

Impaired striatal function in Huntington's disease is due to aberrant p75NTR signaling

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Abbreviations: BDNF, brain-derived neurotrophic factor; dSPN, direct pathway spiny projection neuron; HD, Huntington's disease; iSPN, indirect pathway spiny projection neuron; LTP, long term potentiation; mHtt, mutant huntingtin; NGF, nerve growth factor; NMDA, N-methyl D-aspartate; NR2B, N-methyl D-aspartate receptor subtype 2B; NT3, neurotrophin 3; NT4, neurotrophin 4; p75NTR, p75 neurotrophin receptor; PI3K, phosphoinositide-3 kinase; PKB, protein kinase B; PTEN, phosphatase and tensin homolog deleted on chromosome 10; TrkB, tyrosine kinase receptor B.

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Impaired TrkB receptor signaling underlies corticostriatal dysfunction in Huntington's disease. J. L. Plotkin, M. Day, J.D. Peterson, Z. Xie, G.J. Kress, I. Rafalovich, J. Kondapalli, T.S. Gertler, M. Flajolet, P. Greengard, M. Stavarache, M.G. Kaplitt, J. Rosinski, C.S. Chan and D.J. Surmeier. (2014). *Neuron* 83 (1):178-188.

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Huntington's disease (HD) is a rare genetic neurodegenerative disorder for which there is currently no cure. Early hyperkinetic motor symptoms are consistent with reduced activity of indirect pathway striatal projection neurons (iSPNs) responsible for suppression of unwanted actions. Our recent work suggests that one of the factors contributing to this deficit is impaired brain-derived neurotrophic factor (BDNF) signaling that regulates the strength of iSPN excitatory synapses. Specifically, we found that BDNF-dependent corticostriatal synaptic long-term potentiation (LTP) was lost in iSPNs from 2 genetic models of HD, just as they began to robustly manifest motor symptoms. This deficit was not attributable to problems in BDNF production, delivery or receptor binding. Rather, the plasticity deficit stemmed from enhanced signaling through p75 neurotrophin receptors (p75^{NTRs}) and the phosphatase and tensin homolog (PTEN), leading to antagonism of intracellular TrkB cascades and LTP. This study suggests HD therapeutics should target p75^{NTR} signaling, not TrkB.

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder caused by a polyglutamine expansion in the coding region of the huntingtin gene.¹ Among the brain regions most conspicuously affected are the striatum and cerebral cortex.² In fact, progressive loss of enkephalin expression, a marker of indirect pathway striatal projection neurons (iSPNs), and ultimately striatal neuronal atrophy, are among the most well described HD pathologies.³ According to

the classical model of basal ganglia motor control,^{4,5} impaired iSPN function should compromise the ability to suppress unwanted movements, consistent with the choreic motor symptoms associated with early to mid-stage HD.^{2,6}

In recent years, the impairment of iSPNs has been attributed to diminished trophic support of the striatum by the cerebral cortex.⁷ Specifically, the delivery of cortically made brain-derived neurotrophic factor (BDNF) to the striatum has been posited to be responsible for iSPN atrophy. Despite the wide acceptance of this model, there are key aspects of it that have not been tested. In particular, it has never been shown that BDNF release by cortical axons and binding to postsynaptic TrkB receptors is down-regulated in HD models at the point in time when motor symptoms begin to emerge. To address this need, we developed a novel synaptic plasticity protocol that requires engagement of TrkB signaling cascades by BDNF. In wild-type iSPNs, LTP induction at axospinous corticostriatal synapses requires co-activation of TrkB receptors, N-methyl-D-aspartate (NMDA) receptors, and A2a adenosine receptors. In conjunction with patch clamp recording and 2 photon laser scanning microscopy, 2 photon uncaging of glutamate or optogenetic activation of cortical terminals can be used to monitor LTP induction on a spine-by-spine basis. This type of LTP is only seen at spines that have cortical synapses, consistent with the cortex being the predominant source of striatal BDNF.⁸ The ability to support this TrkB-dependent synaptic plasticity was progressively lost in iSPNs in brain slices from BACHD and Q175 mouse models of HD. By the time HD mice were 6 months of age – an age when the motor

symptoms are very evident – LTP in iSPNs was completely lost but still normal in neighboring direct pathway SPNs (dSPNs).

Why was BDNF/TrkB-dependent synaptic plasticity lost in HD mice? One explanation would be that BDNF production and delivery to iSPNs (but not dSPNs) is impaired. As difficult as this is to envision given the overlap in the cortical projections to these 2 cell types,^{9,10} it is possible. However, using qPCR primers recognizing 6 regions spanning the BDNF gene, normalized to a panel of at least 6 reference genes, we found no evidence of altered cortical BDNF expression in 6 month-old HD mice; nor did we find altered TrkB expression in either dSPNs or iSPNs in these models. Furthermore, there was no deficit in cortical or striatal BDNF protein at this age and activity-dependent phosphorylation of striatal TrkB was unperturbed. Thus, diminished BDNF production, delivery or activation of TrkB cannot be the culprit.

Why did our results ostensibly differ from those of previous reports?^{7,11,12} One possibility is that the procedure used to calibrate estimates of mRNA abundance using quantitative PCR in previous studies yielded spurious results. Nearly all studies reporting changes in BDNF were normalized to a single variable reference gene, either GAPDH or β -actin; both transcripts can be affected by a variety of factors which are undesirable for a 'housekeeping' gene. We avoided this situation by using a weighted average of at least 6 mRNAs.¹³ Another possibility is that the level of mutant huntingtin

(mHtt) expression is critical to phenotype; there is no doubt for example that high levels of mHtt can disrupt axonal trafficking of BDNF.¹⁴ The level of expression needed to have a measurable impact might not be reached in the heterozygous BACHD and Q175 models used in our work. Indeed, in homozygous Q175 mice, cortical expression of BDNF was reduced at 6 months of age. Lastly, the stage in the evolution of the disease might be critical to phenotype. Our work focused on ages when symptoms were becoming clear. But as the disease evolves, other regions and mechanisms might come into play. It is important to remember that the cerebral cortex and the basal ganglia are part of an interdependent neural network. Nevertheless, from the therapeutic standpoint, the earliest events in a pathophysiological cascade should be the best targets.

Although BDNF expression and delivery to the striatum were not altered in HD mice, TrkB signaling was, as determined by phosphorylation of its downstream target, protein kinase B (PKB, also known as Akt). Surprisingly however, signaling elements downstream of TrkB appeared to be intact. Rather, the inability of TrkB activation to induce LTP was due to attenuated signaling through an immediate player in the TrkB cascade: phosphoinositide-3 kinase (PI3K). This deficit was traced back to p75^{NTR}. While overall p75^{NTR} mRNA and protein expression did not appear to be altered, expression of its downstream target PTEN was increased. Thus, p75^{NTR} signaling was amplified in iSPNs from HD mice (Fig. 1). Indeed, the capacity to support

LTP was completely restored in BACHD iSPNs by inhibiting p75^{NTR} and PTEN activity.

p75^{NTR}s can be activated by a number of ligands, including nerve growth factor (NGF), pro-NGF, neurotrophin 3 (NT3), neurotrophin 4 (NT4), and even BDNF itself, albeit less effectively than TrkB. This raises the possibility that, in HD, BDNF is stepping on both the accelerator (TrkB) and the brake (p75^{NTR}s) at the same time. This is consistent with other work showing that the balance between TrkB and p75^{NTR} signaling is altered in HD and that this imbalance can lead to synapse-specific adaptations.¹⁷ If this is the case, compounds that are agonists at TrkB, but not p75^{NTR}s, could be effective therapeutics.^{18,19} The other possibility is that p75^{NTR} signaling is being driven in HD by one of the other agonists at this receptor.

Regardless of the activation mechanism of p75^{NTR}s, their signaling is amplified by increased PTEN expression in symptomatic HD iSPNs. It is interesting to speculate about other potential consequences elevated PTEN activity may have. A well-described synaptic pathology in HD mouse models is increased insertion of N-methyl D- aspartate receptor subtype 2B (NR2B)-containing extrasynaptic NMDA receptors.²⁰ Besides shunting PI3K signaling by dephosphorylating PtdIns(3,4,5)P₃ into PtdIns(4,5)P₂, PTEN physically interacts with NR2B containing NMDARs, enhancing extrasynaptic NMDAR function.²¹ If such an interaction is at play in iSPNs, PTEN may represent another means of enhancing extrasynaptic NMDARs in HD, and the neurotoxic cascades extrasynaptic NR2B receptors are associated with.

Though pathological p75^{NTR} signaling in HD iSPNs seems to be a consequence of elevated PTEN expression, it is a poor therapeutic target because of its role as a tumor suppressor.²² Interestingly, a recent epidemiological study showed that the incidence of cancer is lower in HD patients,²³ a phenomenon that is consistent with elevated PTEN expression. p75^{NTR} is a much better therapeutic target as it is developmentally downregulated in most parts of the brain, suggesting it is

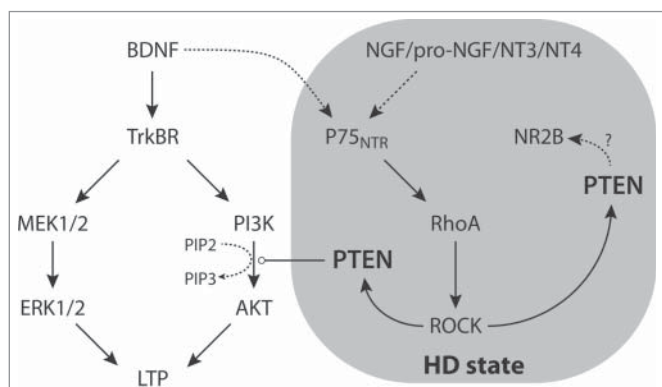


Figure 1. Diagram of TrkB and p75^{NTR} signaling in iSPNs in normal and HD conditions.

dispensable. Although we don't know of a selective p75^{NTR} antagonist, conditional knockouts of p75^{NTR} in adult mice are feasible, making their role, and their viability as a therapeutic target, testable.

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