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THE p75 NEUROTROPHIN RECEPTOR AUGMENTS SURVIVAL SIGNALING IN THE STRIATUM OF PRE-SYMPTOMATIC *Q175^{WT/HD}* MICE

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Abstract

Huntington's disease (HD) is a dominantly inherited neurodegenerative disorder characterized by a constellation of motor, cognitive, and psychiatric features. Striatal medium spiny neurons, one of the most affected populations, are dependent on brain-derived neurotrophic factor (BDNF) anterogradely transported from the cortex for proper function and survival. Recent studies suggest both receptors for BDNF, TrkB and p75 neurotrophin receptor (p75), are improperly regulated in the striata of HD patients and mouse models of HD. While BDNF–TrkB signaling almost exclusively promotes survival and metabolic function, p75 signaling is able to induce survival or apoptosis depending on the available ligand and associated co-receptor. We investigated the role of p75 in the *Q175* knock-in mouse model of HD by examining the levels and activation of downstream signaling molecules, and subsequently examining *Hdh^{+/Q175};p75^{-/-}* mice to determine if p75 represents a promising therapeutic target. In *Hdh^{+/Q175};p75^{+/+}* mice, we observed enhanced survival signaling as evidenced by an increase in phosphorylation and activation of Akt and the p65 subunit of NFκB in the striatum at 5 months of age and an increase in XIAP expression compared to *Hdh^{+/+};p75^{+/+}* mice; this increase was lost in *Hdh^{+/Q175};p75^{-/-}* mice. *Hdh^{+/Q175};p75^{-/-}* mice also showed a decrease in Bcl-XL expression by immunoblotting compared to *Hdh^{+/Q175};p75^{+/+}* and *Hdh^{+/+};p75^{+/+}* littermates. Consistent with diminished survival signaling, DARPP-32 expression decreased both by immunoblotting and by immunohistochemistry in *Hdh^{+/Q175};p75^{-/-}* mice compared to *Hdh^{+/+};p75^{+/+}*, *Hdh^{+/Q175};p75^{+/+}*, and *Hdh^{+/+};p75^{-/-}* littermates. Additionally, striatal volume declined to a greater extent in *Hdh^{+/Q175};p75^{-/-}* when compared to *Hdh^{+/Q175};p75^{+/+}* littermates at 12 months, indicating a more aggressive onset of degeneration. These data suggest that p75 signaling plays an early role in augmenting pro-survival signaling in the striatum and that disruption of p75 signaling at a pre-symptomatic age may exacerbate pathologic changes in *Hdh^{+/Q175}* mice.

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Keywords

Huntington's; BDNF; p75; Q175

INTRODUCTION

Huntington's disease (HD) is an adult-onset, progressive, and invariably fatal neurodegenerative disease characterized by a constellation of motor, cognitive, and psychiatric features. HD is dominantly inherited and caused by a CAG repeat expansion in the *huntingtin* gene (Group, 1993). There are only limited symptomatic treatment options and no disease-modifying therapies. Striatal medium spiny neurons (MSNs) are one of the earliest and most affected populations of neurons (Walker, 2007). MSNs are dependent on cortically derived neurotrophic factors, such as brain-derived neurotrophic factor (BDNF), anterogradely transported to the striatum for normal development, function, and maintenance (Mizuno et al., 1994; Baquet et al., 2004; Baydyuk et al., 2013).

Huntingtin protein (HTT) regulates cortical BDNF transcription, with mutated HTT (mHTT) being impaired in its ability to regulate BDNF (Zuccato et al., 2001). The *BDNF* gene encodes as many as eight promoters, with promoters I, II, and IV being most highly transcriptionally active and well-studied (West et al., 2014). BDNF transcription from promoter II depends largely on the activity of the repressor element 1/neuron-restrictive silencer element (RE1/NRSE) present in this promoter of the *BDNF* gene. This sequence recruits RE1 silencing transcription factor/neuron-restrictive silencing factor (REST/NRSF), which actively silences transcription (Timmusk et al., 1999). HTT sequesters REST in the cytoplasm, preventing it from binding to the RE1/NRSE, and subsequently leading to higher levels of BDNF transcription from promoter II. mHTT is less able to sequester REST in the cytoplasm, leading to reduced transcription of BDNF from promoter II (Zuccato et al., 2003). Consistent with these studies, in several mouse models of HD and in post-mortem HD brains, lower levels of cortical BDNF expression were reported (Zuccato et al., 2008). In addition, HTT may be involved in the anterograde trafficking of BDNF from the cortex to the striatum (Gauthier et al., 2004), which is also consistent with the lower levels of BDNF observed in the striatum of several animal models of HD and in post-mortem HD brains (Ferrer et al., 2000; Her and Goldstein, 2008). BDNF protein levels can be difficult to measure due to the lack of antibodies of sufficient specificity and sensitivity. To overcome this difficulty, Ma et al. crossed the Q175 mouse line to a line expressing BDNF with an HA epitope tag. Using highly sensitive and specific HA antibodies, a decrease in BDNF protein levels in the striatum of Q175 mice at 6 months of age was observed (Ma et al., 2015). A decrease in BDNF levels and signaling in the striatum is proposed to contribute to the pathogenesis of HD (Zuccato and Cattaneo, 2007).

There are two known BDNF receptors, Tropomyosin receptor kinase B (TrkB) and p75 neurotrophin receptor (p75). Both TrkB and p75 are expressed in the striatum. Signaling downstream of TrkB activation almost exclusively promotes survival and metabolic function, and disruption of TrkB signaling is likely detrimental to MSN functional integrity (Gupta et al., 2013; Baydyuk and Xu, 2014). While TrkB levels and trafficking were studied in

multiple animal models of HD and in post-mortem HD brains, p75 has been examined only recently. p75 mRNA levels were reported to be elevated in post-mortem HD striata (Zuccato et al., 2008), and striatal p75 protein levels were reported as elevated in two mouse models of HD (Brito et al., 2013). Unlike TrkB, signaling downstream of p75 is highly context-dependent and may support neuronal survival or contribute to neuronal dysfunction and death (Kraemer et al., 2014). p75 can be activated by all four neurotrophins, pro-forms of neurotrophins, and other ligands (Hempstead, 2002; Nykjaer et al., 2005).

As a member of the TNF family of death receptors, p75 is capable of inducing apoptosis when complexed with pro-apoptotic co-receptors or adaptor proteins, such as sortilin and NRIF (Nykjaer et al., 2004). p75 can also interact with Trk receptors, however, and these interactions are crucial for maximal survival signaling in several cell types (Ceni et al., 2010; Kommaddi et al., 2011; Matusica et al., 2013; Negrini et al., 2013). p75 stabilizes activated Trk receptors on the membrane, extending survival signaling (Makkerh et al., 2005). p75 is also capable of promoting survival signaling independently of Trk receptors through activation of nuclear factor kappa B (NFκB) (Carter et al., 1996). Based on the ability of p75 to complement Trk signaling and to promote survival signaling independently of Trk activation, we hypothesized that p75 signaling augments survival signaling in the striata of HD mouse models.

To explore the role of p75 in HD, we crossed a germline p75 knock-out mouse into the Q175 mouse model of HD. The Q175 mouse is a knock-in model containing approximately 200 glutamine repeats in the closely related murine homolog of *htt* (*hdh*). These mice develop behavioral phenotypes and pathological changes beginning at 6–12 months of age (Smith et al., 2014). In order to investigate signaling changes and the function of p75 prior to the onset of visible pathology, we examined mice at 5 months of age to determine whether p75 had a compensatory protective function for MSNs and again at 12 months of age to determine the pathological consequences of the loss of p75. We examined the effect of p75 deletion on pro-survival signaling in the following genotypes *Hdh*^{+/+};*p75*^{+/+}, *Hdh*^{+/Q175};*p75*^{+/+}, *Hdh*^{+/Q175};*p75*^{-/-}, and *Hdh*^{+/+};*p75*^{-/-}. Our data suggested that p75 plays an early role in augmenting survival signaling in the striatum and that disruption of p75 signaling at a pre-symptomatic age may exacerbate pathologic changes in *Hdh*^{+/Q175} mice.

EXPERIMENTAL PROCEDURES

Mice

Heterozygous Q175 (*Hdh*^{+/Q175}) knock-in mice (C57Bl/6 background) and wild-type (*Hdh*^{+/+}) littermate controls were obtained from Jackson Laboratories (Stock #027410, Bar Harbor, ME, USA). CAG repeat numbers were in the range of 176–201 (reported by Jackson Laboratories). For initial experiments, mice arrived in one cohort at 5 months of age, with an equal number of males and females used for biochemical analyses. A separate cohort of *Hdh*^{+/Q175} mice were used for breeding to produce all additional animals. p75 deletion was achieved by crossing *Hdh*^{+/Q175} mice with *p75*^{-/-} mice in a mixed background (C57Bl/6 and 129/Sv) (Bogenmann et al., 2011) for two successive generations to produce the necessary genotypes. All mice examined were littermates. For the initial *Hdh*^{+/Q175} and *Hdh*^{+/+} experiments (Figs. 1 and 2), eight mice of each genotype were examined. For the

Hdh^{+/Q175;p75^{-/-}} experiments, 10–12 mice of each genotype were used for western blotting, five to seven mice of each genotype were used for 7-month IHC, and four mice of each genotype were used for 12-month volume measurements. In all figures, the number of mice analyzed is indicated within each graph. Mice were housed with access to food and water *ad libitum* under a 12:12-h light/dark cycle. All animal procedures were performed in accordance with National Institutes of Health guidelines and were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Michigan.

Striata dissections

Mice were anesthetized by isoflurane, euthanized by decapitation, and striata were rapidly isolated using a Zivic instruments mouse brain slicer. Striata were immersed in 300 μ L of immunoprecipitation buffer (10% glycerol, protease inhibitors, sodium vanadate in Tris buffered saline (TBS), pH 6.8) with each hemisphere of the striatum treated individually. Tissues were homogenized rapidly (Tissuelyser II, Qiagen) and detergent-extracted (Addition of nonidet p-40 to 1%) by incubation for 30 min, with rotation, at 4 °C. To produce whole-cell lysates (WCLs), one striatal hemisphere from each animal was mixed with 300 μ L 2 $\times$ sodium dodecyl sulfate (SDS) sample buffer (20% glycerol, 4% SDS, 1% β -mercaptoethanol (β -ME), and bromophenol blue in TBS, pH 6.8), boiled for 6 min, resolved by 4–20% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and transferred to polyvinylidene fluoride (PVDF) membranes.

Immunoblotting and antibodies

Membranes were incubated with blocking solution (3% bovine serum albumin (BSA) or 4% milk) in TBS-T (Tris buffered saline, pH 7.4, with 0.1% Tween-20) and incubated overnight with the appropriate primary antibodies in blocking solution. The blots were washed, incubated with appropriate HRP-linked secondary antibodies (Jackson ImmunoResearch, West Grove, PA, USA), washed again, and visualized using an enhanced chemiluminescent substrate (Advansta and ThermoScientific, Waltham, MA, USA). The following primary antibodies were used: anti-p75 (1:2000 Promega, Madison, WI, USA, catalog G323A), anti-p75 (1:2000 Millipore, Billerica, MA, USA, catalog 07-476), anti-p75 (1:2000 Advanced Targeting Systems, San Diego, CA, USA, AB-N01AP), anti-TrkB (1:1000 BD biosciences, San Jose, CA, USA, catalog 610101), anti-actin (1:2000 Iowa Hybridoma Bank, Iowa City, Iowa, USA, catalog JLA 20-9), anti-Bcl-XL (1:4000 Abcam, Cambridge, United Kingdom, catalog ab 178844), anti-XIAP (1:1000 Abcam, catalog ab28151), anti-DARPP-32 (1:2000 Abcam, catalog ab40801), anti-phospho-cJun (1:1000 catalog 9261), anti-phospho-JNK (1:1000 catalog 9251), anti-phospho-Akt (1:1000 Y243/241, catalog 4058), anti-phospho-Src (1:1000 Y416, catalog 2101), and all non-phosphorylated versions of these proteins (1:1000 Cell Signaling Technologies, Danvers, MA, USA). All immunoblots were quantified using ImageJ software (NIH) and graphed as mean $\pm$ SEM.

Perfusion and immunohistochemistry (IHC)

Seven- to eight- and 12–13-month-old mice were terminally anesthetized with isoflurane and perfused transcardially with heparinized saline (0.1%) followed by 4% paraformaldehyde (PFA) in phosphate buffer. Brains were removed and post-fixed in 4% PFA for 1 h before cryoprotection in 30% sucrose. Brains were frozen in isopentane chilled

over dry ice and stored at -80°C until use. Coronal sections were cut at $60\text{ }\mu\text{m}$ on a cryostat (CM1950, Leica), and stored in cryoprotection solution at -20°C until use. For IHC, sections were rinsed in phosphate-buffered saline (PBS) and endogenous peroxidases were quenched in 1% hydrogen peroxide for 10 min. After rinses, sections were incubated in 2.5% normal donkey serum (Jackson labs) and MOM (Vector, for NeuN-staining) in 0.3% Triton X-100 in PBS. Sections were then incubated for 15–18 h at RT with either anti-NeuN (1:10,000; Millipore) or anti-DARPP-32 (1:300,000; Abcam). After washing, sections were incubated with a biotinylated secondary antibody (anti-mouse or rabbit, 1:500; Jackson ImmunoResearch). Staining of tissue-bound antibodies was visualized using a standard peroxidase-based method (Vectastain Elite, Vector Labs) with the 3,3-diaminobenzidine chromogen (DAB, Sigma, St. Louis, MO, USA).

Image analysis and cell counting

Immunohistochemical images were collected using a Zeiss Axiovert 200M inverted microscope at $10\times$. All conditions were imaged using identical microscope settings and entire striata were tiled together for analysis. From a randomized starting point, every sixth slice ($60\text{-}\mu\text{m}$ thickness) containing the striata was imaged. An average of six slices per animal were imaged, and these results were averaged to provide one value per animal. For volume measurements, contours were drawn around the striatum on every sixth slice, and volume was estimated using the Cavalieri volume estimation method. An experimenter blinded to genotype made all measurements.

Statistics

All results are expressed as mean $\pm$ SEM. Experimental data from *Q175;p75* mice were analyzed by one-way analyses of variance (ANOVAs) followed by post hoc Tukey–Kramer minimum significant differences comparison. Experimental data from *Q175* mice were analyzed by Student *t*-tests. A value of $p < 0.05$ was accepted as denoting statistically significant differences. To justify parametric testing, all data sets were tested for normality using the Shapiro–Wilk test for normality. Sampling was random, and all samples are independent. Homogeneity of variance was determined using the Brown–Forsythe test.

RESULTS

p75 and TrkB levels unchanged at 5 months in the striatum of Q175 mice

Although the levels of p75 were reported to be increased in some animal models of HD, levels of p75 have not been examined in *Hdh^{+/Q175}* mice before 12 months of age. We examined p75 protein levels in striata of *Hdh^{+/+}* and *Hdh^{+/Q175}* mice from WCLs at 5 months of age (Fig. 1). This age was chosen because it is a pre-symptomatic time point and may provide insight into pathophysiological changes occurring before onset of overt pathology. p75 levels from WCLs were not altered (Fig. 1A, C). TrkB levels were examined previously in these mice at 6, 12, and 16 months, showing an increase in TrkB in the striatum at 12 months and no change in TrkB levels at 6 or 16 months in the striatum (Smith et al., 2014). In agreement with this finding, no significant difference in TrkB protein levels was observed in the striata of *Hdh^{+/+}* and *Hdh^{+/Q175}* mice at 5 months of age (Fig. 1A, C). These levels, however, only reflect the total amount of TrkB protein in striata and do not

represent activation or surface levels. Taken together, in the striata of 5-month-old *Hdh^{+/-}/Q175* mice, there was no significant change in striatal levels of either p75 or TrkB when compared to *Hdh^{+/-}* mice.

Apoptotic signaling pathways were not altered in the striatum of *Hdh^{+/-}/Q175* mice at 5 months

In some cellular contexts, signaling through p75 leads to activation of apoptotic signaling pathways, caspase activation, and programmed cell death (Kraemer et al., 2014). To determine if apoptotic signaling was enhanced at 5 months in *Hdh^{+/-}/Q175* mice as compared to *Hdh^{+/-}* mice, we examined the level of activation of c-Jun N-terminal kinase (JNK) and cJun, as well as expression levels of phosphatase and tensin homolog (PTEN). Activation of JNK leads to phosphorylation and activation of cJun, which then leads to transcription of pro-apoptotic genes (Putcha et al., 2001; Freeman et al., 2004). Activation of JNK also leads to activation of pro-death members of the Bcl-2 family, such as Bim and Bax (Bogoyevitch and Kobe, 2006). PTEN inhibits the PI3K/Akt signaling pathway (Datta et al., 1999). PTEN levels and JNK and cJun phosphorylation are increased by pro-apoptotic p75 activation (Kraemer et al., 2014). WCLs of *Hdh^{+/-}* and *Hdh^{+/-}/Q175* mice were examined by p-JNK, JNK, and p-cJun immunoblotting. Phosphorylation of cJun leads rapidly to its own transcriptional upregulation and therefore, normalizing to total cJun levels can lead to an underrepresentation of the extent of cJun activation (Eilers et al., 1998). The 46-kDa isoform of JNK was analyzed as it was significantly more abundant in our samples than the 54-kDa isoform. When comparing *Hdh^{+/-}/Q175* mice to *Hdh^{+/-}* littermates, there were no differences in striatal JNK or cJun phosphorylation (Fig. 2A, B, C). Likewise, PTEN immunoblotting did not reveal any significant differences between *Hdh^{+/-}/Q175* mice and *Hdh^{+/-}* mice (Fig. 2A, D). Given that the activation of JNK and cJun and the levels of PTEN were unchanged, there does not appear to be an increase in p75-related apoptotic signaling in the striatum of *Hdh^{+/-}/Q175* mice at 5 months of age.

Akt activation was increased in the striatum of *Hdh^{+/-}/Q175* mice at 5 months

While p75 signaling can lead to apoptosis, it can also lead to survival when activated independently or when paired with the correct co-receptors and downstream effectors. To examine whether survival pathways were affected in *Hdh^{+/-}/Q175* striata at 5 months of age, we examined phosphorylation of Src on Tyr416 and Akt on Ser473. Src can be phosphorylated on Tyr416 following TrkB activation and this phosphorylation event induces Src catalytic activity, enhancing cell survival (Huang and McNamara, 2010). By immunoblotting, there was no change in Src activation in the striata of *Hdh^{+/-}/Q175* mice as compared to *Hdh^{+/-}* mice (Fig. 2A, E). Akt activation, which can be monitored via Akt phosphorylation at Ser473, promotes survival by inactivating Bad and Caspase 9, among other pro-apoptotic proteins (Datta et al., 1999). We examined Akt phosphorylation because it can be activated by TrkB and p75 independently (Datta et al., 1999; Kraemer et al., 2014). Phosphorylation of Akt at Ser473 was significantly increased in the striata of *Hdh^{+/-}/Q175* mice when compared to *Hdh^{+/-}* littermates (Fig. 2A, F). This increase in the activation of Akt in the striata of *Hdh^{+/-}/Q175* mice at 5 months of age suggests augmented survival signaling.

Analysis of p75 and TrkB in *Hdh^{+/-}Q175;p75* mice

After surveying survival and apoptotic signaling pathways in *Hdh^{+/-}Q175* mice and *Hdh^{+/-}* littermates, we evaluated the functional role of p75 in the observed pro-survival signaling. To address this question, *Hdh^{+/-}Q175* mice were crossed into a germ-line knock-out of p75 (Bogenmann et al., 2011), and the following genotypes were examined at 5 months of age: *Hdh^{+/-};p75^{+/-}*, *Hdh^{+/-}Q175;p75^{+/-}*, *Hdh^{+/-}Q175;p75^{-/-}*, and *Hdh^{+/-};p75^{-/-}*. In this mixed background, we again examined p75 levels by direct immunoblotting (WCL). In attempting to probe the WCLs for p75 using a commonly reported p75 antibody (Promega), our results yielded a non-specific band at the same size as p75 that was present equally in *p75^{+/-}* and *p75^{-/-}* extracts (Fig. 3A panel 1). To combat this issue, we used an alternative antibody (Advanced Targeting Systems, ATS) to measure levels of p75 in WCLs (Fig. 3A panel 2). At 5 months, no significant change in levels of p75 in the striatum of *Hdh^{+/-}Q175;p75^{+/-}* mice when compared to *Hdh^{+/-};p75^{+/-}* littermates was detected (Fig. 3A, B). There was no p75 protein detected in WCLs from *Hdh^{+/-};p75^{-/-}* or *Hdh^{+/-}Q175;p75^{-/-}* mice as expected (Fig. 3A panel 2). The levels of TrkB were also examined in these four genotypes by immunoblotting WCLs for TrkB. As seen in Fig. 1, there was no change in the levels of TrkB in *Hdh^{+/-}Q175;p75^{+/-}* mice compared to *Hdh^{+/-};p75^{+/-}* mice. While there appeared to be a slight decrease in TrkB in both *Hdh^{+/-}Q175;p75^{-/-}* and *Hdh^{+/-};p75^{-/-}* mice, this difference was not statistically significant (Fig. 3A, C).

Increased survival signaling in *Hdh^{+/-}Q175* mice is abolished by p75 deletion

To determine mechanistically whether p75 was important for the increase in survival signaling observed in *Hdh^{+/-}Q175* mice at 5 months of age, Akt activation was examined as in Fig. 2. Similar to the increase seen in *Hdh^{+/-}Q175* mice, Akt activation was also increased in *Hdh^{+/-}Q175;p75^{+/-}* mice compared to *Hdh^{+/-};p75^{+/-}* control littermates (Fig. 4B). This increase was completely abolished in *Hdh^{+/-}Q175;p75^{-/-}* mice (Fig. 4A, B). Because Akt activation was increased in *Hdh^{+/-}Q175* mice (Fig. 2A, F), we decided to also examine activation of NFκB. NFκB activation is capable of promoting survival downstream of p75 activation in multiple cell types (Mattson et al., 1997; Middleton et al., 2000; Vicario et al., 2015), but does so independently of TrkB activity (Carter et al., 1996). The p65 subunit of NFκB can be phosphorylated on several different serine residues, all leading to slightly different transcriptional outcomes. We examined phosphorylation of p65 at Ser276 and at Ser536 because these two serines are important for more potent and longer NFκB-dependent transcriptional activation (Neumann and Naumann, 2007). p65 subunit phosphorylation at both residues was increased in *Hdh^{+/-}Q175;p75^{+/-}* mice compared to *Hdh^{+/-};p75^{+/-}* mice. This increase was completely abolished in *Hdh^{+/-}Q175;p75^{-/-}* mice (Fig. 4A, C, D). B-cell lymphoma-extra large (Bcl-XL) and X-linked inhibitor of apoptosis (XIAP) are inhibitors of apoptosis known to be transcriptionally up-regulated by activation of NFκB (Stehlik et al., 1998; Chao et al., 2011). Interestingly, levels of both Bcl-XL and XIAP were significantly lower in *Hdh^{+/-}Q175;p75^{-/-}* mice when compared to *Hdh^{+/-}Q175;p75^{+/-}* mice (Fig. 5). We also examined the expression of Bim, a pro-apoptotic BH3-only Bcl2 family member that is induced during programmed cell death (Putcha et al., 2001). We did not see any alteration in the expression of Bim between *Hdh^{+/-};p75^{+/-}*, *Hdh^{+/-}Q175;p75^{+/-}*, *Hdh^{+/-}Q175;p75^{-/-}*, or *Hdh^{+/-};p75^{-/-}* mice (data not shown). Taken together, these data suggest that at pre-

symptomatic ages in *Hdh^{+/Q175}* mice p75 upregulates pro-survival signaling pathways, and that loss of this p75-mediated augmentation leads to decreased survival signaling.

Striatal dysfunction is accelerated in *Hdh^{+/Q175}; p75^{-/-}* mice

Striatal dysfunction and eventual degeneration is a hallmark in patients with HD and in some animal models of HD. Healthy MSNs express high levels of dopamine and cAMP-regulated phosphoprotein, MW 32 kDa (DARPP-32), which plays a critical role in the processing of dopamine signaling in the striatum. Decreased expression of DARPP-32 is a pathological marker of several diseases affecting striatal function, including HD (Bibb et al., 2000; Dellen et al., 2000). A previous examination of DARPP-32 levels in *Hdh^{+/Q175}* mice showed DARPP-32 to be significantly reduced in *Hdh^{+/Q175}* mice compared to *Hdh^{+/+}* littermates at 12 months of age, but not at earlier time points (Smith et al., 2014). In agreement with prior work, we did not observe a change in DARPP-32 expression in *Hdh^{+/Q175};p75^{+/+}* mice at 5 months by western blot, nor at 7 months by IHC, when compared to *Hdh^{+/+};p75^{+/+}* littermates (Fig. 6). Interestingly, DARPP-32 expression decreased significantly in *Hdh^{+/Q175};p75^{-/-}* mice both by western blot at 5 months and by IHC at 7 months compared to *Hdh^{+/+};p75^{+/+}*, *Hdh^{+/Q175};p75^{+/+}*, and *Hdh^{+/+};p75^{-/-}*, suggesting an earlier onset of striatal dysfunction (Fig. 6).

Striatal volume decreased in *Hdh^{+/Q175};p75^{-/-}* mice at 12 months of age

We evaluated striatal volume using NeuN IHC at 7 and 12 months of age. Differences in striatal volume have been reported at 12 months of age in *Hdh^{+/Q175}* mice, but not earlier (Smith et al., 2014). No significant difference was observed in striatal volume in any genotype examined at 7 months of age (Fig. 7). As the density of NeuN+ cells also does not change (data not shown), overt neuronal cell loss does not seem to have begun in these mice at this time point. At 12 months of age, however, striatal volume is significantly decreased in *Hdh^{+/Q175};p75^{+/+}* mice compared to *Hdh^{+/+};p75^{+/+}*, as reported previously. Importantly, striatal volume is further decreased in *Hdh^{+/Q175};p75^{-/-}* mice compared to both *Hdh^{+/+};p75^{+/+}* and *Hdh^{+/Q175};p75^{+/+}* mice. This decrease demonstrates a more severe degeneration in the striatum of *Hdh^{+/Q175};p75^{-/-}* mice compared to *Hdh^{+/Q175};p75^{+/+}* littermates.

DISCUSSION

In this study, we examined the extent to which p75 is involved in the early striatal response to mutant *Hdh* and effects of the loss of early p75 expression on later stage neuronal survival. *Hdh^{+/Q175};p75^{+/+}* mice showed increased activation of Akt, increased phosphorylation of the p65 subunit of NFκB at two sites, and increased XIAP expression at 5 months of age when compared to *Hdh^{+/+};p75^{+/+}* littermates. These increases were abolished in *Hdh^{+/Q175};p75^{-/-}* mice, suggesting that p75 is essential for this enhanced survival signaling. Additionally, *Hdh^{+/Q175};p75^{-/-}* mice showed a decrease in Bcl-XL expression compared to *Hdh^{+/Q175};p75^{+/+}* and *Hdh^{+/+};p75^{+/+}* littermates, as well as a decrease in DARPP-32 expression, consistent with a loss of survival signaling and an earlier onset of dysfunction in *Hdh^{+/Q175};p75^{-/-}* mice. Finally, *Hdh^{+/Q175};p75^{-/-}* showed decreased striatal volume at 12 months of age compared to both *Hdh^{+/+};p75^{+/+}* and *Hdh^{+/Q175};p75^{+/+}*

littermates. These data suggest a role for p75 in augmenting survival signaling in the striata of *Hdh^{+/Q175}* mice, and that the loss of this early pro-survival signaling leads to earlier onset of striatal dysfunction/degeneration.

While these data support a role for p75 in augmenting survival signaling prior to onset of dysfunction in the striata of *Hdh^{+/Q175}* mice, it is possible that the role of p75 changes with disease progression, acts in a region-specific manner, and/or differs with animal model. Plotkin et al. demonstrated that the inhibition of p75 rescued the loss of synaptic plasticity seen in the striatum of symptomatic BACHD mice (2014). The BACHD model used in this study differs substantially from the *Hdh^{+/Q175}* mice used in this study, both in symptom onset and disease course, and this paper was examining a specific type of synaptic plasticity, while we focused on biochemical and histological changes. In addition, Brito et al. reported rescued learning and memory deficits in Q111 mice by decreasing the level of p75 expression in the hippocampus (2014). These two studies suggest a potentially pathogenic role for p75 in HD at symptomatic disease stages and/or possibly in a regionally specific, or animal model-specific fashion. To address whether p75 signaling can switch from survival-promoting to dysfunctional later in disease progression, or whether p75 functions differently in different populations of cells, conditional p75 mice should be utilized in future experiments. Crossing conditional p75 mice into varied Cre recombinase-expressing strains will allow for cell-type-specific and temporal-specific deletion. It is important to acknowledge that, unlike our results, other studies have reported an increase in p75 protein and mRNA levels in the striatum of mouse models of HD and HD post-mortem brains, respectively (Zuccato et al., 2008; Brito et al., 2013). The mouse models used in these prior studies were a truncation model (R6/1) and a knock-in model (Q111). The R6/1 model differs considerably from the heterozygous *Hdh^{+/Q175}* mice. R6/1 is more rapidly progressive, and the truncated mutation is transgenically expressed rather than being expressed from the endogenous locus (Brooks et al., 2011; Pouladi et al., 2013). We were unable to achieve specific labeling with the p75 antibody used in the Q111 and human post-mortem studies, and therefore a direct comparison with these studies is difficult (Brito et al., 2013, 2014). Interestingly, in the same Q175 model of HD used in this study, p75 levels were reported to be decreased at 12 months of age in both the heterozygous and homozygous mutants when compared to WT animals (Ma et al., 2015). p75 levels in the striatum, therefore, may vary depending on which HD mouse model is investigated.

Considering these data along with the growing literature involving p75 and HD, it is clear that p75 signaling in HD mouse models is more complicated than initially appreciated. There is interest in developing p75 antagonists as a disease-modifying treatment for HD. Antagonizing p75 prior to dysfunction may be counter-productive, however, and developing p75-specific antagonists may only be helpful later in the course of the disease. An effective early intervention could take advantage of augmenting and/or prolonging this prosurvival signaling via p75. Additionally, it will be important to determine which cell type(s) express(es) p75 in the striatum of *Hdh^{+/Q175}* mice. The simplest model would be cell-autonomous signaling in MSNs leading to increased activation of Akt and NFκB and followed by an increase/normalization in survival targets of Akt and NFκB, such as XIAP and Bcl-XL. This process, however, may not necessarily be cell-autonomous. Other non-neuronal cell types present in the striatum, such as astrocytes, microglia, and

oligodendrocytes could express p75 and provide some type of extrinsic support for the MSNs. Some expression of p75 in MSNs has been reported previously (Brito et al., 2014). A recent study, however, indicates that the majority of p75 in the striatum is expressed in immature oligodendrocytes, suggesting at least some p75 signaling is non-cell autonomous (Ma et al., 2015). Additionally, this enhanced survival signaling may be through p75 alone, through p75/TrkB complexes, or through p75 complexed with a different receptor. Ultimately, if this potential compensatory mechanism can be better understood, it may be possible to find novel targets to augment the brain's natural defenses, prolonging normal function, and delaying, or even preventing, the onset of dysfunction.

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Abbreviations

Bcl-XL	B-cell lymphoma-extra large
BDNF	brain-derived neurotrophic factor
DARPP-32	dopamine and cAMP-regulated phosphoprotein, MW 32 kDa
HD	Huntington's disease
HTT	Huntingtin protein
IHC	immunohistochemistry
JNK	c-Jun N-terminal kinase
mHTT	mutated HTT
MSN	medium spiny neuron
NFκB	nuclear factor kappa B
p75	p75 neurotrophin receptor
PBS	phosphate-buffered saline
PFA	paraformaldehyde
PTEN	phosphatase and tensin homolog
RE1/NRSE	repressor element 1/neuron-restrictive silencer element
SDS	sodium dodecyl sulfate
TBS	Tris-buffered saline
TrkB	Tropomyosin receptor kinase receptor B
WCL	whole-cell lysate
XIAP	X-linked inhibitor of apoptosis

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Fig. 1.

Analysis of TrkB and p75 in the striatum of *Hdh*^{+/Q175} mice at 5 months. A half of the striata from 5-month-old mice were used for whole-cell lysates (WCLs). (A) Western blots for p75 and TrkB from the IPs (top two panels) and from actin are shown. (B, C) Quantifications of (A) are graphed as mean $\pm$ SEM. Actin levels were used as a loading control, and protein levels are normalized to *Hdh*^{+/+} animals processed on the same blots. No significant differences in p75 or TrkB levels between *Hdh*^{+/+} and *Hdh*^{+/Q175} mice were observed (*t*-test; *p* > 0.05).

Fig. 2. Biochemical analysis of apoptotic and survival signaling in the striatum of *Hdh*^{+/Q175} mice at 5 months. WCLs of the striata from 5-month-old mice of the stated genotypes were analyzed by immunoblotting. (A) Western blots were run on WCLs using the stated antibodies. (B–F) Quantifications of (A) were performed as in Fig. 1. No significant differences were seen between *Hdh*^{+/+} and *Hdh*^{+/Q175} mice in JNK, cJun, or Src activation, or in PTEN levels. A significant increase in Akt activation was observed. (*t*-test; *p* < 0.05).

Fig. 3.

Examination of p75 and TrkB association and expression in the striatum of *Hdh^{+/Q175};p75^{-/-}* mice at 5 months. A half of the striata from 5-month-old mice of the stated genotypes were used to produce WCLs. (A) Western blots for WCLs using the stated antibodies. The WCLs were tested for p75 using two separate antibodies, as listed. (B, C) Quantifications of (A). Actin levels were used as a loading control, and protein levels were normalized to WT animals processed on the same gel. Graphs are mean $\pm$ SEM. No significant change in p75 levels was observed in Q175 mice compared to WT littermates. A

significant decrease was observed in WCL TrkB levels in both *Hdh^{+/Q175};p75^{-/-}* and *Hdh^{+/+};p75^{-/-}* compared to WT littermates. (ANOVA; $p < 0.05$).

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Fig. 4.

Activation of Akt and NFκB is increased in the striatum of *Hdh*^{+/Q175}; *p75*^{+/+} mice and not *Hdh*^{+/Q175}; *p75*^{-/-} mice at 5 months. WCLs from 5-month-old mice of the stated genotypes were examined by immunoblotting. (A) Western blots were run on WCLs using the stated antibodies. (B–D) Immunoblots were normalized and quantified as in Fig. 1. A significant increase in phosphorylation of Akt at Ser473 and NFκB at Ser276 and Ser536 was observed in *Hdh*^{+/Q175}; *p75*^{+/+} mice, and this increase was abolished in *Hdh*^{+/Q175}; *p75*^{-/-} mice (ANOVA; $p < 0.05$).

Fig. 5.

XIAP and Bcl-XL expression decreased in the striatum of *Hdh^{+/Q175};p75^{-/-}* mice compared to *Hdh^{+/Q175};p75^{+/+}* mice at 5 months. (A) WCLs from a half of the striata of the stated genotypes at 5 months of age were tested using the listed antibodies. (B, C) Quantifications of (A). A significant decrease in XIAP and Bcl-XL expression was observed in *Hdh^{+/Q175};p75^{-/-}* mice compared to *Hdh^{+/Q175};p75^{+/+}* littermates (ANOVA; $p < 0.05$).

Fig. 6. DARPP-32 expression decreased in the striatum of *Hdh^{+/Q175};p75^{-/-}* mice compared to *Hdh^{+/+};p75^{+/+}* mice. (A) WCLs from a half of the striata of the stated genotypes at 5 months of age were probed for the listed antibodies. (B). Quantification of (A). (C–F) Immunohistochemistry was performed on the striata of 7-month-old mice of the stated genotype for DARPP-32 as described in the methods at 10 $\times$. (G–J) Images at 64 $\times$. Decreased levels of DARPP-32 were observed with WB and IHC in the striatum of *Hdh^{+/Q175};p75^{-/-}* mice compared to *Hdh^{+/+};p75^{+/+}*, *Hdh^{+/Q175};p75^{+/+}*, and *Hdh^{+/+};p75^{-/-}* littermates.

Fig. 7.

Striatal volume is decreased in the striatum at 12 months. NeuN immunohistochemistry was performed on the striata of 7–8- and 12–13-month-old mice of the stated genotypes as described in the methods. (A) Striatal volume quantifications of 7–8-month-old mice. No significant differences were observed in either neuronal density or striatal volume in any genotype. (B) Striatal volume quantifications of 12–13-month-old mice. * denotes significant decrease compared to *Hdh*^{+/+}; *p75*^{+/+} littermates. & denotes significant decrease compared to *Hdh*^{+/+}; *Q175*; *p75*^{+/+} littermates, # denotes significant decrease compared to

Hdh^{+/+}; *p75*^{-/-} littermates, and ^ denotes significant decrease compared to the same genotype at 7 months (ANOVA; $p > 0.05$).

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