Sudden death due to paralysis and synaptic and behavioral deficits when Hip14/Zdhhc17 is deleted in adult mice

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Abstract

Background: Palmitoylation, the addition of palmitate to proteins by palmitoyl acyltransferases (PATs), is an important regulator of synaptic protein localization and function. Many palmitoylated proteins and PATs have been implicated in neuropsychiatric diseases, including Huntington disease, schizophrenia, amyotrophic lateral sclerosis, Alzheimer disease, and X-linked intellectual disability. HIP14/DHHC17 is the most conserved PAT that palmitoylates many synaptic proteins. Hip14 hypomorphic mice have behavioral and synaptic deficits. However, the phenotype is developmental; thus, a model of post-developmental loss of Hip14 was generated to examine the role of HIP14 in synaptic function in the adult.

Results: Ten weeks after Hip14 deletion (iHip14Δ/Δ), mice die suddenly from rapidly progressive paralysis. Prior to death the mice exhibit motor deficits, increased escape response during tests of anxiety, anhedonia, a symptom indicative of depressive-like behavior, and striatal synaptic deficits, including reduced probability of transmitter release and increased amplitude but decreased frequency of spontaneous post-synaptic currents. The mice also have increased brain weight due to microgliosis and astrogliosis in the cortex.

Conclusions: Behavioral changes and electrophysiological measures suggest striatal dysfunction in iHip14Δ/Δ mice, and increased cortical volume due to astrogliosis and microgliosis suggests a novel role for HIP14 in glia. These data suggest that HIP14 is essential for maintenance of life and neuronal integrity in the adult mouse.

Keywords: Huntington's disease, Palmitoylation, Palmitoyl acyltransferase, HIP14, DHHC17

Background

In recent years palmitoylation has emerged as an important regulator of protein localization and function, particularly in neurons [1, 2]. Palmitoylation is the reversible addition of long chain fatty acids, typically palmitate, to proteins at cysteine residues [3, 4]. It is mediated by DHHC-domain containing palmitoyl acyltransferases (PATs) that palmitoylate proteins at cysteine residues via a thioester bond [5, 6]. Many PATs have been implicated in diseases of the nervous system, including Huntington disease (HD), an autosomal dominant fatal neurodegenerative disease; schizophrenia; amyotrophic lateral sclerosis; Alzheimer disease; and X-linked intellectual disability [1, 2].

Palmitoylation is the only reversible lipid modification, and this reversibility is analogous to phosphorylation, where enzyme-mediated addition and removal of palmitate allows for rapid cycling of palmitate on some proteins, providing an additional level of regulation of localization and function [7]. Indeed, in neurons, palmitoylation has been shown to regulate localization of many synaptic proteins. For example, palmitoylation of post-synaptic density protein 95 (PSD95) is required for its synaptic localization, and its palmitoylation undergoes cycles of de/repalmitoylation that regulate PSD95 nanoclusters within the synapse [8]. Palmitoylation also regulates the synaptic insertion/removal of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA)}
subunits GluA1 and GluA2, and of N-methyl-D-aspartate receptor (NMDAR) subunits GluN2A and GluN2B [9, 10]. Huntington in interacting protein 14 (HIP14 or ZDHHC17) is the most highly conserved of the 23 human PATs. It palmitoylates many synaptic proteins, including cysteine string protein (CSP), GluA1, GluA2, PSD95, synaptosomal-associated protein 25 (SNAP25), synaptotagmin 1 (SYT1), the large conductance calcium- and voltage-activated potassium BK channel (KCNMA1) STREX isoform, and the HD disease-causing protein huntingtin (HTT) [2]. It has recently become more apparent that HIP14 is an important regulator of synaptic function. Indeed, Hip14 knockdown reduces PSD95 clustering in neurons [6] and in Drosophila melanogaster HIP14 is required for CSP targeting to synaptic vesicles and, in turn, pre-synaptic exocytosis [11]. Interestingly, in an HD mouse model HIP14 is less active [12, 13] and the constitutive Hip14-deficient mouse (Hip14<sup>ggt/ggt</sup>) has behavioral, neuropathological, and synaptic dysfunction reminiscent of HD [12, 14, 15].

The Hip14<sup>ggt/ggt</sup> mouse is a hypomorph expressing ~10% of endogenous HIP14 protein [16, 17] and the phenotype is developmental, as neurodegeneration occurs during late embryogenesis. Thus, we sought to determine the consequences of complete loss of Hip14 in the adult animal and its effect on synaptic defects and neuronal degeneration. An inducible Hip14-deficient mouse model was generated, and Hip14 deletion was induced in the young adult mouse.

Results

Generation of post-development Hip14-deficient mice

Hip14 “conditional knockout” (Hip14<sup>F/F</sup>) mice (Fig. 1a–d) were crossed to ubiquitously expressed tamoxifen (TM)-inducible Cre recombinase (Cre-ER<sup>T2</sup>)-expressing transgenic mice [18]. Hip14 deletion was induced in Hip14<sup>F/F</sup>,Cre<sup>+</sup> mice at 6 weeks of age by TM treatment (iHip14<sup>F/A</sup> herein) to allow mice a month to recover from TM toxicity prior to any behavior testing per- formance. Both female and male iHip14<sup>F/A</sup> mice were approximately 10% smaller than wild-type (WT) vehicle (VEH)-treated and iHip14<sup>F/F</sup> control mice (Fig. 2a and b). To assess global nervous system and motor function, spontaneous activity was assessed during the dark phase. iHip14<sup>F/A</sup> mice were hyperactive during exploration of a novel environment (increased distance traveled, Fig. 2c, and ambulatory time, d).

Motor coordination and sensorimotor gating deficits in iHip14<sup>F/A</sup> mice

To determine if loss of HIP14 in the adult mouse results in neurological dysfunction, motor function was assessed. Motor coordination of iHip14<sup>F/A</sup> mice was tested on rotarod and climbing tests [20]. iHip14<sup>F/A</sup> mice had motor coordination deficits on the rotarod compared to control WT VEH and iHip14<sup>F/F</sup> mice (Fig. 3a). As the rotarod performance is a trained test where mice learn to stay on the rotarod, it is less sensitive to motor dysfunction than the spontaneous test of motor coordination: climbing [21, 22]. There was also a dramatic reduction in the number of climbing events in these mice (Fig. 3b) but no change in the number of rearing events, indicative of motivation to explore the apparatus (Fig. 3c). Taken together, these data indicate motor dysfunction.

Schizophrenia and other neurological disorders were recently shown to be enriched for palmitoylated proteins [1]. Pre-pulse inhibition (PPI) is a test of sensorimotor gating, partly mediated by the striatum [20, 23]. PPI deficits are associated with schizophrenia and other psychiatric disorders as well as HD [24]. When a quieter tone (the pre-pulse) is played prior to a loud stimulus (the startle pulse), mice with intact sensorimotor gating will startle less than they would to the loud startle stimulus alone [25]. iHip14<sup>F/A</sup> mice showed impaired pre-pulse inhibition at all pre-pulse levels that was significant at 2, 4, and 9 dB above background with a trend at 16 dB compared to control mice (Fig. 3d), indicating impaired sensorimotor gating and potential striatal dysfunction.

Increased escape response and anhedonia in iHip14<sup>F/A</sup> mice

As it is becoming increasingly evident that palmitoylation is important in neuropsychiatric disorders [1, 2], the impact of loss of HIP14 on psychiatric phenotypes such as depression and anxiety was assessed [21, 26]. iHip14<sup>F/A</sup> mice were tested in the Porsolt forced swim test for depression [26–28]. Interestingly, iHip14<sup>F/A</sup> mice spent dramatically less time immobile during forced swimming than controls (Fig. 4a). During behavior testing iHip14<sup>F/A</sup> mice were observed to be very reactive to the experimenter and testing conditions, having explosive responses to both. Thus, rather than truly reflecting an antidepressive effect, these data in the forced swim test are
consistent with the hyperactivity and reactivity to testing observed in these mice.

Anxiety-like behavior was assessed in the open field exploration test, a well-established test of anxiety-like behaviors in rodents [21, 29]. iHip14Δ/Δ mice explored the brightly lit open field to the same extent as control mice, as measured by distance traveled (Fig. 4b), but spent less time in the center of the field (Fig. 4c), suggesting an increase in anxiety-like behaviors in these mice.

To confirm anxiety in iHip14Δ/Δ mice, the mice were tested using the elevated plus maze (EPM) test for anxiety [30, 31]. Surprisingly, the iHip14Δ/Δ mice spent more time in the open arms of the EPM than the control WT VEH or iHip14F/F mice (Fig. 4d), suggesting decreased anxiety,
opposite to the findings from the open field testing. The iHip14Δ/Δ mice did not explore the EPM as much as the control mice (Fig. 4e), likely because they spent more time dipping their head off the edge of the open arms of the maze (Fig. 4f), again suggesting decreased anxiety. These data suggest an anxiolytic phenotype rather than the anxiogenic phenotype suggested by the open field exploration test. Alternatively, the iHip14Δ/Δ mice may be trying to escape the testing apparatus; i.e., they spend more time exploring the edges of the open field box trying to find a way out, and in the EPM they dip their heads off the open arms trying to escape the maze. This interpretation would also be consistent with their reactivity to handling and increased time spent immobile in the forced swim test.

To separate anxiety-like behavior from increased escape response, a modified light-dark box test was designed that completely removed any possibility of escape, where one side was dark and the other side was brightly lit, and both sides were completely enclosed. The iHip14Δ/Δ mice spent the same amount of time in the light box as the control mice (Fig. 4g). These data suggest iHip14Δ/Δ mice are not anxious per se but have an increased escape response.

To delineate escape response from depressive-like behavior, the iHip14Δ/Δ mice were tested using the sucrose preference test for anhedonia-like behavior (the inability to experience pleasure), as anhedonia is a major symptom of depression [26, 32]. The sucrose preference test is performed in the home cage with no experimenter present, thus eliminating the confound of increased escape response. The iHip14Δ/Δ mice consumed the same amount of fluid (Fig. 4g) but had decreased preference for sucrose compared to the control mice (Fig. 4h), indicating anhedonia and suggesting a depressive-like phenotype.

**Increased forebrain weight, increased cortical volume, and decreased corpus callosum volume in iHip14Δ/Δ mice**

To determine the effect of loss of HIP14 in the adult mouse on brain morphology and neurodegeneration, neuropathological assessments were performed. Increased brain weight was observed in iHip14Δ/Δ mice (Fig. 5a). This increase was restricted to the forebrain (Fig. 5b), as there was no change in cerebellar weight compared to
dramatic increase in both GFAP (Fig. 5g and i) and IBA1 staining intensity, respectively. There was a significant increase in cortical volume, astrocytes and corpus callosum volume, indicating loss of white matter (Fig. 5e), potentially due to axonal degeneration or loss of myelination.

To understand what factors may account for the observed increase in cortical volume, astrocytes and microglia were assessed by glial fibrillary acidic protein (GFAP) and ionized calcium-binding adapter molecule 1 (IBA1) staining intensity, respectively. There was a dramatic increase in both GFAP (Fig. 5g and i) and IBA1 (Fig. 5h and j) staining intensity in the cortex of iHip14Δ/Δ mice compared to controls, indicating significant astrogliosis and microgliosis, respectively.

Impaired synaptic transmission in the striatum of iHip14Δ/Δ mice

Since palmitoylation has been implicated in localization of synaptic proteins and in synaptic signaling and HIP14 was previously shown to be important for striatal physiology and striatal processing during motor behaviors [14, 15], the synaptic properties of medium-sized spiny neurons (MSNs) in the striatum of iHip14Δ/Δ mice were examined [14, 33–35] by making current- and voltage-clamp recordings in the dorsal striatum. We observed no significant effect of loss of Hip14 on eitherMSN resting membrane potential or rheobase (Fig. 6a–c); the amount of current injection required to initiate action potential firing. Membrane capacitance, an indirect measure of cell-surface area, was also similar between groups (Fig. 6d). Thus, MSN membrane potential, excitability, and cell size appear to be unaltered by loss of Hip14 in adulthood.

To assay excitatory synaptic function, AMPAR-mediated spontaneous excitatory post-synaptic currents (sEPSCs) were recorded from MSNs held at -70 mV in the presence of picROTOXIN, a γ-aminobutyric acid A (GABA A) receptor antagonist. There was a significant decrease in the frequency and a significant increase in the amplitude of sEPSCs recorded from iHip14Δ/Δ MSNs (Fig. 6e and f) compared to controls. These data demonstrate synaptic dysfunction in iHip14Δ/Δ mice and suggest a reduction in the number of excitatory synapses and/or a reduction in transmitter release probability with additional AMPARs at the synapses or more glutamate released per synaptic vesicle.

To assess transmitter release probability from cortical afferents onto MSNs in the striatum, a stimulating electrode was placed 200–250 μm dorsal to the recorded cell, various inter-pulse intervals were applied, and the paired pulse ratio (PPR) was calculated. MSNs from iHip14Δ/Δ mice had increased PPRs compared to MSNs from control mice (Fig. 6g and h). These data are indicative of a lower probability of transmitter release and are consistent with the reduction in sEPSC frequency, further suggesting synaptic dysfunction.

Reduced survival due to rapidly progressing paralysis in iHip14Δ/Δ mice

As mice were being aged for longitudinal behavior studies, a dramatic decrease in survival of iHip14Δ/Δ mice, beginning at about 16 weeks of age or 10 weeks post-Hip14 deletion, was observed (Fig. 7a). Typically, all mice appeared healthy prior to sudden death. Six mice were found with hind limb paralysis prior to being euthanized for other reasons.
purposes. Post-mortem examination of 14 iHip14Δ/Δ mice revealed signs of paralysis in 13, including splayed hind limbs and clenched front paws. One iHip14Δ/Δ mouse found with hind limb paralysis was monitored by video (Additional file 1). The paralysis progressed rapidly over 5 h beginning with the hind limbs. Initially, the mouse did not appear distressed and was able to move around the cage and eat. Paralysis progressed until the mouse could no longer move and it was euthanized. A second iHip14Δ/Δ mouse was found almost completely paralyzed and was video monitored for a few minutes until it went into respiratory arrest and died (Additional file 2). These data indicate that iHip14Δ/Δ mice have dramatically reduced survival due to rapidly progressing paralysis and sudden death.

Two iHip14Δ/Δ mice survived past 20 weeks of age (10%). One of these mice reached a humane endpoint due to wasting at 43 weeks of age and was euthanized. At the time of euthanasia, it weighed 30% less than its control littersmates. The brain was harvested for biochemistry to assess HIP14 protein levels to ensure complete loss of HIP14. Indeed, negligible HIP14 protein was detected, indicating that efficient recombination occurred in this mouse (Fig. 7b).

**Discussion**

The most striking phenotype of the iHip14Δ/Δ mice is the rapidly progressing hind limb paralysis leading to sudden death. This was highly unexpected, as there is no survival deficit of Hip14+/-/+ mice. HIP14 is the most highly conserved PAT, with 99% protein sequence identity between human and mouse and 88% between human and zebrafish as well as 100% conservation of the DHHC active site domain from human to chicken [2]. This high sequence conservation suggests an essential function for the protein, which is supported by the phenotype of iHip14Δ/Δ mice. The constitutive Hip14-deficient Hip14+/-/+ mouse has HD-like neurological deficits [12, 20, 25]; thus, iHip14Δ/Δ mice were expected to develop a similar phenotype. However, the severe phenotype of iHip14Δ/Δ mice shows that HIP14 is crucial for the life of the adult mouse. Hip14+/-/+ mice develop early onset neurological disease, and these mice express 10% of the endogenous levels of HIP14 in all cells [16, 17], whereas complete loss in >90% of cells in iHip14Δ/Δ adult mice causes a severe phenotype, including sudden death. Thus, complete loss of HIP14 is likely not compatible with survival. It will be interesting to see what happens if HIP14 is fully deleted from conception. However, there may also be developmental compensation that occurs when HIP14 is deleted from conception, likely by other PATs, which cannot occur when HIP14 is deleted in the adult animal.

The iHip14Δ/Δ mice have motor coordination deficits similar to those of Hip14+/-/+ mice. The motor deficits are dramatic, particularly in the spontaneous climbing
modified light-dark box test that eliminated any avenues for escape. However, anxiety and escape response are likely associated. Thus, it is possible that they become anxious when they are unable to escape a novel environment [36]. Overall, the increased escape response phenotype of iHip14Δ/Δ mice agrees with rodent striatal lesion models with enhanced escape response behavior, providing further evidence of an essential role for HIP14 in striatal function [37].

Interestingly, iHip14Δ/Δ mice have increased forebrain weight due to microgliosis and astrogliosis in the cortex. This may be a downstream response to neuron or circuit dysfunction or may suggest a novel role for HIP14 in glial cell function. Also, although there is clear striatal dysfunction in iHip14Δ/Δ mice, there was no change in striatal volume, unlike the striatal atrophy observed in iHip14Δ/Δ mice [12, 38]. One possible explanation for this discrepancy is that iHip14Δ/Δ mice die before sufficient striatal neuron death for detection by stereology has occurred. There was, however, a decrease in corpus callosum volume, suggesting decreased white matter and potentially axonal degeneration or demyelination.

Further evidence for striatal dysfunction was apparent in the physiology of MSNs. Although there is no change in membrane excitability or surface area in iHip14Δ/Δ MSNs, they did display aberrant synaptic transmission. The increase in sEPSC amplitude, decrease in sEPSC frequency, and increased PPR suggest a lower probability for escape. However, anxiety and escape response are likely associated. Thus, it is possible that they become anxious when they are unable to escape a novel environment [36]. Overall, the increased escape response phenotype of iHip14Δ/Δ mice agrees with rodent striatal lesion models with enhanced escape response behavior, providing further evidence of an essential role for HIP14 in striatal function [37].
of transmitter release but more AMPARs at excitatory synapses in the striatum and/or more glutamate released per synaptic vesicle to the same number of AMPARs. Loss of palmitoylation at either palmitoylation site of GluA1 or GluA2 AMPAR subunits would increase their synaptic expression, which could contribute to these phenotypes [9].

HIP14 has been shown to be a “hub” protein with many interacting partners, and it shares many interactors (not specifically substrates) with HTT, also a “hub” protein with many interactors [39]. In addition to being a PAT, HIP14 has also been shown to have other, non-PAT-related, functions in MAP kinase signaling and magnesium/manganese transport [40–42]. Thus, the phenotype of these mice may be due to loss of palmitoylation of one or multiple crucial proteins, may result from loss of one of these other functions of HIP14, or may be caused by a combination of all these factors.

Conclusions
This is the first study, to our knowledge, to examine the “conditional knockout” of a DHHC PAT and conclusively demonstrates that HIP14 is essential for life and neuronal integrity. The iHip14FR/Δ mice have a severe phenotype, different than that of the Hip14Δ/Δ mice, that results in sudden death, striatal dysfunction, and significant astrogliosis and microgliosis. These data highlight the importance of this PAT to neurological function and suggest that palmitoylation is an essential protein modification.

Methods

Generation of inducible Hip14 knockout mice
Xenogen Biosciences (now Taconic Biosciences, Rensselaer, NY, USA) generated the Hip14 “floxed” mice (Hip14FR) on the FVB/N background strain using a gene targeting strategy where exon 2 was selected as the conditional deletion region, as deletion of this region leads to a frameshift mutation and multiple premature stop codons (Fig. 1a–d). The 5’ and 3’ homology arms and the conditional knockout region (cKO) were amplified from bacterial artificial chromosome DNA and inserted into the targeting vector at the indicated restriction enzyme sites such that the cKO region was flanked by loxP sites (Fig. 1a). A positive selection neo cassette was included and flanked by flippase (Fp) recognition target (FRT) sites, and a negative selection cassette diphtheria toxin A (DTA) was also included to select against random insertion (Fig. 1a). Male FVB/N embryonic stem cells were electroporated with the targeting vector and selected using G418 (Geneticin) resistance and screened for homologous recombination at the 5′ and 3′ homology arms with the WT allele (Fig. 1b) by restriction enzyme digest, southern blot, and PCR. The neo cassette was then removed in positive clones by electroporation with Flp recombinase to mediate recombination between the FRT sites and generate the recombinated Hip14FR allele (Fig. 1c). Neo cassette deletion was confirmed by G418 sensitivity and PCR. Hip14FR embryos were then injected into C57BL/6 j blastocysts to generate male chimeras that were bred with FVB/N females. Resulting white coat progeny indicated germline transmission, and those mice were genotyped using the following primers: the forward primer in the 5’ homology arm in intron 1 (5’−GGAGAGATGGTAGGAAAGCTCGTACC−3’) and the reverse primer in the cKO region in intron 1 upstream of the first loxP site (5’−GGAGAAAGCATGCAAGCAGCTCTTTCT−3’).

Hip14FR mice were then crossed to mice expressing Cre-ERT2 under the human ubiquitin ligase C promoter, a promoter that will result in ubiquitous Cre expression in all cell types [18] (The Jackson Laboratory, Bar Harbor, ME, USA). The Cre-ERT2 transgene expresses Cre recombinase fused to a mutated form of the estrogen receptor that is not activated by estrogen but is activated by the estrogen analog tamoxifen (TM) [18]. This generated mice in which Hip14 can be deleted at any time point (Hip14FR/Δ;Cre-ERT2). The primers used to genotype at the Cre-ERT2 transgene were 5’−GCCTCTCGGCAGTAAAAACTATC−3’ and 5’−GTGAAACAGCTTTGCTGTCACTT−3’. Gene deletion was induced using a 5-day TM treatment paradigm by giving a single intraperitoneal injection once a day for 5 days at a dose of 0.2 mg TM/g body weight in 98% corn oil with 2% ethanol (iHip14FR/F and iHip14FR/Δ) or vehicle alone (WT VEH) as previously described [18]. Mice were treated with TM at 6 weeks of age.

Quantitative real-time PCR
Total RNA was isolated from −80 °C frozen tissues using the RNeasy mini kit (Qiagen, Venlo, The Netherlands). RNA was treated with DNAsé I (Life Technologies, Carlsbad, CA, USA) to remove residual genomic DNA. cDNA was prepared from 1 μg total RNA using the SuperScript III First-Strand Synthesis System (Life Technologies, Carlsbad, CA, USA). Quantitative RT-PCR (qPCR) on the mouse Hip14 gene using primers spanning exons 1 and 2 (5’−ACCCGAGGAATCACCACAGA−3’ and 5’−TACATCGAACCCGCTTCCACC−3’) was performed using Power SYBR Green PCR Master Mix (Applied Biosystems, Life Technologies, Carlsbad, CA, USA) and the ABI 7500 Fast Real-Time PCR System (Applied Biosystems, Life Technologies, Carlsbad, CA, USA) under default conditions. Each sample was run in triplicate. Expression levels for mRNA were normalized to β-actin.

Antibodies
The primary antibodies used were HIP14 polyclonal antibody (in house, 1:400 for immunoblotting), β-tubulin monoclonal antibody (T8328, Sigma, RRID:AB_1844090,
the total time the mouse spent moving while making automated readouts were recorded. Ambulatory time is move about and explore for half an hour. A number of ing chamber (27 × 27 × 20.3 cm) and allowed to freely to the room, mice were placed in the center of the test-ning chamber following 1 h of acclimatization described [20]. Briefly, no later than 1 h after the begin-

Tissue lysis and western blotting analysis
Tissues were homogenized on ice for 5 min in one volume 1% SDS TEEN (TEEN: 50 mM Tris pH 7.5, 1 mM EDTA, 1 mM EGTA, 150 mM NaCl, and 1× complete protease inhibitor cocktail [Roche]) and subsequently diluted in four volumes 1% TritonX-100 TEEN for 5 min for further homogenization. Samples were sonicated once at 20% power for 5 s to shear DNA, and the insoluble material was removed by centrifugation at 14,000 rpm for 15 min.

Proteins in cell lysates were heated at 70 °C in 1× NuPAGE LDS sample buffer (Invitrogen) with 10 mM dithiothreitol (DTT) before separation by SDS-PAGE. After transfer of the proteins onto a nitrocellulose membrane, immunoblots were blocked in 5% milk TBS (TBS: 50 mM Tris pH 7.5, 150 mM NaCl). Primary antibody dilutions of HIP14 polyclonal antibody and β-tubulin monoclonal antibody in 5% BSA PBST (bovine serum al-

Behavior
All behavioral testing was performed with the tester blind to genotype. All testing was performed at 3 months of age (7 weeks post-TM injection). All of the apparatuses were cleaned between mice with 70% ethanol.

Spontaneous activity
Spontaneous activity in the dark was measured using the Med Associates activity monitoring system (Med Associates Inc., St Albans, VT, USA) as previously de-

Rotarod and climbing
Fixed-speed and accelerating rotarods were used to assess motor coordination (Ugo Basile, Comerio, Italy) as previ-

Pre-pulse inhibition (PPI)
PPI was performed using the Startle Response System (San Diego Instruments, San Diego, CA, USA) as previ-

Porsolt forced swim test
The Porsolt forced swim test was used to assess depressive-like behavior and was performed as previ-

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were allowed to acclimatize to the bottles for 1 week. Libitum access to food and to two water bottles. Mice were single housed in a full-size cage and were given compression, as previously described [26, 44]. Briefly, mice the loss of pleasure-seeking behaviors, a symptom of de-
 Sucrose preference was used to test for anhedonia, or Sucrose preference measure of anxiety-like behavior. The total time spent in the light box was scored as a recorded using a video camera through the light box side. Freely explore the apparatus, and their activity was re-
 The light-dark box was used to test for anxiety in an environment where escape was not possible, i.e., a completely enclosed environment. The Gemini Avoidance System (San Diego Instruments) was used for this purpose; no cues or shocks were used. The door between the two chambers was kept open so mice could freely explore both sides of the box, and on one side a light was shone through the transparent door to create a brightly lit light box. The door on the other side was blacked out to create a dark box. Mice were allowed to freely explore the apparatus, and their activity was recorded using a video camera through the light box side. The total time spent in the light box was scored as a measure of anxiety-like behavior.

Sucrose preference
Sucrose preference was used to test for anhedonia, or the loss of pleasure-seeking behaviors, a symptom of depression, as previously described [26, 44]. Briefly, mice were single housed in a full-size cage and were given ad libitum access to food and to two water bottles. Mice were allowed to acclimatize to the bottles for 1 week. On day 7 the water in one of the bottles was replaced with a 2% sucrose solution and the mice and both bottles were weighed. Twenty-four hours later the bottles were weighed again and the total fluid and sucrose intake were calculated as g/kg of body weight. Sucrose preference was calculated as follows: sucrose preference = (sucrose intake/total fluid intake) × 100.

Neuropathology
All neuropathological studies were conducted as previously described [12, 31]. Mice were anesthetized by intraperitoneal injection of 2.5% avertin and intracardially perfused with ice-cold 4% paraformaldehyde. Brains were harvested and post-fixed in 4% paraformaldehyde for 24 h at 4 °C, and then cryopreserved in 30% sucrose in phosphate-buffered saline (PBS). To determine the brain weight, the olfactory bulbs, paraflocculi, and brain stem were removed prior to weighing. The cerebellum was then removed and weighed separately. Forebrain weight was calculated as brain weight minus cerebellum weight. The forebrain was then flash frozen on dry ice, mounted with Tissue-TEK O.C.T. compound (Sakura Finetek, Torrance, CA, USA), and sectioned coronally on a cryostat (Microm HM 500 M) into 25-μm free-floating sections. Sections were stored until immunohistochemical processing in PBS with 0.01% sodium azide.

A series of 25-μm sections spaced 200 μm apart spanning the striatum were processed for stereological volumetric assessments by staining with NeuN antibody (1:1000, Millipore MAB377) overnight at room temperature to stain all neuronal nuclei. Sections were then stained with biotinylated anti-mouse antibody for 2 h (1:1000, Vector Laboratories BA-9200) and the signal was amplified using the Vectastain ABC kit for 30 min (1:1000, Vector Laboratories) and then detected with 3,3′-diaminobenzidine (DAB, Thermo Scientific). StereolInvestigator software (Microbrightfield Bioscience, Williston, VT, USA) was used to determine striatal, cortical, and corpus callosum volumes by tracing the perimeter of the desired structures; the volumes were determined using the Cavalieri principle.

Two additional series of sections described above were used for glial fibrillary acidic protein (GFAP) and ionized calcium-binding adapter molecule 1 (IBA1) immunohisto-logy. Sections were blocked with 3% H2O2 in PBS for 30 min, then washed with PBS. Sections were then blocked with 5% normal goat serum (NGS) in PBS-Triton for 30 min. Sections were incubated overnight at room temperature in either monoclonal mouse anti-GFAP-Cy3 antibody (1:500, Sigma-Aldrich) or polyclonal rabbit anti-IBA1 antibody (1:500, Wako), both solutions made in 1% NGS and PBS-Triton. Sections were then incubated for 2 h at room temperature in either biotinylated goat anti-mouse IgG antibody (1:500, Vector) or biotinylated goat anti-rabbit IgG antibody (1:500, Vector), both solutions
made in 1% NGS and PBS-Triton. Lastly, sections were incubated with the Vectastain ABC kit (1:1000, Vector) for 30 min and developed with DAB (1:10, Sigma-Aldrich) for 2 min. Sections were mounted on Superfrost Plus microscope slides (Fisher) prior to analysis. Sections were imaged on a Zeiss Axioplan 2 imaging system with a 5x Zeiss Plan-Neofluar objective using a Photometrics Cool Snap HQ camera. Analyses were done using MetaMorph software version 6.3 (Universal Imaging Corporation, Bedford Hills, NY). After delineating the cortex for each image, labeling of GFAP and IBA1 was identified using the threshold held at a constant level with background excluded for all images and then analyzed using the “integrated morphology” feature. Relative levels of GFAP and IBA1 staining were calculated as the sum of the integrated optical density (IOD) for each image divided by the area of the region selected, then multiplied by the sampling interval (8) and section thickness (25 μm). No staining was observed in a negative control without primary antibody [31].

Electrophysiology
Mice were transferred to the University of British Columbia (UBC) Animal Research Unit approximately 4–5 weeks following TM injections and all electrophysiological experiments were performed on mice that were approximately 3 months old. Electrophysiological analyses were performed as previously described [14]. Briefly, mice were anesthetized with isoflurane and brains were quickly removed and immediately placed in an ice-cold cutting solution that contained (in millimoles): 125 NaCl, 2.5 KCl, 25 NaHCO3, 1.25 NaH2PO4, 2.5 MgCl2, 0.5 CaCl2, and 10 glucose. Coronal blocks containing the striatum were then cut on a vibratome (Leica VT1200S) at 400 μm. Stratal sections were transferred to artificial cerebrospinal fluid (ACSF), which was the same as the cutting solution except that it contained 1 mM MgCl2 and 2 mM CaCl2, and were heated to approximately 32 °C for 30–45 min.

Following recovery, slices were transferred to a recording chamber with ACSF perfused at a rate of 2–3 ml/min. In stratal sections, spiny projection neurons in the dorsal striatum were targeted for recording [14]. For excitatory post-synaptic current (EPSC) recordings in the stratum, picrotoxin (50 μM) was added to the ACSF, but tetrodotoxin (TTX, 0.5 mM) was omitted, as we have previously shown that most EPSCs in our coronal slice preparation are action potential-independent [45]. However, while largely action potential-independent, these are referred to as spontaneous EPSCs (sEPSCs) in the manuscript to indicate the lack of TTX. Glass pipettes (3–6 MΩ) were filled with a potassium gluconate (KGlut) internal solution for sEPSC recordings [46]. The liquid junction potential (theoretical = −15.6 mV) was left uncorrected. sEPSCs were filtered at 1 kHz and digitized at 20 kHz. Where applicable, glutamate release was evoked by an ACSF-filled glass pipette (1 MΩ) placed 200–250 μm dorsal to the recorded cell. Paired pulse ratios (PPRs) were obtained at a −70 mV holding voltage, and various interpulse intervals were applied with a stimulus intensity known to generate a response approximately 30–40% of the maximal response. Basic membrane properties were obtained within 60 s following break-in by monitoring the current response to a 10-mV voltage step applied in the membrane test feature in Clampex 10 (Molecular Devices, Sunnyvale, CA, USA). All electrophysiological recordings were acquired and analyzed using the pClamp 10 software bundle.

Statistics
Data were analyzed using the Student’s t test, one-way ANOVA, or two-way ANOVA as indicated using Prism 5 software where all post hoc tests in ANOVA analyses used Bonferroni’s multiple comparison test. Error bars indicate standard error of the mean and in all graphs the mean is indicated. * p < 0.05, ** p < 0.01, *** p < 0.001. In all cases, except for Fig. 6, the number of replicates (N) refers to the number of individual mice used and is considered to mean biological replicates. In Fig. 6, N refers to the number of cells analyzed from a total of 4 mice per genotype. ANOVA values and exact numbers are listed in Additional file 3.

Additional files

Additional file 1: Rapidly progressing paralysis in an iHip14+/+/ mouse over the course of 5 h. An iHip14+/− mouse found with hind limb paralysis was monitored by video. The paralysis progressed rapidly over 5 h, beginning with the hind limbs, from where the mouse was able to move around the cage and eat until it could no longer move and was euthanized. The video lasts a total of 7 min, with the first minute from the beginning of the 5 h and the rest of the video just before the mouse was euthanized. (MOV 12680 kb)

Additional file 2: Sudden death due to rapidly progressive paralysis in an iHip14+/− mouse. A second iHip14+/− mouse was found almost completely paralyzed and was video monitored for a few minutes until it went into respiratory arrest and died. (MOV 6931 kb)

Additional file 3: One-way and two-way ANOVA values and replicates. (XLSX 40 kb)

Additional file 4: Individual data values for experiments where N < 6. (XLSX 44 kb)

Abbreviations
(DNAJC5): DnaJ heat shock protein 40 homolog; ACSF: Artificial cerebrospinal fluid; AMPAR: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; ANOVA: Analysis of variance; CKO: Conditional knockout region of Hip14; CSP: Cysteine string protein; DARPP-32: Dopamine- and cAMP-regulated neuronal phosphoprotein (PPP1R1B); Dr4HC: Asp-His-His-Cys; DTA: Diphtheria toxin A; EPM: Elevated plus maze; EPSC: Excitatory post-synaptic current; Flp: Flippase recombinase; FRT: Flippase recognition target; FVB/N: FVB/NJ mouse strain, Friend virus B NIH Jackson; G418: Geneticin; GABA, γ-amino butyric acid A (receptor); GluA1: AMPA receptor subunit 1 (GRA1); GluA2: AMPA receptor subunit 2 (GRIA2); GluN2A: NMDA receptor
subunit 2A (GRIN2A); GluN2B: NMDA receptor subunit 2B (GRIN2B); HD: Huntington disease; HIP14: Huntington interacting protein 14 (ZDHHC17); HTT: HD disease-causing protein huntingtin; lGluP: Locus of X-over P1, 34 bp Cre recombinase sequence from P1 bacteriophage; MAP: Mitogen-activated protein; MSN: Medium spiny neurons; NMDAR: N-methyl-D-aspartate receptor; PAT: Palmitoyl acyltransferase; PPI: Pre-pulse inhibition; PPR: Paired pulse ratio; PSD95: Post-synaptic density protein 95 (DLG4); SNAP25: Synaptosomal-associated protein 25; STREX BK: Stress-regulated exon splice variant of the calcium- and voltage-activated potassium channel; SYT1: Synaptotagmin 1; TM: Tannoxifen; WT: Wild type.

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Availability of data and materials
The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request. Individual data values in those experiments with N < 6 are given in Additional file 4.

Authors’ contributions
SS conceived of the project, analyzed and interpreted all biochemical and neuropathological data; produced, analyzed, and interpreted all behavior and survival data; assisted with the analysis and interpretation of the electrophysiological data; prepared all of the figures; and wrote the manuscript. MMP produced, analyzed, and interpreted all electrophysiological data and wrote the corresponding Methods sections. LAR assisted with the interpretation of the electrophysiological data. MRH assisted with the conception of the project, the experimental design, interpretation of the results, and the writing of the manuscript. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

Ethics approval
All procedures and animal work were approved by the University of British Columbia Committee on Animal Care in protocols A12-0063 and A16-0130.

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