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Huntingtin Controls Neurotrophic Support and Survival of Neurons by Enhancing BDNF Vesicular Transport along Microtubules

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Summary

Polyglutamine expansion (polyQ) in the protein huntingtin is pathogenic and responsible for the neuronal toxicity associated with Huntington's disease (HD). Although wild-type huntingtin possesses antiapoptotic properties, the relationship between the neuroprotective functions of huntingtin and pathogenesis of HD remains unclear. Here, we show that huntingtin specifically enhances vesicular transport of brain-derived neurotrophic factor (BDNF) along microtubules. Huntingtin-mediated transport involves huntingtin-associated protein-1 (HAP1) and the p150^{Glued} subunit of dynactin, an essential component of molecular motors. BDNF transport is attenuated both in the disease context and by reducing the levels of wild-type huntingtin. The alteration of the huntingtin/HAP1/p150^{Glued} complex correlates with reduced association of motor proteins with microtubules. Finally, we find that the polyQ-huntingtin-induced transport deficit results in the loss of neurotrophic support and neuronal toxicity. Our findings indicate that a key role of huntingtin is to promote BDNF transport and suggest that loss of this function might contribute to pathogenesis.

Introduction

Polyglutamine repeat expansions are the cause of inherited neurodegenerative disorders (Ross, 2002) including Huntington's disease (HD). HD is characterized by uncontrolled movements, personality changes, and dementia and causes the death of patients within 10–20 years after the appearance of the first symptoms. The neuropathology in HD involves a significant dysfunction and death of neurons, particularly the medium spiny neurons of the striatum. Neuronal loss, however, could be secondary to neuronal dysfunction. In support, motor and cognitive deficits have been detected in patients and in murine models of HD before any neurodegeneration is detected (Lione et al., 1999; Smith et al., 2000).

The pathogenic mechanisms induced by polyQ-huntingtin (polyQ-htt) are still not clearly understood but could involve the gain of a new toxic function and/or the loss of beneficial activities of wild-type htt (Cattaneo et al., 2001; Ross, 2002). Htt is a brain-enriched protein that is essential for embryonic development and neurogenesis (White et al., 1997; Zeitlin et al., 1995). Htt possesses antiapoptotic properties, as demonstrated by an increased programmed cell death during development in mice lacking htt (Zeitlin et al., 1995). These beneficial properties of wild-type htt are necessary in adults, as late inactivation of htt in a conditional mouse model leads to progressive neurodegeneration (Dragatsis et al., 2000). Wild-type htt is protective in neuronal cultures (Cattaneo et al., 2001) and against death induced by polyQ-htt in vivo (Leavitt et al., 2001).

Although htt is observed in the nucleus, it is predominantly found in the cytoplasm where it associates with vesicular structures and microtubules (MTs). In fact, htt associates with various proteins that could play a role in intracellular trafficking (Harjes and Wanker, 2003). In particular, htt interacts with the huntingtin-associated protein-1 (HAP1), a protein that is transported in axons and associates with p150^{Glued} dynactin subunit, an essential component of the dynein/dynactin microtubule-based motor complex (Block-Galarza et al., 1997; Engelender et al., 1997; Li et al., 1995, 1998; Schroer, 1996). Thus, htt could participate in the vesicular transport. Consistent with these studies, htt and other pathogenic polyQ-containing proteins affect fast axonal transport (Gunawardena et al., 2003; Szebenyi et al., 2003). However, the molecular mechanisms leading to defect in transport and the consequences on the degeneration of specific neurons were not established.

The antiapoptotic properties and the emerging role of htt in intracellular trafficking led us to hypothesize that htt could play a crucial role in controlling the transport of neurotrophic factors. Among these, brain-derived neurotrophic factor (BDNF) is of particular importance for HD, as BDNF is produced by cortical neurons and transported from the cortex to the striatum (Altar et al., 1997), where it acts as a prosurvival factor for the striatal neurons (Baquet et al., 2004; Saudou et al., 1998). Moreover, BDNF levels are decreased in the brain of HD patients (Ferrer et al., 2000). While this decrease has been linked to a decrease in BDNF transcription (Zuccato et al., 2001), it could also be explained by a defect in the transport of this neurotrophic factor.

To examine whether htt could control neuronal sur-

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vival by promoting the transport of vesicles containing BDNF, we analyzed the distribution and dynamics of BDNF-eGFP-containing vesicles in real time by ultrafast 3D videomicroscopy followed by deconvolution. BDNF-eGFP has biochemical and biological characteristics indistinguishable from those of BDNF (Hartmann et al., 2001; Kohara et al., 2001). We demonstrate here that htt is a processivity factor that increases the transport efficiency of BDNF-containing vesicles along MTs. In the pathological situation, BDNF transport is affected as polyQ-htt via the HAP1 protein disrupts the association of key components of the motor machinery to MTs. Reduced BDNF transport leads to a decrease in neurotrophic support and to neurotoxicity, which are both rescued by wild-type htt. These results demonstrate that the antiapoptotic properties of htt are linked to the ability of htt to promote transport of BDNF in the brain.

Results

Huntingtin Regulates the Dynamics of BDNF-Containing Vesicles

We first analyzed the distribution of endogenous wild-type and polyQ-htt with BDNF in neuronal cell lines derived from knockin mice (Trettel et al., 2000). These cell lines carry either two copies of wild-type htt (wild-type neuronal cells, +/+) or two copies of mutant htt (homozygous mutant neuronal cells, 109Q/109Q). 3D deconvolution microscopy revealed a partial colocalization of endogenous BDNF with htt, but no obvious differences were found with polyQ-htt (Figure 1A). This partial colocalization was confirmed biochemically (see Supplemental Figure S1 at http://www.cell.com/cgi/content/full/118/1/127/DC1).

We next examined the effect of htt upon the dynamics of BDNF-containing vesicles using fast 3D videomicroscopy followed by deconvolution. NG108-15 neuroblastoma cells were transfected with BDNF-eGFP and either wild-type full-length htt (wt-FL-htt), polyQ-FL-htt or the corresponding empty vector. We monitored the movement of vesicles by acquiring images in 3D. After deconvolution, a 2D reconstruction of each time point was performed. The overlapping of vesicles in comparison to their previous localization was drastically decreased by expression of wt-FL-htt (Figure 1B). In contrast, when htt contained the polyQ expansion, vesicular movement was comparable to the BDNF-eGFP alone. We calculated the mean percentage of colocalization of vesicles between two successive time points (every 1.5 s) for the first ten acquisitions. We found the percentage of static vesicles to be significantly reduced from 43.3% to 23.7% by the expression of wt-FL-htt (Figure 1C).

We tracked BDNF-containing vesicles in NG108-15 cells and analyzed the influence of wt or polyQ-FL-htt on vesicle velocities (Figure 1D). Velocities were determined by comparing the x and y coordinates of one vesicle every 1 or 2 s over 1 min and were typically calculated for each condition from two to three independent transfections with a total of about 1000–5000 measures in 5–17 independent cells. Under control conditions, BDNF vesicles moved at an average velocity of 8.62 μm/min, which was comparable to previously reported values (Kohara et al., 2001). Expression of wt-FL-htt induced a significant increase in the mean vesicular velocity, while polyQ-FL-htt did not. We also determined the pausing time of vesicles corresponding to the time vesicles spend without moving and observed that expression of wt-FL-htt but not polyQ-FL-htt significantly decreased this value (Figure 1E). Analysis of velocity distribution showed that wt-FL-htt significantly increased the number of vesicles that have a velocity greater than 42 μm/min (see Supplemental Figure S2 at *Cell* web site).

To unequivocally confirm that the stimulatory effect on vesicular transport is due to the presence of htt, we decreased htt expression by small interfering RNAs (siRNAs; Figure 1F). Tracking of moving vesicles revealed that a reduction in htt increased the percentage of static vesicles and reduced their velocities while increasing their pausing times (Figures 1G).

Thus, we demonstrate that one function of htt is to enhance intracellular transport of BDNF-containing vesicles. This function is altered when htt expression is reduced or when htt contains the polyQ expansion.

BDNF Vesicular Transport Is Specifically Altered in HD

To establish whether our findings are relevant to HD pathogenesis, we analyzed BDNF dynamics in primary cultures of cortical neurons. In comparison to the control condition, wt-FL-htt but not polyQ-FL-htt significantly increased the mean velocity of vesicles (Figure 2A and Supplemental Movies S1, S2, and S3).

We next assessed BDNF dynamics in more physiological conditions using neuronal cell lines derived from knockin mice where a CAG expansion has been inserted into the endogenous mouse htt gene (Trettel et al., 2000). These cell lines carry either two copies of wild-type htt (wild-type neuronal cells, +/+), one copy of wild-type htt and one of mutant htt (heterozygous neuronal cells, +/109Q), or two copies of mutant htt (homozygous mutant neuronal cells, 109Q/109Q). These cell lines reflect the closest situation to HD patients, as, in these cells, wild-type or polyQ-htt are expressed at endogenous levels. The mean velocity of BDNF vesicles was significantly decreased in +/109Q and 109Q/109Q cells compared to +/+ cells (Figure 2B). Interestingly, the alteration in transport was similar in heterozygous and homozygous mutant neuronal cells. This finding is in agreement with the genetic dominance in HD and suggests that mutant polyQ-htt could act in a dominant manner to alter transport.

Finally, by ectopically expressing wt or polyQ-FL-htt in 109Q/109Q cells, we tested whether the loss of intracellular transport of BDNF in polyQ-htt condition could be rescued by wild-type htt (Figure 2C). Expression of wild-type htt increased velocity in 109Q/109Q cells, while polyQ-htt had no effect.

In addition to htt, polyQ expansion in unrelated proteins is the cause of other neurodegenerative disorders, including spinal and cerebellar ataxias such as SCA3 and SCA7. Therefore, we tested the effect of wild-type and polyQ forms of ataxin-3 and -7 on BDNF transport. Strikingly, these proteins had neither a stimulatory nor an inhibitory effect on BDNF transport (Figure 2D). This

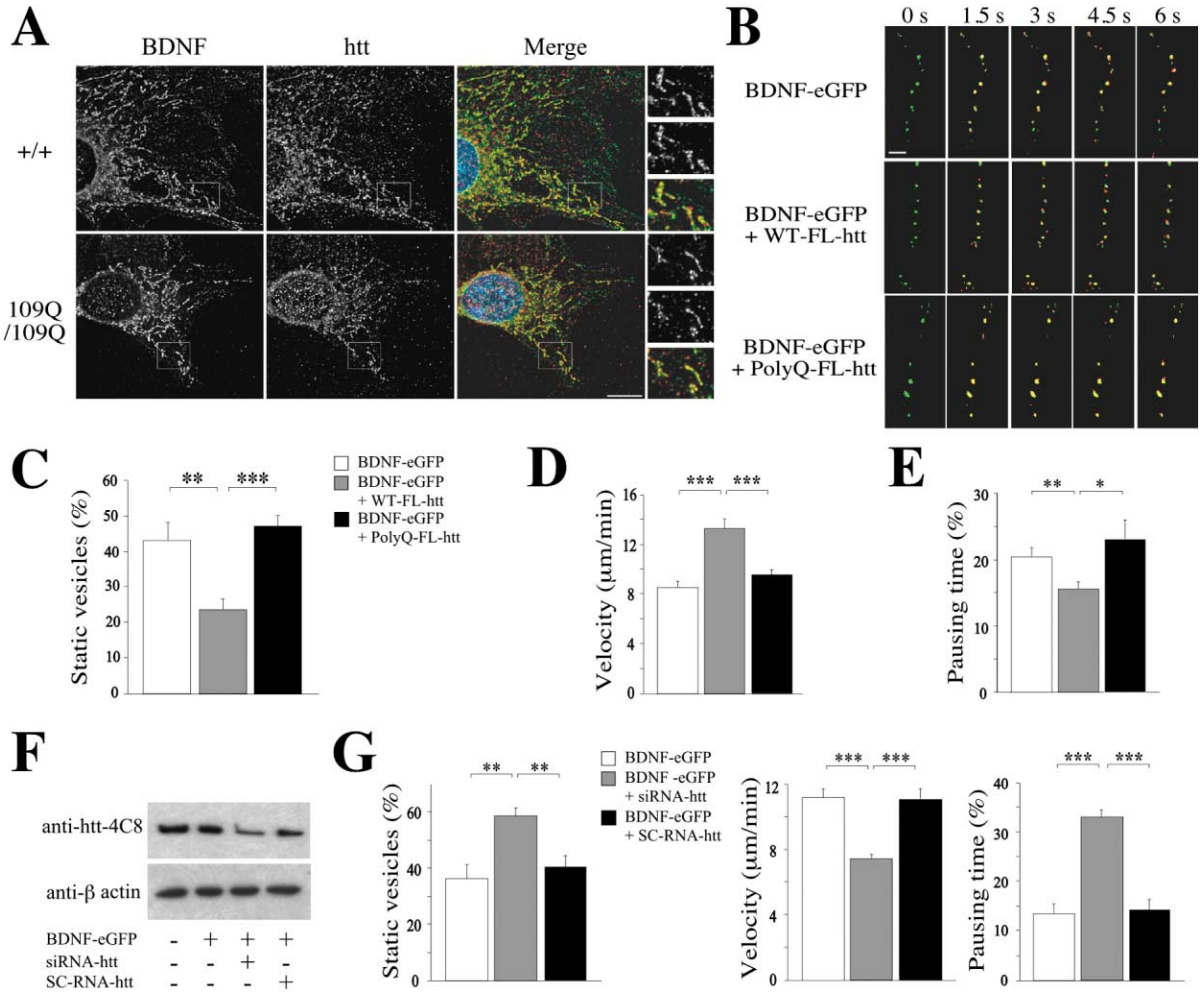

Figure 1. Htt Colocalizes with BDNF and Stimulates BDNF Vesicular Trafficking in Neuronal Cells

(A) Colocalization (yellow) of endogenous BDNF (green) and htt (red, 4E6 antibody) in +/+ and 109Q/109Q neuronal cells. Similar colocalization is observed between BDNF and wild-type or polyQ-htt. The small right panels correspond to a 2 × enlargement of the boxes present in the low magnification panels. (B) Overlapping of BDNF vesicles at different time points. Static vesicles appear in yellow and are reduced in the presence of wt-FL-htt. Scale bar, 5 μm. (C) Wild-type htt reduces the percentage of static vesicles. Percentage of static vesicles in control condition (220 measures, 11 cells), with wt-FL-htt (180 measures, nine cells), and with polyQ-FL-htt (160 measures, eight cells). (D) Wild-type but not polyQ-htt increases vesicles' velocities. Mean velocities were calculated without null values that correspond to pauses. (E) Wild-type but not polyQ-htt decreases the pausing time of BDNF vesicles. Dynamic parameters in (D) and (E) were calculated from 5416 measures for BDNF-eGFP (13 cells), 3666 measures for wt-FL-htt (16 cells), and 3968 measures for polyQ-FL-htt (11 cells). (F) Transfection of NG108-15 cells with siRNAs directed against mouse htt (siRNA-htt) leads to a significant decrease in htt levels compared to the corresponding scramble RNA (SC-RNA-htt). (G) Reduction in htt levels increases the percentage of static vesicles and reduces their velocities while increasing their pausing time (BDNF-eGFP, 2541 measures, nine cells; siRNA-htt, 4047 measures, ten cells; SC-RNA-htt, 2520 measures, eight cells). Statistical analyses revealed significant differences for mean velocities, pausing, and percentage of static vesicles in NG108-15 cells and in silencing experiments (*$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$).

result further demonstrates the specificity of htt in the transport of BDNF vesicles.

We tested whether transport of other organelles such as mitochondria is affected in HD using a mitochondrial marker. While 109Q/109Q cells showed a decreased BDNF vesicular velocity compared to +/+ cells (Figure 2B), the transport of mitochondria was unaffected in HD mutant cells (Figure 2E). Although we can not exclude that other types of transport are affected in 109Q/109Q cells, our findings indicate that the function/dysfunction of htt is specific, at least in part, to the transport of BDNF.

Huntingtin Increases the Efficiency of Microtubule-Based Transport

We next investigated the mechanisms by which htt mediates a stimulatory effect on BDNF trafficking. BDNF vesicles were localized along MTs and distributed in a graded fashion from the perinuclear region to the plasma membrane (Figure 3A). After depolymerizing MTs with nocodazole, BDNF vesicles were randomly distributed. In nocodazole-treated cells, BDNF vesicular movement was completely blocked (Figure 3B), demonstrating that BDNF transport, as observed in our experimental condi-

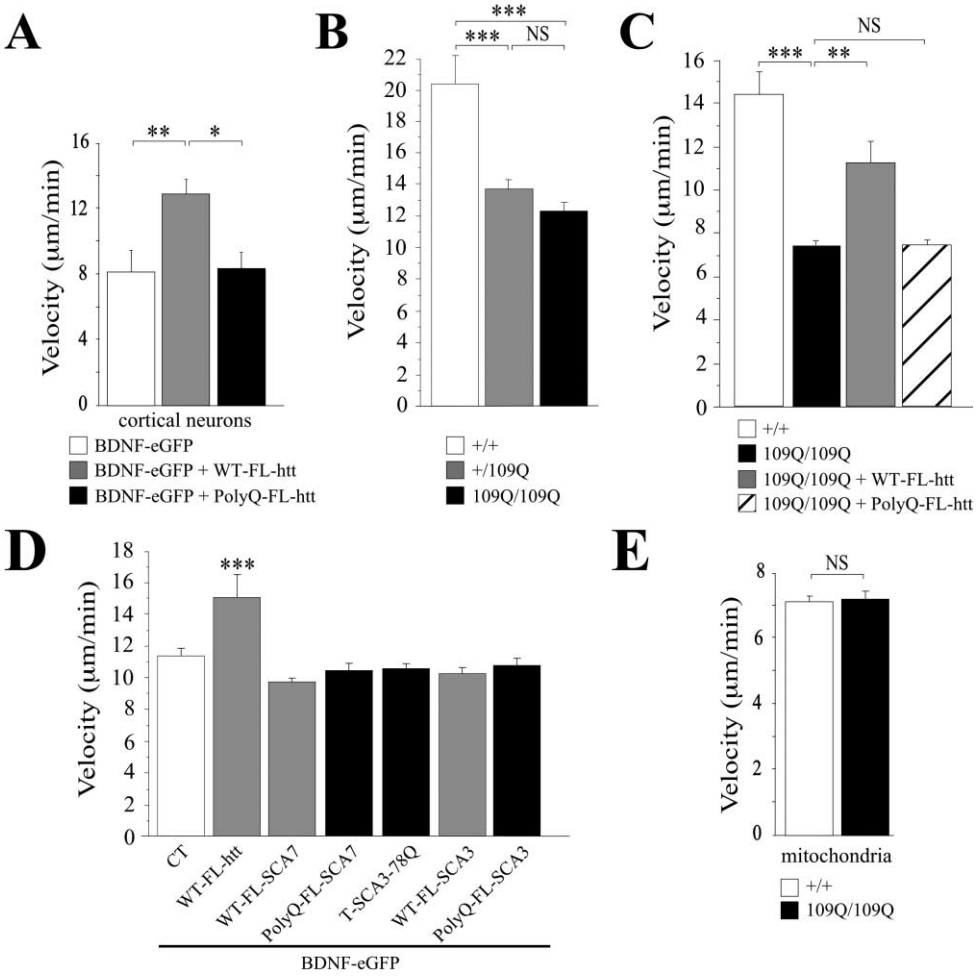

Figure 2. BDNF Transport Is Specifically Altered in Huntington's Disease and Is Rescued by Expression of Wild-Type htt

(A) Wt-FL-htt but not polyQ-FL-htt increases the mean velocity of BDNF-containing vesicles and decreases their pausing time in primary cultures of cortical neurons (BDNF-eGFP, 1330 measures, 11 cells; wt-FL-htt: 2020 measures, ten cells; or polyQ-FL-htt, 477 measures, three cells) (see Supplemental Movies S1, S2, and S3). (B) Mean velocities of moving vesicles are altered in +/109Q cells (7780 measures, 25 cells) and 109Q/109Q cells (5066 measures, 23 cells) in comparison to +/+ cells (4168 measures, 17 cells). (C) BDNF transport deficiency in 109Q/109Q cells (2040 measures, nine cells; 2106 measures, eight +/+ cells) is restored by the expression of wt- (2360 measures, eight cells) but not polyQ-FL-htt (2456 measures, eight cells). (D) BDNF transport is not altered by other polyQ-disorder proteins. Velocity of BDNF vesicles is not modified in NG108-15 cells transfected with wt-FL-ataxin-7 (2729 measures, eight cells) or polyQ-ataxin-7 (3698 measures, nine cells) or in cells expressing either truncated polyQ-ataxin-3 (2746 measures, seven cells), wt-FL-ataxin-3 (2319 measures, six cells), or polyQ-ataxin-3 (3551 measures, eight cells). Control, 2354 measures, seven cells; and wt-FL-htt, 1908 measures, five cells. (E) Mitochondrial dynamic is not altered in HD mutant neuronal cells (+/+ cells, 7557 measures, 16 cells; 109Q/109Q cells, 7211 measures, 25 cells) (*$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$).

tions, is an MT-based transport. It is worth noting that the alteration in BDNF transport in HD cells is unlikely to be the consequence of a major polyQ-induced cytoskeletal disorganization, as actin and MT networks as well as the distal end of the growing MTs were comparable between +/+ and 109Q/109Q cells (Supplemental Figure S3).

In order to quantify transport efficiency along MTs, we investigated the influence of htt on two parameters. The first one, "persistence," relies on the ability of a given organelle to move and persist in a directional movement and is calculated as the ratio between the total distance covered by the particle and the shortest possible distance between its initial and final points (Cordonnier et al., 2001). Persistence was lower in neuroblastoma cells expressing wt-FL-htt than in control cells or cells expressing polyQ-FL-htt (Figure 3C), indicating that wt-FL-htt induces a more direct transport of vesicles along the MT network. Second, we calculated the effect of htt on the distance traveled by vesicles between two pauses. This parameter was used to demonstrate that dynactin is a processivity factor for the cytoplasmic motor protein dynein (King and Schroer, 2000). When expressing the mean velocity as a function of the distances, wt-FL-htt increased the distance traveled between two pauses independently of velocity (Figure 3D). By contrast, polyQ-FL-htt had no effect. In conclusion, this demonstrates that a function of htt is to enhance the efficiency of BDNF vesicles transport along MTs.

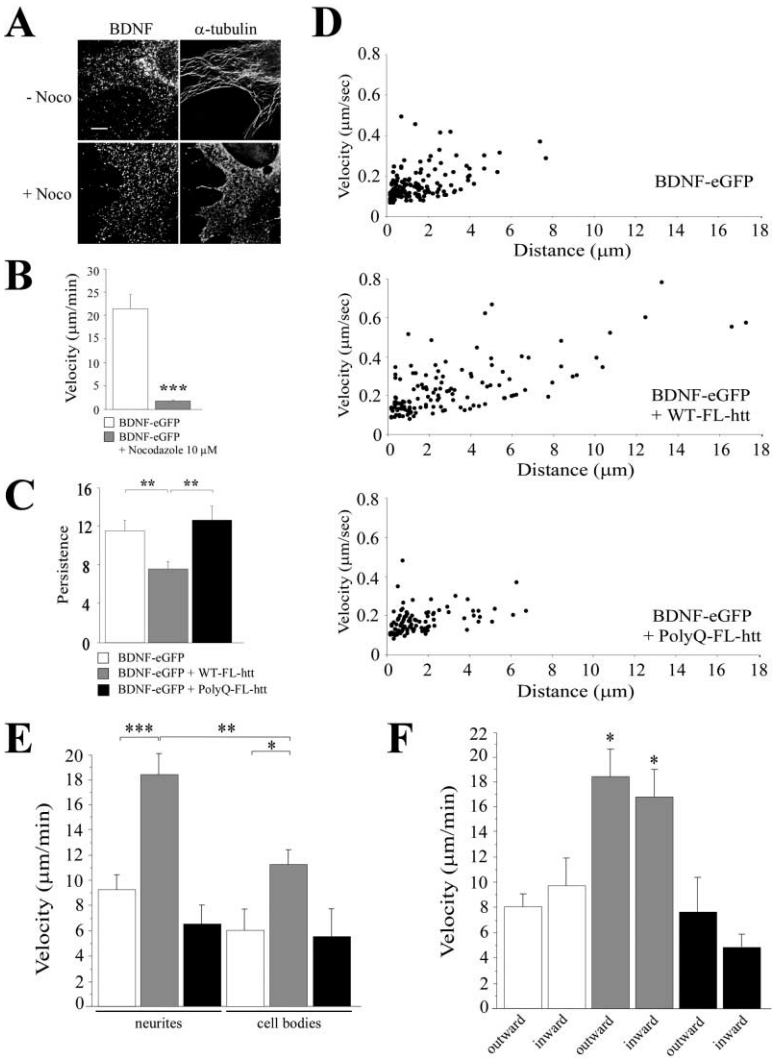

Figure 3. Wt- but Not polyQ-FL-htt Increases the Efficiency of MT-Based Transport

(A) Immunostaining for BDNF and α-tubulin in neuronal cells without (upper panels) and with nocodazole treatment (lower panels). Scale bar, 5 μm. (B) Nocodazole treatment significantly reduces the mean velocity of moving vesicles in +/+ neuronal cells (control, 1218 measures, five cells; Noco, 2188 measures, four cells). (C) Persistence of BDNF vesicles is reduced in cells transfected with BDNF-eGFP and wt-FL-htt (117 tracks, 17 cells) in comparison to cells transfected with BDNF-eGFP and polyQ-FL-htt (83 tracks, 11 cells) or with BDNF-eGFP alone (108 tracks, 12 cells). (D) Scatter plots of the translocation velocities of BDNF-containing vesicles as a function of the distance traveled between two pauses. (E) Htt enhances BDNF transport in neurites and in cell bodies of cortical neurons. Parameters in cell body and neurites are, respectively, from BDNF-eGFP, 796 and 381 measures in five cells; wt-FL-htt, 1239 measures, ten cells; 564 measures, eight cells; and polyQ-FL-htt, 208 and 248 measures, three cells. (F) Htt stimulates outward/anterograde and inward/retrograde movements in cortical neurons. Outward and inward parameters are, respectively, from BDNF-eGFP, 238 and 143 measures, five cells; wt-FL-htt, 281 measures, eight cells; 283 measures, seven cells; and polyQ-FL-htt, 116 and 132 measures, three cells (*$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$).

We next investigated BDNF dynamics in cell body versus neurites in cortical neurons (Figure 3E). While htt stimulated BDNF transport both in neurites and in cell bodies, this stimulatory effect was especially evident in neurites. As we observed the movements of BDNF to be bidirectional (see Supplemental Movie S4), we also asked whether, in neurites, transport was selectively regulated by htt in one or both directions. In axons, MTs are orientated unidirectionally with all plus ends pointing toward the growth cone. However, in dendrites, MTs are organized in both directions with plus ends pointing both distally and toward the cell body (Baas et al., 1988). As we could not distinguish between axons and dendrites in our assay, we determined the velocity of BDNF vesicles moving toward the tips of the neurites (outward) or moving to the cell body (inward) (Figure 3F). Htt enhanced the velocity of BDNF vesicles that moved outward or inward, and both movements were affected equally by polyQ. To relate the outward/inward movement to the organization of the MT network in axons and dendrites, we analyzed the directionality of MT growth in cortical neurons using end binding protein 3 (EB3) coupled to GFP. EB3 is neuron-specific plus end-tracking protein (Stepanova et al., 2003). In agreement with previous studies, 70%–80% of the observed EB3 displacements in neurons were toward the tips of the neurites (data not shown), strongly indicating that most of the outward and inward movements correspond to anterograde and retrograde movements, respectively, according to the MTs' organization.

Huntingtin-Mediated Vesicular Transport Requires HAP1

Htt interacts with HAP1, which could play a role in intracellular transport. We analyzed the consequences of HAP1 expression on BDNF-eGFP dynamics. Strikingly, HAP1 specifically induced a dramatic redistribution of BDNF-containing vesicles into large cytoplasmic bodies with a partial to complete disappearance of individual vesicles (Figure 4A and Supplemental Figure S4). HAP1 was localized at the periphery of these BDNF immunoreactive cores. These structures resembled HAP1-containing organelles, described as stigmoid bodies in normal brains (Gutekunst et al., 1998) (Supplemental Figure S5). HAP1 induced the recruitment of wt- or polyQ-FL-htt and p150^{Glued} into these BDNF-containing bodies (Fig-

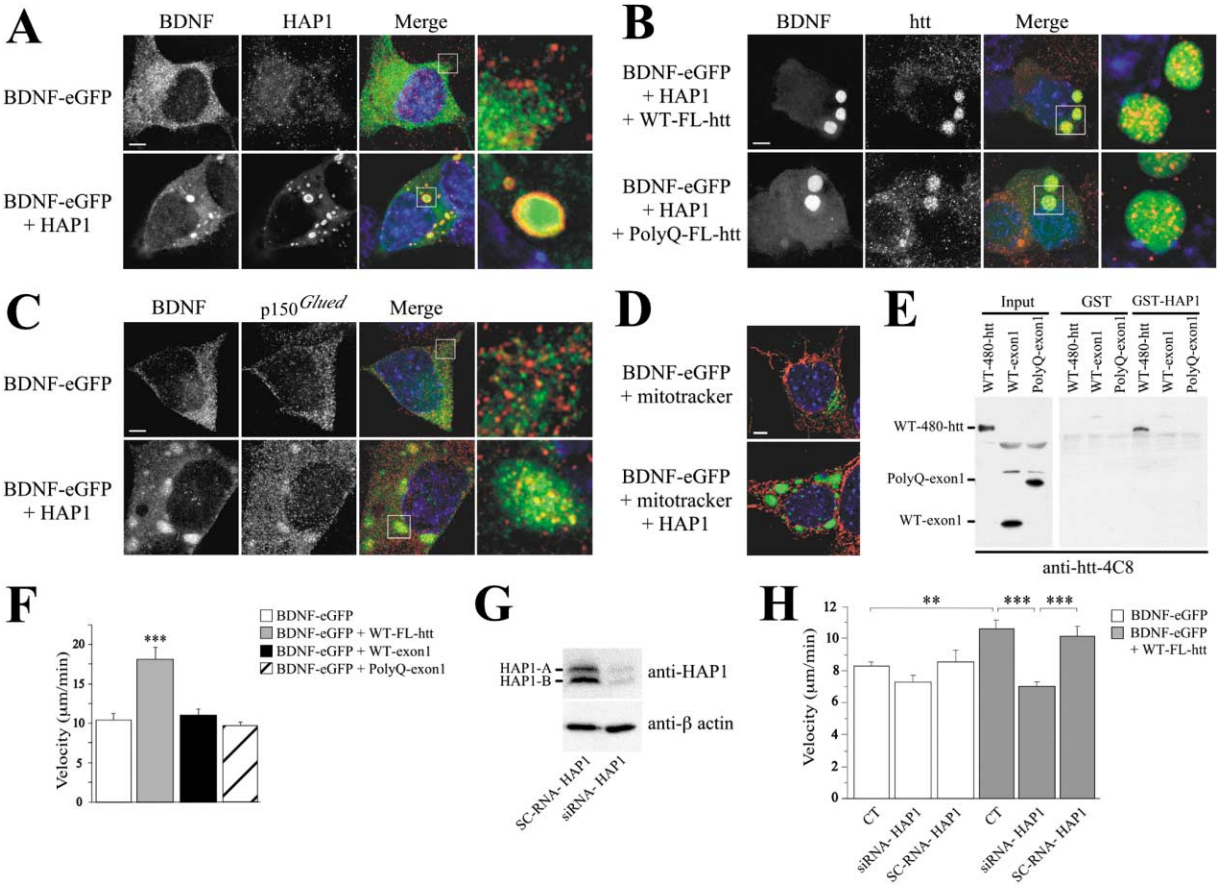

Figure 4. HAP1 Is Required for htt-Mediated Transport of BDNF

(A) HAP1 delocalizes BDNF-containing vesicles. NG108-15 cells were transfected with BDNF-eGFP and HAP1 or its corresponding empty vector and immunostained for BDNF (green) and HAP1 (red). (B) HAP1/BDNF-containing bodies recruit htt. NG108-15 cells were cotransfected with BDNF-eGFP, HAP1, and wt- or polyQ-FL-htt and immunostained for BDNF (green) and htt (red). The right panels in (A) and (B) correspond to a 6 × enlargement of the small boxes present in the merge panels. (C) p150^{Glued} and BNDF-eGFP are relocalized when coexpressed with HAP1. Compare subcellular distribution of p150^{Glued} (red) and BNDF (green) in the absence (upper panels) or presence of HAP1 (lower panels). The right panels correspond to a 6 × enlargement of the small boxes present in the merge panels. (D) HAP1 induces the relocalization of BDNF (green) but not of mitochondria (red). Scale bar, 5 μm. (E) Exon1 of htt does not interact with HAP1. GST-HAP1 pull-down experiments were performed with the first 480 amino acids of htt or with the wt- and polyQ-exon1 fragments. (F) Exon1 of htt does not stimulate transport. Mean velocities of moving vesicles in NG108-15 cells after transfection with wt-FL-htt (2645 measures, nine cells), wt-exon1-htt (3425 measures, 11 cells), or polyQ-exon1-htt (4442 measures, 12 cells). Control, 3633 measures, nine cells. (G) Transfection of NG108-15 cells with siRNAs directed against HAP1 (siRNA-HAP1) but not corresponding scrambled RNA (SC-RNA-HAP1) leads to a significant decrease in HAP1 levels. (H) Reduction in HAP1 levels reduces BDNF velocity and blocks the htt-induced stimulation of transport. Velocities were calculated from 2808 measures for BDNF-eGFP (eight cells), siRNA-HAP1 (2178 measures, eight cells), SC-RNA-HAP1 (1491 measures, seven cells), wt-FL-htt (2152 measures, eight cells), wt-FL-htt and siRNA-HAP1 (2853 measures, nine cells), and wt-FL-htt and SC-RNA-HAP1 (2664 measures, nine cells) (**$p < 0.01$ and ***$p < 0.001$).

ures 4B and 4C) but had no effect on mitochondria (Figure 4D). Finally, the formation of the HAP1-induced BDNF bodies depended on the integrity of the MT network and was minus end-directed as sensitive to the overexpression of p50/Dynamitin (Supplemental Figures S6 and S7).

We sought to establish whether truncated versions of htt that do not interact with HAP1 still activate transport of BDNF. In contrast to an N-terminal fragment of htt containing the first 480 amino acids, exon1 of htt (first 67 amino acids) did not bind HAP1 (Figure 4E) (Bertaux et al., 1998) and had no effect on transport (Figure 4F). Finally, htt-mediated stimulation of transport was blocked when HAP1 protein levels were reduced by siRNA (Figures 4G and 4H). These results demonstrate that HAP1 is essential for the stimulatory effect of htt on BDNF trafficking.

Huntingtin/HAP1/p150^{Glued} Dynactin Complex Is Altered in HD

The finding that HAP1, htt, and p150^{Glued} relocalized in the BDNF-containing bodies led us to assess the status of these proteins in the pathological situation by sucrose gradient fractionation. Cytosolic cell extracts from +/+ and 109Q/109Q cells were fractionated on a linear sucrose gradient (7.5%–25%). Wild-type htt comigrated with p150^{Glued} and dynein, suggesting that a fraction of soluble wild-type htt is present in the p150^{Glued}/dynein complex (Figure 5A) (Li et al., 1998). In addition, we also found a peak of htt in lower-density fractions, indicating

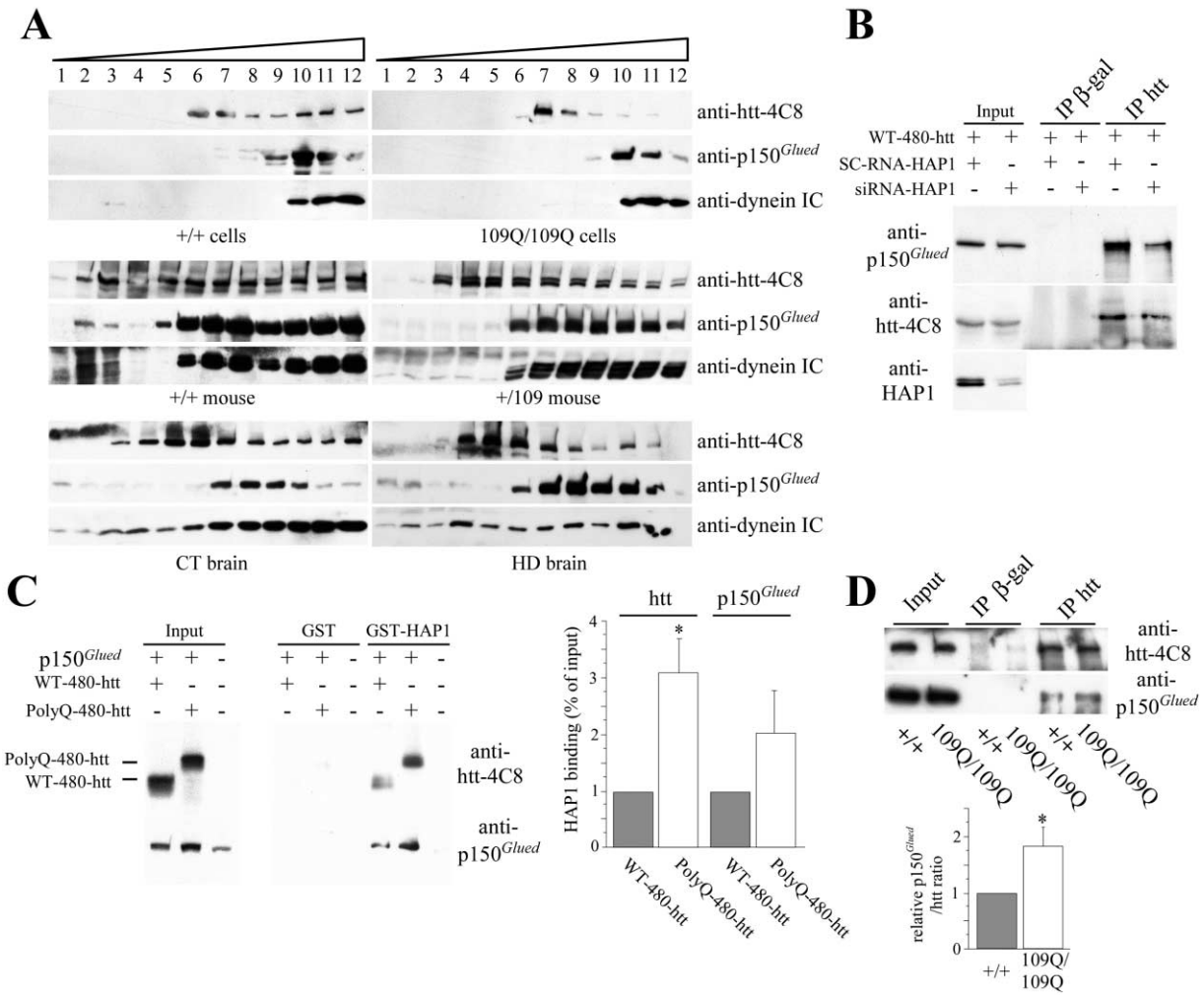

Figure 5. Htt/HAP1/p150^{Glued} Complex Is Altered in HD

(A) Comigration of htt with p150^{Glued} and dynein in sucrose gradients is modified by polyQ expansion in 109Q/109Q cells, in brain from +/109Q mice, and in brain from HD patients (two HD individuals were tested). (B) Htt binds p150^{Glued} via HAP1. Extracts from cells transfected with 480-17Q and siRNA-HAP1 or corresponding scramble were immunoprecipitated (IP) using an anti-htt antibody, revealing a decreased immunoprecipitation of p150^{Glued} when HAP1 levels are decreased. (C) Interaction of htt and p150^{Glued} is enhanced by the polyQ expansion. GST-HAP1 pull-down experiments were performed in the presence or absence of p150^{Glued} and wild-type or polyQ-htt. The graph represents the quantitative assessment of htt (n = 4) and p150^{Glued} (n = 3) binding to HAP1. (D) Immunoprecipitation experiments in 109Q/109Q cells show enhanced interaction of p150^{Glued} with polyQ-htt. Cell extracts were immunoprecipitated using anti-htt or anti-β-galactosidase (control) antibodies and immunoprobed with anti-htt or anti-p150^{Glued} antibodies. The graph represents the quantitative assessment of the optical density of p150^{Glued} and htt and is expressed as a p150^{Glued}/htt ratio (n = 4) (*$p < 0.05$).

that htt may also exist as a free component of this complex. Interestingly, in the pathological situation, most of polyQ-htt sedimented in these lower-density fractions. We confirmed this result in more physiological situations in HD mice and in HD individuals. In HD patients, a shift in the migration of dynein IC compared to p150^{Glued} was observed, suggesting a disruption of the dynein/dynactin complex. Together, our results in cellular and mouse models of HD as well as in HD patients suggest that p150^{Glued}, htt, and dynein may be involved in the same cytoplasmic protein complex and that this complex is altered in HD.

We examined whether htt binds to p150^{Glued} and whether this binding is direct or requires HAP1 by immunoprecipitation experiments. Htt interacted with p150^{Glued}, and this interaction depended on the presence of HAP1 (Figure 5B), showing that htt forms a complex in cells with p150^{Glued} via HAP1.

We next analyzed the influence of the polyQ expansion on the htt/HAP1/p150^{Glued} complex. PolyQ-htt bound HAP1 with a higher affinity compared to wild-type htt (Figure 5C) (Li et al., 1995). Strikingly, p150^{Glued} binding to HAP1 was also increased when htt contained the pathological expansion. In a more physiological situation, the ability of anti-htt antibodies to immunoprecipitate the p150^{Glued} protein was enhanced in the 109Q/109Q neuronal cells in comparison to wild-type cells (Figure 5D). We also observed this enhanced binding of p150^{Glued} to htt in postmortem brain extracts from HD patients compared to control individuals (data not

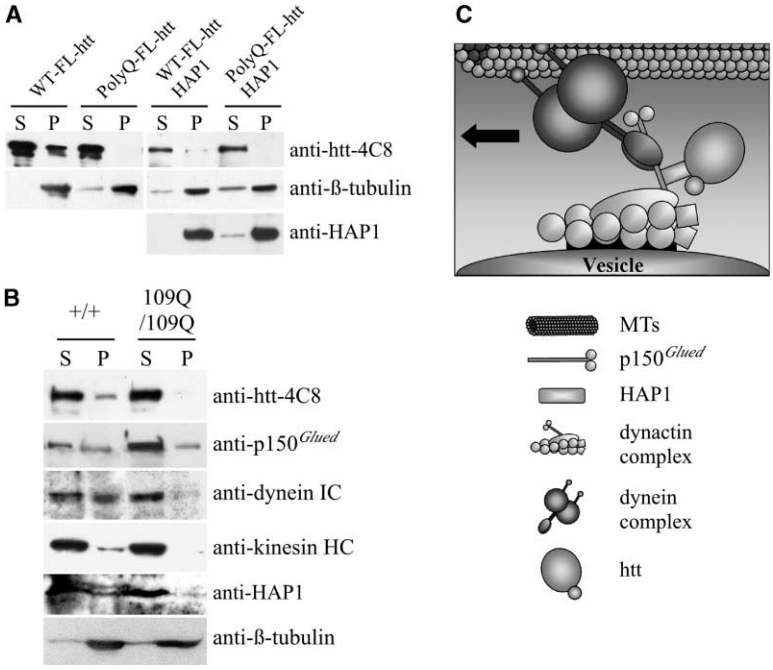

Figure 6. PolyQ Expansion Inhibits htt, HAP1, and Motor Proteins' Association to MTs

(A) Htt and HAP1 association to MTs is reduced by polyQ expansion. MT preparations from cells expressing wt-FL-htt or polyQ-FL-htt in absence (left panel) or in presence of HAP1 (right panel) were immunoprobed for htt, β-tubulin, and HAP1. S and P, supernatant and pellet fractions.

(B) Htt and motor proteins do not associate to MTs in HD neuronal cells. MT preparations from +/+ or 109Q/109Q cells were analyzed for the presence of htt, p150^{Glued}, dynein IC, kinesin HC, and β-tubulin.

(C) Htt's function in transport.

shown). In conclusion, the presence of polyQ expansion in htt leads to an increased interaction between polyQ-htt, HAP1, and p150^{Glued} proteins.

PolyQ-Huntingtin Inhibits HAP1/p150^{Glued} Dynactin Binding to Microtubules

We next assessed the association of htt to MTs by preparing taxol-stabilized MT fractions. Htt was mainly found in the supernatant fraction, but a significant percentage was found in the pellet fraction (Figure 6A, left panel) (Hoffner et al., 2002; Tukamoto et al., 1997). PolyQ-htt was not detectable in the pellet fraction. We analyzed the distribution of HAP1 to MTs by cotransfecting cells with htt and HAP1. While HAP1 was normally enriched in the pellet fraction, a significant fraction of HAP1 redistributed to the supernatant fraction in the presence of polyQ-htt (Figure 6A, right panel).

Using 109Q/109Q cells, we found that binding of endogenous htt and p150^{Glued} to MTs was reduced (Figure 6B). In addition, the association of key proteins of the molecular motor complexes such as dynein intermediate chain (IC) and kinesin heavy chain (HC) to MTs was compromised in homozygous mutant cells.

These results demonstrate that the increased binding of polyQ-htt to HAP1 leads to a reduced association of key components of the motors to MTs (Figure 6C). As these components play a crucial role in regulating transport along the MTs, this reduced association to MTs is likely to be responsible for the decreased transport of BDNF-containing vesicles.

The PolyQ-Huntingtin-Induced Transport Deficit Results in a Loss of Neurotrophic Support and Toxicity: Rescue by Wild-Type Huntingtin

We addressed the consequences of an altered MT-dependent transport on the ability of neuronal cells to release BDNF by treating cells with nocodazole. Nocodazole had no effect on the amount of BDNF released after depolarization (Figure 7A). To specifically test the effect of transport on BDNF release, cells were depolarized, treated with nocodazole for 30 min, and depolarized a second time (Figure 7B). In control cells, a recovery period of 30 min was sufficient to reconstitute a releasable pool similar to the first one (Figures 7C and 7D). However, in the nocodazole-treated cells, the release of BDNF was completely inhibited. This result shows that inhibition of BDNF transport abrogates the ability of cells to refill the releasable pool of BDNF vesicles following depolarization.

Having established the experimental conditions to assay a BDNF release that is strictly dependent upon transport, we investigated the effect of polyQ expansion. To make sure that the observed effect on BDNF release was not due to a defect in BDNF production, we ectopically expressed BDNF under the control of CMV promoter and found similar levels of intracellular BDNF in +/+ and 109Q/109Q cells (data not shown). We next measured in these cells the release of BDNF as in Figure 7B. While the amount of BDNF released after K1 was similar between +/+ and 109Q/109Q cells, the amount of BDNF in K2 was significantly reduced in mutant cells, resulting in a decreased K2/K1 release ratio (Figure 7E). This demonstrates that 109Q/109Q cells present a transport-dependent deficit of BDNF release.

The alteration in the transport of BDNF in 109Q/109Q cells is rescued by wt-FL-htt (Figure 2C). Therefore, we investigated whether expression of wt-FL-htt in 109Q/109Q cells can also rescue the defect of BDNF release. In contrast to the expression of polyQ-htt, expression of wild-type htt restored the ability of 109Q/109Q neuronal cells to release BDNF (Figure 7E). We conclude that, in HD cells, the loss of htt function in the transport of BDNF vesicles results in the inability of these cells to release BDNF.

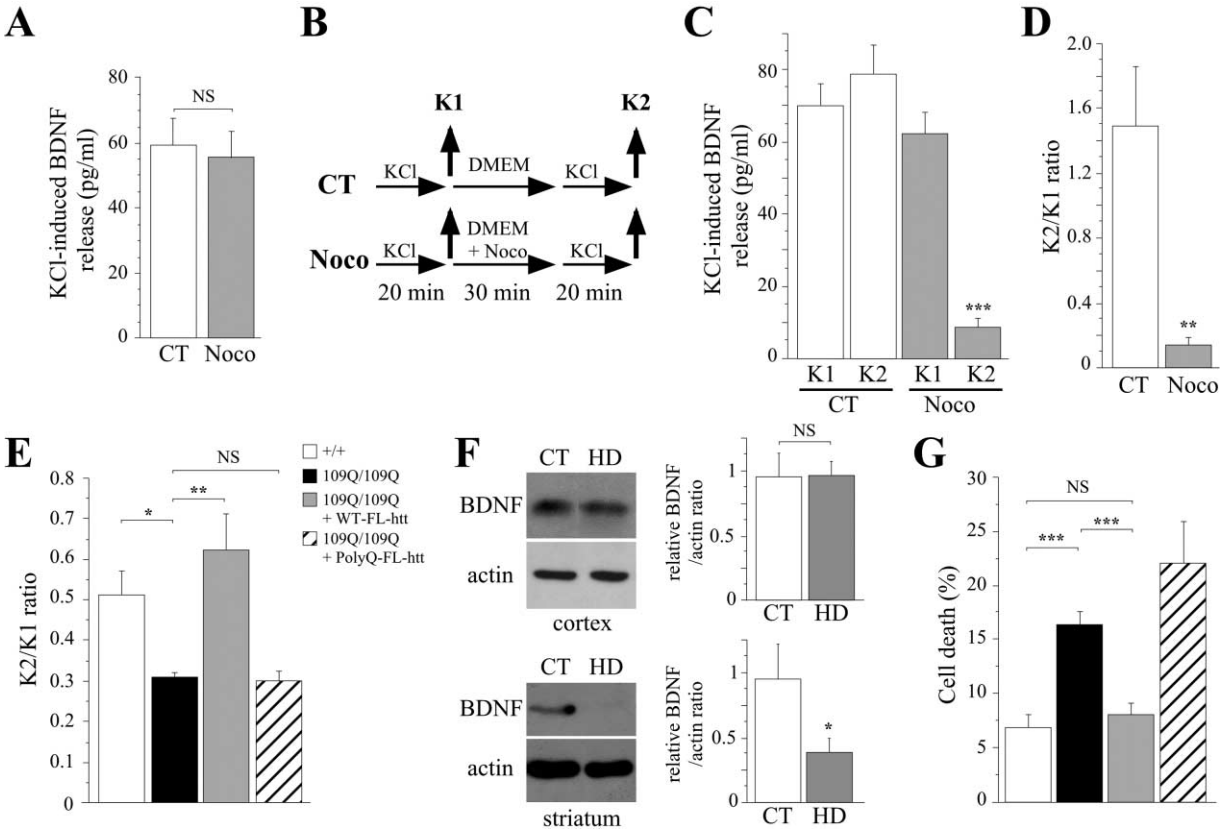

Figure 7. Disruption of htt-Mediated Transport of BDNF Results in a Loss of BDNF Release and an Increased Cell Death that Are Both Rescued by Wild-Type htt

(A) BDNF release after KCl-induced depolarization is not affected by nocodazole treatment. (B) Description of the protocol used to assess the transport-dependent release of BDNF. (C) Transport-dependent BDNF release is significantly blocked by nocodazole treatment. (D) Transport-dependent BDNF release can also be represented as a K2/K1 ratio. (E) Transport-dependent BDNF release is reduced in 109Q/109Q neuronal cells and is rescued by expression of wt but not polyQ-htt. (F) BDNF levels are reduced in the striatum but not in the cortex of HD patients. Representative immunoblots of BDNF and actin are shown. Graphs represent the normalized BDNF protein levels in the cortex (upper graph, CT, n = 3; HD, n = 10) and in the striatum (lower graph, CT, n = 7; HD, n = 10). (G) Neuronal death is statistically increased in 109Q/109Q neuronal cells and is rescued by expression of wt but not polyQ-htt (*$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$).

To confirm our hypothesis, we determined BDNF levels in cortical and striatal postmortem brain extracts from control and HD patients. BDNF levels were reduced in the striatum but not in the cortex of HD patients (Figure 7F) (Ferrer et al., 2000). This is in agreement with a defect in the transport of BDNF by the cortico-striatal projecting neurons.

Our findings of the reduced transport and subsequent reduced release in BDNF led us to investigate whether homozygous mutant neuronal cells are more susceptible to death and whether cell death can be rescued by overexpressing wt-FL-htt. We observed a statistically significant increased cell death in 109Q/109Q cells in comparison to +/+ cells (Figure 7G). Moreover, wild-type but not polyQ-htt rescued cell death in 109Q/109Q cells.

Discussion

Huntingtin Controls Anterograde and Retrograde Transport of BDNF Vesicles

We demonstrate that the increased association of polyQ-htt to HAP1 and p150^{Glued} leads to the depletion of HAP1, p150^{Glued}, dynein IC, and kinesin HC from MTs. p150^{Glued} and dynein IC are key components of the molecular motors (Vale, 2003). Indeed, dynactin is a factor that regulates the processivity of the cytoplasmic dynein. In vitro, addition of dynactin to dynein increases the motility of polystyrene beads along MTs while this movement is reduced by incubation with anti-p150^{Glued} antibodies (King and Schroer, 2000). Therefore, a reduction in the amount of these proteins at the MTs is likely to decrease intracellular transport. In support, disruption of the dynein/dynactin complex by overexpression of the p50 dynamitin or mutations in dynein cause axonal transport inhibition and neurodegeneration in mouse (Burkhardt et al., 1997; Hafezparast et al., 2003; LaMonte et al., 2002). Moreover, the inability of dynactin to bind to MTs is responsible for an autosomal dominant form of lower motor neuron disease in humans (Puls et al., 2003).

We also observed a reduced association of kinesin HC to MT in HD knockin mouse. Kinesins are motor proteins responsible for anterograde movement of various cargoes (Kanai et al., 2000). This is in agreement with our observation that htt stimulates both anterograde and retrograde transport of BDNF vesicles (Figure 3F and

Supplemental Movie S4). Interestingly, a cDNA encoding a human kinesin-like protein was pulled down by the yeast two-hybrid system using HAP1 as bait (Engelender et al., 1997). We subsequently found this cDNA to be 99% identical to the human kinesin heavy chain isoform 5C, a neuron-specific kinesin (Kanai et al., 2000). Thus, HAP1 could directly interact not only with $p150^{Glued}$ but also with kinesins and, as demonstrated for $p150^{Glued}$ itself (Deacon et al., 2003), participate in both the anterograde and retrograde transport of vesicles.

Our observation that expression of HAP1 clusters BDNF vesicles but not mitochondria supports the notion that htt specifically stimulates transport of BDNF. Interestingly, overexpression of Milton, a *Drosophila* ortholog of HAP1 (Stowers et al., 2002), leads to the redistribution of mitochondria. Therefore, HAP1 and Milton show specificity in the type of cargoes they are transporting. This also suggests the existence in human of an HAP1 homolog that could specifically transport mitochondria.

PolyQ-Induced Loss of htt Function and Axonal Poisoning

Our results are consistent with studies that demonstrated an alteration of transport by polyQ-containing proteins (Gunawardena et al., 2003; Lee et al., 2004; Piccioni et al., 2002; Szebenyi et al., 2003). Indeed, the authors proposed that axonal transport could be affected by at least two mechanisms. One could be specific to the function of htt in intracellular transport, and the other mechanism could be general to all polyQ-containing proteins and would involve axonal blockage (i.e., axonal poisoning). Here, we dissected the molecular mechanisms by which htt controls intracellular transport of BDNF. We believe these specific mechanisms to be distinct from those involved in axonal poisoning for several reasons. First, we observed this deficit in transport only with htt and not with other polyQ-containing proteins. Second, in our experiments, no microscopically visible aggregates could be detected (data not shown), suggesting that soluble polyQ-htt is unable to stimulate transport. Finally, we found that wt-FL-htt stimulates transport while a reduced intracellular transport is observed after RNA interference. These experiments were performed independently of the pathological context. They further support a role for wild-type htt in intracellular transport and that this function is mitigated in the disease situation.

Our results suggest that, in the early course of the disease, the function of htt in transport is lost via a direct effect of the polyQ expansion on the ability of htt to interact properly with the HAP1 protein. In later stages of the disease, neuritic aggregates accumulate and will also contribute to reduce axonal transport. Finally, our observation that transport is reduced but not completely blocked or stopped is in agreement with the fact that, in HD, disease progresses several years before symptoms appear in patients.

Loss of htt's Function in Transport by polyQ Expansion and Proteolysis

We have demonstrated that polyQ-htt acts in a dominant manner to alter transport in heterozygous neuronal cells for HD mutation. In agreement with the genetic dominance in HD, we found the alteration in transport to be similar in heterozygous and homozygous neuronal cells. We could not observe such dominant effect of polyQ-htt in overexpressing conditions (compare Figures 1D and 2A to Figure 2B). This discrepancy might be related to the experimental conditions. Indeed, in contrast to overexpressing cells, neuronal cells generated from knockin HD mice express wild-type and mutant htt at endogenous levels and thus reflect more closely the pathological situation observed in HD patients. In good agreement, our biochemical experiments demonstrate that polyQ-htt depletes motor proteins from MTs similarly in homozygous cells and HD patients. In conclusion, htt is a positive regulator of BDNF transport, and abnormal polyQ expansion in htt results in the loss of its function in transport by a dominant effect.

A key step during the cascade of molecular events leading to the death of neurons in the brain is the cleavage of htt into smaller N-terminal fragments. Indeed, htt is the substrate of several proteases that generate various N-terminal fragments containing the expanded polyQ stretch (DiFiglia, 2002). We show that a N-terminal fragment of htt corresponding to exon1 is unable to stimulate intracellular transport of BDNF-containing vesicles. Therefore, one of the consequences of wild-type htt proteolysis could be to inactivate the function of htt in transport. These results are consistent with the observations that truncated htt has lost its antiapoptotic properties (Cattaneo et al., 2001). As proteolysis of normal htt is also observed in HD brains (DiFiglia, 2002), this cleavage, by reducing BDNF transport and thus neurotrophic supply, could enhance the neurodegeneration induced by the polyQ expansion in the mutant allele.

Antiapoptotic Properties of Huntingtin

BDNF that is provided by cortico-striatal projections is essential for the survival of striatal neurons in normal and pathological situation (Altar et al., 1997; Baquet et al., 2004; Saudou et al., 1998). Therefore, a reduction in the transport of BDNF in cortical neurons that has a direct consequence on the ability of these cells to release BDNF will be deleterious for striatal neurons. In support, the amount of BDNF is reduced in the striatum but not in the cortex of HD patients (Ferrer et al., 2000) (Figure 7F).

Our results are consistent with the findings that wt-FL-htt but not polyQ-FL-htt (or N-terminal fragments) possesses antiapoptotic properties (Cattaneo et al., 2001). These antiapoptotic properties have been linked to an increased BDNF transcriptional activity (Zuccato et al., 2001) but could also be related to the capacity of the wt-FL-htt protein to increase the transport of BDNF.

Our findings that intracellular transport is rescued by wt-FL-htt but not polyQ-FL-htt suggest that the measurement of BDNF transport by 3D fast videomicroscopy could be used to screen for compounds able to restore htt function in transport. The identification of such compounds might be of therapeutic interest and/or help in the design of strategies for early therapeutic interventions in HD.

Experimental Procedures

Description of cell culture and cell death assay, plasmids, antibodies, siRNA, brain and mouse tissues, buffers, and statistical analyses may be found in Supplemental Data.

Sucrose Gradients, Cell Fractionation, Immunoprecipitation, and Immunofluorescence Analyses

Cell pellets or brain samples were homogenized in acetate buffer and clarified by centrifuging, and the supernatant was layered on the top of sucrose gradients (3.5 ml, 7.5%–25% in acetate buffer without NP-40). Gradients were centrifuged (10 hr, 30,000 rpm, SW 50.1 rotor) and 12 fractions collected. The same volume of each fraction was analyzed by Western blot.

For MT preparations, cells were treated 1 hr with 1 μM paclitaxel (Sigma) and lysed 15 min at 37°C in PM2G buffer. After centrifugation (30 min, 10,000 rpm), 1 volume of 2% SDS buffer was added to the supernatant, and the pellet was resuspended in 1% SDS buffer, heated 5 min at 100°C, and sonicated before loading.

For immunoprecipitation (IP) experiments, cells were lysed in Triton X-100 1% buffer. Lysates were incubated 1 hr at 4°C with 1 μg of primary antibody and then supplemented with either 50 μl of protein A sepharose (Amersham Pharmacia) for IP with anti-β-galactosidase antibody or protein G agarose (Sigma) for IP with anti-htt (4C8) or anti-$p150^{Glued}$ antibodies.

For GST pull-down, cells were lysed in Triton X-100 1% buffer, and extracts were preincubated (1 hr, 4°C) with GST sepharose beads. The cleared lysate (125 μg) was then incubated 1 hr at 4°C with GST-HAP1 or GST proteins that were produced in bacteria and purified on glutathion sepharose beads.

The method of immunocytochemistry was previously published (Humbert et al., 2002). When stated, prior to fixation, cells were treated (1 hr) with 10 μM nocodazole (Sigma). Pictures of immunostained cells were collected using a 3D microscope and analyzed after subsequent deconvolution.

Videomicroscopy Experiments and Analyses

Videomicroscopy experiments were done two days after transfection. Cells were cotransfected with BDNF-eGFP and various constructs of htt or the corresponding empty vectors with a DNA ratio of 1:4. For the tracking of mitochondria, neuronal cells were incubated 30 min with MitoTracker Red CMXRos (Molecular Probes) prior to experimentation. Live videomicroscopy was carried out using an imaging system previously detailed (Savino et al., 2001). Cells were grown on glass coverslip that was mounted in a Ludin chamber. The microscope and the chamber were kept at 37°C (33°C for knockin cells). Stacks of 12 images with a Z step of 0.3 μm were acquired with a 100 × PlanApo N.A. 1.4 oil immersion objective coupled to a piezo device (PI). Images were collected in stream mode using a Micromax camera (Ropper Scientific) set at 2 × 2 binning with an exposure time of 50–100 ms (frequency of 2–5 s). All stacks were treated by automatic batch deconvolution using the PSF of the optical system. Projections, animations, and analyses (tracking and colocalizations) were generated using the Metamorph software (Universal Imaging Corp.). Dynamics were characterized by tracking positions of eGFP vesicles in cells as a function of time. During tracking, the Cartesian coordinates of the centers of vesicles were used to calculate dynamic parameters (see Supplemental Data for statistical analyses).

BDNF Immunoenzyme Assays

BDNF assays were performed 48 hr after lipofection (Invitrogen). For the nocodazole experiment, cells were treated with 10 μM nocodazole for 30 min, washed, and treated for 20 min with DMEM containing high K^+ (30 mM $CaCl_2$, 30 mM NaCl, 28 mM KCl). For the transport-dependent release, cells were depolarized (K1), treated 30 min with 10 μM nocodazole or DMEM alone (Figure 7C) or 1 hr in DMEM (Figure 7E), washed, and depolarized (K2). The amount of BDNF was measured in supernatants and cell lysates using the BDNF Emax Immunoassay System (Promega).

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