neurodegenerative diseases, including Parkinson, Alzheimer and Huntington (41). Because of the well-established link between subventricular zone (SVZ) neurogenesis and the olfactory system, the observed association of aberrant SVZ neurogenesis and olfactory dysfunction in neurodegenerative diseases (42), the reported regulatory functions of dopaminergic and glutamatergic signaling for neurogenesis (43), as well as the potential implications of the olfactory system for our observations of animal behavior, we investigated SVZ neurogenesis in our model.

While neurodegeneration is characteristic of HD, previous studies observed aberrant neuronal differentiation during embryonic development *in vitro* and *in vivo* in the Hdh-Q111 model (22, 23), as well as in iPS-derived neural precursors from HD patients (21). It is therefore likely that any aberrations on the cellular and molecular level underlying behavioral changes are the product of aberrant neurodevelopmental, rather than neurodegenerative, processes. Consequently, we asked whether mHTT affected neuronal differentiation capacities of SVZ neural stem/progenitor cells (NSCs) during postnatal development. We cultured NSCs from P10 WT, tgHD+/- and tgHD+/+ pups, and differentiated them into glial and neuronal lineages. This resulted in a significant, gene dose dependent reduction in neuronal numbers at all time points (Fig. 3a-c). Analysis of differentiation to mature neuronal phenotypes revealed a reduction in bIII tubulin$^+$ neurons at 7 DIV (Fig. 3b), as well as lower numbers of NeuN$^+$ neurons at 14 and 28 DIV

(Fig. 3b). Likewise, Darpp32+ cell numbers were reduced in tgHD+/+ cultures at 14 and 28 DIV, while we observed an increase at 14 DIV and a decrease at 28 DIV in tgHD+/- cultures (Fig. 3c). Astrocyte numbers were unchanged, but oligodendrocyte counts were significantly increased after differentiation (Fig. 3a,b). These findings demonstrate an increase in oligodendroglial differentiation at the expense of neuron production from SVZ-derived NSC cultures in heterozygous and homozygous tgHD pups. Differentiation of tgHD cultures was accompanied by an increase in apoptotic cells identified by active Caspase 3 staining. This is also reflected in reduced total cell counts in tgHD+/- and tgHD +/+ compared to WT cultures (Fig. 3d).

To determine if neuronal, oligodendroglial and glial differentiation capacity was also affected in BACHD mice, we generated neurospheres from BACHD and WT littermates at E13.5. We detected significant reductions in the total number of cells, MAP2 immunopositive neurons and CNPase immunopositive oligodendrocytes in BACHD cultures compared to WT cultures at 7 DIV, while astrocyte numbers remained unchanged (Fig. 3e).

Because of the profound changes in differentiation capacity between WT and tgHD NSC cultures, we were interested if *in vivo* neurogenesis was affected by the presence of mHTT. To address this, we crossbred tgHD rats with a model expressing the reporter dsRed2 under control of the Dcx promoter (dcx::dsRed2,tgHD)(44). Dcx is a marker of newborn, migratory neurons, and is expressed up to 14 days after birth of new neurons (45). We analyzed sagittal sections of dsRed2-positive WT and tgHD pups, revealing minor differences in RMS and OB volumes, and a significant reduction in the content of

Dcx+ neurons (proportional to the relative fluorescence intensity, heatmaps in Fig. 3f). This corroborates reduced capacity for SVZ neurogenesis in the tgHD model.

Of note, diffusion tensor imaging (46) revealed a trend towards an increase in axial diffusivity, radial diffusivity and mean diffusivity and a reduction in fractional anisotropy in tgHD pups compared to wild type (Fig. S1c).

Low dose LBH589 treatment restores early behavioral, cellular and molecular phenotype in HD

The observed, mHTT-associated aberrations at the molecular, cellular and behavioral levels in these HD models extend previously reported alterations in embryonic development to the early postnatal phase, a time of neuronal maturation and gliogenesis. We next tested whether therapeutic intervention can restore these changes.

HDACi have a long history of use exerting mood stabilizing and anti-epileptic effects. In models of HD, previous studies have demonstrated that the HDACi SAHA (Vorinostat) could reverse motor symptoms and improve phenotypes in the R6/2 model (34, 35). Because of the neuroprotective effects of LBH589 in mouse models of HD (33), and its ability to cross the blood-brain-barrier (47) (Dataset S3), we treated tgHD pups with this HDACi. TgHD rat pups and WT littermates were subjected to four different dose regiments, administered every other day between P8 and P20 (Fig.4a, S2a-b). We analyzed USV at P11, startle response and PPI at P17 and the NCT at P21. Administration of 0.001 mg/kg LBH589 led to significantly increased USV in transgenic

rat pups (Fig 4b). LBH589 had a suppressive effect on prepulse inhibition and startle response independent of the individual genotype at all doses (Fig. 4c). In addition, performance in the NCT was improved in tgHD rats. Duration in the center was reduced and duration in the wall areas increased to match values of vehicle treated WT animals (Fig. 4d). In contrast, LBH589 had no effect on WT animals in this test. Furthermore, average velocity and track length in tgHD pups were restored to WT levels (Fig. S2a), while in the 0.01mg/kg WT group average velocity and track length were significantly decreased compared to vehicle treated WT animals. The improved performance of LBH589-treated tgHD pups in USV and NCT tests indicates that this compound alleviates the reduced anxiety and increased risk taking behavior in these animals.

We have previously demonstrated that HDACi increase neuronal differentiation capacities of NSCs (48). Therefore, we tested whether exposure to LBH589 could restore neuronal differentiation of tgHD NSCs *in vitro*. After testing for optimum dose regimens (Fig. S2c), we found that treatment of differentiating NSC cultures with 10 nM LBH589 for 24 hours restored neuronal differentiation of tgHD cultures to WT levels. Comparing bIII tubulin+ cells at 7 DIV, and NeuN+ and Darpp32+ cells at 14 DIV, we observed a significant increase in neurons at all time points (Fig. 4e), indicating improved regenerative capacities of SVZ stem/progenitor cells after treatment. LBH589 treatment had no effect on total cell numbers in WT controls, but increased tgHD total cell numbers (Fig. 4f).

Similarly, treatment of E13.5-derived BACHD NSC cultures with 10 nM LBH589 increased the percentage of MAP2+ neurons in both WT and BACHD cultures at 7 DIV

(Fig.4g). LBH589 exerted a positive effect in WT cultures already at 1nM, whereas BACHD cultures required 10 nM to increase the percentage of MAP2 cells. Furthermore, treatment increased the percentage of CNPase+ oligodendrocytes at 7 DIV. There were no differences in GFAP+ astrocyte numbers between any treatment conditions in BACHD and WT cultures (Fig. S2d-g).

Transcriptomic and proteomic profiling (Fig. 2, S3, Dataset S2) identified alterations in several pathways relevant to HD pathogenesis and neurodevelopment (Fig. S4). We therefore tested whether *in vivo* LBH589 treatment affected expression of a target gene of the dopamine pathway in medium spiny neurons (RasGRP2), which is downregulated in HD (49). LBH589 treatment increased RasGRP2 expression, and restored protein levels of 2 additional markers of striatal development, DARPP-32 and PPP1R7 (Fig. 4h; S5).

To determine whether LBH589 was capable of restoring neurogenesis *in vivo*, we quantified RMS volume and Dcx staining intensity in vehicle and LBH589 WT and tgHD pups at P21. Continuous administration of 0.001 mg/kg LBH589 from P8-P20 (7 doses) could restore *in vivo* neuronal differentiation and neurogenesis in tgHD pups to WT levels (Fig. 4i).

Thus, early postnatal behavioral, cellular and molecular alterations of HD can be alleviated by low-dose therapy with LBH589.

Discussion

Here we describe behavioral, cellular and molecular changes during a developmental phase of gliogenesis and neuronal maturation that is comparable to the third trimester of human gestation (50). Importantly, these changes can be reversed by treatment with HDACi, which fully restores alterations in neuronal differentiation in HD pups. Hence, these data contribute to the increasing evidence of neurodevelopmental aberrations in genetic animal models of HD and suggest that HD should be considered a neurodevelopmental disorder.

We identify reduced separation-induced ultrasonic vocalization at P10 and increased risk taking behavior (P21) as key features of an early behavioral phenotype in transgenic HD mice and rats. These features may correspond to an anxiolytic phenotype similar to the reduced anxiety-like behavior found in transgenic HD rats at 1 months of age (26) or be attributable to depression-like features as described in 2 month old BACHD mice (51). Dopaminergic and glutamatergic signaling regulate neuronal differentiation during development, and deregulation of these pathways may provide a molecular basis for HD-associated behavioral changes. These species-independent behavioral abnormalities provide a readout for the prodromal phase of HD, which is characterized by neurodevelopmental, rather than neurodegenerative mechanisms. mHTT causes aberrant neuronal differentiation during embryonic development and from iPS cells (21-24). Our findings link alterations in neuronal differentiation and molecular pathways to behavior on the systems level.

These observations support that HD pathology consists of at least three phases: (i) an early phase with successful adaptation to the effects of accumulating mutant Htt, likely to

be outbalanced by changes in early neurodevelopment. This early phenotype impresses with signs of overcompensation, and is followed by an intermediate and largely "silent" phase, which, depending on the number of CAG-repeats at mid-age, shifts into a third phase of overt decompensation and neurodegeneration. The notion that this early phase of HD may be viewed as a slowly progressive neurodevelopmental disorder that ultimately transforms into a neurodegenerative disease is documented in many studies identifying symptoms long before the onset of neurodegeneration in animal models (25-28, 52). In view of this evidence for a "pre-HD-syndrome", it should be mentioned that such changes may not be "disease-like" but instead may reflect a whole spectrum of signs and symptoms attributable to higher glutamine availability in the CNS and/or successful compensation and repair of the very early damage associated with mHTT. The former would be in line with the hypothesis of polyglutamine diseases arising from evolutionary search mechanisms (53) attempting to increase central glutamine storage in the brain, while the latter would imply that e.g. neurotrophic factors are induced, which may affect development in all its dimensions of growth, maturation, and learning. Whether mHTT causes developmental aberrations in gene carriers is still unclear, but recent studies have found differences in growth and development, as well as cranial volume in both adult (32) and juvenile (54), premanifest gene carriers.

Microarray analyses detected deregulation of the dopamine signaling pathway in postnatal tgHD rats. This was corroborated by findings of deficits in the D1/cAMP/PKA/DARPP-32 signaling cascade of P10 tgHD rats. These findings in postnatal tgHD rat brain mirror previous imaging studies in asymptomatic HD gene carriers (55, 56), which revealed a reduction of striatal D1 and D2 receptors. Evidence of

dopaminergic deregulation and receptor imbalance has further been substantiated in molecular studies of postmortem HD brains (57-59) and imaging studies of symptomatic HD patients (60, 61). Previous studies in four-week-old HD mice also demonstrated a reduction of total levels of DARPP-32 and D1 receptor protein levels (62). We previously showed that a significant loss of striatal D1 receptor expression and DARPP-32 positive cells are present in 14 months old male tgHD animals. Beyond deregulation of dopaminergic signaling pathways we observed a significantly reduced NMDA receptor density in the striatum of tgHD P10 rat pups. NMDA-receptor numbers are reduced particularly in the basal ganglia in both symptomatic (63, 64) and presymptomatic (65) HD patients. Early changes in extrasynaptic NMDA receptor signaling and expression have also been reported in 1-month-old presymptomatic YAC72 and YAC128 mice prior to onset of motor dysfunction and neuronal loss (66). Furthermore, there is evidence indicating that mHTT increases NR2B-containing extrasynaptic NMDARs in the striatum of young YAC72 and YAC128 mice (67). By contrast, our own data in P10 rat tissue show a downregulation of NR1, NR2B and NR2C subunits of NMDARs. A pathogenic link between dopamine and glutamate signaling is provided by studies showing that striatal dopamine modulates ionotropic glutamate receptors by altering glutamate currents and by modifying glutamate receptor surface localization (68, 69). Since D1 and NMDA receptors are capable of direct interaction, which affects receptor activity (70), it is possible that the aberrant striatal receptor expression observed in P10 tgHD rats is due to abnormal glutamate/dopamine receptor interactions. Alternatively, downregulation of D1A and NMDA receptors could also suggest a developmental delay in tgHD pups.

SVZ neurogenesis has been shown to affect social behavior (71), and aberrations in olfaction and SVZ neurogenesis are among the earliest symptoms in neurodegenerative diseases (41, 42). SVZ neurogenesis is modulated by dopamine and glutamate (43), thus it is likely that the observed deregulation of both pathways extends to neural stem cells as well. We demonstrate that SVZ-derived NSC cultures show a prominent reduction of their neuronal differentiation in both tgHD and BACHD models, which is corroborated by a reduction of NeuN positive neurons at 14 and 28 DIV in tgHD. The different results in oligodendrocyte differentiation between tgHD and BACHD models may be explained by their different ages at isolation. Oligodendroglial maturation and myelination start around P14 *in vivo*, thus embryonic-derived BACHD cultures may be too early to reflect mHtt effects on the oligodendrocyte lineage. Altered SVZ neurogenesis could be confirmed *in vivo* in P10 tgHD rats. Others have shown the relevance of mHTT during development (22, 23), as well as its impact on differentiation capacities of pluripotent stem-cell derived neural precursors (21, 72). mHTT has been shown to affect proliferation and cell fate of cortical progenitor cells (18). Consequently, the reduced neuronal differentiation observed here is likely due to direct effects of mHTT in neural precursors.

Bystander microenvironmental effects, e.g. from microglia, may contribute as well, as it has been shown that these cells affect SVZ neurogenesis *in vitro* (73). The specific effects of mHTT on postnatal neurogenesis in symptomatic HD models are somewhat controversial. While some studies did not find changes in SVZ proliferation or differentiation in R6/2 or YAC128 mice (74, 75), others detected reduced levels of neurogenesis in the R6/2 model (76, 77) and aberrant neuronal differentiation in tgHD

rats (78). In HD patients, cell proliferation in the SVZ is increased, which correlates with disease grade (79). Importantly, our observations are long before the onset of classical symptoms. Thus, it is conceivable that mHTT reduces generation of new neurons during earlier stages, while SVZ cell proliferation as a reaction to neurodegenerative processes dominates later stages of the disease.

Reduced neurogenesis may ultimately contribute to disease progression, i.e. lack of regeneration of striatal cell loss and dysfunction. This hypothesis is further supported by the observation of reduced numbers of striatal neurons (80) and reduced numbers of MSN (81) in the tgHD rat model.

Transcriptional deregulation is known to be part of the pathogenesis of HD (82, 83). A number of studies showed that HDACi ameliorate neurodegeneration in cell systems and fly models (84), as well as motor deficits in a mouse model of HD (34, 85). Here we treated postnatal tgHD rats with the HDACi LBH589 (86) to intervene with neurodevelopmental changes in a preclinical setting. LBH589 is known to efficiently cross the blood-brain-barrier and has already been tested in preclinical studies (33, 47). Low dose treatment with LBH589 led to a partial reversal of behavioral symptoms and restored aberrant neuronal differentiation in tgHD and BACHD cultures *in vitro* and in tgHD pups *in vivo*. We conclude that very early LBH589 treatment may be a promising novel treatment approach in HD.

Methods

Animals

We used several tgHD litters derived from mating +/- male and female tgHD rats carrying a truncated human *HTT* fragment of 51 CAG repeats under control of the native rat HTT promoter (87). BACHD mouse pups were derived from mating hemizygous male BACHD mice (expressing fl *HTT* with 97 CAG repeats) with female FVB/N dams (88). All animal experiments presented here were approved by local ethical boards (54-2532.1-16/08) and conducted according to local, NIH, and ARRIVE guidelines.

Behavioral phenotyping

Ultrasonic vocalization (USV)

P10 rat and mouse pups were placed into a recording cage in a sound isolation box. The detection sessions were initiated directly thereafter and were always performed by the same experienced experimenter between 8 and 10 am. A maximum of two pups per litter were tested for 5 minutes each. For P10 rats and mice signals were recorded at a 35-250 kHz range using an Avisoft Ultra Sound Gate 116Hb (Avisoft Bioacoustics, Germany). Incoming signals were displayed as a time-event-plot by Avisoft recorder^{TM} software (Avisoft, Germany). For further statistical analysis, parameters “number of events” [n] and “total duration of cues” [s] were analyzed using one-way ANOVA.

Acoustic startle response and prepulse inhibition (PPI)

Startle response and PPI were measured in homozygous (+/+; n ≥ 10), hemizygous (+/-; n ≥ 10) and wild type (-/-; n ≥ 10) tgHD rats, and hemizygous (+/-; n ≥ 10) and wild type (-/-; n ≥ 10) BACHD mice at P17. The test point was validated in preliminary experiments. Pups were placed in a startle chamber and the protocol was conducted as described previously (89). Results were analyzed using one-way ANOVA and are displayed as % inhibition of startle response.

Novel Cage Test (NCT)

The Novel Cage Test was performed in P21 pre-weaning homozygous (+/+; n ≥ 5), hemizygous (+/-; n ≥ 5), and wild type (-/-; n ≥ 5) tgHD rats and hemizygous BACHD and wild type mice as described previously (37). Results were analyzed using one-way ANOVA.

Treatment with HDAC inhibitor (HDACi) LBH589

Pups were genotyped 3 days after birth, divided into groups of 10 according to genotype and marked daily throughout the experiment. The animals were treated with LBH589 (Novartis, Switzerland) using the following doses: 0.001 mg-salt/kg BW; 0.01 mg-salt/kg BW, 0.1 mg-salt/kg BW and 1 mg-salt/kg BW. Control animals were treated with vehicle: 0.03 M lactic acid/5% dextrose in water buffered to pH 4.2-4.5 with 0.1 M NaOH. LBH589 was administered intraperitoneally every 48h beginning at P8 until P20.

The behavioral tests were performed at P11 (ultrasonic vocalization), P17 (startle response and PPI) and P21 (novel cage test). Pups were sacrificed at day 21 for histology.

Double-transgenic model and *in vivo* neurogenesis

tgHD rats were crossbred with a rat model expressing dsRed2 under control of the dcx promoter (Dcx::dsRed2)(90). The resulting offspring were interbred to generate hemizygous tgHD rats that were either homozygous or wild type for dcx-dsRed2 (tgHD+/- dsRed2-/- or tgHD+/- dsRed2+/+). Interbreeding these animals generated tgHD wild type and homozygous offspring that were heterozygous for dcx-dsRed2 (tgHD-/- dsRed+/- or tgHD+/+ dsRed2+/-). Animals from these litters were genotyped at P3 and used for experiments at P10. Pups were anaesthetized on ice and then transcardially perfused using 4% PFA, their brains removed and postfixed in 4% PFA overnight. Fixed brains were washed twice in PBS and then sectioned at 100 microns in the sagittal plane on a Leica VT-1000S vibratome. Serial sections from one hemisphere were mounted onto glass slides, air-dried and coverslipped using Vectashield with DAPI (Vector labs). Low power fluorescent images were taken on a Leica DMLB epifluorescence microscope (Bannockburn, IL) equipped with a CCD camera (Spot Imaging Solutions, Sterling Heights, MI). To obtain full images of brain sections, multiple gray scale images were acquired per section using Spot Advanced software (Spot Imaging Solutions) and merged into a full image and inverted into black-on-white images using Photoshop CS4 (Adobe Systems, San Jose, CA). Merged images were imported into ImageJ and area size and mean pixel values of RMS and OB analyzed.

Data analysis

All data were analyzed with GraphPad Prizm 7.0 (GraphPad Software) and statistical significance determined using appropriate statistical tests, as indicated in the figure legends. A p-value of <0.05 was deemed significant for all analyses. We used the D'Agostino-Pearson method to test for normal distribution of data points.

Additional methods are listed in Supplementary Information.

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Author contributions

FAS, KAR, AP and SvH designed and performed experiments and analyzed data. YKU, ASK, FC, DA, BG, MS, SM, JH, HN, RC performed experiments. MS, MB, OR, AB, LA, SCD, MAP, PS, AO, AA, CZ, AW, RK, SM, IB, AVDL, LR and AP contributed data and reagents. DAS, OR, AO, LR, RC, and AP contributed to the manuscript. FAS, KAR and SvH wrote the manuscript.

References

1. Group THsDCR (1993) A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. . *Cell* 72(6):971-983.
2. Paulsen J (1999) *Understanding Behavior in Huntington's Disease* (Huntington's Disease Society of America, New York).
3. Reiner A*, et al.* (1988) Differential loss of striatal projection neurons in Huntington disease. *Proceedings of the National Academy of Sciences of the United States of America* 85(15):5733-5737.
4. Rosas HD*, et al.* (2003) Evidence for more widespread cerebral pathology in early HD: an MRI-based morphometric analysis. *Neurology* 60(10):1615-1620.
5. Davies SW*, et al.* (1997) Formation of neuronal intranuclear inclusions underlies the neurological dysfunction in mice transgenic for the HD mutation. *Cell* 90(3):537-548.
6. DiFiglia M*, et al.* (1997) Aggregation of huntingtin in neuronal intranuclear inclusions and dystrophic neurites in brain. *Science* 277(5334):1990-1993.
7. Martindale D*, et al.* (1998) Length of huntingtin and its polyglutamine tract influences localization and frequency of intracellular aggregates. *Nat Genet* 18(2):150-154.
8. Andrew SE*, et al.* (1993) The relationship between trinucleotide (CAG) repeat length and clinical features of Huntington's disease. *Nat Genet* 4(4):398-403.
9. Rubinsztein DC, Barton DE, Davison BC, & Ferguson-Smith MA (1993) Analysis of the huntingtin gene reveals a trinucleotide-length polymorphism in the region of the gene that contains two CCG-rich stretches and a correlation between decreased age of onset of Huntington's disease and CAG repeat number. *Human molecular genetics* 2(10):1713-1715.
10. Humbert S (2010) Is Huntington disease a developmental disorder? *EMBO Rep* 11(12):899.
11. Wiatr K, Szlachcic WJ, Trzeciak M, Figlerowicz M, & Figiel M (2018) Huntington Disease as a Neurodevelopmental Disorder and Early Signs of the Disease in Stem Cells. *Mol Neurobiol* 55(4):3351-3371.
12. Bhide PG*, et al.* (1996) Expression of Normal and Mutant Huntingtin in the Developing Brain. *The Journal of Neuroscience* 16(17):5523-5535.
13. Jeong SJ*, et al.* (2006) Huntingtin is localized in the nucleus during preimplanatation embryo development in mice. *International journal of developmental neuroscience : the official journal of the International Society for Developmental Neuroscience* 24(1):81-85.
14. Duyao MP*, et al.* (1995) Inactivation of the mouse Huntington's disease gene homolog Hdh. *Science* 269(5222):407-410.

15. Nasir J*, et al.* (1995) Targeted disruption of the Huntington's disease gene results in embryonic lethality and behavioral and morphological changes in heterozygotes. *Cell* 81(5):811-823.
16. Zeitlin S, Liu JP, Chapman DL, Papaioannou VE, & Efstratiadis A (1995) Increased apoptosis and early embryonic lethality in mice nullizygous for the Huntington's disease gene homologue. *Nat Genet* 11(2):155-163.
17. Conforti P*, et al.* (2018) Faulty neuronal determination and cell polarization are reverted by modulating HD early phenotypes. *Proceedings of the National Academy of Sciences of the United States of America* 115(4):E762-E771.
18. Molina-Calavita M*, et al.* (2014) Mutant huntingtin affects cortical progenitor cell division and development of the mouse neocortex. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 34(30):10034-10040.
19. Saudou F & Humbert S (2016) The Biology of Huntingtin. *Neuron* 89(5):910-926.
20. Raymond LA*, et al.* (2011) Pathophysiology of Huntington's disease: time-dependent alterations in synaptic and receptor function. *Neuroscience* 198:252-273.
21. Consortium HDi (2017) Developmental alterations in Huntington's disease neural cells and pharmacological rescue in cells and mice. *Nat Neurosci* 20(5):648-660.
22. Molero AE*, et al.* (2009) Impairment of developmental stem cell-mediated striatal neurogenesis and pluripotency genes in a knock-in model of Huntington's disease. *Proceedings of the National Academy of Sciences of the United States of America* 106(51):21900-21905.
23. Molero AE*, et al.* (2016) Selective expression of mutant huntingtin during development recapitulates characteristic features of Huntington's disease. *Proceedings of the National Academy of Sciences of the United States of America* 113(20):5736-5741.
24. Arteaga-Bracho EE*, et al.* (2016) Postnatal and adult consequences of loss of huntingtin during development: Implications for Huntington's disease. *Neurobiology of disease* 96:144-155.
25. Hickey MA, Gallant K, Gross GG, Levine MS, & Chesselet MF (2005) Early behavioral deficits in R6/2 mice suitable for use in preclinical drug testing. *Neurobiology of disease* 20(1):1-11.
26. Nguyen HP*, et al.* (2006) Behavioral abnormalities precede neuropathological markers in rats transgenic for Huntington's disease. *Human molecular genetics* 15(21):3177-3194.
27. Mochel F*, et al.* (2012) Early alterations of brain cellular energy homeostasis in Huntington disease models. *The Journal of biological chemistry* 287(2):1361-1370.
28. Jin J*, et al.* (2015) Early white matter abnormalities, progressive brain pathology and motor deficits in a novel knock-in mouse model of Huntington's disease. *Human molecular genetics* 24(9):2508-2527.
29. Duff K, Paulsen JS, Beglinger LJ, Langbehn DR, & Stout JC (2007) Psychiatric symptoms in Huntington's disease before diagnosis: the predict-HD study. *Biol Psychiatry* 62(12):1341-1346.

30. Penney JB, Jr., *et al.* (1990) Huntington's disease in Venezuela: 7 years of follow-up on symptomatic and asymptomatic individuals. *Mov Disord* 5(2):93-99.
31. Paulsen JS, *et al.* (2008) Detection of Huntington's disease decades before diagnosis: the Predict-HD study. *J Neurol Neurosurg Psychiatry* 79(8):874-880.
32. Nopoulos PC, *et al.* (2011) Smaller intracranial volume in prodromal Huntington's disease: evidence for abnormal neurodevelopment. *Brain : a journal of neurology* 134(Pt 1):137-142.
33. Chopra V, *et al.* (2016) LBH589, A Hydroxamic Acid-Derived HDAC Inhibitor, is Neuroprotective in Mouse Models of Huntington's Disease. *J Huntingtons Dis* 5(4):347-355.
34. Hockly E, *et al.* (2003) Suberoylanilide hydroxamic acid, a histone deacetylase inhibitor, ameliorates motor deficits in a mouse model of Huntington's disease. *Proceedings of the National Academy of Sciences of the United States of America* 100(4):2041-2046.
35. Mielcarek M, *et al.* (2011) SAHA decreases HDAC 2 and 4 levels in vivo and improves molecular phenotypes in the R6/2 mouse model of Huntington's disease. *PloS one* 6(11):e27746.
36. Hogarth P, Lovrecic L, & Krainc D (2007) Sodium phenylbutyrate in Huntington's disease: a dose-finding study. *Mov Disord* 22(13):1962-1964.
37. Marques JM, Olsson IA, Ogren SO, & Dahlborn K (2008) Evaluation of exploration and risk assessment in pre-weaning mice using the novel cage test. *Physiology & behavior* 93(1-2):139-147.
38. Zabel C, *et al.* (2009) A large number of protein expression changes occur early in life and precede phenotype onset in a mouse model for huntington disease. *Molecular & cellular proteomics : MCP* 8(4):720-734.
39. Metzler M, *et al.* (2010) Phosphorylation of huntingtin at Ser421 in YAC128 neurons is associated with protection of YAC128 neurons from NMDA-mediated excitotoxicity and is modulated by PP1 and PP2A. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 30(43):14318-14329.
40. Lewandowski NM, *et al.* (2013) Regional vulnerability in Huntington's disease: fMRI-guided molecular analysis in patients and a mouse model of disease. *Neurobiology of disease* 52:84-93.
41. Barresi M, *et al.* (2012) Evaluation of olfactory dysfunction in neurodegenerative diseases. *Journal of the neurological sciences* 323(1-2):16-24.
42. Winner B, Kohl Z, & Gage FH (2011) Neurodegenerative disease and adult neurogenesis. *Eur J Neurosci* 33(6):1139-1151.
43. Young SZ, Taylor MM, & Bordey A (2011) Neurotransmitters couple brain activity to subventricular zone neurogenesis. *Eur J Neurosci* 33(6):1123-1132.
44. Schrodl F, *et al.* (2014) Rat choroidal pericytes as a target of the autonomic nervous system. *Cell Tissue Res* 356(1):1-8.
45. Brown JP, *et al.* (2003) Transient expression of doublecortin during adult neurogenesis. *The Journal of comparative neurology* 467(1):1-10.
46. Blockx I, *et al.* (2012) Microstructural changes observed with DKI in a transgenic Huntington rat model: evidence for abnormal neurodevelopment. *NeuroImage* 59(2):957-967.

47. Pipalia NH*, et al.* (2011) Histone deacetylase inhibitor treatment dramatically reduces cholesterol accumulation in Niemann-Pick type C1 mutant human fibroblasts. *Proceedings of the National Academy of Sciences of the United States of America* 108(14):5620-5625.
48. Siebzehnrubl FA*, et al.* (2007) Histone deacetylase inhibitors increase neuronal differentiation in adult forebrain precursor cells. *Exp Brain Res* 176(4):672-678.
49. Crittenden JR*, et al.* (2010) CalDAG-GEFI down-regulation in the striatum as a neuroprotective change in Huntington's disease. *Human molecular genetics* 19(9):1756-1765.
50. Semple BD, Blomgren K, Gimlin K, Ferriero DM, & Noble-Haeusslein LJ (2013) Brain development in rodents and humans: Identifying benchmarks of maturation and vulnerability to injury across species. *Prog Neurobiol* 106-107:1-16.
51. Hult Lundh S, Nilsson N, Soylu R, Kirik D, & Petersen A (2013) Hypothalamic expression of mutant huntingtin contributes to the development of depressive-like behavior in the BAC transgenic mouse model of Huntington's disease. *Human molecular genetics* 22(17):3485-3497.
52. L'Episcopo F*, et al.* (2016) GSK-3beta-induced Tau pathology drives hippocampal neuronal cell death in Huntington's disease: involvement of astrocyte-neuron interactions. *Cell death & disease* 7:e2206.
53. Brusilow WS (2006) Is Huntington's a glutamine storage disease? *The Neuroscientist : a review journal bringing neurobiology, neurology and psychiatry* 12(4):300-304.
54. Lee JK*, et al.* (2012) Measures of growth in children at risk for Huntington disease. *Neurology* 79(7):668-674.
55. Weeks RA, Piccini P, Harding AE, & Brooks DJ (1996) Striatal D1 and D2 dopamine receptor loss in asymptomatic mutation carriers of Huntington's disease. *Ann Neurol* 40(1):49-54.
56. Ginovart N*, et al.* (1997) PET study of the pre- and post-synaptic dopaminergic markers for the neurodegenerative process in Huntington's disease. *Brain : a journal of neurology* 120 (Pt 3):503-514.
57. Filloux F*, et al.* (1990) Nigral dopamine type-1 receptors are reduced in Huntington's disease: a postmortem autoradiographic study using [3H]SCH 23390 and correlation with [3H]forskolin binding. *Exp Neurol* 110(2):219-227.
58. Richfield EK, O'Brien CF, Eskin T, & Shoulson I (1991) Heterogeneous dopamine receptor changes in early and late Huntington's disease. *Neuroscience letters* 132(1):121-126.
59. Glass M, Dragunow M, & Faull RL (2000) The pattern of neurodegeneration in Huntington's disease: a comparative study of cannabinoid, dopamine, adenosine and GABA(A) receptor alterations in the human basal ganglia in Huntington's disease. *Neuroscience* 97(3):505-519.
60. Sedvall G*, et al.* (1994) Dopamine D1 receptor number--a sensitive PET marker for early brain degeneration in Huntington's disease. *Eur Arch Psychiatry Clin Neurosci* 243(5):249-255.
61. Turjanski N, Weeks R, Dolan R, Harding AE, & Brooks DJ (1995) Striatal D1 and D2 receptor binding in patients with Huntington's disease and other choreas. A PET study. *Brain : a journal of neurology* 118 (Pt 3):689-696.

62. Bibb JA, *et al.* (2000) Severe deficiencies in dopamine signaling in presymptomatic Huntington's disease mice. *Proceedings of the National Academy of Sciences of the United States of America* 97(12):6809-6814.
63. Greenamyre JT, *et al.* (1985) Alterations in L-glutamate binding in Alzheimer's and Huntington's diseases. *Science* 227(4693):1496-1499.
64. Young AB, *et al.* (1988) NMDA receptor losses in putamen from patients with Huntington's disease. *Science* 241(4868):981-983.
65. Albin RL, *et al.* (1990) Abnormalities of striatal projection neurons and N-methyl-D-aspartate receptors in presymptomatic Huntington's disease. *N Engl J Med* 322(18):1293-1298.
66. Milnerwood AJ, *et al.* (2010) Early increase in extrasynaptic NMDA receptor signaling and expression contributes to phenotype onset in Huntington's disease mice. *Neuron* 65(2):178-190.
67. Cowan CM, *et al.* (2008) Polyglutamine-modulated striatal calpain activity in YAC transgenic huntington disease mouse model: impact on NMDA receptor function and toxicity. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 28(48):12725-12735.
68. Dunah AW & Standaert DG (2001) Dopamine D1 receptor-dependent trafficking of striatal NMDA glutamate receptors to the postsynaptic membrane. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 21(15):5546-5558.
69. Andre VM, Cepeda C, & Levine MS (2010) Dopamine and glutamate in Huntington's disease: A balancing act. *CNS Neurosci Ther* 16(3):163-178.
70. Lee FJ & Liu F (2004) Direct interactions between NMDA and D1 receptors: a tale of tails. *Biochem Soc Trans* 32(Pt 6):1032-1036.
71. Gheusi G, Ortega-Perez I, Murray K, & Lledo PM (2009) A niche for adult neurogenesis in social behavior. *Behavioural brain research* 200(2):315-322.
72. Nguyen GD, Gokhan S, Molero AE, & Mehler MF (2013) Selective roles of normal and mutant huntingtin in neural induction and early neurogenesis. *PloS one* 8(5):e64368.
73. Walton NM, *et al.* (2006) Microglia instruct subventricular zone neurogenesis. *Glia* 54(8):815-825.
74. Gil JM, Leist M, Popovic N, Brundin P, & Petersen A (2004) Asialoerythropoietin is not effective in the R6/2 line of Huntington's disease mice. *BMC neuroscience* 5:17.
75. Simpson JM, *et al.* (2011) Altered adult hippocampal neurogenesis in the YAC128 transgenic mouse model of Huntington disease. *Neurobiology of disease* 41(2):249-260.
76. Kohl Z, *et al.* (2010) Impaired adult olfactory bulb neurogenesis in the R6/2 mouse model of Huntington's disease. *BMC neuroscience* 11:114.
77. Fedele V, Roybon L, Nordstrom U, Li JY, & Brundin P (2011) Neurogenesis in the R6/2 mouse model of Huntington's disease is impaired at the level of NeuroD1. *Neuroscience* 173:76-81.
78. Kandasamy M, *et al.* (2015) Reduction in subventricular zone-derived olfactory bulb neurogenesis in a rat model of Huntington's disease is accompanied by striatal invasion of neuroblasts. *PloS one* 10(2):e0116069.

79. Curtis MA, *et al.* (2003) Increased cell proliferation and neurogenesis in the adult human Huntington's disease brain. *Proceedings of the National Academy of Sciences of the United States of America* 100(15):9023-9027.
80. Kantor O, *et al.* (2006) Selective striatal neuron loss and alterations in behavior correlate with impaired striatal function in Huntington's disease transgenic rats. *Neurobiology of disease* 22(3):538-547.
81. Bode FJ, *et al.* (2008) Sex differences in a transgenic rat model of Huntington's disease: decreased 17beta-estradiol levels correlate with reduced numbers of DARPP32+ neurons in males. *Human molecular genetics* 17(17):2595-2609.
82. Cha JH (2007) Transcriptional signatures in Huntington's disease. *Prog Neurobiol* 83(4):228-248.
83. Nguyen HP, *et al.* (2008) Age-dependent gene expression profile and protein expression in a transgenic rat model of Huntington's disease. *Proteomics Clin. Appl.* 2:1638-1650.
84. Steffan JS, *et al.* (2001) Histone deacetylase inhibitors arrest polyglutamine-dependent neurodegeneration in Drosophila. *Nature* 413(6857):739-743.
85. Ferrante RJ, *et al.* (2003) Histone deacetylase inhibition by sodium butyrate chemotherapy ameliorates the neurodegenerative phenotype in Huntington's disease mice. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 23(28):9418-9427.
86. Beckers T, *et al.* (2007) Distinct pharmacological properties of second generation HDAC inhibitors with the benzamide or hydroxamate head group. *International journal of cancer. Journal international du cancer* 121(5):1138-1148.
87. von Horsten S, *et al.* (2003) Transgenic rat model of Huntington's disease. *Human molecular genetics* 12(6):617-624.
88. Gray M, *et al.* (2008) Full-length human mutant huntingtin with a stable polyglutamine repeat can elicit progressive and selective neuropathogenesis in BACHD mice. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 28(24):6182-6195.
89. Urbach YK, Bode FJ, Nguyen HP, Riess O, & von Horsten S (2010) Neurobehavioral tests in rat models of degenerative brain diseases. *Methods Mol Biol* 597:333-356.
90. Couillard-Despres S, *et al.* (2006) Targeted transgene expression in neuronal precursors: watching young neurons in the old brain. *Eur J Neurosci* 24(6):1535-1545.

Figure Legends

Figure 1: Early behavioral phenotyping in transgenic models of Huntington's disease. Rat (SPRDtgHD) and mouse pups (BACHD) were screened for a behavioral phenotype between P10 and P21. Number and total duration of ultrasonic calls in rat (wild type (WT), hemizygous (HET), homozygous (HOM), n>30) (**A**) and mouse (wild type (WT), hemizygous (BACHD), n>7) (**B**) pups were determined at P10. Transgenic pups emitted significantly less calls (tgHD rat pups: HOM (+/+) vs WT (-/-): $p<0.0001$; HET (+/-) vs WT (-/-): $p=0.0003$; BACHD mouse pups: BACHD (+/-) vs WT (-/-): $p=0.05$) of shorter duration (tgHD rat pups: HOM (+/+) vs WT (-/-): $p<0.0001$; HET (+/-) vs WT (-/-): $p=0.0221$; BACHD mouse pups: BACHD (+/-) vs WT (-/-): $p=0.0256$). Startle response and PPI were measured at P17. Transgenic HD rat pups showed a significant loss of prepulse inhibition at a prepulse of 80 dB white noise ($p=0.0266$, n>8) (**C**), while there were no differences observed in BACHD mice at this age (**D**; n=10). Altered exploration and risk behavior was detected in transgenic pre-weaning rats (**E**) as well as in transgenic mice (**F**) at P21 using the Novel Cage test. Time spent in the center of the cage was significantly increased in homozygous rat (**E**) (tgHD rat: HOM (+/+) vs WT (-/-): $p=0.0108$; n=5) and BACHD mouse (**F**) (BACHD mouse: BACHD (+/-) vs WT (-/-): $p=0.0257$; n>8) pups, while time spent in the wall area was significantly decreased (tgHD rat: HOM (+/+) vs WT (-/-): $p=0.0102$; BACHD mouse: BACHD (+/-) vs WT (-/-): $p=0.0266$). A time course graph illustrates the development of the behavioral phenotype of the transgenic tgHD rat (**G**) and BACHD (**H**) mice. Data represent means ± SEM. Statistical analyses using ANOVA (*$p< .05$, **$p< .01$; ***$p< .001$ vs WT pups).

Figure 2: Molecular analyses of tgHD P10 striata. Microarray analysis of rat striata at P10 (WT vs HOM, n=5 each) demonstrated clusters of up- (yellow) and downregulated (blue) gene expression (**A**). Ingenuity pathway analysis revealed 17 target mRNAs associated with "behavior", 7 of which (#) were validated by qRT-PCR (p values from ANOVA vs WT; n>8) (**B**). Network analysis indicated deregulation of dopaminergic signaling (**C**, relevant pathways highlighted in yellow). Rat P10 striatal mRNA-expression of DARPP-32 (HOM vs WT: p=0.00465), PKA (HOM vs WT: p=0.0069; HET vs WT: p=0.0009) and dopamine receptor D1A (HOM vs WT: p=0.0067; HET vs WT: p=0.0209) is downregulated, whereas the expression of dopamine transporter Slc6a3 is upregulated (HOM vs: p=0.0065). Data are displayed as mean normalized expression (% of WT; ANOVA, n>8) (**D**). DARPP-32 downregulation (HOM vs WT: p=0.0044; two-tailed T-test, n>5) could be confirmed in Western Blot studies. Phosphorylation levels of DARPP-32 (Thr^{34} and Thr^{75}) in P10 striata were not significantly different in WT and transgenic pups (**E**). Receptor autoradiography revealed a significant reduction of dopamine receptor D1A (HOM vs WT: p=0.0067) and NMDA receptor (HOM vs WT: p=0.0071) density in the striata of transgenic rat pups (two-tailed T-test, n=5). Representative autoradiographs of coronal sections of P10 rats are presented (**F**). Immunohistochemistry confirmed reduced expression of DARPP32 and TH in the striatum of P10 tgHD pups (representative images from n=10 pups) (**G**). Data represent means ± SEM (*p< .05, **p< .01; ***p< .001 vs WT pups).

Figure 3: Cellular analysis of tgHD P10 neurogenesis. Quantitative analysis of P10 rat neurosphere differentiation demonstrated reduced neuronal (bIII-tubulin, green, upper panels) and increased oligodendroglial (CNP, green, lower panels) differentiation, while astroglial (GFAP, red, upper panels) fates were unaffected (scale bar = 20 μm applies to all images). Graphical representation indicates percentages ± SEM of individual cell types (**A**). 7 days after induction of differentiation (DIV), neuron numbers were significantly decreased (HOM vs WT: $p<0.0001$; HET vs WT: $p=0.0307$), while oligodendrocyte counts were increased (HOM vs WT: $p<0.0001$; HET vs WT: $p=0.0481$). Astrocyte numbers were not affected significantly. Numbers of mature, NeuN expressing neurons were significantly reduced at 14 DIV and 28 DIV (HOM vs WT: $p<0.0001$; HET vs WT: $p<0.0001$) (**B**). Similarly, DARPP32+ cell counts were significantly reduced at both time points (14 DIV HOM vs WT: $p=0.0009$; HET vs WT: $p=0.0132$; 28 DIV HOM vs WT: $p=0.0168$; HET vs WT: $p=0.0168$) (**C**). The differentiation phenotype was accompanied by an increase in apoptosis (HOM vs WT: $p<0.0001$; HET vs WT: $p=0.0120$), which is reflected in lower cell counts after differentiation (HOM vs WT: $p<0.0001$; HET vs WT: $p<0.0001$) (**D**) (A-D: ANOVA, n=3 independent experiments with 10 replicates). Differentiation of BACHD embryonic (E13.5) neurospheres revealed significantly lower numbers of total cells, MAP2+ and CNPase+ cells compared to WT cultures (ANOVA, n=8). GFAP+ astrocyte counts were unchanged (**E**). *In vivo* changes in neuronal differentiation are reflected in the RMS and OB of P10 Dcx-dsRed2-tgHD double transgenic pups (Mann-Whitney test, n=7; upper panels inverted fluorescence images, lower panels heatmaps of fluorescence intensity). While the volume of RMS and OB was not significantly different, the mean fluorescence

intensity (proportional to neuronal numbers) was significantly reduced in both structures (RMS HOM vs WT: p=0.0105; OB HOM vs WT: p=0.0012) (**F**).

Figure 4: Treatment of early phenotype using HDAC inhibitor LBH589. tgHD HOM pups and WT controls were treated with the HDACi LBH589 from P8 to P20 (n=10 each). Behavioral readouts were USV (P11), startle response and PPI (P17), and NCT (P21). Four different doses (0.001; 0.01; 0.1 and 1.0 mg/kg BW) were administered i.p. every other day (**A**). The numbers of ultrasonic calls were significantly increased in transgenic animals treated with LBH589 compared to vehicle controls (0.001 mg/kg: p=0.0390; 0.01 mg/kg: p=0.1002; 0.1 mg/kg: p=0.0005). Increase of total duration in transgenic animals didn't reach significance except for the 0.1 mg/kg group (0.001 mg/kg: p=0.2561; 0.01 mg/kg: p=0.1949; 0.1 mg/kg: p=0.0016; ANOVA, n>10) (**B**). Prepulse inhibition was suppressed in animals treated with LBH589 in all doses tested compared to WT (ANOVA, n>3) (**C**). LBH589 treatment significantly decreased duration in center areas and increased duration in wall areas in transgenic rats compared to corresponding vehicle treated transgenic animals (ANOVA, n>4). LBH589 had no significant effect on WT animals (**D**). Data represent means ± SEM. Significant effects vs WT (**p*< .05, ***p*< .01; ****p*< .001) and treatment effects vs vehicle control (#*p*< .05, ##*p*< .01; ###*p*< .001). LBH589 treatment of differentiating neurosphere cultures restored tgHD HOM neuron numbers to WT levels (representative images of cultures treated with 10 nM LBH for 24 hours; scale bar = 20 μm applies to both images). TuJ^+ neurons were counted after 7 days and $NeuN^+$ and $Darpp32^+$ neurons were quantified

after 14 days. TuJ^+, $NeuN^+$ and $Darpp32^+$ cells were within the range of wild type cultures (HOM DMSO vs LBH, TuJ p=0.0024, NeuN p<0.0001, DARPP32 p=0.0140; WT not significant; ANOVA, n=3 independent experiments with 10 replicates) (**E**). LBH treatment resulted in increased cell counts in transgenic, but not wild type cell cultures (HOM DMSO vs LBH: p=0.0424) (**F**). Percentage of MAP2 immunopositive neurons were significantly increased with 10 nM LBH589 in WT and BACHD E13.5 neurosphere cultures (ANOVA, n=8; ***p<0.001, **** p<0.0001). Representative photomicrograph showing MAP2+ neurons (green) and DAPI (blue) from WT and BACHD cultures (scale bar, 100 μm) (**G**). LBH treatment (0.001 mg/kg) increased striatal RasGRP2 gene expression and DARPP32 and PPP1R7 protein levels in P10 tgHD pups, but not WT littermates (TG vs WT: *p<.05, **p<.01, WT DMSO vs WT LBH: #p<.05 ANOVA, n>6) (**H**). Heatmaps of dcx-stained immunofluorescence micrographs from P21 tgHD pups highlight SVZ-RMS neurogenesis *in vivo* (scale bar, 100 μm). LBH treatment restored both RMS volume (p=0.0159 HOM treated vs. vehicle) and dcx fluorescence intensity (p=0.0029 HOM treated vs. vehicle) in tgHD pups to WT levels (7 injections of 0.001 mg/kg compared to vehicle control; two-way ANOVA, n>3) (**I**).