Ganglioside GM1 induces phosphorylation of mutant huntingtin and restores normal motor behavior in Huntington disease mice

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Huntington disease (HD) is a progressive neurodegenerative monogenic disorder caused by expansion of a polyglutamine stretch in the huntingtin (Htt) protein. Mutant huntingtin triggers neural dysfunction and death, mainly in the corpus striatum and cerebral cortex, resulting in pathognomonic motor symptoms, as well as cognitive and psychiatric decline. Currently, there is no effective treatment for HD. We report that intraventricular infusion of ganglioside GM1 induces phosphorylation of mutant huntingtin at specific serine amino acid residues that attenuate huntingtin toxicity, and restores normal motor function in already symptomatic HD mice. Thus, our studies have identified a potential therapy for HD that targets a post-translational modification of mutant huntingtin with critical effects on disease pathogenesis.

Huntington disease (HD) is an inherited neurodegenerative monogenic disorder caused by the expansion of a polyglutamine stretch beyond 36 residues in the amino-terminal domain of huntingtin (Htt), a protein expressed in most tissues and cells. The mutation causes huntingtin to acquire toxic conformation/s and to affect neuronal function and viability. Medium-sized spiny neurons in the corpus striatum are most affected, but neurodegeneration also occurs in the cerebral cortex and, to a minor extent, in other brain areas, resulting in motor and psychiatric symptoms, as well as cognitive decline.

The cellular and molecular mechanisms underlying HD pathogenesis are complex. Both loss and gain of function of mutant huntingtin contribute to cause a wide array of neuronal dysfunctions affecting cell signaling, gene transcription, axonal transport, cell and mitochondrial metabolism as well as neurotransmission (1).

In recent years, a breakthrough in HD research has been the discovery that posttranslational modifications of mutant Htt are crucial modulators of mutant Htt toxicity (2–4). Phosphorylation at various serine residues prevents cleavage of mutant huntingtin into more toxic fragments, decreases neural cell death in vitro (5–10), and/or restores Htt functions that are compromised by the mutation (8, 11). The most dramatic effects have been described for huntingtin phosphorylation at serine 13 and serine 16. These two amino acid residues are part of the highly conserved amino-terminal “N17” domain of huntingtin, a domain that regulates huntingtin intracellular localization and association to cellular membranes (12, 13), as well as kinetics of mutant huntingtin aggregation (14, 15). Phosphomimetic mutations of serine 13 and serine 16 by aspartic or glutamic acid substitution (S13D and S16D or S13E and S16E) decrease the toxicity of mutant huntingtin fragments in vitro (10, 16). In line with these studies, expression of a phosphomimetic (S13D and S16D) mutant form of expanded full-length huntingtin in a BACHD transgenic mouse model was shown to result in a normal phenotype, with no detectable signs of HD pathology by 12 mo (17).

These findings suggest that pharmacological interventions that modulate cell signaling and mutant huntingtin phosphorylation might slow down or even stop disease progression.

Recently, we and other groups reported that levels of GM1, a ganglioside involved in cell signaling (18), are decreased in HD models (19–21), in fibroblasts isolated from HD patients (19), and in postmortem human HD brain samples (20, 21). Gangliosides are sialic acid-containing glycosphingolipids highly abundant in the brain, where they exert a plethora of important cell regulatory functions (18). They are major components of membrane microdomains known as “lipid rafts” (22) and are important players in cell signaling (23) and cell–cell interaction (24). By influencing membrane properties and/or by direct interaction with membrane proteins, gangliosides modulate the activity of many tyrosine kinase (25–28) and neurotransmitter receptors (29), ion channels (30, 31), and downstream cell signaling pathways. In addition, gangliosides regulate axon–myelin communication and the maintenance of myelinated axons in the adult central and peripheral nervous systems (32–34).

Consistent with the pivotal role of gangliosides in the nervous system and in cell signaling, defects in their biosynthetic pathway lead to a severe infantile neurodegenerative disorder characterized by progressive brain atrophy, chorea, and epilepsy (35), symptoms that are also common to the juvenile form of HD (36).

We hypothesized that in HD, decreased GM1 levels contribute to neuronal dysfunction and disease pathogenesis. Supporting this hypothesis, we demonstrated that restoring normal GM1 levels in an HD neural cell line stimulates the activation of prosurvival cell signaling pathways and provides protection from apoptosis. As a corollary, inhibiting GM1 synthesis in wild-type striatal cells recapitulates the increased susceptibility to apoptosis that is observed in HD neuronal cells (19).

In this study, we have explored the therapeutic potential of restoring GM1 levels in a transgenic HD mouse model. We demonstrate that GM1 infusion abrogates the motor deficit of yeast artificial chromosome (YAC)128 mice, an effect that is accompanied by increased expression and activation of striatal dopamine and adenosine 3',5'-monophosphate-regulated phosphoprotein (32 kDa) (DARPP-32), as well as phosphorylation of huntingtin at serine 13 and serine 16, in vivo.

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Results

Chronic Infusion of GM1 Restores Normal Motor Behavior in YAC128 Mice

To assess the therapeutic potential of GM1 in HD, and to avoid potential confounding effects from peripheral drug administration and limited blood–brain barrier permeability, we developed a protocol for chronic intraventricular infusion of the ganglioside in a well-characterized transgenic mouse model of HD, the YAC128 mouse. YAC128 mice express the full-length mutant huntingtin protein and recapitulate the motor deficit and other salient features of the human pathology (37). YAC128 and wild-type littermates were infused with GM1 or vehicle (artificial cerebrospinal fluid, CSF) for 28 d. Motor behavior was analyzed before and at various time points during the infusion period.

Chronic infusion of GM1 restored normal GM1 levels in the striatum and the cortex of YAC128 mice (Fig. S1). At the time when the treatment with ganglioside GM1 was initiated, 5-mo-old YAC128 mice already showed severe motor impairment compared with wild-type (WT) littermates (Fig. 1 *A*, *C*, and *D*, time point –4 in all graphs), as assessed using a battery of motor behavior tests. These included the rotarod, a gold standard in the qualitative analysis of motor behavior in HD mice, as well as the horizontal ladder walking test and the narrow beam, two highly discriminatory motor tests for the assessment of cortical and subcortical dysfunction (38, 39). Remarkably, after 2 wk of GM1 intraventricular infusion, normal motor function was recovered in YAC128 mice compared with WT littermates, in all of the tests performed (Fig. 1). At fixed rotarod speed (20 rpm), YAC128 mice were able to remain on the rotarod for less than 30 s. As expected, intraventricular infusion of artificial CSF vehicle did not improve their performance. On the contrary, YAC128 mice that received GM1 improved dramatically and by the end of the treatment could finish the 60-s test like most of the WT mice (Fig. 1*A*). As this test was only moderately challenging for WT mice and we wanted to assess the true extent of recovery in YAC128 mice, we repeated the test with an accelerating rotarod, in

conditions that were very challenging even for WT mice (from 4 to 40 rpm in 1 min). Even in these conditions, GM1-treated YAC128 mice performed similarly to WT littermates (differences were not statistically significant) (Fig. 1*B*). Complete rescue of normal motor functions was also observed when YAC128 mice were tested in the ladder walking task (Fig. 1*C* and Movie S1) and in the narrow beam test (Fig. 1*D* and Movie S2), which require balance and fine motor skills (38, 39). The beneficial effects of GM1 extended up to 14 d after discontinuation of the treatment (Fig. S2). No effects on motor behavior were observed in WT mice infused with GM1 or in YAC128 mice infused with the artificial CSF vehicle (Fig. 1). Importantly, no evident signs of treatment-related toxicity or illness were detected at any time during the experimental protocol.

GM1 Increases DARPP-32 Expression and Activation in the Striatum of YAC128 Mice

DARPP-32 is highly enriched in medium-sized spiny neurons and its down-regulation is an early marker of neuronal dysfunction in HD (40). Normalization of motor behavior in GM1-treated YAC128 mice correlated with increased striatal expression of DARPP-32 (Fig. 2). Treatment with GM1 also restored normal levels of DARPP-32 phosphorylation at threonine 34 in the striatum of YAC128 mice, suggesting overall normalization of cell signaling and/or dopaminergic pathways in medium spiny neurons (41, 42).

GM1 Triggers Phosphorylation of Huntingtin at Serine 13 and Serine 16

The dramatic effects exerted by GM1 in YAC128 mice suggest that the ganglioside might decrease overall mutant huntingtin toxicity. As phosphomimetic mutations of serine 13 and serine 16 result in abrogation of mutant huntingtin toxicity in a BACHD model (17), we tested whether GM1 could increase phosphorylation of huntingtin at these two critical amino acid residues. Using a phospho-specific antibody (pN17) (16) that recognizes the amino-terminal N17 domain of huntingtin (the first 17 amino

Fig. 1. GM1 restores normal motor behavior in YAC128 mice. Behavioral tests were conducted at the indicated time, on 5-mo-old YAC128 and WT mice, before and during GM1 chronic brain infusion. Each data point represents the average performance $\pm$ SD of six mice. (*A*) Rotarod test at fixed speed (20 rpm for 60 s). YAC128 mice treated with GM1 showed progressive improvement and, by the end of the treatment, were able to finish the test like most WT mice. The horizontal gray line in the graph marks the test endpoint. (*B*) Accelerating rotarod (4–40 rpm in 1 min). In this challenging test, YAC128 mice treated with GM1 performed as well as WT mice (differences between WT and GM1-treated YAC128 mice were not statistically significant). (*C*) Horizontal ladder test. The ability of mice to cross a horizontal ladder with irregular rung pattern was analyzed. A score was assigned to each type of footfall and other mistakes made by the mice according to ref. 38. (*D*) Narrow beam test. Motor performance was scored as the mice walked along a narrow beam (100 cm long, 0.75 cm wide). # $P < 0.01$ (YAC128 vs. WT); * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (GM1-treated vs. CSF-treated YAC128).

acids) only when serine 13 and serine 16 are phosphorylated, we detected a significant increase of huntingtin phosphorylation at serines 13 and 16 in primary cultures of neurons isolated from YAC128 or WT littermates, by immunocytochemistry (Fig. 3A) and immunoblotting (Fig. S3A), after incubation with GM1 for 5 h. Similar results were obtained using immortalized striatal homozygous knock-in cells expressing mutant huntingtin (STHdh^{111/111} cells; Fig. 3B). Thus, GM1 can induce phosphorylation of huntingtin at those critical residues both in normal and HD cells. Treatment with the ganglioside triggered huntingtin phosphorylation also in fibroblasts derived from HD patients (Fig. 3C and Fig. S3B), demonstrating that the underlying mechanism is shared between animal and human models and that a GM1-based therapy is likely to have beneficial effects in HD patients.

We confirmed that GM1 induces phosphorylation at serines 13 and 16 by incubation of striatal knock-in cells (STHdh^{111/111}) with GM1, followed by immunoprecipitation of mutant huntingtin with a polyclonal anti-huntingtin antibody (16) and immunoblotting with three phospho-specific antibodies: pN17 (16) and anti-pSer13 and anti-pSer16 antibodies (10), which recognize phosphoserine 13 and phosphoserine 16, respectively (Fig. 3D). Increased huntingtin phosphorylation at serine 13 and serine 16 after treatment with GM1 was also detected after direct immunoprecipitation of phosphohuntingtin using pN17 antibodies (Fig. 3E). More phosphohuntingtin was immunoprecipitated with pN17 after treatment with GM1 and the results were confirmed by immunoblotting with anti-pSer13 and anti-pSer16 antibodies.

Interestingly, on SDS/PAGE analysis, phosphohuntingtin consistently migrated at a much higher apparent molecular weight (hmv-Htt) (Fig. 3D and E) than the monomeric full-length huntingtin (FL-Htt), regardless of the length of the polyglutamine stretch (Fig. 3F and Fig. S3), and consistent with the formation of a high molecular-weight protein complex or huntingtin dimers. All anti-huntingtin and anti-phosphohuntingtin antibodies used in this study recognized the complex, although mAb2166 with low

affinity. A positive signal with mAb2166 was seen in most cases only after overexposure of the immunoblot (Fig. 3E and Fig. S4). This is not surprising, as binding of mAb2166 antibodies has been shown to be affected by huntingtin conformational changes induced by expansion of the polyQ stretch (43) and/or huntingtin posttranslational modifications (10).

Phosphorylation of huntingtin was required for the formation and/or stabilization of the high molecular-weight huntingtin complex, both in wild-type and HD cells, because dephosphorylation of the cell lysate with shrimp alkaline phosphatase (SAP) resulted in a decrease of the high molecular-weight form of huntingtin and the increase of monomeric full-length huntingtin levels (Fig. 3F).

Confirming our *in vitro* studies, GM1 treatment resulted in a significant increase in huntingtin phosphorylation at serine 13 and 16 *in vivo* in the cortex and striatum of both YAC128 and WT mice that were chronically infused with GM1 (Fig. 4).

Discussion

The complexity and variety of cellular pathways that are affected in HD has long hampered the development of effective therapies for this disease. In recent years, the discovery that posttranslational modifications can dramatically affect mutant huntingtin toxicity has opened the door to novel potential therapeutic strategies that target cell signaling pathways and factors responsible for critical huntingtin protein modifications.

GM1 is a lipid with important modulatory roles in the nervous system (18). In HD, decreased levels of this and other gangliosides, as we recently reported (19), are likely to affect cell signaling and neuronal function. Our previous studies showed that in a knock-in cell model expressing full-length mutant huntingtin, decreased levels of ganglioside GM1 contributed to heightened cell susceptibility to apoptosis. Restoring normal GM1 levels reverted the phenotype of HD cells to normal, suggesting that GM1 could have a therapeutic action in HD models. The *in vivo* studies herein described strongly support this conclusion. To date, GM1 is the only treatment that leads to a complete rescue of the motor phenotype in an HD mouse model, even after disease onset. At the time when the treatment was initiated, YAC128 mice already displayed profound motor deficits (Fig. 1), yet GM1 infusion was able to restore normal motor functions in 14 d of treatment. Interestingly, GM1-treated YAC128 mice performed significantly better than CSF-treated mice for over 14 d after discontinuation of the treatment (Fig. S2). These results suggest that GM1 is able to decrease mutant huntingtin toxicity and to correct abnormal homeostatic mechanisms that develop over time and that are responsible for the overall malfunction, and eventually death, of HD neurons. These conclusions are also supported by our data showing that GM1 restores normal levels of DARPP-32 expression and phosphorylation in the striatum of YAC128 mice (Fig. 2). Decreased DARPP-32 expression is an early sign of neuronal dysfunction in the R6/2 mouse model of HD (40), and has also been reported in YAC128 mice (44). DARPP-32 plays a crucial role at the crossroad of dopaminergic and other signaling pathways, which converge to determine overall expression level and phosphorylation state (resulting in specific protein functions) of DARPP-32 in medium spiny neurons. In turn, DARPP-32 integrates neuronal responses to a variety of neurotransmitters and stimuli and modulates striatum output pathways (41, 42, 45).

Restoring normal GM1 concentration in the brain of YAC128 mice likely resulted in a plethora of beneficial effects, leading to the therapeutic endpoints described in this study. The extent of such effects also suggests that GM1 targets early steps in the cascade of pathogenic events triggered by mutant huntingtin in HD brains. Indeed, we found that GM1 affects mutant huntingtin itself, by increasing phosphorylation at huntingtin serine 13 and serine 16 (Figs. 3 and 4). *In vitro* studies have shown that concomitant phosphorylation of these two amino acid residues

Fig. 2. GM1 restores striatal expression of DARPP-32 in YAC128 mice. Representative immunoblot and Li-Cor Odyssey densitometric analysis of DARPP-32 expression and activation (p-DARPP-32) in WT and YAC128 mice. GM1 infusion for 28 d restores normal levels of DARPP-32 and active p-DARPP-32 in YAC128 mice. Each lane of the immunoblot represents one individual mouse. Graphs show the densitometric analysis performed on three (WT GM1) or six (all other experimental groups) mice per group. Bars represent the mean values $\pm$ SD. * P < 0.05; ** P < 0.01.

Fig. 3. GM1 administration elicits huntingtin phosphorylation at serine 13 and serine 16. (A) Representative confocal microscopy images and relative quantitative analysis of primary striatal neurons isolated from wild-type (WT) and YAC128 mice and incubated for 5 h with 50 μ M GM1 (+) or vehicle (–). Neurons were immunostained with anti-phospho-N17 (pN17) antibody, which recognizes the amino-terminal N17 peptide of huntingtin phosphorylated at amino acid residues serine 13 and serine 16, and with DAPI to visualize nuclei. (B) Representative epifluorescence microscopy images and quantitative analysis of immortalized knock-in striatal progenitor cells expressing mutant huntingtin (STHdh^{111/111}) and treated as in A. (C) Confocal microscopy images and quantitative analysis of primary fibroblasts from HD patients treated as in A. Graphs in A–C show pN17 immunostaining mean fluorescence intensity (MFI) per pixel $\pm$ SD, calculated over a minimum of 100 cells per experimental group. ** P < 0.01; *** P < 0.001. (D and E) Analysis of mutant huntingtin phosphorylation state by immunoprecipitation and immunoblotting. Striatal knock-in cells (STHdh^{111/111}) were incubated with 50 μ M GM1 for the indicated time (10m, 10 min). Mutant huntingtin was immunoprecipitated from equal amounts of total cell lysates using a rabbit polyclonal anti-huntingtin antibody (N17) (in D), phospho-specific pN17 antibodies (in E), or nonspecific rabbit IgG antibodies as negative control (D). Total lysate from cells expressing wild-type huntingtin (STHdh^{7/7}) was loaded in the same gel as reference. All immunoprecipitated material (IP) was immunoblotted with the indicated phospho-specific and anti-huntingtin antibodies. Phosphohuntingtin could not be detected in the total lysates (input lanes, 30 μ g of proteins loaded) due to the proximity of the highly immune-reactive immunoprecipitated material in adjacent lanes. The results of reprobing the input lanes only, after cutting the membrane, are shown in D, Right. An increase in the phosphorylation of huntingtin at serine 13 and serine 16 after treatment with GM1 is evident in both immunoprecipitated material and input total cell lysates. The graph in D shows the densitometric analysis of huntingtin phosphorylation in the input lysates of two independent experiments. A Ponceau red-stained band in the membrane was used as loading control. S, stacking portion of the gel. (F) Wild-type and mutant huntingtin in total cell lysate from striatal knock-in cells (STHdh^{7/7} and STHdh^{111/111}) was dephosphorylated using shrimp alkaline phosphatase (SAP). Dephosphorylation resulted in a dramatic change in huntingtin electrophoretic mobility, with increased amount of protein migrating at the lower apparent molecular weight (FL-Htt) and a decreased amount of high molecular-weight species (hmw-Htt). The graph shows the ratio between hmw-Htt and FL-Htt before and after dephosphorylation with SAP.

Fig. 4. GM1 infusion induces huntingtin phosphorylation in vivo. Total protein lysates were prepared from the cortex and the striatum of YAC128 mice and WT littermates chronically infused with artificial cerebrospinal fluid (CSF) or GM1 in CSF for 28 d (six mice per group). (A) Representative immunoblots showing increased phosphorylation of huntingtin at serine 13 and serine 16 in GM1-infused brains, as detected using pN17 antibody. Each lane corresponds to the lysate from one mouse. (B) Li-Cor Odyssey infrared densitometric analysis of phosphohuntingtin normalized over talin (loading control). Bars represent the mean $\pm$ SD of six mice per experimental group. * $P < 0.05$; *** $P < 0.001$.

decreases the toxicity of mutant huntingtin (10, 16). In vivo, phosphomimetic mutations at the same amino acids protect BACHD transgenic models from the development of HD-like pathology. Thus, mutant huntingtin phosphorylation at these critical sites might explain, at least in part, the therapeutic action of GM1 in HD mice.

It has been proposed that huntingtin phosphorylation at serine 13 and serine 16 might decrease mutant huntingtin toxicity by changing protein conformation (16, 17), huntingtin function (16), and/or rate of huntingtin degradation (10). In a recent study, N-terminal fragments of mutant huntingtin in which serine 16 was changed to aspartic acid (mimicking phosphorylation at serine 16), were shown to preferentially accumulate and form aggregates in the cell nucleus. Although the consequences of huntingtin serine 16 phosphorylation on cell viability were not assessed in that study, it was proposed that phosphorylation at this amino acid residue might be important for toxicity mediated by mutant huntingtin fragments (46). Thus, the consequences of huntingtin phosphorylation at serine 16 on cell functions and viability might depend on whether only serine 16 (46) or both serine 13 and 16 (16, 17, 47, and this study) are phosphorylated, on protein context (full-length huntingtin versus N-terminal fragments) and, potentially, on the co-occurrence of other concomitant posttranslational modifications of mutant huntingtin (2, 10, 17, 48). Elucidating the signaling pathways involved in regulating huntingtin posttranslational modifications and their cross-talk will be of utmost importance.

In our studies we observed that huntingtin phosphorylated at serine 13 and serine 16 consistently migrated at higher molecular weight than predicted, consistent with the formation of an SDS-insoluble protein complex or dimer. The ability of huntingtin to form homodimers has been predicted by computational analysis and demonstrated in a yeast two-hybrid system using huntingtin fragments (49), and in preparations of full-length huntingtin purified from an insect cell expression system (50). Currently, the relevance of the formation of a high molecular-weight huntingtin complex or protein dimerization to huntingtin function is unknown.

We found that the ability to detect the high molecular-weight form of huntingtin by immunoblotting depends on protein transfer conditions (*Materials and Methods*), perhaps explaining

why only in a few other studies this form of huntingtin was detected (but not highlighted) (10, 51, 52).

In conclusion, our data support the use of GM1 in clinical setting for the treatment of HD. In this regard, previous clinical trials in which the ganglioside was used for the treatment of other conditions (35, 53–55) demonstrated that GM1 is safe to use in patients, even when administered by intraventricular infusion (56), and potential adverse effects are rare (35).

Materials and Methods

Animal Models. YAC128 mice were purchased from The Jackson Laboratory and maintained in our animal facility at the University of Alberta. All procedures on animals were approved by the University of Alberta's Animal Care and Use Committee and were in accordance with the guidelines of the Canadian Council on Animal Care. All in vivo experiments were performed in 5- to 6-mo-old male YAC128 mice and WT littermates that showed no sign of distress and weighed no less than 26 g and no more than 34 g.

Chronic GM1 Administration in Vivo. A microcannula was stereotactically inserted into the right ventricle (1.25 mm right lateral and 0.6 mm posterior to bregma, 3 mm deep) of anesthetized mice, and connected to an osmotic pump (Alzet; model 2004) that was implanted s.c. on the back of the mouse. The pump infused a solution of 3.6 mM GM1 in artificial CSF into the brain ventricle at a constant rate (0.25 μ L/h) for 28 d. On the basis of volume and rate of synthesis/renewal of the mouse CSF (57), these conditions result in a concentration of 50 μ M GM1 in the mouse CSF at equilibrium. Experiments were performed with both natural GM1 (highly purified, $\geq 98\%$, from bovine brain; Alexis) and synthetic GM1 provided by Seneb BioSciences Inc., with virtually identical results. Animals were monitored on a daily basis for signs of treatment-related toxicity, such as poor grooming, lethargy, loss of body weight, and abnormal behavior.

Huntingtin Immunoprecipitation and Immunoblotting. Cells were incubated with 50 μ M GM1 for 10 min or 5 h and then lysed in 20 mM Tris, pH 8.0, 150 mM NaCl, 1% Triton X-100, 0.5% Na-deoxycholate, 0.1% SDS, 1 mM EDTA, 1 mM EGTA, containing protease inhibitor mixture (1:100; Sigma-Aldrich), 50 μ M proteasomal inhibitor MG132 (Sigma-Aldrich), and phosphatase inhibitors (20 mM NaF, 2 mM Na_3VO_4). Cells were homogenized through a 26-gauge syringe needle and sonicated three times, 10 s each, followed by incubation on ice for 30 min. Lysates were centrifuged at $10,000 \times g$ for 10 min to remove debris. Immunoprecipitation was performed on 0.8 mg of total lysate using polyclonal anti-huntingtin (N17) or anti-phosphohuntingtin (pN17) (16) complexed to protein G sepharose (Zymed, Invitrogen). The immunoprecipitated protein was resolved on 6% SDS/PAGE and transferred overnight on PVDF membrane (Millipore) in 25 mM Tris, 192 mM glycine containing 0.05% SDS and 16% methanol. These transfer conditions allow for optimal transfer of high molecular-weight forms of huntingtin. Huntingtin detection

was performed by immunoblotting with polyclonal anti-huntingtin (PW0595 and N17; 1:10,000), polyclonal anti-pN17 antibody (1:10,000 (16), monoclonal anti-huntingtin (mAb2166, 1:5,000; Chemicon), polyclonal anti-pSer13 (1:1,000), and anti-pSer16 (1:500 (10). HRP-conjugated secondary antibodies were used at 1:10,000 dilution (Bio-Rad Laboratories). Protein bands were detected by ECL Plus and quantitated with Quantity One software (Bio-Rad Laboratories).

Statistical Analysis. Two-way ANOVA followed by Bonferroni posttest was used to compare treatment groups in the accelerated rotarod, narrow, beam, and horizontal ladder tests, as well as in the analysis of DARPP-32 expression. The Wilcoxon test (nonparametric) was used to analyze data from the rotarod task at fixed speed. Two-tailed *t* test was used to compare the

mean fluorescence intensity of GM1-treated versus GM1-untreated samples in immunocytochemistry experiments and in all other experiments.

Additional methods are described in [SI Materials and Methods](#).

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