Metformin reverses early cortical network dysfunction and behavior changes in Huntington’s disease


1 Institute for Pathophysiology, FTN, Mainz
2 Institute for Human Genetics, University Medical Center, FTN, Mainz
3 German Center for Neurodegenerative Diseases (DZNE), Bonn
4 Department for Neurology, University Medical Center, FTN, Mainz
5 Division of Neurosciences, Ninewells Hospital and Medical School, Dundee
6 Mouse Behavior Unit, University Medical Center, FTN, Mainz
7 Department of Neuroproteomics, Max-Delbrück-Center, Berlin

*,** equal contribution

Corresponding authors: Albrecht Stroh and Susann Schweiger

To whom correspondence should be addressed:

Susann Schweiger (MD) schweigs@uni-mainz.de; Langenbeckstr. 1
55131 Mainz, Germany; tel: +49 6131 17-5788

Albrecht Stroh (PhD); albrecht.stroh@unimedizin-mainz.de; Hanns-Dieter-Hüsch-Weg 19, 55128 Mainz, Germany; tel: +49 6131 39-21347

Short title: Rebalancing early network alterations in Huntington’s disease
Keywords: Huntington disease, *in vivo* calcium imaging, cortical microcircuits, neuronal hyperactivity, metformin
Abstract

Catching primal functional changes in early, “very far from disease onset” (VFDO) stages of Huntington’s disease is likely to be the key to a successful therapy. Focusing on VFDO stages, we assessed neuronal microcircuits in premanifest Hdh150 knock-in mice. Employing in vivo two-photon Ca\(^{2+}\) imaging, we revealed an early pattern of circuit dysregulation in the visual cortex- one of the first regions affected in premanifest Huntington’s disease - characterized by an increase in activity, an enhanced synchronicity and hyperactive neurons. These findings are accompanied by aberrations in animal behavior. We furthermore show that the anti-diabetic drug metformin diminishes aberrant Huntingtin protein load and fully restores both, early network activity patterns and behavioral aberrations. This network-centered approach reveals a critical window of vulnerability far before clinical manifestation and establishes metformin as a promising candidate for a chronic therapy starting early in premanifest Huntington’s disease pathogenesis long before the onset of clinical symptoms.
Introduction

Huntington’s disease is caused by the expansion of a CAG repeat in the open reading frame of the huntingtin gene (HTT), which translates into an expanded glutamine stretch in the aberrant, mutant protein (mHTT). Huntington’s disease has primarily been described as a late-onset neurodegenerative disease. However, it is preceded in its premanifest period by a prolonged presymptomatic followed by a prodromal phase with hardly detectable and unspecific symptoms occurring far before classical Huntington’s disease becomes apparent (Ross et al. 2014). These symptoms include reduced impulse control, social disengagement, low conversational participation, reduction of the concentration span and decline of clearly defined cognitive domains (Stout et al. 2011, Duff et al. 2007, Ross et al. 2014, Labuschagne et al. 2016) and are accompanied by slight changes of cortical network topology and functional connectivity in resting state fMRI measures (Harrington et al. 2015, Wolf et al. 2012). Importantly, such subtle network dysregulations may occur even earlier than the described prodromal symptoms in a very far from disease onset (VFDO) premanifest stage (Fig. 1a). This stage reaches back more than 15-20 years before motor symptoms become visible in patients’ lives and far before protein aggregates and neurodegeneration are observed.

In Huntington’s disease occurrence of such very early changes is supported by the observation of early deficits in premanifest Huntington’s disease mutation carriers, such as loss of phosphodiesterase 10A in the occipital lobe up to 47 years prior to disease onset (summarized in (Wilson et al. 2017)). Also, the ability to perform complex visuospatial orientation, such as visual search, seems to be altered even in pre-manifest stages far from clinical diagnosis (Labuschagne et al. 2016). We hypothesize that primal functional changes in the VFDO stage of premanifest Huntington’s disease open up very early vulnerable windows for disease development and preventive therapy prior to neuronal loss and may also provide

It seems that the visual cortex is one of the first regions that are functionally affected during disease development in Huntington’s disease (Labuschagne et al. 2016, Dogan et al. 2013). In order to identify primal network changes we have here established a network-centered approach and focused on microcircuit function in layer II/III of the visual cortex at an early premanifest stage in a mouse model of Huntington’s disease corresponding to the VFDO stage in premanifest Huntington’s disease. We have used two-photon imaging using fluorescent indicators of intracellular Ca\(^{2+}\) in order to resolve the functional architecture of intact cortical microcircuits \textit{in vivo} with single neuron resolution (Grienberger and Konnerth 2012). We show that already at the early age of 10-15 weeks (young adults compared to humans), the entire cortical microcircuit shifts towards a more excitable state characterized by a complex change in neuronal activity pattern and hyperactive neurons. These findings are accompanied by changes in animal behavior including a decrease in anxiety.

No effective and curative treatment has been developed for Huntington’s disease so far (Frank 2014). A chronic drug-therapy that commences early on in VFDO stages in premanifest Huntington’s disease and already ameliorates early dysregulations as the potential origin of pathogenic processes and disease spreading is therefore a promising and necessary strategy. In Huntington’s disease animal models, even short term reduction of protein load through RNA interference and antisense strategies has beneficial effects on disease phenotypes and progression lasting several months after intervention (Stanek et al. 2014, DiFiglia et al. 2007, Keiser, Kordasiewicz, and McBride 2016). We have recently shown that mRNAs carrying CAG repeats bind to a protein complex containing the ubiquitin ligase midline 1 (MID1) in a repeat size dependent manner. Through ubiquitination MID1 regulates PP2A (protein phosphatase 2A) and mTOR (mechanistic target of rapamycin) activities and the translation of
associated mRNAs (Krauss et al. 2013, Griesche et al. 2016). Disruption of the
MID1/PP2A/mTOR protein complex leads to an increase of PP2A activity, a decrease of
mTOR activity and a reduction of translation rates of mRNAs with expanded CAG repeats
(Krauss et al. 2013).

We show here that the type II diabetes drug metformin interferes with the
MID1/PP2A/mTOR protein complex and significantly reduces the translation rate of Htt
mRNA, resulting in a reduction of aberrant Htt protein production in vitro and in-vivo in the
Hdh150 mouse model. Notably, in Hdh150 mice in vivo metformin, when given early in the
VFDO stage, and chronically in the drinking water, fully reverses both, early neuronal
network dysregulations and behavioral aberrations.
Results

Increase in cortical neuronal network activity in presymptomatic Huntington’s disease mice.

Since the visual cortex is one of the first regions affected by the disease (Dogan et al. 2013, Labuschagne et al. 2016), we focused on layer 2/3 of the visual cortex of 12 weeks old, heterozygous knock-in mice expressing expanded Htt with 150 glutamine repeats (Hdh150), in the lightly anesthetized mouse. This time corresponds to the VFDO in presymptomatic Huntington’s disease (Fig. 1a, b). We focused our analysis on male mice, thereby minimizing the influence of hormonal fluctuations on network activity. This approach is in line with a recent study in the field of Alzheimer’s disease (Iaccarino et al. 2016), using males only. We employed two-photon Ca2+ imaging in vivo using the synthetic Ca2+ indicator Oregon-Green BAPTA1 (OGB-1) AM to monitor the suprathreshold activity of a neuronal microcircuit with cellular resolution, typically comprising around 200 neurons (Kerr, Greenberg, and Helmchen 2005) (Fig. 1c, Fig. 1- figure supplement 1a, b). Events from astrocytes, which are also stained by OGB-1, were excluded from this neuronal network based on their temporal dynamics (Fig. 1- figure supplement 1c-f, Fig. 1 – source data 1).

By imaging at different depths, we found a similar spatial extent of OGB-1 staining in the visual cortex of Hdh150 and control wild-type (WT) mice (Fig. 1- figure supplement 1b). Figure 1- Movie supplement 1 shows two-photon images acquired from the pial surface illustrating a homogenous OGB-1 staining in the layers 1, 2 and 3 of the mouse visual cortex.

We observed no difference in the density of stained cells (Fig. 1c and 1e, Fig. 1 – source data 1, WT: 1413 ± 74 cell/mm² (n= 11 mice), Hdh150 1456 ± 90 cell/mm² (n= 10 mice), Mann-Whitney test p = 0.8).
To identify changes in microcircuit activity, we assessed spontaneous activity in vivo in the cortical microcircuit of WT and Hdh150 mice, which reliably reflects the functional microarchitecture of sensory cortical areas (Miller et al. 2014). Fig. 1 – Movie supplement 2 shows an example of in vivo two-photon Ca\(^{2+}\) imaging exhibiting ongoing spontaneous activity in layer 2/3 of mouse visual cortex with single-cell resolution. Both WT and Hdh150 mice exhibited silent and active cells (Fig. 1d), but a significantly higher proportion of active neurons was detected in Hdh150 mice, indicating a more active network (Fig. 1f, Fig. 1 – source data 1, WT: 65.1 ± 3.5 % (n=8 mice), Hdh150: 77.1 ± 3.5 % (n=6 mice), unpaired t-test p < 0.05). No spatial clustering of silent or active cells could be observed in either group (Fig. 1d).

Next, we analyzed the frequency of Ca\(^{2+}\) transients and activity patterns in the population of active neurons (Figure 1-source data 2). Notably, the frequency of Ca\(^{2+}\) transients was significantly higher in Hdh150 compared to WT mice (Fig. 1g-h, Fig. 1 – source data 1, WT: 0.74 ± 0.06 trans/min (n=8 mice), Hdh150: 1.2 ± 0.14 trans/min (n=6 mice), Mann-Whitney test, p < 0.01). No difference was found in the mean area under the curve (AUC) of Ca\(^{2+}\) transients between WT and Hdh150 mice (Fig. 1i, Fig. 1 – source data 1, WT: 39.8 ± 3.9 (dF/f)*s, Hdh150: 39.9 ± 3.6 (dF/f)*s, unpaired t-test, p = 0.98) indicating that on average, the mean number of underlying action potentials for each individual calcium transient was not different in Hdh150 mice.

We furthermore analyzed the distribution of neurons according to their Ca\(^{2+}\) transient frequency. The cumulative probability distribution of the activity of individual neurons in Huntington’s disease mice was shifted towards higher frequencies indicating that the overall neuronal activity transitioned towards a more excitable network (Fig. 1j, Fig. 1 – source data 1, two-way ANOVA p < 0.0001). We sub-classified the active neurons into three functional...
subgroups according to their transient frequency: low, medium and hyperactive (Fig. 1k, Fig. 1 – source data 1). Notably, we identified a unique subgroup in VFDO Hdh150 mice: hyperactive neurons (Fig. 1l, Fig. 1 – source data 1, Chi-square test p < 0.01). This subgroup was accompanied by a reduction in the number of neurons with low activity, which corroborates the shift of the microcircuit activity.

In later stages of Alzheimer’s disease, hyperactive cells were shown to cluster near amyloid plaques (Busche et al. 2008). A similar scenario might occur in Huntington’s disease. To clarify whether the cortex of VFDO Hdh150 mice is affected by mHtt aggregates - a later stage Huntington’s disease hallmark - we performed immunohistochemistry in Hdh150 and WT animals. We observed only diffuse and non-aggregated Htt immunoreactivity in the cytoplasm of neurons in cortical areas of both WT and Hdh150 mice (Fig. 1 – figure supplement 2a) in accordance with previous studies (Lin et al. 2001). In addition, no reactive astrocytes were observed (Fig. 1 – figure supplement 2b) and only few cells that were stained for activated caspase-3, an apoptotic marker, in cortical areas of WT and Hdh150 mice (Figure 1 – figure supplement 2c) as previously observed (Yu et al. 2003). In order to test whether hyperactive cells cluster in the Hdh150 mouse model, spatial distance between every pair of neurons was quantified (Fig. 1 – figure supplement 3c, d, Fig. 1 – source data 1). We observed no significant differences between the permutations of functional subgroups in premanifest Hdh150 and control mice, reflecting a rather homogenous distribution of all functional subgroups (Fig. 1 – figure supplement 3c, d, Fig. 1 – source data 1, Mann-Whitney, not significant, see table 1 for p-values). This finding was confirmed in randomized data in which cell location was kept but functional identity was randomly permutated (Figure 1 – figure supplement 3a, b and d, Fig. 1 – source data 1). Taken together, clustering of hyperactive cells could not be observed in VFDO Hdh150 animals and cortical hyperactivity is independent of mHtt aggregation, apoptotic cell death or astrogliosis at this presymptomatic VFDO stage.
Increased synchronicity and cortical dysfunction in premanifest VFDO Huntington’s disease mice

An important aspect of neuronal information processing is the optimization of encoding strategies. In the visual cortex, encoding of information is characterized by sparse and precisely timed neuronal activity. Cortical activity is defined by transiently co-active ensembles of neurons acting as a functional unit (Miller et al. 2014). To capture these spatiotemporal dynamics in the microcircuit, we analyzed synchronicity of the transients between all pairs of neurons (Fig. 2a) by calculating Pearson’s correlation coefficient (Pearson’s r) for every pair. First, we confirmed that the level of synchronicity within a healthy cortical microcircuit is drastically higher than the random synchronicity in shuffled data (Fig. 2b, Fig. 2 – source data 1, WT: 0.024 ± 0.005, WT rand: -0.0003 ± 0.0003, Mann-Whitney test p < 0.0001). Notably, the synchronicity was even higher in Hdh150 mice compared to healthy controls (Fig. 2b, Fig. 2 – source data 1, WT: 0.02 ± 0.005, Hdh150: 0.04 ± 0.006, Mann-Whitney test p < 0.05).

Next, we compared the synchronicity level in all functional subgroups (Fig. 2c, Fig. 2 – source data 1). The pairs involving the low activity subgroups (LL and LM) showed no differences in WT and Hdh150 mice (Mann-Whitney test, in LL p = 0.5 and in LM p = 0.7). However, Pearson’s r increased significantly for the medium-to-medium (MM) pairs in Hdh150. Pearson’s r was even higher for medium-hyperactive (MH) and hyperactive-hyperactive (HH) compared to low-low (LL) pairs (Mann-Whitney test compared to LL in Hdh150 mice : in MM p < 0.05, in MH and HH p < 0.01).

To assess whether the increased synchronicity in the VFDO Hdh150 mice occurred merely due to the higher number of transients, we used randomized data with unchanged frequency but temporally shuffled transients (Fig. 1 – figure supplement 3a and b). Pearson’s r in the randomized data was nearly zero for all, including the high frequency subgroups in WT and
Hdh150 mice (Fig. 2 – figure supplement 1a and b, Fig. 2 – source data 1). This finding argued against the possibility that the increased Pearson's r in the experimental data occurred by chance.

We next asked whether functional ensembles with a high level of synchronicity are located in spatial vicinity to each other, by testing whether Pearson's r changed with physical distance between the pairs of neurons (Fig. 2d). An inverse linear relationship was observed between Pearson’s r and the pairwise distance in both the WT and Hdh150 mice (Fig. 2d, Fig. 2 – source data 1, two-way ANOVA p < 0.0001, WT vs Hdh150) suggesting that two closely located neurons have a higher probability to fire together. This is consistent with similar findings in the forelimb motor cortex of head-restrained mice (Dombeck, Graziano, and Tank 2009). Randomization of the data abolished the inverse relationship between Pearson's r and distance (Fig. 2e, Fig. 2 – source data 1, two-way ANOVA p = 0.3, WT rand vs Hdh150 rand).

Since many studies, especially in premanifest mutation carriers, have linked Huntington’s disease pathology to changes in metabolism (Damiano et al. 2010, Duan, Jiang, and Jin 2014, Jin and Johnson 2010, Labbadia and Morimoto 2013, Mochel and Haller 2011), hyperactivity might mirror metabolic dysregulation in subgroups of cortical cells. We used mitochondrial respiration as a marker of metabolic functionality and quantified mitochondrial respiration using high-resolution respirometry of cortical tissues. Mitochondrial respiration was unchanged in cortical tissue of Hdh150 mice suggesting that the observed neuronal hyperactivity occurs prior to metabolic changes (Fig. 2 – figure supplement 2a-c, Fig. 2 – source data 1, Mann-Whitney test, not significant, see table 1 for p-values).

Behavior changes in premanifest VFDO Huntington’s disease mice
Next, we asked if hyperactivity and increased synchronicity of cortical networks are associated with behavioral changes in the VFDO animals. A visual discrimination task did not show any aberrations in the Hdh150 animals (Fig. 3 – figure supplement 1a-d; Fig. 3 – movie supplement 1, Fig. 3 – source data 1, two-way ANOVA, not significant, see table 1 for p-values). In contrast, in an open field test premanifest VDFO Hdh150 animals moved significantly more to the center than WT littermates suggesting anxiolytic effects of the VFDO changes (Fig. 3a, b, Fig. 3 – source data 1, Mann-Whitney test, p = 0.03). Distance travelled (as a measure of motility) did not differ between groups (Fig. 3 – figure supplement 1, Fig. 3 – source data 1, Mann-Whitney test, not significant, see table 1 for p-values).

Taken together, we have found network hyperactivity in the cortex of VFDO Hdh150 mice together with anxiolytic behavior.

**Metformin reduces mutant Htt protein load**

Based on our previous observations that the MID1/PP2A/mTOR protein complex regulates the translation of mHtt protein (Krauss et al. 2013) and that treatment with metformin interferes with the MID1 complex (Kickstein et al. 2010), we hypothesized that metformin inhibits the MID1/PP2A/mTOR-mediated protein synthesis of mutant mHtt and is therefore a promising candidate molecule to reduce mHtt load and reverse Huntington’s disease associated symptoms.

In order to test for an effect of metformin on mHtt protein load and aggregation, HEKT cells stably expressing FLAG-tagged exon 1 of human mHTT carrying 83 CAG repeats were treated with 1 mM or 2.5 mM metformin, or with vehicle for 48 hours. Aggregation was quantified in a filter retardation assay. Metformin reduced the amount of aggregated FLAG-HTT in a concentration dependent manner (Fig. 4a, Fig. 4 – source data 1, Mann-Whitney test, control vs 2.5 mM metformin p = 0.02).
To test whether the metformin effect on human exon 1 mHTT aggregates is mediated by a blockade of the MID1 protein complex, we depleted MID1 by siRNA mediated knockdown in the cell line expressing FLAG-HTT exon 1 with 83 CAG repeats, in presence or absence of metformin. While depletion of MID1 reduced mHTT aggregation, no additive effect of metformin treatment on mHTT aggregates was observed, suggesting that MID1 and metformin indeed act through the same pathway (Fig. 4b, Fig. 4 – source data 1, Mann-Whitney test, control siRNA vs MID1 siRNA p= 0.009; control siRNA vs MID1 siRNA + metformin p = 0.02).

We had shown previously that the MID1/PP2A/mTOR protein complex regulates the translation efficiency of the human 

HTT mRNA in a repeat dependent manner (Krauss et al. 2013). We therefore looked at a possible influence of metformin on the protein synthesis rate of mHTT exon 1 protein using a previously described FRAP (Fluorescence recovery after photo bleaching) - based assay that allows monitoring of protein translation rates in living cells (Krauss et al. 2013). We detected a clear reduction in the protein synthesis rate of a GFP-Htt fusion protein carrying 49 repeats in the metformin treated samples in a concentration dependent manner in primary neurons (Fig. 4c, Fig. 4 – source data 1, RM two-way ANOVA p = 0.008). This effect was confirmed in N2A cells (Fig. 4 – figure supplement 1a, Fig. 4 – source data 1, RM two-way ANOVA p = 0.03). To further support the contribution of the MID1/PP2A/mTOR protein complex and PP2A activity to this effect, the GFP-Htt transfected cells were subsequently either (i) mock treated, (ii) treated with only metformin, (iii) treated with ocdadaic acid (OA), or (iv) co-treated with metformin and OA. OA is an inhibitor of PP2A activity. As expected, OA significantly increased translation rates of the 

GFP-Htt reporter mRNA and metformin did not have a reducing effect on the translation rates in cells co-treated with OA suggesting that indeed the metformin effect is mediated by PP2A activity (Fig. 4d, Fig. 4 – source data 1, RM two-way ANOVA p = 0.002).
To analyze metformin effects on early signs of pathology \textit{in vivo}, VFDO Hdh150 mice were fed with, or without 5 mg/ml metformin in the drinking water. Metformin did not significantly reduce drinking volume (Fig. 4 – figure supplement 1b, Fig. 4 – source data 1, RM two-way ANOVA not significant, see table 1 for p-values). After three weeks of treatment we looked at phosphorylation pattern of the PP2A / mTOR target S6 and the amount of mHtt protein relative to wildtype Htt in whole brain tissue. The metformin treated group showed a significant reduction of S6 phosphorylation suggesting an increase in PP2A activity (Fig. 4e and f, Fig. 4 – source data 1, unpaired t-test p = 0.05). At the same time a slight tendency (not significant) of reduced mHtt was detected suggesting that metformin has an influence on mHtt expression (Fig. 4 – figure supplement 1c and d, Fig. 4 – source data 1, unpaired t-test not significant, see table 1 for p-values). A significant reduction of mHtt expression however became clearly visible after 11 weeks of treatment in cortical tissue (Fig. 4g-j, Fig. 4 – source data 1, unpaired t-test, mHtt/wtHtt p = 0.05, mHtt/Gapdh p = 0.002, wtHtt/Gapdh p = 0.9).

\textbf{Metformin reverses signs of Huntington’s disease pathology}

Our data suggest metformin as a promising molecule to interfere with VFDO Huntington’s disease biochemical and cellular pathological changes. We initially used a \textit{C. elegans} model of polyQ-mediated diseases to test whether metformin effectively ameliorates disease symptoms in an easily controllable model with short lifespan. The \textit{C. elegans} worms carry a transgene encoding YFP-tagged Q40 polypeptide in body wall muscle cells (Morley et al. 2002). Adult Q40::YFP nematodes exhibit intracellular aggregates of polyglutamine-containing protein and develop progressive paralysis over time, which is reflected in significantly reduced motility. We counted aggregates in metformin treated and untreated nematodes. Moreover, we assessed their motility in a liquid thrashing experiment, in which worms are placed in liquid and the frequency of lateral swimming (thrashing) movement is analyzed as a measure of motility. We found that 5 days metformin treatment reduced the
number of intracellular inclusion bodies significantly and rescued motility impairment (Fig. 4 – figure supplement 2a and b, Fig. 4 – source data 1, Mann-Whitney, p < 0.0001 ). Since bacteria can metabolize metformin (Cabreiro et al. 2013), we confirmed the results on heat-inactivated OP50 bacteria (Fig. 4 – figure supplement 2c, Fig. 4 – source data 1, Mann-Whitney test, control vs 5 mM metformin p = 0.008, control vs 10 mM metformin p < 0.0001). siRNA mediated knock-down of arc-1, the C.elegans MID1 homolog, leads to a reduction of inclusion bodies and improved motility similar to the metformin effects (Fig. 4 – figure supplement 2d and e, Fig. 4 – source data 1, Mann-Whitney test, p < 0.0001 ) and confirms that the MID1/PP2A/mTOR protein complex underlies metformin effects.

We then asked whether metformin could rescue the altered cortical activity in vivo in Hdh150 mice. Metformin treatment did not affect the density of OGB-1 stained cells (Fig. 5 – figure supplement 1a, b, Fig. 5 – source data 1, WT: 1413 ± 74 cells/mm² (n = 11 mice), Hdh150: 1456 ± 90 cells/mm² (n = 10 mice), WT met: 1351 ± 65 cells/mm² (n = 9 mice) and Hdh150 met: 1448 ± 60 cells/mm² (n = 6 mice), unpaired t-test, not significant, see table 1 for p-values). Notably, 3 weeks metformin treatment in the drinking water completely restored the proportion of active cells (Fig. 5b, Fig. 5 – source data 1, Hdh150: 77.2 ± 3.5% (n = 6 mice), Hdh150 met: 64.4 ± 4.1 % (n = 6 mice), Mann-Whitney test p < 0.05) as well as the average frequency of Ca²⁺ transients (Fig. 5a, c, Fig. 5 – source data 1, Hdh150: 1.2 ± 0.1 trans/min (n = 6 mice), Hdh150 met: 0.7 ± 0.06% (n = 6 mice), Mann-Whitney test p < 0.05). The individual traces of treated Hdh150 animals were indistinguishable from untreated WT animals (Fig. 5a, Fig. 5 – figure supplement 1c). Importantly in these experiments metformin is specific to dysregulated network components. Only the AUC of calcium transients was slightly yet significant affected in WT mice which might be due to an increase of baseline calcium concentration induced by the activation of MID1/PP2A/mTOR signaling pathway (Fig. 5 – figure supplement 1c, Fig. 5 – source data 1). One of the hallmarks of the VFDO Hdh150 mice was the distinct functional subgroup of hyperactive neurons (Fig. 1). We thus
assessed the effect of metformin treatment on the relative proportions of low, normal and hyperactive subgroups. Treatment with metformin in Hdh150 mice led to the complete abolishment of the hyperactive subgroup (Fig. 5d, Fig. 5 – source data 1, two-way ANOVA p < 0.0001 and Fig. 5e Fig. 5 – source data 1, Chi-square test not significant, see table 1 for p-values) and restored the relative proportion of functional subgroups. The spatial distribution of functional subgroups remained unchanged by metformin treatment (Fig. 5 – figure supplement 1 f, Fig. 5 – source data 1, Mann-Whitney test, not significant, see table 1 for p-values). This complete restoration of dysregulated microcircuit activity was also evident in the cumulative frequency distribution (Fig. 5d, Fig. 5 – source data 1) and all other network measures found to be aberrant in young Hdh150 animals such as synchronicity (Fig. 5f and g, Fig. 5 – figure supplement 1d-e, Fig. 5 – source data 1).

Accompanying the network dysfunction, we had observed anxiolytic behavior in the VFDO Hdh150 mice (see Fig. 3a-b). Treatment with metformin fully reversed the anxiolytic behavior(Fig. 5h and i, Fig. 5 – source data 1, Mann-Whitney test, WT vs Hdh150 p = 0.002, Hdh150 vs Hdh150 met p = 0.002, Hdh150 vs WT met p = 0.03, WT vs Hdh150 met p = 0.8).

In conclusion, our data suggest that metformin, by interfering with the translation rate of mHtt protein, reduces protein load in cell culture and in vivo and reverses early Huntington’s disease-related network dysregulations as well as cognitive and anxiety-related behavioral aberrations in mice. Furthermore, usage of metformin in adult C.elegans, a model of polyQ disease also significantly influences inclusion bodies formation and motility.
Discussion

Here, we have identified a dysregulation of spontaneous neuronal activity in the visual cortex in a very early stage of a mouse model of Huntington’s disease that corresponds to a very early stage in the premanifest stage in Huntington’s disease mutation carriers (Fig. 1a, b) which might indeed be a distinct pathological disease stage which is very far from disease onset (VFDO). Correspondingly, the visual cortex is one of the first structures affected in patients (Labuschagne et al. 2016, Dogan et al. 2013). This dysregulation is characterized by an increase in cortical network activity patterns, the emergence of a functional subgroup of hyperactive neurons, and enhanced synchronicity. Overall visual cortex functioning seems to be preserved at this early time point of Huntington’s disease course. Network changes are accompanied by subtle behavior alterations including an anxiolytic phenotype, suggesting that at least part of the brain-wide circuitry exhausted its compensational reserve. Anxiety-related abnormalities including anxiolytic behavior have previously been described in the preclinical phase in several other rodent Huntington’s disease models ((Nguyen et al. 2006), reviewed in (Pouladi, Morton, and Hayden 2013)). So far, changes described here represent the earliest identified abnormalities in cortical pathophysiology and behavior in heterozygous Hdh150 animals, which closely resemble the human disease (Lin et al. 2001, Heng et al. 2007, Brooks et al. 2012, Tallaksen-Greene et al. 2005). Furthermore we show that the type II diabetes drug metformin inhibits the translation of mHtt protein and thereby decreases mHtt protein load in vitro and in vivo. Promisingly, this leads to a complete restoration of VFDO network activity patterns, as well as behavior abnormalities under chronic metformin therapy.

Our data report primal changes in cortical network function in the VFDO stage of Huntington’s disease. A recognition of the network as a pathophysiological entity has recently been suggested in the context of Alzheimer’s disease (Busche et al. 2008, Iaccarino et al.
Moreover, focus has shifted away from the mechanisms accompanying neuronal and network degeneration and instead moved towards small and subtle functional changes at very early stages of the disease when irrecoverable damage to the network has not yet occurred (Busche and Konnerth 2016). Indeed, hyperactive neurons are associated with both advanced and early stages of Alzheimer’s disease, independent of plaque formation (Busche et al. 2012, Busche et al. 2008). In addition, evidence points towards hyperactive neurons preventing the cortex-wide propagation of slow oscillations in early Alzheimer’s disease (Busche et al. 2015).

We here describe a similarly distinct hyperactive phenotype in very early stages of Huntington’s disease. We conclude that neuronal hyperactivity may be a principle mechanism that develops early not only in Alzheimer’s disease but also in other neurodegenerative diseases. Thus, the notion of early network dysregulation as a therapeutic target may have broad implications.

Similar to early stages of Alzheimer’s disease hyperactive neurons in the Hdh150 animals emerge in the absence of aggregate formation. Also hyperactive cells do not cluster, and reactive astrocytes or cells with activated caspase 3 as a marker of early apoptosis are not found in these early stages of Huntington’s disease. Furthermore, cortical neurons did not exhibit metabolic dysregulation as measured in a mitochondrial respiration assay. Therefore, we may postulate, that the cortex merely responds to early pathophysiological events already commencing in subcortical regions, e.g. the striatum. This is well in line to the current emerging hypotheses of disease progression in Alzheimer’s disease. Young Alzheimer’s disease animals develop hyperactivity in a plaque-independent fashion first in the hippocampus (possibly due to higher vulnerability of the hippocampus in Alzheimer’s disease), followed by a similar hyperactivity pattern in the cortex later in the disease process (Busche et al. 2012, Busche et al. 2008). Furthermore, in Alzheimer’s disease patients,
degeneration of cortical projection targets of the hippocampus is associated with hippocampal
cyberactivity indicating a connectivity-based spread of network dysregulations eventually
leading to neurodegeneration (Putcha et al. 2011). Our data indeed suggest an altered activity
in subcortical drivers, since unspecific alteration of excitability in individual neurons is
unlikely to lead to an increase in synchronicity, but rather would result in a random increase
in firing.

Our data was collected at very early stages of the disease in the Hdh150 mouse model, which
corresponds to the VFDO stage in Huntington’s disease patients. The importance of
expression of mutant Htt protein during very early phases for disease development has been
demonstrated in the BACHD:CAG-Cre\textsuperscript{ERT2} mouse (Molero et al. 2016). With the help of
tamoxifen treatment expression of mutant Htt was turned off early postnatally. Still the typical
symptoms of Huntington’s disease at 3 and 9 months of age were observed in the animals. We
conclude that only at the VFDO stages, when cellular and network degeneration have not yet
been established, preventive strategies will be most effective; only then can we still rescue
small homeostatic shifts, prevent spreading and potentially stabilize network function.

Phenotype reversal could be demonstrated in a tetracyclin dependent conditional mouse
model for Huntington’s disease. Both, neuropathological findings and behavior aberrations
were found to disappear when mHtt protein production was stopped through a tet-off
regulation mechanism in the adult animal (Yamamoto, Lucas, and Hen 2000). Additional
support for the beneficial effect of suppression of aberrant protein production on the
Huntington’s disease phenotype stems from several studies with RNA interference (siRNA),
or antisense oligonucleotides showing that gene suppression reducing mHtt protein load by 40
% or more, are sufficient to significantly ameliorate the Huntington’s Disease phenotype
2014). These studies have demonstrated, that (i) the earlier suppression takes place the more
robust and beneficial effects on behavior phenotypes are [reviewed in (Keiser, Kordasiewicz, and McBride 2016)] and (ii) that even transient suppression of Htt protein during early disease stages was sufficient to obtain long-term effects on the disease phenotype lasting for months, far beyond the treatment period (Lu and Yang 2012, Kordasiewicz et al. 2012).

However, developing siRNA and antisense oligonucleotides technologies into therapeutics for clinical use is difficult and a long way to go. Difficulties here include toxicity and modes of delivery: so far oligonucleotides have to be regularly injected into the cerebrospinal fluid, which is a huge effort for patients and physicians. Furthermore, a short N-terminal fragment of the mHtt protein, mHttex1p, that is produced by incomplete exon 1 splicing and a short poly-adenylated mRNA in several animal models as well as in Huntington’s disease patients rather than full-length mHtt protein was found to be particularly pathogenic. Its occurrence correlates well with age of onset and severity of the disease. This short mRNA is difficult to target by oligonucleotide strategies (Neueder et al. 2017, Sathasivam et al. 2013). We show here that a well-known, widely used, orally delivered small compound, metformin, suppresses mHtt production by targeting both, full-length and mHttex1p, in vitro and in vivo and thereby significantly reduces mHtt protein load, which makes it a very promising candidate for chronic early onset Huntington’s disease therapy.

Metformin is an FDA-approved, inexpensive biguanide that has been used in patients with Type II diabetes for decades and is under discussion for cancer preventive therapy (Demir et al. 2014, Micic et al.). Very recently, metformin has been shown to rescue core phenotypic features in a mouse model for fragile X-syndrome, a neurodevelopmental disorder, by normalizing ERK signalling (Gantois et al. 2017).

Metformin had been brought in as a promising compound in Huntington’s disease previously. It has been found to protect cells from the toxicity of mutant Huntingtin protein in a cell culture model (Jin et al. 2016). In a study on R6/2 animals, a very aggressive model for...
Huntington’s disease, Ma and colleagues had found a significant effect on survival rates and hind clasping in male animals only when given metformin in the drinking water starting from week 5 (Ma et al. 2007). In relation to the phenotype in the R6/2 animals that develops severe aberrations from 4 weeks on this is a late time point and would be placed in the motor phase stage when projected to the phenotypic time line given in Fig 1a. We hypothesize here that preventive treatment at a very early stage is important to substantially and stably influence the disease. This is in agreement with observations in a mouse model for spinocerebellar ataxia I, another neurodegenerative disease based on CAG expansion and studies with antisense oligonucleotides in a Huntington’s disease model. Both studies show that gene suppression has more stable effects on the phenotype when performed early enough (Kordasiewicz et al. 2012, Rubinsztein and Orr 2016, Zu et al. 2004). In line with that only two phenotypic features had been found to react on late metformin treatment - survival rates and frequency of clasping- in the R6/2 animals. Also and again as expected, effect size on animal survival was quite small (p = 0.02). Gender differences in response rate seen in this study can possibly be explained by gender differences in disease development and progression at the motor stage, which had been observed in several mouse models (Menalled et al. 2009). When disease progression differs, differences in blood brain barrier permeability can be expected (reviewed in (Sweeney, Sagare, and Zlokovic 2018)) which then is likely to lead to gender specific variation in bioavailability of metformin in the brain. In contrast to this study we here show that in vivo metformin has highly significant effects (p = 0.03 to p < 0.0001) already on primal changes in the very early, VFDO phases of Huntington’s disease making metformin a promising compound for the development of a therapeutic scheme that is based on early prevention of pathology development. While we focused on male mice in this study, to reduce physiological variability due to hormone fluctuations, at the VFDO stage brain barrier changes are not expected to influence bioavailability of metformin.
Metformin was suggested to lead, through AMPK activation, to a reduction of mHtt aggregates in vitro (Walter et al. 2016, Vazquez-Manrique et al. 2016). In our previous work however we have shown that in cortical neurons metformin does not induce phosphorylation of the AMPK target ACC at all and only when given chronically it induces phosphorylation of AMPK itself in vitro. When giving 5 mg/ml metformin in the drinking water for 16-24 days to wildtype animals, while phosphorylation of S6 is significantly reduced, AMPK phosphorylation does not change in whole brain extracts (Kickstein et al. 2010). This indicates that AMPK activation is likely to depend on the dose. In WT animals as in preclinical Huntington’s disease animals the blood-brain barrier is intact, which limits bioavailability of metformin in the brain. Metformin concentrations needed to influence mTOR/PP2A activity seem to be significantly lower than those needed to influence AMPK activity. Like in the Kickstein et al. paper, in the present study, we used 5 mg/ml metformin in the drinking water, a concentration at which AMPK activation is not expected, but as we show here in the Hdh150 animals, metformin has a significant effect on the phosphorylation of the mTOR/PP2A target S6.

The effect of Htt loss on brain function is still under debate. SiRNA studies suggest that postnatal reduction of endogenous Htt protein is well tolerated (summarized in (Keiser, Kordasiewicz, and McBride 2016)). However, conditional knock-out animals with a perinatal loss of around 40% of Htt protein in the forebrain show a neurodegenerative phenotype (Dragatsis, Levine, and Zeitlin 2000). Likewise, depletion of Htt protein in the adult brain leads to progressive behavior deficits (Dietrich et al. 2017). We demonstrate here that metformin has a very specific effect on the expression of mHtt protein only, leaving wildtype Htt that is produced from the non-mutated allele in dominant Huntington’s disease untouched (Fig. 4). This makes metformin the only compound available at present with a specific effect on only mutant but not wildtype Htt protein.
In support of an effect of metformin in Huntington’s disease patients an \textit{in silico} comparison of cognitive performance of Huntington’s disease patients treated with metformin was performed. In this study using the Enroll patient cohort, it was shown that diabetic Huntington’s disease patients in the manifest stage treated with metformin had a better cognitive performance than Huntington’s disease patients not treated with metformin (Hervas et al. 2017).

Our data indicate that metformin treatment reverses all cortical network dysregulations \textit{in vivo} in the premanifest VFDO Hdh150 mice including functional sub-group distribution, frequency and synchronicity. Regaining network stability shows promise for ameliorating the molecular pathophysiology, probably by activating intrinsic repair mechanisms as shown in the context of Alzheimer’s disease (Iaccarino et al. 2016, Keskin et al. 2017). Following this network-centered view, restoration of network functions might also prevent secondary damage to the neuronal microcircuit. We therefore propose a shift in experimental treatment strategies: rather than exploring single pathways for target-, we might also consider re-balancing network function in the VFDO stages of the disease.

Taken together, our data provides evidence for the existence of a pathophysiological entity very far from onset of the manifest disease (VFDO) characterized by early homeostatic changes of network activity and, associated with that, subtle behavior alterations. The data also strongly support the observation, that similar to humans the disease in mice develops over a long period of time. This provides an early critical window of vulnerability and gives opportunities for early therapeutic interference with disease development. So far, all attempts to develop a causative therapy for Huntington’s disease have been unsuccessful [summarized in (Crook and Housman 2011, Clabough 2013)]. In terms of therapeutic intervention, consideration should be given to a chronic treatment of mutation carriers, which covers the critical windows of vulnerability, as early as in the VFDO stages. Such a strategy avoids
delaying intervention until clinical signs of the disease are evident, implying that substantial brain damage has already occurred. Our data suggest that metformin has the potential to reduce mHtt protein load and substantially influence the early development of pathology and, as seen in a *C. elegans* model, protein aggregation and movement aberrations which are pathognomonic for later disease stages. It is an inexpensive substance, well known in long-term clinical usage and has a defined, relatively benign spectrum of side effects. Prescription to mutation carriers from young adulthood on (or even earlier) is possible and will cover these newly discovered critical windows of opportunity for therapy.
### Materials and Methods

<table>
<thead>
<tr>
<th>Reagent type (species) or resource</th>
<th>Designation</th>
<th>Source or reference</th>
<th>Identifier(s)</th>
<th>Additional information</th>
</tr>
</thead>
<tbody>
<tr>
<td>genetic reagent (<em>C. elegans</em>)</td>
<td><em>C. elegans</em> strain AM141, genotype rmIs133</td>
<td>University of Minnesota</td>
<td>AM141 (WormBase ID) RRID:WB - STRAIN: AM141</td>
<td></td>
</tr>
<tr>
<td>genetic reagent (<em>M. Musculus</em>)</td>
<td>Hdh150</td>
<td>Jackson Laboratory</td>
<td>#004595 RRID:IM SR_JAX:004595</td>
<td>Only males were used</td>
</tr>
<tr>
<td>cell line (<em>H. sapiens</em>)</td>
<td>HEK 293T/17</td>
<td>Scherzinger et al., 1997</td>
<td>CRL-11268 RRID:CV CL_1926</td>
<td></td>
</tr>
<tr>
<td>cell line (<em>M. Musculus</em>)</td>
<td>Neuro-2A</td>
<td>ATCC</td>
<td>ATCC® CCL131 RRID:CV CL_0470</td>
<td></td>
</tr>
<tr>
<td>cell line (<em>M. Musculus</em>)</td>
<td>primary cortical neurons isolated from NMRI (Janvier)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>transfected construct</td>
<td>pEGFP-C1-Httex1</td>
<td>Krauss et al., 2013</td>
<td>9661 RRID:AB _2341188</td>
<td></td>
</tr>
<tr>
<td>antibody</td>
<td>rabbit anti-activated-caspase-3</td>
<td>Cell signaling</td>
<td>1 to 500</td>
<td></td>
</tr>
<tr>
<td>antibody</td>
<td>mouse anti-NeuN</td>
<td>Millipore</td>
<td>MAB377 RRID:AB _2298772</td>
<td>1 to 500</td>
</tr>
<tr>
<td>antibody</td>
<td>rabbit anti-GFAP</td>
<td>Dako</td>
<td>Z0334 RRID:AB _1001338</td>
<td>1 to 1500</td>
</tr>
<tr>
<td>antibody</td>
<td>rabbit anti-Htt</td>
<td>Abcam</td>
<td>ab109115 RRID:AB _1086308</td>
<td>WB: 1:850, IHC: 1:200</td>
</tr>
<tr>
<td>antibody</td>
<td>Alexa 546 goat anti-rabbit</td>
<td>Invitrogen</td>
<td>A11035 RRID:AB _143051</td>
<td>1 to 300</td>
</tr>
<tr>
<td>antibody</td>
<td>Alexa 647 goat anti-mouse</td>
<td>Invitrogen</td>
<td>A21235 RRID:AB _141693</td>
<td>1 to 300</td>
</tr>
<tr>
<td>antibody</td>
<td>Cy2 donkey</td>
<td>Jackson</td>
<td>711-225-</td>
<td>1 to 300</td>
</tr>
</tbody>
</table>
### Antibody Uses

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Type</th>
<th>Supplier</th>
<th>Catalog Number</th>
<th>RRID:AB</th>
<th>Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alexa 488 goat anti-rabbit</td>
<td>Life technologies</td>
<td>A11008</td>
<td>RRID:AB_2340612</td>
<td>1 to 200</td>
<td></td>
</tr>
<tr>
<td>anti-FLAG M2- Peroxidase</td>
<td>Sigma-Aldrich</td>
<td>A8592</td>
<td>RRID:AB_143165</td>
<td>1 to 3000</td>
<td></td>
</tr>
<tr>
<td>rabbit anti-actin</td>
<td>Sigma-Aldrich</td>
<td>A2066</td>
<td>RRID:AB_476693</td>
<td>1 to 2000</td>
<td></td>
</tr>
<tr>
<td>rabbit anti-pS6</td>
<td>Cell signaling</td>
<td>2215</td>
<td>RRID:AB_2630325</td>
<td>1 to 2000</td>
<td></td>
</tr>
<tr>
<td>mouse anti-GAPDH</td>
<td>Abcam</td>
<td>ab8245</td>
<td>RRID:AB_2107448</td>
<td>1 to 2000</td>
<td></td>
</tr>
<tr>
<td>HRP-anti-mouse</td>
<td>Dianova</td>
<td>115-035-072</td>
<td>RRID:AB_2338507</td>
<td>1 to 6000</td>
<td></td>
</tr>
<tr>
<td>HRP-anti-rabbit</td>
<td>Dianova</td>
<td>305-036-003</td>
<td>RRID:AB_2337936</td>
<td>1 to 6000</td>
<td></td>
</tr>
<tr>
<td>goat anti-rabbit IgG, AlexaFluor 488 conjugate</td>
<td>Life technologies</td>
<td>A11008</td>
<td>RRID:AB_143165</td>
<td>1 to 200</td>
<td></td>
</tr>
</tbody>
</table>

### Sequence-Based Reagent

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Sequence</th>
<th>Supplier</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>primers 5'-CCC ATT CAT TGC CTT GCT GCT AGG-3' and 5'-CCT CTG GAC AGG GAA CAG</td>
<td>Sigma-Aldrich</td>
<td>custom</td>
<td></td>
</tr>
<tr>
<td>siRNA AATTGACAG AGGAGTGTG ATC</td>
<td>Qiagen</td>
<td>custom</td>
<td></td>
</tr>
<tr>
<td>siRNA CACCGCAUC CUAGUAUCA CACTT</td>
<td>Qiagen</td>
<td>custom</td>
<td></td>
</tr>
<tr>
<td>siRNA CAGGAUUAC AACUUUUA GGAATT</td>
<td>Qiagen</td>
<td>custom</td>
<td></td>
</tr>
<tr>
<td>siRNA</td>
<td>Qiagen</td>
<td>custom</td>
<td></td>
</tr>
<tr>
<td>Chemical Compound, Drug</td>
<td>Sequence-based Reagent</td>
<td>Qiagen</td>
<td>Custom</td>
</tr>
<tr>
<td>-------------------------</td>
<td>------------------------</td>
<td>--------</td>
<td>--------</td>
</tr>
<tr>
<td>Hoechst33342</td>
<td>siRNA TTGAGTGAG</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CGCTATGAC AAA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fluoromount</td>
<td>siRNA TAGAACGTG</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ATGAGTCAT CAT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fluoroshield Mounting Medium</td>
<td>non siRNA AATTCTCCG</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>AACGTGTCA CGT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PBS tablettts</td>
<td>natural donkey serum</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Abcam ab7475 RRID: AB_23372 58</td>
<td>4-2%</td>
<td></td>
</tr>
<tr>
<td>PBS tabletts</td>
<td>natural goat serum</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Abcam ab7481 RRID: 253 2945</td>
<td>4-2%</td>
<td></td>
</tr>
<tr>
<td>PBS tabletts</td>
<td>natural sheep serum</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Abcam ab7489 RRID: AB_23350 34</td>
<td>20%</td>
<td></td>
</tr>
<tr>
<td>PBS tabletts</td>
<td>xylocaine AstraZeneca</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PUN0804 40</td>
<td>2%</td>
<td></td>
</tr>
<tr>
<td>PBS tabletts</td>
<td>isoflurane AbbVie</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8506 CHEBI:60 15</td>
<td>1-1.55%</td>
<td></td>
</tr>
<tr>
<td>PBS tabletts</td>
<td>PBS Life technologies</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>18912-014</td>
<td>1 M</td>
<td></td>
</tr>
<tr>
<td>chemical compound, drug</td>
<td>paraformaldehyde</td>
<td>Life technologies</td>
<td>15710 CHEBI:31 962</td>
</tr>
<tr>
<td>-------------------------</td>
<td>------------------</td>
<td>------------------</td>
<td>---------------------</td>
</tr>
<tr>
<td>Oregon-Green BAPTA1 AM</td>
<td>Molecular probes</td>
<td>O6807</td>
<td>1 mM</td>
</tr>
<tr>
<td>EGTA</td>
<td>Sigma-Aldrich</td>
<td>E4378</td>
<td>CHEBI:30 740</td>
</tr>
<tr>
<td>MgCl2</td>
<td>Sigma-Aldrich</td>
<td>M2670</td>
<td>CHEBI:86 345</td>
</tr>
<tr>
<td>K-lactobionate</td>
<td>Sigma-Aldrich</td>
<td>L2398</td>
<td>CHEBI:55 481</td>
</tr>
<tr>
<td>Taurine</td>
<td>Sigma-Aldrich</td>
<td>T0625</td>
<td>CHEBI:15 891</td>
</tr>
<tr>
<td>KH$_2$PO$_4$</td>
<td>Sigma-Aldrich</td>
<td>P5655</td>
<td>CHEBI:63 036</td>
</tr>
<tr>
<td>HEPES</td>
<td>Sigma-Aldrich</td>
<td>H3375</td>
<td>CHEBI:42 334</td>
</tr>
<tr>
<td>Sucrose</td>
<td>Sigma-Aldrich</td>
<td>S0389</td>
<td>CHEBI:17 992</td>
</tr>
<tr>
<td>BSA</td>
<td>Sigma-Aldrich</td>
<td>A6003</td>
<td>1 g/L</td>
</tr>
<tr>
<td>Malate</td>
<td>Sigma-Aldrich</td>
<td>M1000</td>
<td>CHEBI:66 50</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>Sigma-Aldrich</td>
<td>P2256</td>
<td>CHEBI:50 144</td>
</tr>
<tr>
<td>Glutamate</td>
<td>Sigma-Aldrich</td>
<td>G1626</td>
<td>CHEBI:64 243</td>
</tr>
<tr>
<td>ADP</td>
<td>Sigma-Aldrich</td>
<td>A2754</td>
<td>CHEBI:16 761</td>
</tr>
<tr>
<td>Succinate</td>
<td>Sigma-Aldrich</td>
<td>S2378</td>
<td>CHEBI:63 686</td>
</tr>
<tr>
<td>FCCP</td>
<td>Sigma-Aldrich</td>
<td>C2920</td>
<td>CHEBI:75 458</td>
</tr>
<tr>
<td>Rotenone</td>
<td>Sigma-Aldrich</td>
<td>R8875</td>
<td>CHEBI:28 201</td>
</tr>
<tr>
<td>Antimycin A</td>
<td>Sigma-Aldrich</td>
<td>A8674</td>
<td>CHEBI:22</td>
</tr>
<tr>
<td>chemical compound, drug</td>
<td>MgSO₄</td>
<td>Sigma-Aldrich</td>
<td>203726 CHEBI:32599</td>
</tr>
<tr>
<td>------------------------</td>
<td>-------</td>
<td>---------------</td>
<td>-------------------</td>
</tr>
<tr>
<td>chemical compound, drug</td>
<td>NaCl</td>
<td>Sigma-Aldrich</td>
<td>S3014 CHEBI:26710</td>
</tr>
<tr>
<td>chemical compound, drug</td>
<td>Na₂HPO₄</td>
<td>Sigma-Aldrich</td>
<td>S3264 CHEBI:34683</td>
</tr>
<tr>
<td>chemical compound, drug</td>
<td>oligofectamine</td>
<td>Thermo-Fisher</td>
<td>12252-011 0.2%</td>
</tr>
<tr>
<td>chemical compound, drug</td>
<td>metformin</td>
<td>MP Biomedicals</td>
<td>157805 CHEBI:6802 5mg/ml</td>
</tr>
<tr>
<td>chemical compound, drug</td>
<td>urea</td>
<td>Roth</td>
<td>2317.3 CHEBI:16199 48%</td>
</tr>
<tr>
<td>chemical compound, drug</td>
<td>Tris</td>
<td>Roth</td>
<td>4855.2 CHEBI:9754 15mM</td>
</tr>
<tr>
<td>chemical compound, drug</td>
<td>Glycerin</td>
<td>Roth</td>
<td>3783.1 CHEBI:17754 8.7%</td>
</tr>
<tr>
<td>chemical compound, drug</td>
<td>SDS</td>
<td>Roth</td>
<td>2326.1 CHEBI:8984 1%</td>
</tr>
<tr>
<td>chemical compound, drug</td>
<td>mercaptoehanol</td>
<td>Roth</td>
<td>4227.3 CHEBI:41218 1%</td>
</tr>
<tr>
<td>chemical compound, drug</td>
<td>protease inhibitors</td>
<td>Roche</td>
<td>04 693 116 001 1 tablet per 50 ml</td>
</tr>
<tr>
<td>chemical compound, drug</td>
<td>Phosstop</td>
<td>Roche</td>
<td>04 906 837 001 2 tablets per 10 ml</td>
</tr>
<tr>
<td>software, algorithm</td>
<td>GraphPad Prism</td>
<td>GraphPad Prism</td>
<td>RRID:SC R_002798 [<a href="http://www.graphpad.com/">http://www.graphpad.com/</a>]</td>
</tr>
<tr>
<td>software, algorithm</td>
<td>MATLAB R2011a</td>
<td>Mathworks</td>
<td>RRID:SC R_001622 [<a href="https://www.mathworks.com">https://www.mathworks.com</a>]</td>
</tr>
<tr>
<td>software, algorithm</td>
<td>Code use for Calcium transient analysis</td>
<td>this paper</td>
<td>the code is enclosed as a source file</td>
</tr>
<tr>
<td>software, algorithm</td>
<td>LaVision BioTec ImSpector microscopy software</td>
<td>LaVision BioTec</td>
<td>RRID:SC R_015249 [<a href="https://www.lavisionbiotec.com/">https://www.lavisionbiotec.com/</a>]</td>
</tr>
</tbody>
</table>
Animals

All experimental procedures were performed in accordance with institutional animal welfare guidelines and were approved by the state government of Rhineland-Palatinate, Germany (G14-1-010 and G14-1-017). WT littermates and heterozygous Hdh\(^{(CAG)150}\) mice (Hdh150, RRID:IMSR_JAX:004595) carrying an extended CAG sequence (~150) replacing the normal length CAG sequence in mouse Htt gene were obtained by crossing Hdh150 heterozygous with WT mice (Lin et al. 2001).

Male mice at 10-15 weeks of age were used to examine the change in neuronal network activity prior to disease onset and at 14-17 weeks of age to examine the effect of the \textit{in vivo} metformin treatment. Male mice at 12-16 weeks of age were used for behavior studies. Male mice at 13 weeks of age were used for immunohistochemistry. The mice were kept under specific-pathogen-free conditions on a 12 h light / 12 h darkness cycle with free access to water and food. Mice were genotyped using the primers 5’-CCC ATT CAT TGC CTT GCT GCT AGG-3’ and 5’-CCT CTG GAC AGG GAA CAG TGT TGG-3’ (Sigma-Aldrich) producing 379- and 829-bp-long fragments for WT and mutant alleles, respectively.

\textbf{Surgery for \textit{in vivo} two-photon Ca\textsuperscript{2+} imaging}
Mice were prepared for in vivo imaging under isoflurane (1-1.5% in pure O₂, AbbVie). Anesthesia depth was assessed by monitoring pinch withdrawal and respiration rate. Body temperature was kept at 37°C with a heating pad (ATC 200, World precision instruments). Local anesthesia (2% xylocaine, AstraZeneca) was applied to the scalp. A custom-made recording chamber was glued to the skull with cyanoacrylic glue (UHU) followed by dental cement (Paladur, Heraeus). Then, a craniotomy of 1.5 x 1.5 mm was performed using stainless steel drill bits. The position of the primary visual cortex was located according to brain atlas coordinates (Bregma -3 to -4.5 mm, 2-3 mm lateral to the midline) (Paxinos, Franklin, and Franklin 2001). After surgery, the mouse was subjected to the two-photon imaging setup.

**Two-photon Ca²⁺ imaging**

The fluorescent Oregon-Green BAPTA1 AM (OGB-1 AM, O6807, Molecular Probes) was bulked-loaded in the visual cortex as described previously (Stosiek et al. 2003). Anesthesia level was continuously monitored by keeping the breathing rate at 100-110 breaths/min. High-speed two-photon Ca²⁺ imaging was performed in layer 2/3 (150 to 350 µm from the pial surface) with an upright LaVision BioTec TrimScope II resonant scanning microscope, equipped with a Ti:sapphire excitation laser (Chameleon Ultra II, Coherent) and a 25x (1.1 N.A., MRD77220, Nikon) or 40x (0.8 N.A., NIRAPO, Nikon) objective. The laser was tuned to 800 nm and fluorescence emission was isolated using a band-pass filter (525/50, Semrock) and detected using a GaAsP photomultiplier tube (PMT; H7422-40, Hamamatsu). The TriM Scope II scan head, equipped with a resonant scanner, imaged time-lapses (512x512 pixels, ~440x440 µm field of view) at a maximum frame rate of 30.4 Hz. Time lapses were recorded for 5-8 minutes on average. Imspector software (LaVision BioTec) was used for microscope control and image acquisition.

**Determining cell number and analysis of Ca²⁺ transients**
First, the number of cells loaded with OGB-1 was manually counted in ImageJ (National Institutes of Health). The area containing all the cells was traced freehand and calculated by the software. Functional data were analyzed using custom-written functions in MATLAB R2011a (Mathworks, Natick, MA, U.S.A.) and Igor Pro 6.22-6.37 (Wavemetrics, Inc., Lake Oswego, OR, U.S.A.). The code is attached as Figure 1-source data 2. Regions of interest (ROIs) were hand-drawn by tracing the outlines of OGB1-positive neurons. Fluorescence intensities were quantified by averaging pixels inside each ROI for every image in a sequence. The fluorescence values were normalized by user-defined baseline. Specifically, dF/F was defined as the following: 
\[
\frac{dF}{F} = \left( \frac{\text{mean fluorescence inside an ROI}}{\text{mean user-defined baseline}} - 1 \right) \times 100
\]
where the baseline is defined as a mean fluorescence from a 1-3 s silent period in the same ROI. The peak of a Ca\textsuperscript{2+} transient was defined as the first derivative to crossed zero, and the second derivative to be negative, and where the amplitude to be greater than 3 standard deviations (SD) above the mean. The peak location was corrected manually where necessary. Each dF/F trace, sampled at 15.2-30.4 Hz sampling frequency, was preprocessed by binomial Gaussian smoothing (20-40 iterations) followed by a high pass filter. The baseline was estimated as the median of activity-free 10 s period preceding each peak. The foot and the tail of Ca\textsuperscript{2+} transients were determined as the first data point that fell within 0.5 SD of the baseline before and after the peak, respectively. The area under the curve was trapezoidal and measured between the foot and the tail. A distribution histogram of neurons according to their Ca\textsuperscript{2+} transient frequency was used to segregate neurons into three functional subgroups. The definition of hyperactive neurons (> 3 trans/min) was determined by the absence of neurons above this limit in WT (0.4 ± 0.3%). The criterion used for low active neurons was set to comprise ~25% of the WT neuronal population (25.8 ± 3.9%). The distance between two neurons was calculated by Pythagorean theorem after the x,y coordinate was determined for each ROI.
The randomization of experimental data comprised two steps: first, each raster plot was reassigned to a randomly selected ROI; then, the location of the individual raster event was shuffled randomly, except no spikes were allowed to occur within 1 s of each other.

**Immunohistochemistry**

Mice were anesthetized with a mixture of ketamine/xylocaine and perfused transcardially with 4% paraformaldehyde (#15710, Life technologies) in 0.1M phosphate buffer and brains were post-fixed. 50-µm-thick sections were sliced using a HM650 V vibratome (ThermoFisher) and collected in phosphate buffer saline (PBS; Life technologies). Floating sections were incubated for 1 h with PBS containing 4% natural goat serum (NGS, ab7481, Abcam) or 4% natural donkey serum (NDS, ab7475, Abcam) and 1% Triton X-100 (Sigma-Aldrich) at room temperature (RT, 22°C). Slices were then incubated for 48 h at 4°C with primary antibodies against the apoptotic marker-cleaved caspase-3 (1:500; rabbit polyclonal; 9661, Cell signaling), neuronal marker NeuN (1:500; mouse monoclonal; MAB377, Millipore) or astrocytic marker GFAP (1:1500; rabbit polyclonal; Z0334, Dako). Slices were incubated for 2 h at RT with secondary antibody Alexa 546 goat anti-rabbit (1:300, A11035, Invitrogen), Alexa 647 goat anti-mouse (1:300, A21235, Invitrogen) or Cy2 donkey anti-rabbit (711-225-152, Jackson Immuno Research). Primary and secondary antibodies were diluted in PBS containing 2% NGS or 2% NDS and 0.2% Triton X-100. After staining, brain slices were mounted with Fluoroshield Mounting Medium (ab104135, Abcam).

For Htt staining, brains were embedded in tissue tek (Sakura) and frozen on dry ice with 100% ethanol. 5-10 µm sagittal sections were sliced and subjected to antigen retrieval by being placed in 10 mM sodium citrate buffer at 84°C or 90-95°C for 15-20 min and rinsed with TBS-Triton-X (0.3%, Roth). Subsequently, sections were blocked with 20% sheep or horse serum in TBS-Triton-X for 1 h at RT. Primary antibody (Htt: 1:200, rabbit monoclonal, ab109115, Abcam) was diluted in TBS-Triton-X and incubated overnight at 4°C. Secondary antibody (1:200, goat anti-rabbit AlexaFluor 488, A11008, Life technologies) in TBS-Triton-
X was incubated for 2 h at RT. Afterwards, sections were embedded in fluoromount (Sigma-Aldrich) including Hoechst33342 (1:1000, B2261, Sigma-Aldrich). Mounted slices were analyzed with a confocal laser-scanning microscope (Leica SP8).

**Sample preparation for respirometry experiments**

Experimental animals were sacrificed by cervical dislocation immediately before OXPHOS analysis. Brains were micro-dissected on ice and specimens weighed on an analytical balance (Sartorius, CPA1003S; Germany). The micro-dissected brain regions were directly transferred into ice-cold mitochondrial respiration medium MiR05 (EGTA 0.5 mM, MgCl$_2$ 3 mM, K-lactobionate 60 mM, taurine 20 mM, KH$_2$PO$_4$ 10 mM, HEPES 20 mM, sucrose 110 mM, BSA 1 g/L, adjusted to pH 7.1) (Kuznetsov et al. 2000). The tissue was then homogenized in a pre-cooled 1.5 ml tube with a motorized pestle in MiR05 medium with 10 strokes. Resulting homogenates containing 10 mg tissue wet weight were suspended in 100 µl of ice-cold MiR05 and later 20 µl (2 mg) from the 100 µl tissue suspension was added to each chamber of the Oxygraph-2k, Oroboros Instrument containing 2 ml of MiR05 for OXPHOS analysis (Holmstrom et al. 2012). All chemicals were purchased from Sigma-Aldrich, Germany. The optimized motorized pestle preparation of brain tissue yields a high degree of permeabilization as evident by the minimal effect of digitonin titrations on OXPHOS capacity. Therefore, digitonin is not necessary for this protocol.

**High-resolution respirometry in tissue**

Tissue homogenates were transferred into calibrated Oxygraph-2k (O2k, Oroboros Instruments, Innsbruck, Austria) 2 ml chambers. Oxygen polarography was performed at 37 ± 0.001 °C (electronic Peltier regulation) in O2k chambers and oxygen concentration (µM) as well as oxygen flux per tissue mass (pmol O$_2$·s$^{-1}$·mg$^{-1}$) were recorded real-time using DatLab software (Oroboros Instruments Innsbruck, Austria). A multisubstrate protocol was used to sequentially explore the various components of mitochondrial respiratory capacity. The homogenate was suspended in MiR05, added to the Oxygraph-2k glass chambers and the
O$_2$ flux was allowed to stabilize. A substrate-uncoupler-inhibitor titration (SUIT) protocol was applied to assess qualitative and quantitative mitochondrial changes in Hdh150 transgenic mice and unaffected controls. After stabilization, LEAK respiration was evaluated by adding the complex I (CI) substrates malate (2 mM), pyruvate (10 mM) and glutamate (20 mM). The maximum oxidative phosphorylation (OXPHOS) capacity with CI substrates was attained by the addition of ADP+Mg$^{2+}$ (5 mM) (CI$_{OXP}$HOS). For evaluation of maximum OXPHOS capacity of the convergent input from CI and complex II (CII) at saturating ADP-concentration, the CII substrate succinate (10 mM) was added (CI+CII$_{OXP}$HOS). We then uncoupled respiration to examine the maximal capacity of the electron transport system (ETS or CI+II$_{ETS}$) using the protonophore, carbonylcyanide 4 (trifluoromethoxy) phenylhydrazone (FCCP; successive titrations of 0.2 μM until maximal respiration rates were reached). We then examined consumption in the uncoupled state solely due to the activity of complex II by inhibiting complex I with the addition of rotenone (0.1 μM; ETS CII or CII$_{ETS}$). Finally, electron transport through complex III was inhibited by adding antimycin A (2 μM) to obtain the level of residual oxygen consumption (ROX) due to oxidating side reactions outside of mitochondrial respiration. The O$_2$ flux obtained in each step of the protocol was normalized by the wet weight of the tissue sample used for the analysis and in addition ROX was subtracted from the fluxes in each run to correct for non-mitochondrial respiration (Hollis et al. 2015). All respiration experiments comprise 2-3 counterbalanced blocks across days. All substrates and inhibitors used were obtained from Sigma.

**Visual discrimination task**

WT and presymptomatic VFDO Hdh150 mice (13-15 weeks of age) were isolated and food deprived for 24 h. Subsequently, they were placed into an operant chamber with a touchscreen including two windows and a food dispenser on the opposite wall (Med Associates Inc; St. Albans, USA). In order to keep animals motivated to perform the task, their daily food intake
was adjusted to maintain body weight at 75-80% of their initial body weight during the course of the experiment. The experiment consisted of 3 phases:

1) mice were trained to collect a food pellet from the dispenser twice on day 1, 2) mice were trained to nose poke the touchscreen to obtain food pellet reward. They needed to collect and consume the pellet to proceed to the next trial. One daily session was either 30 minutes or 70 trials. The touch training was over when mice reached 70 trials on three consecutive days. 3) For the visual discrimination task, the screen presented two stimuli (pair 1: black vs. white or pair 2: black vs. grey), one correct, one false, randomly presented left or right. The mice were trained to nose poke the correct stimulus, whereupon a pellet was released. Again, they needed to collect and consume the pellet to proceed to the next trial. One daily session was either 30 min or 100 trials. The task was successful when the mice reached 70% correct trials on three consecutive days.

**Open field test**

Mice were not habituated to the set-up. Each mouse was removed from its home cage and put into a holding box next to the testing box. Subsequently, the mouse was put into the testing box facing the rear wall. The mice had time to explore the area for 5 minutes. Time in the center, which was determined as 10 cm away from each wall of the box, was measured automatically by EthoVision XT 8.5, when the center-point of the mouse moved into it.

**Cell lines and filter retardation assay**

For all cell lines used in this study the identity has been authenticated and mycoplasma contamination has been tested and excluded.

HEKT cell lines (ATCC, RRID:CVCL_1926) stably expressing FLAG-tagged HTT-exon 1 with either 51 or 83 CAG repeats under the control of a Tet-off promotor as well as the filter retardation assay were described previously (Scherzinger et al. 1997).

For the filter retardation assay cells were either transfected with a pool of MID1 specific siRNA oligonucleotides (AATTGACAGAGGAGTGATGATC,
CACCGCAUCCUAGUAUCACACTT, CAGGAUUACAACUUUUAGGAATT,
TTGAGTGAGCGCTATGACAAA, AAGGTGATGAGGCTTCGCAAA,
TAGAACGTGATGAGTCATCAT

or non-silencing control oligonucleotides (AATTCTCCGAACGTGTCACGT) using Oligofectamine (Thermo Fisher Scientific) or treated with metformin at a final concentration of 1mM and 2.5mM for 24h hours. Cell lysates were soaked through a filter membrane and aggregates were detected using monoclonal anti-FLAG M2-Peroxidase (HRP) antibodies (Sigma-Aldrich). Signals were quantified using the Fiji Software.

FRAP (fluorescence recovery after photobleaching)-based assay

Neuro-2A (a mouse neuroblastoma cell line, ATCC, RRID:CVCL_0470) cells or murine primary cortical neurons (prepared from NMRI mice E14.5 as described previously (Kickstein et al. 2010)) were transfected with constructs expressing Htt exon1 with 49CAG repeats fused to GFP (vector pEGFP-C1-HTTex1; an N-terminal GFP tag) the day before analysis. Cells were analyzed in a previously established FRAP-based assay to monitor translation in living cells (Krauss et al. 2013) using a Zeiss LSM700 confocal microscope. In brief, in contrast to standard FRAP, the GFP-signal of the entire cell was bleached using a 488 argon laser and fluorescence recovery was imaged over a time frame of 4 h. The fluorescence signal was quantified as the sum of the pixel over the cell area, and the resulting total cell fluorescence was normalized to the post-bleach signal, which was set to 100%. Fluorescence recovery curves represent mean ± SEM of at least 35 cells.

Caenorhabditis elegans

The following C. elegans strains was used: strain AM141, genotype rmIs133 [unc-54p::Q40::YFP] (RRID:WB-STRAIN:AM141). AM141 worms express YFP that is linked to a polyglutamine stretch of 40 glutamines (Q40) in the muscle cells of the body wall. In the early lifetime of the worms YFP-Q40 is soluble and it aggregates gradually over time. This strain is used as a model for polyglutamine diseases like Huntington´s disease, since the 40Q
represents a pathological range of the polyglutamine stretch.

For the treatment with metformin, NGM plates were seeded with OP50 bacteria and dried overnight. For heat inactivation OP50 bacteria were incubated at 70°C for 30min. Metformin in a concentration of 5mM, 10mM or 500mM was added the next day and dried again before usage. Worms were then put onto the plates and aggregates and liquid trashing were quantified after 5 days.

Nematodes were synchronized by hypochloride treatment. At day 5 of adulthood the worms were analyzed. Aggregates were counted under a fluorescence stereo microscope after anesthetizing the worms with 25mM Levamisol on a coverslip. For each experiment 15-20 animals were counted. In addition liquid thrashing was analyzed in 10-15 animals per experiment. Therefore, single worms were transferred into one drop of M9 buffer (3 g KH$_2$PO$_4$, 6 g Na$_2$HPO$_4$, 5 g NaCl, 1 ml 1 M MgSO$_4$, H$_2$O to 1 liter) and the rhythmic bending of the worm around its body axis was counted for 30sec. Each experiment was conducted at least three times.

**Western blots**

For western blotting mice were sacrificed and brains were grinded and shaked in magic mix (48% urea, 15mM TRIS-HCl (pH7.5), 8.7% glycerin, 1% SDS, 1% mercaptoethanol, complete protease inhibitors (Roche), Phosstop (Roche)) at 4°C with add-on homogenization (QIAshredder). Afterwards, samples were boiled at 95°C and 30 µg protein lysate (40µg for Htt blot) was loaded on a 10% SDS PAGE gel, resolved (overnight at 100 V for separation of mHtt from wtHtt) and blotted onto a PVDF membrane (BioRad) using TransBlot Turbo (BioRad). Membranes were then blocked with 1% BSA (pS6) or 5% milk (Actin, Gapdh, Htt) in PBS-Tween20 (Roth) and incubated with primary antibody (Actin: 1:2000, A2066, Sigma Aldrich; pS6: 1:2000, 2215, cell signaling; Gapdh: 1:2000, ab8245, abcam; Htt: 1:850, ab109115, abcam) in blocking buffer overnight. Membranes were then washed 3 times with PBS-Tween20 and subsequently incubated with secondary antibody (1:6000, for Htt 1:4000,
Donkey anti-rabbit or anti-mouse, Jackson Immuno Research) for 1h in blocking buffer. Subsequently, membranes were again washed three times with PBS-Tween20. Chemiluminescent detection was done by using Western Lightning Plus-ECL (PerkinElmer). Visualisation was performed on a ChemiDoc MP Imaging System (Biorad). Quantification of resulting bands was performed using Image Lab (version 5.2.1).

**Metformin treatment in vivo**

Both the Hdh150 and WT mice received chronic metformin (MP Biomedicals, LLC; France) administration (5 mg/ml in the drinking water) freshly prepared every day for 3 weeks starting from an age of 9-10 weeks.

**Statistics**

Statistical significance was tested in GraphPad Prism (GraphPad Software Inc., La Jolla, CA, USA). Table 1 contains all details concerning statistical tests used (name of the test, p-values, F values and degree of freedom). * p < 0.05, ** p < 0.01, ***p<0.001 and **** p < 0.0001. For all data, we first tested for normal distribution using the one-sample Kolmogorov-Smirnov test. In case that the null hypothesis of a normal distribution could not be rejected (for p>0.05), we employed a parametric test, if H₀ could be rejected (for p<0.05) we used non-parametric tests: Mann-Whitney U test for non-parametric data and t test for parametric data. Pearson’s correlation coefficient was used on raster plots that were temporally binned (328 ms per bin) to compare activity patterns between pairs of neurons. Box-and-whisker plots indicate the median (line) of average values from multiple time-lapses, the 25-75th percentiles (box) and the 10-90th percentiles (whiskers). Graphs show mean ± s.e.m (standard error of the mean).

**Data availability**

Values used in figures are available on Dryad Digital repository (doi:10.5061/dryad.g3b5272) and the code used for the analysis of calcium imaging is in Figure 1-source data 2.

**Acknowledgement**
The authors thank all members of the Methner, Schweiger and Stroh labs for advice and helpful discussions. We thank Zeke Barger and Andrea Kronfeld for help in the analysis of two-photon $\text{Ca}^{2+}$ imaging data. We also thank Ulrich Schmidt for help in behavior experiments and Cheryl Ernest, Ina Vorberg, Dan Ehninger, Simon Rumpel and Oliver Tuescher for proofreading the manuscript. We acknowledge the Caenorhabditis Genetics Center for the worm strain.

**Funding**

This study was funded by the European Huntington Disease Network (A.S. and A.M.), the Focus Program Translational Neurosciences (FTN; I.A., M.W., Su.S., A.S., A.M.) and the BMBF (Eurostars) to AS and was supported by Tenovus Scotland.
References


Rubinsztein, D. C., and H. T. Orr. 2016. "Diminishing return for mechanistic therapeutics with neurodegenerative disease duration?: There may be a point in the course of a neurodegenerative condition where therapeutics targeting disease-causing mechanisms are futile." Bioessays 38 (10):977-80. doi: 10.1002/bies.201600048.


Figure 1: In vivo two-photon Ca^{2+} imaging in layer 2/3 of visual cortex reveals a hyperactive neuronal activity pattern prior to disease onset.

a, b. Top right, Illustrations indicating the visual cortex (blue area) in human (a) and mouse (b) brains. The brains are not drawn to scale. Bottom, timeline of Huntington’s disease progression in human and Hdh150 mouse model of Huntington’s disease. The Huntington’s disease onset was age 30-50 years in humans and ~70 weeks in Hdh150 mice. We conducted our experiments during a very early pre-symptomatic phase, far prior to mHtt aggregates and motor symptoms. VFDO: very far from disease onset.

c. Representative two-photon images of OGB-1 AM staining in the layer 2/3 of the visual cortex of WT and Hdh150 mice. Scale bar: 70 µm.

d. Color-coded maps of silent (black) and spontaneously active (orange) neurons in WT (left) and Hdh150 (right) mice. Dashed lines represent the boundaries of blood vessels (original images in Fig. 1c). Scale bar: 100 µm.

e. Density of stained cells in the layer 2/3 of the visual cortex in WT and Hdh150 mice. Unpaired t-test, p = 0.71.

f. Increased proportion of spontaneously active neurons in Hdh150 mice. Unpaired t-test, p < 0.05.

g. Representative traces of spontaneous Ca^{2+} transients (red) of ten neurons recorded in vivo in WT and Hdh150 mice. Vertical scale bars: 40% dF/F.

h. Increased frequency of Ca^{2+} transients in spontaneously active neurons of Hdh150 mice. Silent neurons were excluded, as in subsequent panels. Mann-Whitney test, p < 0.01

i. Quantification of area under the curve (AUC) of the Ca^{2+} transients. Unpaired t-test, p = 0.98

j. Cumulative frequency distribution of Ca^{2+} transients in WT (dark grey) and Hdh150 (red) mice. Top, colored categorization of neurons according to their Ca^{2+} transient frequencies. Two-way ANOVA, group: p < 0.0001, time: p < 0.0001, Interaction: p < 0.0001.
k. Color-coded categorization of neurons according to their $\text{Ca}^{2+}$ transient frequency: “low” ($< 0.3$ trans/min, blue; silent neurons excluded), “medium” (0.3-3 trans/min, orange) and “hyper” ($> 3$ trans/min, red). Each peak is marked by an asterisk.

l. Relative proportion of low, medium and hyperactive neurons in layer 2/3 of the visual cortex in WT (left) and Hdh150 (right) mice. Chi-square test, $p < 0.01$
Figure 2: Presymptomatic Hdh150 mice exhibit an increased synchronicity of cortical microcircuits

a. Color-coded Pearson’s r matrices calculated from representative recordings of WT (left) and Hdh150 (right) mice. Silent cells were excluded from the analysis. Right, color-coded scale of Pearson’s r values.

b. Overall Pearson’s correlation coefficient (Pearson's r) in WT (dark grey) and Hdh150 (red) for experimental (filled) and randomized (open) raster data. Mann-Whitney test, WT vs. Hdh150 p< 0.05; WT vs. WT rand p<0.0001; Hdh150 vs. Hdh150 rand p<0.0001

c. Pearson’s r for combinations of neuronal pairs (LL: low-low, LM: low-medium, LH: low-hyper, MM: medium-medium, MH: medium-hyper, HH: hyper-hyper) in WT (dark grey) and Hdh150 (red) mice. * pairwise comparisons between a pair of WT and Hdh150 mice. # comparisons of functional subgroup pairs to the low-low pair within the same genotype. The pairs involving hyperactive neurons could only be analyzed in Hdh150 mice. Mann-Whitney test, WT vs. Hdh150 mice: MM p < 0.05 in Hdh150 mice; compared to LL: MM p < 0.05, MH p < 0.01, HH p < 0.01

d, e. Relationship between Pearson’s r and distance between neuronal pairs in WT (black) and Hdh150 (red) mice (d) and randomized data (e). Lines represent the linear fit of WT and Hdh150 experimental data. Two way ANOVA (d) Genotype: p < 0.0001 Distance: p=0.97, Interaction: p=0.3 , (e) Genotype=0.35, p=0.3, Interaction: p=0.8
Figure 3: Presymptomatic VFDO Hdh150 mice exhibit anxiolytic behavior

a. Representative travel pathways of WT (left) and presymptomatic Hdh150 (right) mice analyzed in a 5 minutes open field test.

b. Increased explorative behavior of Hdh150 animals compared to the WT mice. Mann-Whitney test, p < 0.05.
Figure 4: Metformin reduces translation rates of mutant HTT through MID1/PP2A protein complex \textit{in vitro} and decreases both S6 phosphorylation and mutant Htt protein load in Hdh150 animals

a. FLAG-HTT detected on a filter retardation assay after treatment with and without 1mM and 2.5 mM metformin. Quantification on right panel. Mann-Whitney test, control vs. 1 mM metformin $p = 0.08$; control vs. 2.5 mM metformin, $p < 0.05$.

b. Stable cell line expressing FLAG-HTT exon1 with 83 CAG repeats transfected with \textit{MID1}-specific siRNAs or control siRNAs in the presence or absence of 2.5 mM metformin. FLAG-HTT detected on a filter retardation assay. Efficiency of the knock-down including Actin as a loading control is shown on a western blot (left panel). Quantification of filter retardation assay on right panel. Mann-Whitney test, control siRNA vs. MID1 siRNA $p < 0.01$; control siRNA vs. MID1 siRNA + metformin $p < 0.05$.

c. Protein translation rate of GFP-tagged mutant Htt exon1 (49 CAG repeats) in primary cortical neurons measured in a FRAP-based assay, over a time frame of 4 hours. Curves show the GFP-signal intensity over time in mock-treated (control) and metformin (1 mM and 2.5 mM) treated cells. Curves represent means, shadowed areas standard deviations. Repeated measures two way ANOVA, treatment $p < 0.01$, time $p < 0.0001$; interaction $p < 0.0001$.

d. Protein translation rate measured in a FRAP-based assay (see c) Curves show the GFP-signal intensity over time in mock-treated (control), metformin (2.5 mM) treated, ocadaic acid (OA) treated and metformin + OA treated cells. Shadowed areas show SEM. Repeated measures two way ANOVA, treatment $p < 0.01$; time $p < 0.0001$, interaction $p < 0.0001$.

e. Transgenic Hdh150 mice received metformin containing water (5mg/ml, Hdh150 + metformin) or pure water (Hdh150) over a period of 3 weeks. Whole brain lysates were analyzed for the phosphorylation of S6, the expression of total S6, mHtt and wtHtt on western blots. Representative western blots are shown.

f. Quantification of pS6 relative to S6. Unpaired t-test, $p < 0.05$. 
mHtt and wt Htt proteins of prefrontal cortex lysates analyzed on western blots after 11 weeks treatment with metformin (5mg/ml, Hdh150 + metformin) or pure water (Hdh150).

Representative western blots are shown.

Quantification of mHtt relative to wtHtt. Treatment of 5 mg/ml metformin in the drinking water showed a significant reduction of mHtt protein compared to water control treatment.

Unpaired t-test p < 0.05

Quantification of mHtt relative to Gapdh. Unpaired t-test, p < 0.01.

Quantification of wtHtt relative to Gapdh. Unpaired t-test, p = 0.88.

Figure 5: Metformin treatment reverses pathological neuronal network activity and behavioral abnormalities in presymptomatic VFDO Hdh150 mice.

a. Representative traces of spontaneous Ca\(^{2+}\) transients of ten neurons recorded in vivo in WT and Hdh150 mice after metformin treatment. Vertical scale bar: 40% dF/F.

b. Relative proportion of spontaneously active neurons in WT (dark grey), Hdh150 (red), WT metformin-treated (light grey) and Hdh150 metformin-treated (light red) mice. Mann-Whitney test, WT vs. Hdh150, p < 0.05; Hdh150 vs. Hdh150 met, p < 0.05; Hdh150 vs. WT met, p < 0.05.

c. Significant reduction in the spontaneous Ca\(^{2+}\) transient frequency to WT levels in Hdh150 mice after metformin treatment (red vs. light red). Mann-Whitney test, WT vs. Hdh150, p < 0.01; Hdh150 vs. Hdh150 met, p < 0.01; Hdh150 vs. WT met, p < 0.01.

d. Cumulative frequency distributions of Ca\(^{2+}\) transients in WT (dark grey), Hdh150 (red), metformin-treated WT (light grey) and metformin-treated Hdh150 (light red) mice. Top, color-coding of active neurons by frequency. Two way ANOVA test, Group: p < 0.0001; Time: p < 0.0001; Interaction: p < 0.0001.

e. Pie charts showing the relative proportion of low (blue), medium (orange) and hyperactive (red) neurons in the layer 2/3 of the visual cortex in WT (top) and Hdh150 (bottom) mice after metformin treatment. Chi-square test, p = 0.62, Chi-square = 0.24.
f. Comparison of Pearson’s r between a pair of neurons in WT (dark grey), Hdh150 (red), metformin-treated WT (light grey) and metformin-treated Hdh150 (light red) mice. Mann-Whitney test, WT vs. Hdh150, p < 0.05, Hdh150 vs. Hdh150 met, p < 0.01, Hdh150 vs. WT met, p < 0.01.

g. Relationship between pairwise Pearson’s r and pairwise distance in metformin-treated WT (light grey) and Hdh150 (light red) mice. Two way ANOVA test, group p < 0.0001; Distance p = 0.09; Interaction p < 0.001.

h. Representative travel pathways of a metformin-treated WT (left) and pre-symptomatic Hdh150 (right) mice analyzed in a 5 minutes open field test.

i. Decreased in the explorative behavior of metformin-treated Hdh150 animals. Mann-Whitney test, WT vs. Hdh150, p < 0.01; Hdh150 vs. Hdh150 met, p < 0.001; Hdh150 vs. WT met, p < 0.05; WT vs. Hdh150 met, p = 0.8.
Figure 1- figure supplement 1: Ca$^{2+}$ events from astrocytes and neurons show clearly distinct kinetics.

a. Schematic diagram of the two-photon microscope used for high-speed Ca$^{2+}$ imaging in vivo. The synthetic dye OGB-1 AM loaded in the layer 2/3 of mouse visual cortex was excited by pulsed laser light generated by the Ti:sapphire laser ($\lambda = 800$ nm), a resonant scanner enabled video-rate imaging. Emitted light was collected by a photomultiplier tube (PMT).

b. Two-photon images of OGB-1 AM staining captured in vivo at depths between 150 and 270 µm from the pial surface in the visual cortex of WT (top) and Hdh150 (bottom) mice. Scale bar: 70 µm.

c. Two-photon image of OGB-1 AM staining in the layer 2/3 of the visual cortex. Neurons (open squares) show round shapes with no processes. Astrocytes (inverted open triangle) in contrast, are more intensely stained; both the soma and processes can be visualized. Scale bar: 40 µm.

d. Five representative traces recorded for visually identified neurons (left) and astrocytes (right). Putative astrocytes show slower rise time, longer duration, and slower decay. The onset of events is aligned. Scale bars: 40% dF/F.

e, f. Box-and-whisker plots displaying the time to peak (e) and decay time (f) in neurons and astrocytes. Mann-Whitney test, $p < 0.01$. 


Figure 1- figure supplement 2: Cortical hyperactivity is independent of mHtt aggregation, apoptotic cell death or astrogliosis in presymptomatic VFDO Hdh150 mice.

a, b, c. Confocal images of sagittal or coronal sections of 13 weeks old WT and Hdh150 mice stained for Htt proteins (a), glial fibrillary acidic protein (GFAP, b) and neuronal (NeuN, c) and apoptotic (cleaved-caspase 3, c, arrows).

Scale bars: 20 (a), 200 (b) and 100 (c) µm.
Figure 1- figure supplement 3: Randomization of experimental data to assess specific spatial clustering.

a, b. Color-coded spatial distributions and raster plots of the peak of Ca\(^{2+}\) transients in low (blue), medium (orange) and hyperactive (red) neurons recorded \emph{in vivo} in the layer 2/3 of the visual cortex (a, left) and after randomization of the data (b, right). Silent cells are also displayed in the color-coded maps (black).

c. Color-coded spatial distribution of silent (black), low (blue), medium (orange) and hyperactive (red) neurons in WT (left) and Hdh150 (right) mice. The maps are equivalent to Fig. 1d, but active neurons were subdivided into groups based on Ca\(^{2+}\) transient frequency. Dashed line outlines the boundary of blood vessels. Scale bar: 100 µm.

d. Box-and-whisker plot showing the mean pairwise distance for the different type of neuronal pairs (SS: silent-silent, SL: silent-low, SM: silent-medium, SH: silent-hyper, LL: low-low, LM: low-medium, LH: low-hyper, MM: medium-medium, MH: medium-hyper, HH: hyper-hyper) in WT (filled dark grey) and Hdh150 (filled red) mice. The experimental data were compared to randomized data (WT rand and Hdh150 rand, open dark grey and red, respectively). No significant difference was observed between groups in WT and Hdh150 mice. This indicated an absence of spatial clustering among any of the subgroups of active neurons. Mann-Whitney test, not significant, see table 1 for p-values.
Figure 2- figure supplement 1: Randomization of experimental data to assess specific network synchronicity.

a, b. Comparison of Pearson’s r for different functional subgroup pairs in WT experimental and randomized data (a) and Hdh150 data (b), * p < 0.05, ** p < 0.01 and *** p < 0.001, Mann-Whitney test. * p < 0.05, ** p < 0.01, in comparisons of functional subgroup pairs to the low-low pair within the same genotype.
Figure 2- figure supplement 2: Presymptomatic Hdh150 mice did not exhibit alteration of mitochondria respiration.

a, b. Scheme of the Oroboros O₂K respirometer and typical traces obtained from high-resolution respirometry of microdissected cortical tissue from male WT or Hdh150 mice.

c. Box-and-whisker plot depicting the O₂ flow in n = 6 mice per genotype. No difference was observed indicating that mitochondria respiration is similar in the two genotypes. Mann-Whitney test, not significant, see table 1 for p-values.
Figure 3- figure supplement 1: Presymptomatic VFDO Hdh150 mice did not exhibit deficit in visual discrimination test and explorative behavior in novel object recognition test

a. Schema of the visual discrimination task set-up. Left, front view of the touch screen panel. Right, top view of the complete test set-up. A monitor is placed at the end of the unit which simultaneously displays the correct and false choice. Both WT and presymptomatic VFDO Hdh150 mice were trained to choose the correct screen and for each correct choice made, a food pellet was released from a reward dispenser placed on the other end of the unit.

b. Graph representing time course of the training sessions of WT (dark grey) and presymptomatic Hdh150 (red) mice. Training sessions were carried out for both groups; mice that made at least 70% correct choice for three consecutive days were considered for discrimination analysis. After a training period of 7 days, mice were able to perform tasks successfully reaching the 70% criterion (dashed line). Note that no significant difference was observed between WT and Hdh150 mice during the training of visual discrimination task. Repeated measures two-way ANOVA, genotype p = 0.6; time p < 0.0001; interaction p = 0.6.

c, d. Time course of visual performance in WT (dark grey) and Hdh150 (red) mice during visual discrimination first of black and white screens (c) and then of black and grey screens (d). c: Repeated measures two-way ANOVA, genotype p = 0.5; time p < 0.0001; interaction p = 0.03. d: Repeated measures two-way ANOVA, genotype p = 0.8; time p < 0.01; interaction p = 0.9.

e. 9 week old transgenic Hdh150 mice and wildtype littermates received metformin containing (5mg/ml, met) or pure water over a period of 3 weeks. Groups of WT, Hdh150, WT met and Hdh150 met were analyzed in an open field test and total distance travelled was measured. Mann-Whitney test, p = 0.3.
Figure 4- figure supplement 1: Metformin reduces mutant Htt protein translation and does not change drinking behavior of Hdh150CAG animal

a. GFP-tagged mutant (49 CAG repeats- Q49) Htt exon1 was expressed in N2A cells and protein translation rate were measured in a FRAP-based assay, in which the GFP signal of transfected cells is removed by photobleaching and the synthesis rate of freshly translated GFP-tagged protein is measured over a time frame of 4 hours. Curves show the GFP-signal intensity over time in mock-treated (control) and metformin (1 mM and 2.5 mM) treated cells. Shadowed areas show standard deviations. Repeated measures two-way ANOVA, treatment p = 0.03; time p < 0.0001; interaction p < 0.0001.

b. Male, 9 week old Hdh150CAG animals were fed with 5 mg/ml metformin in the drinking water (with metformin) or with pure water (without metformin) and observed over 21 days. The water consumption was monitored every day. Curves represent means, shadowed areas show +/- SEM. Repeated measures two-way ANOVA, treatment p = 0.3; time p = 0.06; interaction p = 1.

c. Transgenic Hdh150 mice received metformin containing water (5mg/ml, Hdh150 + metformin) or pure water (Hdh150) over a period of 3 weeks. Whole brain lysates were analyzed for the expression of mHtt and wtHtt on western blots. Representative western blots are shown.

d. Quantification of mHtt relative to wtHtt. Unpaired t-test, p = 0.18.
Figure 4 – figure supplement 2: Metformin treatment rescues motility impairment in a *C. elegans* model

a. Q40::YFP nematodes were treated with 500 mM of metformin or pure water (control): Images of nematodes with and without metformin treatment for 5 days (left panel): After 5 days of metformin treatment, the number of aggregates was significantly reduced (right panel): Mann-Whitney test, p < 0.0001.

c. Q40::YFP worms were grown on heat-inactivated bacteria on plates pre-treated with either 5 mM or 10 mM of metformin or with pure water (control). After 5d of metformin treatment the number of inclusion bodies was analyzed. Mann-Whitney test, control vs 5 mM metformin, p = 0.008; control vs 10 mM metformin, p < 0.0001.

d., e. RNAi knockdown of the MID1-ortholog arc-1 was performed in Q40::YFP *C. elegans*. After 5 days, the number of inclusion bodies (d) and liquid trashing events (e) were analysed. Mann-Whitney test, p < 0.0001.
Figure 5- figure supplement 1: Metformin treatment does not affect cell density or Ca\(^{2+}\) transient dynamics

a. Two-photon images of OGB-1 AM staining collected in vivo at different depths (from 150 to 270 µm from pial surface) in the visual cortex of WT and Hdh150 mice after metformin treatment. Scale bar: 70 µm.

b. Quantification of the density of stained cells in layer 2/3 of the visual cortex in WT (black), Hdh150 (red, n = 10 animals), metformin-treated WT (light grey) and metformin-treated Hdh150 (light red) mice. No significant difference was found in the cell density across genotypes. Unpaired t-test, p = 0.7.

c. Area under the curve (AUC) of Ca\(^{2+}\) transients in WT (black), Hdh150 (red), metformin-treated WT (light grey) and metformin-treated Hdh150 (light red) mice. Unpaired t-test, WT vs. WT met, p < 0.05.

d. Box-and-whisker plot showing Pearson’s r between different combinations of neuron pairs (LL: low-low, LM: low-medium, LH: low-hyper, MM: medium-medium, MH: medium-hyper, HH: hyper-hyper) in metformin-treated WT (light grey) and Hdh150 (light red) mice. There was no statistical difference between WT and Hdh150 mice after metformin treatment. # a statistical difference between a pair vs. LL within the same genotype. Unpaired t-test, WT met: LL vs. LM, p < 0.05; LL vs. MM, p < 0.0001; Hdh150 met: LL vs. MM, p < 0.01.

e. Comparison of Pearson’s r between different combinations of neuron pairs in WT (dark grey), Hdh150 (red), metformin-treated WT (light grey) and Hdh150 (light red) mice. * a statistical difference between WT within the same functional subgroup. LM WT vs LM Hdh150 and MM WT vs MM Hdh150 p < 0.05.

grey) and Hdh150 (light red) mice. No significant difference between metformin-treated WT and Hdh150 mice could be found. Mann-Whitney test, not significant, see table 1 for p-values.
Figure 1 – Movie supplement 1

*In vivo* two-photon images of mouse visual cortex performed at different depths (indicated in the upper left corner) after multi-bolus loading with OGB-1 AM. Scale bar: 50 µm.

Figure 2 – Movie supplement 2

Representative time-lapse of *in vivo* two-photon Ca$^{2+}$ imaging acquired in layer 2/3 of mouse visual cortex showing single-cell Ca$^{2+}$ transients. Scale bar: 40 µm.

Figure 3 – Movie supplement 1

Example of visual discrimination task performed by a trained mouse, real time.
Figure 1-source data 1: Numerical values of Fig. 1 and associated supplement figures
Figure 1- source data 2 : Code used for the analysis of calcium imaging.
Figure 2-source data 1: Numerical values of Fig. 2 and associated supplement figures
Figure 3-source data 1: Numerical values of Fig. 3 and associated supplement figures
Figure 4-source data 1: Numerical values of Fig. 4 and associated supplement figures
Figure 5-source data 1: Numerical values of Fig. 5 and associated supplement figures
## Table 1: Statistics

<table>
<thead>
<tr>
<th>Figure</th>
<th>test</th>
<th>values</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 1e</td>
<td>Unpaired t test, two-tailed</td>
<td>NS, p = 0.71</td>
<td>WT n = 11 mice; Hdh150 n = 10 mice</td>
</tr>
<tr>
<td>Figure 1f</td>
<td>Unpaired t test, two-tailed</td>
<td>p = 0.023</td>
<td>WT n = 1204 cells in 8 mice; Hdh150 n = 933 cells in 6 mice</td>
</tr>
<tr>
<td>Figure 1h</td>
<td>Mann-Whitney test</td>
<td>p = 0.006</td>
<td>WT n = 765 cells in 8 mice; Hdh150 n = 695 cells in 6 mice</td>
</tr>
<tr>
<td>Figure 1i</td>
<td>Unpaired t test, two-tailed</td>
<td>NS, p = 0.98</td>
<td>WT n = 765 cells in 8 mice; Hdh150 n = 695 cells in 6 mice</td>
</tr>
<tr>
<td>Figure 1j</td>
<td>Two-way ANOVA test</td>
<td>Group: p &lt; 0.0001, Df = 1, F = 85.96, time: p &lt; 0.0001, Df = 16, F = 147, Interaction: p &lt; 0.0001, F = 4.9, Df = 16</td>
<td>WT n = 765 cells in 8 mice; Hdh150 n = 695 cells in 6 mice</td>
</tr>
<tr>
<td>Figure 1l</td>
<td>Chi-square test</td>
<td>p = 0.002, df = 1, Chi-square = 9.127</td>
<td>WT n = 765 cells in 8 mice; Hdh150 n = 695 cells in 6 mice</td>
</tr>
<tr>
<td>Figure 2b</td>
<td>Mann-Whitney test</td>
<td>WT vs Hdh150 p = 0.03; WT vs WT rand p &lt; 0.0001; Hdh150 vs Hdh150 rand p &lt; 0.0001</td>
<td>WT n = 26126 Pearson's r in 8 mice; Hdh150 n = 58050 Pearson's r in 6 mice</td>
</tr>
<tr>
<td>Figure 2c</td>
<td>Mann-Whitney test</td>
<td>WT vs Hdh150 mice: MM p = 0.041 in Hdh150 mice; compared to LL: MM p = 0.0496, MH p = 0.005, HH p = 0.009</td>
<td>WT n = 26126 Pearson's r in 8 mice; Hdh150 n = 58050 Pearson's r in 6 mice</td>
</tr>
<tr>
<td>Figure 2d</td>
<td>Two-way ANOVA test</td>
<td>Group: p &lt; 0.0001, Df = 1, F = 58.20; Distance: p = 0.97, Df = 15, F = 0.44; Interaction: p = 0.33, df = 15, F = 1.13</td>
<td>WT n = 26126 distances in 8 mice; Hdh150 n = 58050 distances in 6 mice</td>
</tr>
<tr>
<td>Figure 2e</td>
<td>Two-way ANOVA test</td>
<td>p = 0.35, Df = 1, F = 0.86</td>
<td>WT rand n = 26126 distances in 8 mice; Hdh150 rand n = 58050 distances in 6 mice</td>
</tr>
<tr>
<td>Figure 3b</td>
<td>Mann-Whitney test</td>
<td>p = 0.031119</td>
<td>WT n = 10 mice; Hdh150 n = 13 mice</td>
</tr>
<tr>
<td>Figure 4a</td>
<td>Mann-Whitney test</td>
<td>control vs 1mM metformin p = 0.084521, control vs 2.5mM metformin p = 0.023231</td>
<td>Control n = 10, 1 mM metformin n = 11, 2.5 mM metformin n = 10.</td>
</tr>
<tr>
<td>Figure 4b</td>
<td>Mann-Whitney test</td>
<td>control siRNA vs MID1 siRNA p = 0.008, control siRNA vs MID1 siRNA + metformin p = 0.015</td>
<td>Control siRNA n = 6, MID1 siRNA n = 6, MID1 siRNA + metformin n = 6.</td>
</tr>
<tr>
<td>Figure 4c</td>
<td>RM two-way ANOVA</td>
<td>Treatment: p = 0.0082, Df = 2, F = 5; Time: p &lt; 0.0001, Df = 47, F = 27.5; Interaction: p &lt; 0.0001, Df = 94, F = 5.9</td>
<td>n_{control} = 47, n_{metformin 1mM} = 44, n_{metformin 2.5mM} = 35</td>
</tr>
</tbody>
</table>
Figure 4d: RM two-way ANOVA

Treatment: \( p = 0.0021, \text{Df} = 3, F = 5.1 \)
Time: \( p < 0.0001, \text{Df} = 47, F = 64.1 \)
Interaction: \( p < 0.0001, \text{Df} = 141, F = 6.1 \)

\( n_{\text{control}} = 46, n_{\text{metformin}} = 49, n_{\text{metformin+OA}} = 51, n_{\text{OA}} = 43 \)

Figure 4f: unpaired t-test

\( p = 0.0473 \)

\( \text{Hdh150 n = 6; Hdh150 metformin n = 6} \)

Figure 4h: unpaired t-test

\( p = 0.0467 \)

\( \text{Hdh150 n = 3; Hdh150 metformin n = 3} \)

Figure 4i: unpaired t-test

\( p = 0.0062 \)

\( \text{Hdh150 n = 3; Hdh150 metformin n = 3} \)

Figure 4j: unpaired t-test

\( p = 0.8766 \)

\( \text{Hdh150 n = 3; Hdh150 metformin n = 3} \)

Figure 5b: Mann-Whitney test

\( \text{WT vs Hdh150 } p = 0.023, \text{Hdh150 vs Hdh150 met } p = 0.03, \text{Hdh150 vs WT met } p = 0.012 \)

\( \text{WT n = 1204 cells in 8 mice; Hdh150 n = 933 cells in 6 mice; WT met n = 1915 cells in 9 mice; Hdh150 met n = 1585 cells in 6 mice} \)

Figure 5c: Mann-Whitney test

\( \text{WT vs Hdh150 } p = 0.006; \text{Hdh150 vs Hdh150 met } p = 0.007; \text{Hdh150 vs WT met } p = 0.008 \)

\( \text{WT n = 765 cells in 8 mice; Hdh150 n = 695 cells in 6 mice; WT met n = 1199 in 9 mice; Hdh150 met n = 1014 cells in 6 mice} \)

Figure 5d: two-way ANOVA test

Group: \( p < 0.0001, \text{Df} = 3, F = 61.80 \)
Time: \( p < 0.0001, \text{Df} = 16, F = 345.9 \)
Interaction: \( p < 0.0001, \text{Df} = 48, F = 3.64 \)

\( \text{WT n = 765 cells 8 mice; Hdh150 n = 695 cells 6 mice; WT met n = 1199 cells 9 mice; Hdh150 met n = 1012 cells 6 mice} \)

Figure 5e: Chi-square test

\( p = 0.62, \text{df} = 1; \text{Chi-square} = 0.24 \)

\( \text{WT n = 765 cells 8 mice; Hdh150 n = 695 cells 6 mice; WT met n = 1199 cells 9 mice; Hdh150 met n = 1012 cells 6 mice} \)

Figure 5f: Mann Whitney test

\( \text{WT vs Hdh150 } p = 0.03; \text{Hdh150 vs Hdh150 met } p = 0.002; \text{Hdh150 vs WT met } p = 0.003 \)

\( \text{WT n = 765 cells 8 mice; Hdh150 n = 695 cells 6 mice; WT met n = 1199 cells 9 mice; Hdh150 met n = 1012 cells 6 mice} \)

Figure 5g: two-way ANOVA test

Group: \( p < 0.0001, \text{Df} = 3, F = 85.96 \)
Distance: \( p = 0.99, \text{Df} = 45, F = 0.58 \)
Interaction: \( p = 0.0007, \text{Df} = 15, F = 2.63 \)

\( \text{WT n = 765 cells 8 mice; Hdh150 n = 695 cells 6 mice; WT met n = 1199 cells 9 mice; Hdh150 met n = 1012 cells 6 mice} \)

Figure 5i: Mann Whitney test

\( \text{WT vs Hdh150 } p = 0.002, \text{Hdh150 vs Hdh150 Met } p = 0.002, \text{Hdh150 vs. WT met } p = 0.02, \text{WT vs Hdh150 Met } p = 0.82 \)

\( \text{WT n = 10; Hdh150 n = 13; WT met n = 6; Hdh150 met n = 8 mice} \)

Figures supplements test values n
Figure 1- figure Mann-Whitney test \( p = 0.002 \)
\( n = 6 \text{ neurons, } n = 6 \text{ astrocytes} \)
<table>
<thead>
<tr>
<th>Figure 1- figure supplement 1f</th>
<th>Mann-Whitney test</th>
<th>p = 0.002</th>
<th>n = 6 neurons, n = 6 astrocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>In WT mice: SS vs SL p = 0.5, SS vs SM p = 0.9, SS vs LL p = 0.1, SS vs LM p = 0.2, SS vs MM p = 0.1, SL vs SM p = 0.4, SM vs LL p = 0.1, SM vs MM p = 0.1, LL vs MM p = 0.9, LM vs MM p = 0.4, LM vs SM, p = 0.2.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>In Hdh150 mice: SS vs SL p = 0.8, SS vs SM p = 0.9, SS vs LL p = 0.9, SS vs LM p = 0.9, SS vs LH p = 1, SS vs MM p = 0.1, SS vs MH p = 0.1, SS vs HH p = 0.4, SL vs SM p = 0.8, SL vs SH p = 0.9, SL vs LL p = 0.9, SL vs LM p = 0.9, SL vs LH p = 0.8, SL vs MM p = 0.5, SL vs MH p = 0.6, SL vs HH p = 0.7, SN vs SH p = 0.6, SM vs LL p = 0.7, SM vs LM p = 0.6, SN vs LH p = 0.4, SM vs MM p = 0.3, SN vs MH p = 0.4, SN vs HH p = 0.6, SH vs LL p = 0.7, SH vs LM p = 1, SH vs LH p = 1, SH vs MM p = 0.4, SH vs MH p = 0.3, SH vs HH p = 0.6, LL vs LM p = 0.9, LL vs LH p = 1, LL vs MM p = 0.5, LL vs MH p = 0.7, LL vs HH p = 0.8, LM vs LH p = 0.6, LM vs MM p = 0.3, LM vs MH p = 0.3, LM vs HH p = 0.5, LH vs MM p = 0.3, LH vs MH p = 0.3, LH vs HH p = 0.5, MM vs MH p = 0.7, NN vs HH p = 0.8, MH vs HH p = 0.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>In WT vs WT rand: WT mice: SS p = 0.5, SL p = 0.7, SM p = 0.3, LL p = 0.3, LM p = 0.8, MM p = 0.1.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>In Hdh150 vs Hdh150 rand: SS p = 0.6, SL p = 0.8, MN vs HH p = 0.8, MH vs HH p = 0.9</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

WT n = 72595 distances 8 mice; Hdh150 n = 132009 distances 6 mice
<table>
<thead>
<tr>
<th>Figure 2- figure supplement 1a</th>
<th>Mann-Whitney test</th>
</tr>
</thead>
<tbody>
<tr>
<td>LL p=0.005; LM p&lt;0.0001; MM p&lt;0.0001</td>
<td>WT n = 26126 Pearson's r in 8 mice</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Figure 2- figure supplement 1b</th>
<th>Mann-Whitney test</th>
</tr>
</thead>
<tbody>
<tr>
<td>LL p=0.004; LM p=0.0006; LH p=0.041; MM p&lt;0.0001; MH p=0.0002; HH p=0.01 In Hdh150 mice, compared to LL: MM p=0.049; MH p=0.005; HH p=0.009</td>
<td>Hdh150 n = 58050 Pearson's r in 6 mice</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Figure 2- figure supplement 2c</th>
<th>Mann-Whitney test</th>
</tr>
</thead>
<tbody>
<tr>
<td>routine p = 0.4, leak p = 0.5, CI p = 0.6, CI+CII p = 0.5, ETS p = 0.2</td>
<td>WT n = 6 mice; Hdh150 n = 6 mice</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Figure 3- figure supplement 1b</th>
<th>RM two-way ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype: p = 0.6, Df = 1, F = 0.3 Time: p&lt;0.0001, Df = 6, F = 86.1 Interaction: p = 0.6, Df = 6, F = 0.7</td>
<td>WT n = 16 mice; Hdh150 n = 13 mice</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Figure 3- figure supplement 1c</th>
<th>RM two-way ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype: p = 0.5, Df = 1, F = 0.5 Time: p &lt; 0.0001, Df = 9, F = 35.4 Interaction: p = 0.03, Df = 9, F = 2.2</td>
<td>WT n = 16 mice; Hdh150 n = 13 mice</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Figure 3- figure supplement 1d</th>
<th>RM two-way ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype: p = 0.8, Df = 1, F = 3.4 Time: p&lt;0.01, Df=6, F=3.4 Interaction: p=0.97, Df=6, F=0.2</td>
<td>WT n = 16 mice; Hdh150 n = 13 mice</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Figure 3- figure supplement 1e</th>
<th>Mann-Whitney test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment p = 0.0342, Df = 2, F = 3.5; Time p &lt; 0.0001, Df = 47, F = 45.3; Interaction p &lt; 0.0001, Df = 94, F = 3.5</td>
<td>WT n = 10; Hdh150 n = 13; WT met n = 6; Hdh150 met n = 8 mice</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Figure 4- figure supplement 1a</th>
<th>RM two-way ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment p = 0.2986, Df = 1, F = 1.9; Time p = 0.0654, Df = 20, F = 1.8; Interaction p = 0.9988, Df = 20, F = 0.3.</td>
<td>n_{control} = 36, n_{metformin 1mM} = 42, n_{metformin 2.5mM} = 44</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Figure 4- figure supplement 1b</th>
<th>RM two-way ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment p = 0.0342, Df = 2, F = 3.5; Time p &lt; 0.0001, Df = 47, F = 45.3; Interaction p &lt; 0.0001, Df = 94, F = 3.5</td>
<td>control n = 7, metformin n = 8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Figure 4- figure supplement 1dc</th>
<th>Unpaired t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>n_{control} = 36, n_{metformin 1mM} = 42, n_{metformin 2.5mM} = 44</td>
<td>Hdh150 n = 4; Hdh150 metformin n = 4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Figure 4- figure supplement 2a</th>
<th>Mann Whitney test</th>
</tr>
</thead>
<tbody>
<tr>
<td>p &lt; 0.0001</td>
<td>control n = 65, metformin n = 65</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Figure 4- figure supplement 2b</th>
<th>Mann Whitney test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q40 vs. Q40 Met p &lt; 0.0001</td>
<td>Q40n = 43, Q40 Met n = 43</td>
</tr>
<tr>
<td>Figure 4- figure supplement 2c</td>
<td>Mann-Whitney test</td>
</tr>
<tr>
<td>Figure 4- figure supplement 2d</td>
<td>Mann-Whitney test</td>
</tr>
<tr>
<td>Figure 4- figure supplement 2e</td>
<td>Mann-Whitney test</td>
</tr>
<tr>
<td>Figure 5- figure supplement 1b</td>
<td>Unpaired t test, two-tailed</td>
</tr>
<tr>
<td>Figure 5- figure supplement 1c</td>
<td>Unpaired t test, two-tailed</td>
</tr>
<tr>
<td>Figure 5- figure supplement 1d</td>
<td>Unpaired t test, two-tailed</td>
</tr>
<tr>
<td>Figure 5- figure supplement 1e</td>
<td>Mann-Whitney test</td>
</tr>
<tr>
<td>Figure 5- figure supplement 1f</td>
<td>Mann-Whitney test</td>
</tr>
</tbody>
</table>

**Figure 4- figure supplement 2c**
- Mann-Whitney test
- Ctrl vs. 5mM p = 0.0078, Ctrl vs. 10mM p < 0.0001
- n = 45

**Figure 4- figure supplement 2d**
- Mann-Whitney test
- p < 0.0001
- Control n = 72, arc-1 RNAi n = 74

**Figure 4- figure supplement 2e**
- Mann-Whitney test
- p < 0.0001
- Control n = 60, arc-1 RNAi n = 62

**Figure 5- figure supplement 1b**
- Unpaired t test, two-tailed
- WT met vs. Hdh150 met p = 0.39, WT vs. WT met p = 0.7, Hdh150 vs. Hdh150 met p = 0.9
- n = 11; Hdh150 n = 10; WT met n = 9; Hdh150 met n = 6 mice

**Figure 5- figure supplement 1c**
- Unpaired t test, two-tailed
- WT vs WT met p = 0.024
- n = 765 cells 8 mice; Hdh150 n = 695 cells 6 mice; WT met n = 1199 cells 9 mice; Hdh150 met n = 1012 cells 6 mice

**Figure 5- figure supplement 1d**
- Unpaired t test, two-tailed
- WT met: LL vs LM p = 0.04 and LL vs MM p < 0.0001; Hdh150 met: LL vs LM p = 0.4, LL vs MM p = 0.004
- n = 57140 Pearson's r in 9 mice; Hdh150 met n = 49535 Pearson's r in 6 mice

**Figure 5- figure supplement 1e**
- Mann-Whitney test
- LM WT vs LM Hdh150; MM WT vs MM Hdh150 p = 0.04
- n = 26126 Pearson's r in 8 mice; Hdh150 n = 58050 Pearson's r in 6 mice; WT met n = 57140 Pearson's r in 9 mice; Hdh150 met n = 49535 Pearson's r in 6 mice

**Figure 5- figure supplement 1f**
- Mann-Whitney test
- SS p = 0.1, SL p = 0.1, SM p = 0.1, LL p = 0.4, LM p = 0.3, MM p = 0.2
- n = 140467 distances in 9 mice; Hdh150 met n = 117485 distances in 6 mice
Figure 1

(a) Human

(b) Mouse (Hdh150 heterozygous)

(c) WT

(d) WT

(e) WT

(f) WT

(g) WT

(h) WT

(i) WT

(j) WT

(k) WT

(l) WT
Figure 2

(a) Scatter plots for WT and Hdh150 showing the correlation between distance and Pearson's r. 
(b) Box plots comparing Pearson's r for WT, WT rand, Hdh150, and Hdh150 rand. 
(c) Bar graphs showing the comparison of Pearson's r (x10^-2) for different conditions. 
(d) Graphs showing the linear fits for WT, Hdh150, and their random variants. 
(e) Scatter plots for distance vs. Pearson's r (x10^-2) for WT, WT rand, Hdh150, and Hdh150 rand.
Figure 3

(a) Open field

WT

Hdh150

(b) % Time in center

WT Hdh150

**
**Figure 4**

(a) Bar graph showing relative protein level of HTT/Actin. Three conditions are compared: control, 1 mM metformin, and 2.5 mM metformin. Error bars indicate standard deviation.

(b) Box plot comparing GFP signal intensity over time post bleaching. Three conditions are shown: control, MID1 siRNA, and MID1 siRNA + Metformin. Error bars indicate interquartile range.

(c) Graph showing GFP signal intensity over time post bleaching for Q49 control, Q49 1 mM metformin, and Q49 2.5 mM metformin.

(d) Graph showing GFP signal intensity over time post bleaching for control, 2.5 mM metformin, 2.5 mM metformin + OA, and OA.

(e) Western blot showing expression of pS6 in Hdh150 with and without metformin.

(f) Bar graph showing intensity of pS6 normalized to Actin. Two conditions are compared: Hdh150 and Hdh150 + metformin.

(g) Western blot showing expression of mHtt and wtHtt with and without metformin.

(h) Bar graph showing intensity of mHtt normalized to Gapdh. Two conditions are compared: Hdh150 and Hdh150 + metformin.

(i) Bar graph showing intensity of mHtt normalized to Gapdh. Two conditions are compared: Hdh150 and Hdh150 + metformin.

(j) Bar graph showing intensity of wtHtt normalized to Gapdh. Two conditions are compared: Hdh150 and Hdh150 + metformin.
Figure 5

a) 

<table>
<thead>
<tr>
<th>n1</th>
<th>n2</th>
<th>n3</th>
<th>n4</th>
<th>n5</th>
<th>n6</th>
<th>n7</th>
<th>n8</th>
<th>n9</th>
<th>n10</th>
<th>50s</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

b) 

% of active cells

<table>
<thead>
<tr>
<th>WT</th>
<th>Hdh150</th>
<th>WT met</th>
<th>Hdh150 met</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


c) 

frequency (trans/min)

<table>
<thead>
<tr>
<th>WT</th>
<th>Hdh150</th>
<th>WT met</th>
<th>Hdh150 met</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

d) 

Cumulative probability (%)

<table>
<thead>
<tr>
<th>WT</th>
<th>Hdh150</th>
<th>WT met</th>
<th>Hdh150 met</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

e) 

Distance (µm)

<table>
<thead>
<tr>
<th>Low</th>
<th>Medium</th>
<th>Hyper</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

f) 

Pearson's r

<table>
<thead>
<tr>
<th>WT</th>
<th>Hdh150</th>
<th>WT met</th>
<th>Hdh150 met</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

g) 

Hyper

<table>
<thead>
<tr>
<th>Low</th>
<th>Medium</th>
<th>Hyper</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

h) 

Open field

<table>
<thead>
<tr>
<th>WT met</th>
<th>Hdh150 met</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

i) 

% Time in center

<table>
<thead>
<tr>
<th>WT</th>
<th>Hdh150</th>
<th>WT met</th>
<th>Hdh150 met</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**Figure 1 - figure supplement 1**

(a) A/D converter → PMT

(b) Images showing different conditions with labels: WT, Hdh150, PMT, TiSa, Pre Chirp, dx, dy, Chirp, A/D converter.

(c) Close-up images of neurons and astrocytes.

(d) Graphs showing time to peak and decay time for neurons and astrocytes with bars indicating significance levels.

(e) Time to peak graph with bars for neurons and astrocytes.

(f) Decay time graph with bars for neurons and astrocytes.
Figure 1 - figure supplement 2

(a) Htt

(b) GFAP

(c) casp3/NeuN

WT

Hdh150
Figure 1 - figure supplement 3

(a) Experimental data

(b) Randomized data

(c) WT

(d) Hdh150

Silent · Low

Medium · Hyper

Distance (µm)

0 50 100 150 200 250

SS

SL

SM

SH

LL

LM

LH

MM

MH

HH

0 50 100 150 200 250

SS

SL

SM

SH

LL

LM

LH

MM

MH

HH

Experimental data

Randomized data

WT exp

WT rand

Hdh150 exp

Hdh150 rand
Figure 2 - figure supplement 1

(a) Pearson's r for WT and WT rand conditions.

(b) Pearson's r for Hdh150 and Hdh150 rand conditions.
Figure 2 - figure supplement 2

(a) Diagram of a polarographic oxygen sensor (POS) setup.

(b) Graph showing O2 concentration (µM) over time for WT and Hdh150 conditions.

(c) Scatter plot comparing O2 flux mt (pmol/s/mg) for WT and Hdh150 conditions.
Figure 3 - figure supplement 1

(a) Visual discrimination task diagram

(b) Visual discrimination task - Training graph

(c) Visual discrimination task graph vs WT vs Hdh150

(d) Visual discrimination task graph vs WT vs Hdh150

(e) Total distance travelled graph vs WT, Hdh150, WT met, Hdh150 met
Figure 4 - figure supplement 1

(a) GFP signal intensity over time [min] post bleaching for Q49 control, Q49 1 mM metformin, and Q49 2.5 mM metformin.

(b) Drinking water [g] / mouse over days, showing no metformin and metformin groups.

(c) Western blot analysis showing Hdh150, metformin - and +, 348 kDa, mHtt, and wtHtt.

(d) Box plot comparing mHtt intensity (normalized to wtHtt) for Hdh150 and Hdh150 + metformin.
Figure 4 - figure supplement 2

a

control

metformin

inclusion bodies count

0

20

40

60

80

100

120

Thrashes / min

5 d

control

5 d

metformin

***

b

0

50

100

150

200

250

300

Thrashes / min

5 d Q40

control

5 d Q40

metformin

***

c

0

10

20

30

40

50

60

70

80

90

100

inclusion bodies count

control

5 mM

10 mM

metformin

***

**

d

0

20

40

60

80

100

120

inclusion bodies count

Q40::YFP control

Q40::YFP arc-1 RNAi

***

e

0

40

80

120

160

200

Thrashes / min

Q40::YFP control

Q40::YFP arc-1 RNAi

***