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

Florian A. Siebzehnrübl1,2, Kerstin A. Raber1,2, Yvonne K. Urbach2, Anja Schulze-Krebs2, Fabio Canneva2, Sandra Moceri2, Johanna Habermeyer2, Dalila Achoui2, Bhavana Gupta2, Dennis A. Steindler2,3, Michael Stephan2, Hau Phuc Nguyen1,2, Olaf Riess2, Andreas Bauer2, Ludwig Aigner2, Sebastien Couillard-Despres3, Martin Arce Pacaur2, Per Svenningsson3, Alexander Osmand4, Alexander Andreadis5, Claus Zabel5, Andreas Weiss5, Rainer Kuhn5, Salima Moussaoui1, Ines Blockx6, Annemie Van der Linden5, Rachel Y. Cheong5, Laurent Roybon5, Åsa Petersén6, and Stephan von Hörsten4,7

Huntington disease (HD) is an autosomal dominant neurodegenerative disorder caused by expanded CAG repeats in the huntingtin gene (HTT). Although 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 premature transgenic HD rats and mice. Reduced ultrasonic vocalization, loss of prepulse 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 LBH589 led to significant improvement in behavioral changes and markers of dopaminergic neurotransmission and complete reversal of aberrant neuronal differentiation in vitro and in vivo. Our data support the notion that neurodevelopmental changes contribute to the prodromal phase of HD and that early, presymptomatic intervention using HDAC inhibitors may represent a promising novel treatment approach for HD.

Significance

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

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1F.A.S. and K.A.R. contributed equally to this work.
2Present address: Department of Human Genetics, Ruhr University, 44801 Bochum, Germany.
3Present address: MV2 Martinsried, Martinsried 82152, Germany.
4To whom correspondence should be addressed. Email: stephan.hoersten@fau.de.

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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 4-wk-old tgHD rats and R6/2 mice, and changes in myelination were found in 14-d-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, presymptomatic gene carriers (32). Thus, the clinical and molecular delineation of the 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 changes in neuronal differentiation 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 induced pluripotent stem cells (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 (51 CAG repeats, adult onset) and BACHD mouse (97 CAG 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 with WT littermates (Fig. 1 A and 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. 1 C and 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 than WT pups, suggesting reduced anxiety and increased risk-taking behavior (Fig. 1 E and F). These combined tests indicate decreased anxiety levels in early postnatal HD rodents. Fig. 1 G 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 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 and Dataset S1). Using the Ingenuity Pathway Analysis software to classify genes according to function, we identified 17 aberrantly regulated candidate genes associated with “behavior” (Fig. 2B and C). qRT-PCR validation confirmed aberrant regulation of seven candidates: angiotensinogen (Agt), ATPase, Ca^{2+} transport (Atp2a2), Forkhead box G1B (FoxG1B), hypocretin (orexin) receptor (Hcrtr2), potassium channel, member 1 (Kcn1), solute carrier family 6, 3 (dopamine transporter) (Slc6a3), and tyrosine hydroxylase (Th) (Fig. 2B). Since the two most up-regulated of these seven 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 up-regulation of Slc6a3 and Th, we found that all three genes were significantly down-regulated 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 toward increased phosphorylation at Thr^{75} and Thr^{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 the dopaminergic receptor D1A (Fig. 2F) and NMDA receptor (NMDAR) density (Fig. 2F and SI Appendix, Fig. S1A). Further Western blot analyses of NMDAR subunits revealed down-regulation of NR1, NR2B, and NR2C (SI Appendix, 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 proteome analysis using 2D electrophoresis and subsequent MALDI-TOF. We found 156 proteins to be significantly altered in pre-HD tgHD rat brains. These could be classified into six Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways, confirming the observed alterations in metabolic pathways (38) and further identifying changes in axon sprouting and neurodevelopmental regulations (Dataset S2). These studies revealed an additional down-regulated 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 disease (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), and 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 iPSC-derived neural precursors from HD patients (21). Therefore, it is likely that any aberrations on the cellular and molecular level underlying behavioral changes are the product of aberrant neurodevelopmental capacity 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. 3 A-C). Analysis of differentiation to mature neuronal phenotypes revealed a reduction in bIII tubulin+ neurons at 7 d in vitro (DIV) (Fig. 3B), as well as lower numbers of NeuN+ neurons at 14 and 28 DIV (Fig. 3B). Likewise, Darp32+ 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. 3 A and 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+/+ cultures compared with 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 2′,3′-cyclic-nucleotide 3′-phosphodiesterase (CNPase)-immunopositive oligodendrocytes in BACHD cultures compared with 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 in whether in vivo neurogenesis was affected by the presence of mHTT. To address this, we crossed b2-galactosidase transgenic rats and transgenic mouse 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 (Dataset S3) (47), we treated tgHD pups with this HDACi. TgHD rat pups and WT littermates were subjected to four different dose regimens, administered every other day between P8 and P20 (Fig. 4A and SI Appendix, Fig. S2 A and 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 PPI 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, the average velocity and track length in tgHD pups were restored to WT levels (SI Appendix, Fig. S24), while in the 0.01 mg/kg WT group the average velocity and track length were significantly decreased compared with 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 increases risk-taking behavior in these animals.

We have previously demonstrated that HDACi increase the 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 optimal dose regimens (SI Appendix, Fig. S2C), we found that treatment of differentiating NSC cultures with 10 nM LBH589 for 24 h 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 at 1 nM, 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 (SI Appendix, Fig. S2–G).

Transcriptomic and proteomic profiling (Fig. 2, SI Appendix, Fig. S3, and Dataset S2) identified alterations in several pathways relevant to HD pathogenesis and neurodevelopment (SI Appendix).
Fig. 4. (A) Treatment of early phenotype using HDAC inhibitor LBHS89. tgHD HOM pups and WT controls were treated with the HDACi LBHS89 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 body weight) were administered i.p. every other day. (B) The numbers of ultrasonic calls were significantly increased in transgenic animals treated with LBHS89 compared with vehicle controls. The increase in the total duration in transgenic animals did not 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). (C) PPI was suppressed in animals treated with LBHS89 in all doses tested compared with WT (ANOVA; n > 3). (D) LBHS89 treatment significantly decreased the time spent in center areas and increased the time spent in wall areas in transgenic rats compared with corresponding vehicle-treated transgenic animals (ANOVA; n > 4). LBHS89 had no significant effect on WT animals. (E) LBHS89 treatment of differentiating neurosphere cultures restored tgHD HOM neuron numbers to WT levels. Shown are representative images of cultures treated with 10 nM LBHS89 for 24 h. (Scale bar, 20 μm in both images.) TuJ+ neurons were counted after 7 d, and NeuN+ and Darpp32+ neurons were quantified after 14 d. TuJ+, NeuN+, and Darpp32+ cells were within the range of WT cultures (ANOVA; n = 3 independent experiments with 10 replicates). (F) LBHS89 treatment resulted in increased cell counts in transgenic but not in WT cell cultures. (G, Right) The percentage of MAP2-immunopositive neurons was significantly increased with 10 nM LBHS89 in WT and BACHD E13.5 neurosphere cultures (ANOVA; n = 8). (Left) A representative photomicrograph showing MAP2+ neurons (green) and DAPI (blue) from WT and BACHD cultures. (Scale bar, 100 μm.) (H) LBHS89 treatment (0.001 mg/kg) increased striatal RasGRP2 gene expression and DARPP32 and PPP1R7 protein levels in P10 tgHD pups but not in WT littermates (ANOVA; n > 6). (I, Left) Heatmaps of dcx-stained immunofluorescence micrographs from P21 tgHD pups highlight SVZ-RMS neurogenesis in vivo. (Scale bar, 100 μm.) (Right) LBHS89 treatment restored both RMS volume and dcx fluorescence intensity in tgHD pups to WT levels (seven injections of 0.001 mg/kg compared with vehicle control; two-way ANOVA; n > 3). Data represent means ± SEM. Significant effects vs. WT (*P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001) and treatment effects vs. vehicle control (*P < 0.05; **P < 0.01; ***P < 0.001).
To determine whether LBH589 was capable of restoring neurogenesis in vivo, we quantified RMS volume and Dcx staining intensity in vehicle- and LBH589-treated WT and tgHD pups at P21. Continuous administration of 0.001 mg/kg LBH589 from P8 to P20 (seven doses) could restore in vivo neuronal differentiation and neurogenesis in tgHD pups to WT levels (Fig. 4f).

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 USV at P10 and increased risk-taking behavior at P21 as key features of an early behavioral phenotype in transgenic HD mice and rats. These features may correspond to an anxious-like phenotype similar to the reduced anxiety-like behavior found in transgenic HD rats at 1 mo of age (26) or may be attributable to depression-like features as described in 2-mo-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 iPSCs (21–24). Our findings link alterations in neuronal differentiation and molecular pathways to behavior on the systems level.

These observations support the notion 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 presents 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 glutamate 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 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 in 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 the postnatal tgHD rats 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 been further substantiated in molecular studies of postmortem HD brains (57–59) and imaging studies of symptomatic HD patients (60, 61). Previous studies in 4-wk-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+ cells is present in 14-mo-old male tgHD animals. Beyond deregulation of dopaminergic-signaling pathways, we observed significantly reduced NMDAR density in the striatum of tgHD P10 rat pups. NMDAR numbers are reduced, particularly in the basal ganglia, in both symptomatic (63, 64) and presymptomatic (65) HD patients. Early changes in extrasynaptic NMDAR signaling and expression have also been reported in 1-mo-old presymptomatic YAC72 and YAC128 mice before the 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 the 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 receptors and NMDARs 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, down-regulation of D1A receptors and NMDARs 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 (1, 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+ 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 the differentiation capacities of pluripotent SC-derived neural precursors (21, 72). mHTT has been shown to affect the 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.
We used several tgHD litters derived from mating male and female or tgHD ≥ rats carrying a truncated human Proc Natl Acad Sci USA 410. We thank J. Stiller, S. Meyer, and C. Galeano for 163. dsRed2 ≥ 1715. 0.05 was 90: 983. | Nat Genet 1715. 0.05 was 90: 983. | Nat Genet 90: 983.

The test point was validated in preliminary experiments. Pups were placed in homozygous (Acoustic startle response and PPI. were displayed as a time 8 and 10 AM. A maximum of two pups per litter were tested for 5 min each. Behavioral Phenotyping. and the University of Florida Institutional Animal Care and Use Committee, ernment of Middle Franconia, Bavaria, Germany (approval no. 54-2532.1-16/08) and the University of Florida Institutional Animal Care and Use Committee, and were conducted according to local, NIH, and ARRIVE guidelines (89).

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 floxed HTT with 97 CAG repeats) with female FVB/N dams (88). All animal experiments presented here were approved by local ethical boards of the District Govern- ment of Middle Franconia, Bavaria, Germany (approval no. 54-2532.1-16/08) and the University of Florida Institutional Animal Care and Use Committee, and were conducted according to local, NIH, and ARRIVE guidelines (89).

1. The Huntington’s Disease Collaborative Research Group (1993) A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington’s disease chromosomes. Cell 72: 971–983.
2. Paulsen J (1999) Understanding Behavior in Huntington’s Disease (Huntington’s Dis- ease Society of America, New York).
3. Reiner A, et al. (1988) Differential loss of striatal projection neurons in Huntington disease. Proc Natl Acad Sci USA 85:5733–5737.
4. Rosas HD, et al. (2003) Evidence for more widespread cerebral pathology in early HD: An MRI-based morphometric analysis. Neurology 60:1615–1620.
5. Davies SW, et al. (1997) Formation of neuronal intranuclear inclusions underlies the neurological dysfunction in mice transgenic for the HD mutation. Cell 90: 537–548.
6. DiFiliga M, et al. (1997) Aggregation of huntingtin in neuronal intranuclear inclusions and dystrophic neurites in brain. Science 277:1990–1993.
7. Marderdale D, et al. (1998) Length of huntingtin and its polyglutamine tract in- fluences localization and frequency of intracellular aggregates. Nat Genet 18: 150–154.
8. Andrew SE, et al. (1993) The relationship between trinucleotide (CAG) repeat length and clinical features of Huntington’s disease. Nat Genet 4:398–400.
9. Rubinstein 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 CGG-rich stretches and a correlation between decreased age of onset of Huntington’s disease and CAG repeat number. Hum Mol Genet 2: 1713–1715.
10. Humbert S (2010) Huntington disease: a neurodegenerative disease? Mol Neurobiol 45: 193–204.
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. J Neurosci Transl Med 11:899.
12. Bhide PG, et al. (1996) Expression of normal and mutant huntingtin in the developing brain. J Neurosci Res 43:490–500.
13. Conforti P, et al. (2018) Faulty neuronal determination and cell polarization are re- verted by modulating HD early phenotypes. Proc Natl Acad Sci USA 115:E762–E771.

Data Analysis. All data were analyzed with GraphPad Prism 7.0 (GraphPad Software), and statistical significance was 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 SI Appendix.

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45. Brown JP, et al. (2003) Transient expression of doublecortin during adult neurogenesis. J Comp Neurol 467:1-10.

46. Vogt I, et al. (2012) Microstructural changes observed with DKO in a transgenic Huntington rat model: Evidence for abnormal neurodevelopment. NeuroImage 95: 957-967.

47. Pipalia NH, et al. (2011) Histone deacetylase inhibitor treatment dramatically reduces cell death in Drosophila. J Neurosci 31:11339-1151.

48. Young Z, Kohl Z, Gage FH (2011) Neurodegenerative disease and adult neurogenesis. Eur J Neurosci 33:1139-1151.

49. Schrodi F, et al. (2014) Rat choroidal pericytes as a target of the autonomic nervous system. Cell Death Dis 5:e3002.

50. Semple BD, Blömgen K, Gimlin K, Ferreiro DM, Noble-Haeusslein LJ (2013) Brain development in rodents and humans: Identifying markers of maturation and vulnerability to injury across species. Prog Neurobiol 106:107-116.

51. Hockly E, et al. (2003) Suberoylanilide hydroxamic acid, a histone deacetylase inhibitor, ameliorates motor deficits in a mouse model of Huntington's disease. Proc Natl Acad Sci USA 100:2041-2046.

52. Hickey MA, Gallant K, Gross GG, Levine MS, Chesselet MF (2005) Early behavioral alterations in synaptic and receptor function. Proc Natl Acad Sci USA 102:19050-19055.

53. Mielcarek M, et al. (2011) SAHA decreases HDAC 2 and 4 levels in vivo and improves alterations in synaptic and receptor function. Proc Natl Acad Sci USA 108:1317-1322.

54. Marques JM, Olsson IA, Ogren SO, Dahlborn K (2008) Evaluation of exploration and social behavior. J Neurosci 28:1496-1499.

55. Young AB, et al. (1988) NMDA receptor losses in putamen from patients with Huntington's disease. Science 241:981-983.

56. Albin RL, et al. (1990) Abnormalities of striatal projection neurons and N-methyl-D-aspartate receptors in pre symptomatic Huntington's disease. N Engl J Med 322:1293-1298.

57. Mochel F, et al. (2012) Early alterations of brain cellular energy homeostasis in Huntington disease models. J Biol Chem 287:1361-1370.

58. Schrödl F, et al. (2014) Rat choroidal pericytes as a target of the autonomic nervous system. Brain 134:137-142.

59. Hult Lundh S, Nilsson N, Soylu R, Kirik D, Petersén Å (2013) Hypothalamic expression of mutant huntingtin in neurodegeneration models. BMC Neurosci 14:114.

60. Sedvall G, et al. (1994) Dopamine D1 receptor number – A sensitive PET marker for early neurodegeneration in Huntington's disease. Eur Arch Psychiatry Clin Neurosci 243:249-255.

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 chorea. A PET study. J Neurosci Res 41:880-968.

62. Crittenden JR, et al. (2010) CalDAG-GEFI down-regulation in the striatum as a neuroprotective marker for the neurodegenerative process in Huntington's disease. Brain 133:503-514.

63. Fillox 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 110:219-227.

64. Richfield EK, O’Brien CF, Eskin T, Shoulson I (1991) Heterogeneous dopamine receptor changes in early and late Huntington disease. Neurosci Lett 132:121-126.

65. Glass M, Dragunow M, Faull RL (2000) The pattern of neurodegeneration in Huntington's disease: A comparative study of cannabinoid, dopamine, adenosine, and GABA(3A) receptor alterations in the human basal ganglia in Huntington's disease. Neurosci 97:505-519.

66. Daval G, et al. (1994) Dopamine D1 receptor number – A sensitive PET marker for early neurodegeneration in Huntington's disease. Eur Arch Psychiatry Clin Neurosci 243:249-255.

67. 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 chorea. A PET study. J Neurosci Res 41:880-968.

68. Dunah AW, Standaert DG (2001) Dopamine D1 receptor-dependent trafficking of striatal D1 receptors. J Neurosci 21:5546-5558.

69. Marnell D, et al. (2010) Early increase in extrasynaptic NMDA receptor signaling and expression contributes to phenotype onset in Huntington's disease mouse. Neurobiol Dis 37:347-355.

70. Noguera GD, Golshan S, Molea AO, Mielcarek M (2013) Selective roles of normal and mutant huntingtin in neural induction and early neurogenesis. PLoS One 8: e63468.

71. Winston M, et al. (2006) Microglia instruct subventricular zone neurogenesis. Nat Neurosci 9:842-852.

72. 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 Neurosci 5:17.

73. Kim JH, et al. (2011) Impaired adult olfactory bulb neurogenesis in the R6/2 mouse model of Huntington's disease: A balancing act. Neurobiol Dis 42:1429-1460.

74. Holm Z, et al. (2010) Impaired adult olfactory bulb neurogenesis in the R6/2 mouse model of Huntington's disease. BMC Neurosci 11:114.

75. Fedele V, Roybon L, Nordström U, Li YJ, Brundin P (2011) Neurogenesis in the R6/2 mouse model of Huntington's disease is impaired at the level of NeurD1. Neuroscience 173:76-81.

76. 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 inactivation of neuroblasts. PLoS One 10:e0136069.

77. Curtis MA, et al. (2003) Increased cell proliferation and neurogenesis in the adult human Huntington's disease brain. Proc Natl Acad Sci USA 100:9023-9027.

78. Kantor O, et al. (2006) Selective striatal neuron loss and alterations in behavior correlate with impaired striatal function in Huntington's disease transgenic rats. Neurobiol Dis 22:538-547.

79. Bird FA, et al. (2008) Sex differences in a transgenic rat model of Huntington's disease: Decreased 17β-estradiol levels correlate with reduced numbers of DARPP32+ neurons in males. Proc Natl Acad Sci USA 105:2295-2296.

80. Cha JH (2007) Transcriptional signatures in Huntington's disease. Proc Neurobiol Dis 20:e0116069.

81. Nguyen GP, et al. (2010) Age-dependent gene expression profile and protein expression changes associated with protection of YAC128 neurons from NMDA-mediated excitotoxicity in a transgenic mouse model of Huntington's disease. J Neurosci 30:1756-1765.

82. Kántor O, et al. (2006) Selective striatal neuron loss and alterations in behavior correlated with NMDA receptor loss in the R6/2 mouse model of Huntington's disease. Neuroscience 133:1756-1765.

83. Nguyen GP, et al. (2010) Age-dependent gene expression profile and protein expression changes associated with protection of YAC128 neurons from NMDA-mediated excitotoxicity in a transgenic mouse model of Huntington's disease. J Neurosci 30:1756-1765.
86. Beckers T, et al. (2007) Distinct pharmacological properties of second generation HDAC inhibitors with the benzamide or hydroxamate head group. *Int J Cancer* 121: 1138–1148.
87. von Hörsten S, et al. (2003) Transgenic rat model of Huntington’s disease. *Hum Mol Genet* 12:617–624.
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. *J Neurosci* 28:6182–6195.
89. Kilkenny C, Browne W, Cuthill IC, Emerson M, Altman DG (2010) Animal research: Reporting in vivo experiments: The ARRIVE guidelines. *Br J Pharmacol* 160: 1577–1579.
90. Urbach YK, Bode FJ, Nguyen HP, Riess O, von Hörsten S (2010) Neurobehavioral tests in rat models of degenerative brain diseases. *Methods Mol Biol* 597: 333–356.
91. Couillard-Després S, et al. (2006) Targeted transgene expression in neuronal precursors: Watching young neurons in the old brain. *Eur J Neurosci* 24:1535–1545.