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Wild-Type Huntingtin Plays a Role in Brain Development and Neuronal Survival

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Abstract

While the role of the mutated Huntington's disease (HD) protein in the pathogenesis of HD has been the focus of intensive investigation, the normal protein has received less attention. Nonetheless, the wild-type HD protein appears to be essential for embryogenesis, since deletion of the HD gene in mice results in early embryonic lethality. This early lethality is due to a critical role the HD protein, called huntingtin (Htt), plays in extraembryonic membrane function, presumably in vesicular transport of nutrients. Studies of mutant mice expressing low levels of Htt and of chimeric mice generated by blastocyst injection of *Hdh–/–* embryonic stem cells show that wild-type Htt plays an important role later in development as well, specifically in forebrain formation. Moreover, various lines of study suggest that normal Htt is also critical for survival of neurons in the adult forebrain.

The observation that Htt plays its key developmental and survival roles in those brain areas most affected in HD raises the possibility that a subtle loss of function on the part of the mutant protein or a sequestering of wild-type Htt by mutant Htt may contribute to HD pathogenesis. Regardless of whether this is so, the prosurvival role of Htt suggests that HD therapies that block production of both wild-type and mutant Htt may themselves be harmful.

Index Entries: Basal ganglia; cortex; development; Huntington's Disease; HD gene; colonization.

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Huntington's Disease and the HD Gene

Huntington's disease (HD) is a dominant hereditary neurodegenerative disorder that afflicts approx 30,000 people in the US, and many more worldwide, with nearly twice as many additional individuals being asymptomatic carriers of the gene defect (Conneally, 1984; Bruyn and Went, 1986; Wilson et al., 1987; Albin and Tagle, 1995). The primary site of neuronal cell loss in HD is the striatal portion of the basal ganglia, with additional significant neuron loss occurring in the cerebral cortex (Vonsattel et al., 1985; De La Monte et al., 1988; Hedreen et al., 1991; Storey et al., 1992; Rosas et al., 2002). The neuron loss is progressive and is accompanied by a cognitive decline and deterioration in the ability to control movement (Bruyn and Went, 1986; Wilson et al., 1987; Albin and Tagle, 1995; Vonsattel et al., 1985). The decline associated with this disease is fatal, typically about 20 yr after onset in adults. The gene whose mutation is responsible for HD codes for a 350 kDa protein that is known as huntingtin (Htt) or the HD protein (Huntington's Disease Collaborative Research Group, 1993). The mutation in the HD gene that causes the disease involves an expansion of a CAG repeat in exon 1 of the gene beyond the normal 10–35 repeat range (Albin and Tagle, 1995), resulting in a protein that contains an abnormally long polyglutamine tract in its N-terminus.

The HD gene defect is generally thought to act as a "gain-of-function" mutation, and several lines of evidence are commonly offered for this view (Sharp and Ross, 1996; Ross, 2002). First, HD shows autosomal dominant inheritance, which is typically associated with a gain-of-function mutation. Second, hemizygous inactivation of the HD gene does not cause HD symptoms in humans or mice, despite a reduction in HD gene expression to half of normal (Ambrose et al., 1994; Duyao et al., 1995; Nasir et al., 1995; Zeitlin et al., 1995), Finally, nullizygous mutant mice die *in utero* (Duyao et al., 1995; Nasir et al., 1995; Zeitlin et al., 1995), whereas humans that are homozygous for the HD mutation are born and have generally not been found to show clear differences from HD heterozygotes in disease onset or progression (Myers et al., 1989; Wexler et al., 1987). While the precise nature of the deleterious gain-of-function caused by the polyglutamine expansion in mutant Htt remains uncertain, a number of possibilities have been raised, including transcriptional dysregulation (Kegel et al., 2002; Luthi-Carter et al., 2002; Ross, 2002), proteosomal dysfunction (Chai et al., 1999; Bence et al., 2001), induction of autophagy (Kegel et al., 2000; Petersén et al., 2001), and induction of apoptosis (Sanchez et al., 1999; Zeron et al., 2002). As part of the effort to identify the putative harmful gain-of-function, considerable attention has focused on the means by which the mutant protein in its free or aggregated form might affect the function of other key intracellular proteins or processes so as to cause damaging consequences (DiFiglia et al., 1997; Davies et al., 1997; Nucifora et al., 2001; Luthi-Carter et al., 2002). Because of its presumed irrelevance to HD pathogenesis, far less attention has been devoted to the role of wild-type Htt in normal cellular functions. The authors review recent evidence that the normal protein plays key roles in forebrain development and neuronal survival, and discuss the possibility that these roles may be relevant to HD pathogenesis and treatment. The HD protein of humans and its orthologs in nonhumans is referred to as huntingtin for simplicity (SWIS-PROT database; http://us.expasy.org/sprot).

Regional and Cellular Localization of Huntingtin

The mRNA coding for the Htt protein and Htt itself are widely distributed throughout brain, and almost no brain region is devoid of huntingtin-containing perikarya—although glial cells typically do not appear to contain signifi-

cant levels of Htt (Strong et al., 1993; Sharp and Ross, 1996; Li et al., 1993; Landwehrmeyer et al., 1995; Bhide et al., 1996; Sapp et al., 1997; Vonsattel and DiFiglia, 1998; Fusco et al., 1999), with the notable exception of astrocytes in the arcuate nucleus of postpartum rats (Hebb et al., 1999). Large neuronal perikarya tend to be richer in Htt than medium-sized or small neuronal perikarya, and Htt-positive neurons are especially abundant in the telencephalon and thalamus, but seemingly sparse in the hypothalamus. Within telencephalon, the highest density of Htt-rich neurons is in neocortex, in which pyramidal neurons of layers 3 and 5 are especially rich (Fig. 1), and in hippocampus, in which the pyramidal neurons of CA2–CA3 are labeled intensely for Htt. The vast majority of striatal neurons are, however, moderate in Htt, but scattered large neurons in striatum and the large neurons of globus pallidus, the ventral pallidum, basal nucleus of Meynert, and the entopeduncular nucleus are rich (Fig. 1). The disease-producing mutation in the HD gene does not appear to affect its regional expression in brain (Landwehrmeyer et al., 1995; Schilling et al., 1995; Bhide et al., 1996; Sapp et al., 1997; Vonsattel and DiFiglia, 1998). Thus, while the widespread distribution of Htt in brain indicates that it possesses a role in the functioning of many brain neurons, this function is not limited to the brain regions and neurons that are the major target of HD, and Htt expression is not obviously selectively impaired in the regions or neurons most affected by the HD mutation.

At the cellular level Htt is found in the cytoplasm of neuronal perikarya, in dendrites, and to seemingly a lesser extent in axons and terminals (Vonsattel and DiFiglia, 1998). Immunolabeling and immunoprecipitation studies indicate that Htt associates with vesicle membranes and microtubules, and may therefore be involved in intracellular vesicular transport, membrane trafficking, or the endosomal-lysosomal protein degradation pathway (DiFiglia et al., 1995; Gutekunst et al., 1995; Sharp et al., 1995; Wood et al., 1996; Sapp et al., 1997; Velier et al., 1998; Vonsattel and DiFiglia, 1998). Nuclear localization of full-length wild-type

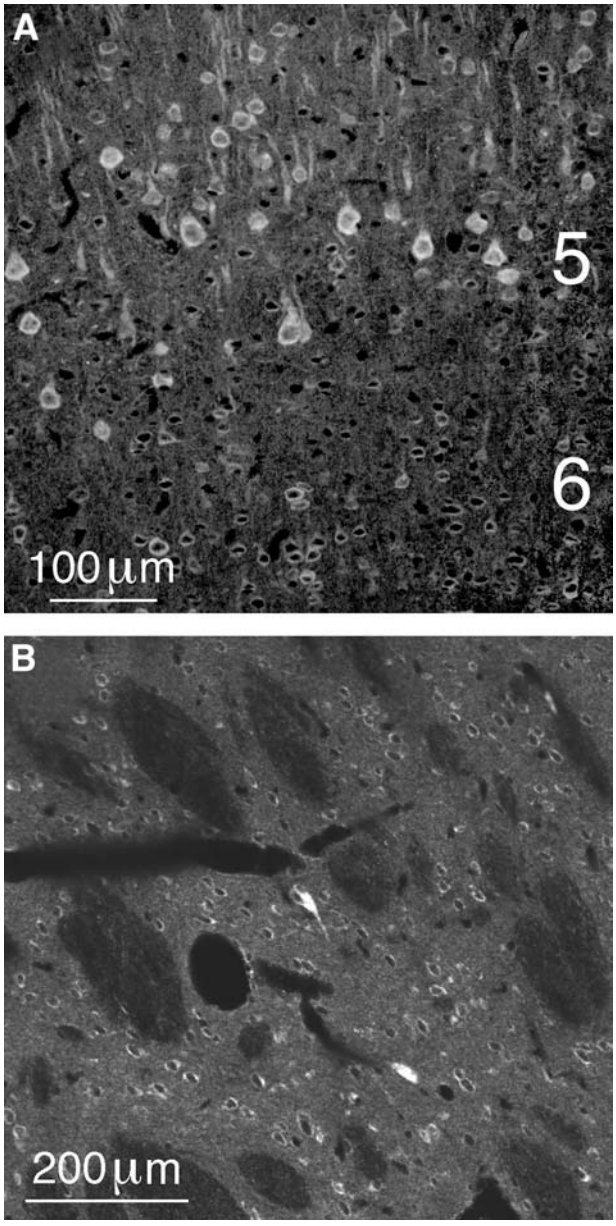

Fig. 1. Images of immunofluorescence labeling for huntingtin (Htt) in rat cortex **(A)** and rat striatum **(B),** taken using confocal laser scanning microscopy and tyramide signal amplification. The field shown in A depicts intense labeling of pyramidal neurons in layer 5 of cortex, while B shows scattered large striatal neurons intensely labeled for huntingtin, and medium-sized striatal neurons that are only moderately labeled.

Htt has also been reported for neurons (Dorsman et al., 1999; Wilkinson et al., 1999). In fibroblasts, Htt has been shown to be localized to the Golgi complex and perinuclear tubulovesicular membranes (Ross et al., 1998; Velier et al., 1998). Ko et al. (2001) recently suggested, based on studies using antibodies directed against different epitopes of wild-type Htt, that Htt may play diverse roles in cellular function. Presumably as a reflection of this diversity, they found that different epitopes of Htt are immunohistochemically detectible in different subcellular compartments, implying differential processing or folding of Htt for its role in the different compartments. These various studies of Htt localization, however, have not yet provided definitive evidence for a specific role in any subcellular compartment. Similarly, while numerous proteins that interact with Htt have been identified, they are themselves rather diverse in function and do not unequivocally clarify the intracellular role or roles of wild-type Htt (Ross et al., 1998; Cattaneo et al., 2001).

Role of Huntingtin During Early Embryonic Development

While studies of Htt localization have provided clues but no certain answers regarding the function of wild-type Htt, genetic manipulations in mice have indicated that Htt is critical for embryogenesis, craniofacial formation, and forebrain development. For example, homozygous deletion of the mouse homolog of the HD gene *(Hdh)* results in early embryonic lethality (Duyao et al., 1995; Nasir et al., 1995; Zeitlin et al., 1995). The basis of this effect appears to be increased apoptosis in the embryonic ectoderm shortly after the onset of gastrulation, resulting in embryo death between embryonic d 8.5 and 10.5. The nullizygous embryos are developmentally retarded and become increasingly disorganized prior to death. Early embryonic lethality, however, can be evaded in chimeric mouse embryos generated by injecting null-*Hdh* embryonic stem (ES) cells into wild-type host blastocysts (Dragatsis et al., 1998). Embryos created by this method, even those that are extensively colonized by *Hdh–/–* cells, can develop and survive well beyond birth, demonstrating that the critical function of Htt in early development lies in the extraembryonic tissues (Dragatsis et al., 1998). Although a role of Htt in the functioning of extraembryonic membranes derived from the trophoblast lineage (such as the extraembryonic ectoderm) was not excluded by these studies, the overall results favor the interpretation that *Hdh* deletion mainly interferes with embryo viability by impairing the nutritive function of the visceral endoderm (Dragatsis et al., 1998).

By contrast, viable chimeras possessing cells with homozygous *Hdh* deletion cannot be readily generated by the early embryo aggregation method of chimera production (Reiner et al., 2001). The apparent participation of Htt in vesicular trafficking (DiFiglia et al., 1995; Sharp et al., 1995; Wood et al., 1996) may be important in the transport of nutrients across the extraembryonic membranes, and disruption of this function may be the basis of the deleterious effect of Htt deletion on the extraembryonic membranes (Dragatsis et al., 1998). Huntingtin with a polyglutamine expansion in the range causing HD, however, does not critically disrupt this function, since humans with a homozygous HD mutation do not exhibit embryonic lethality (Wexler et al., 1987; Myers et al., 1989; Gusella and MacDonald et al., 1996), and also because a transgene expressing Htt with such a polyglutamine expansion rescues *Hdh*-null mice from embryonic lethality (Hodgson et al., 1999; Leavitt et al., 2001). Additionally, one normal Htt allele is sufficient in humans or mice for development and postpartum life that is largely or entirely indistinguishable from normal (Ambrose et al., 1994; Duyao et al., 1995; Nasir et al., 1995; Zeitlin et al., 1995; Persichetti et al., 1996). By contrast, the inability to produce viable chimeras containing *Hdh–/–* cells using the method of early

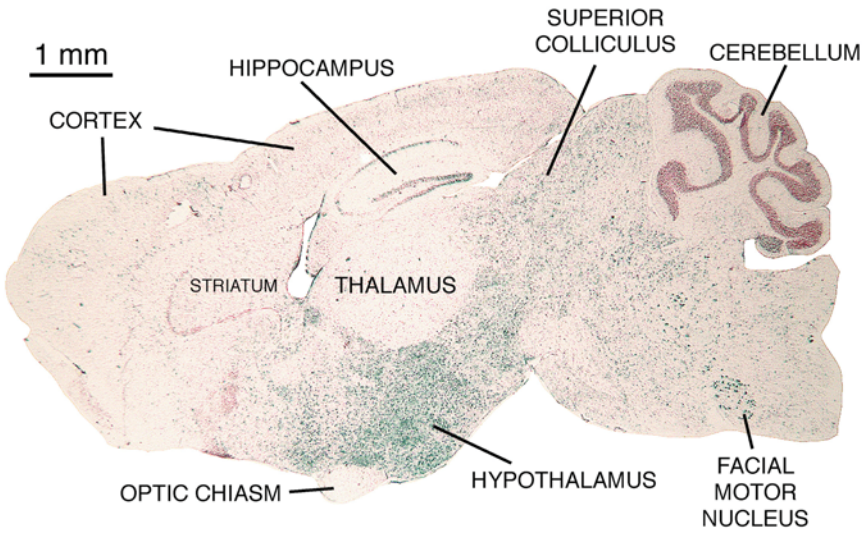

Fig. 2. Image of a sagittal section from one of the chimeric mice created by blastocyst injection of *Hdh–/–* ES cells, stained (blue) to reveal the location of the X-gal-positive *Hdh –/–* cells that have colonized the brain. The tissue was counterstained with neutral red. This animal (ES13) was among those chimeric mice possessing *Hdh –/–* cells that were sacrificed prior to 1 yr due to signs of morbidity. The green/blue X-gal labeling shows that *Hdh –/–* cells are most abundant in hippocampus, preoptic area, hypothalamus, midbrain, and hindbrain, but scarce in cortex, striatum, and thalamus.

embryo aggregation suggests that even limited colonization of the extraembryonic membranes by *Hdh–/–* cells may impair their function so severely as to be lethal to the developing embryo.

Role of Huntingtin in Brain Development

While Htt levels that are 50% of normal (as would occur with hemizygous *Hdh* deletion) do not obviously hinder development, reduction of Htt to levels lower than 50% of normal have been found to result in severe developmental malformations. For example, while early lethality is bypassed in mutant mice with a hypomorphic *Hdh* mutation (which reduced expression of the affected allele to about one-third of wild-type levels), defective neurogenesis, profound malformations of cortex and striatum, telencephalic ventricular enlargement, and agenesis of fiber tracts were evident in late-term embryos and postpartum mice with such a mutation (White et al., 1997). Thus, Htt clearly seems critical for cortical and striatal development. The brain defects in the more severely affected mice were accompanied by craniofacial deformities, and the various developmental abnormalities tended to result in perinatal lethality.

The results of the author's studies on chimeras created by the blastocyst-injection method provide some further insights into the role of wild-type Htt in brain development. It was found that *Hdh–/–* cells in the hypothalamus, the midbrain, the cerebellar granule cell layer, and the hindbrain of such chimeras can typically participate in the normal formation of the brain (Fig. 2; Table 1); that in these regions *Hdh–/–* neurons develop normal morphology and transmitter content (Fig. 3); and that they can survive for over 1 yr

Table 1
Behavioral Traits and Distribution of *Hdh–/–* Cells in Several Major Brain Regions in a Representative Set of *Hdh–/–* <–> Wild-Type Chimeric Mice Created by Blastocyst Injection of *Hdh–/–* ES Cells

Trait	Chimera ES7	Chimera ES8	Chimera ES1	Chimera ES2	Chimera ES11	Chimera ES13
Behavioral abnormalities	–	–	+	+	++	+++
Structures with very low *Hdh–/–* ES cell colonization that were poorly correlated with behavioral abnormalities						
Hdh–/– cells in cerebral cortex	–	+	–	+	+	+
Hdh–/– cells in thalamus	–	–	–	–	–	–
Hdh–/– cells in cerebellar PCL	–	–	–	–	–	–
Structures with high *Hdh–/–* ES cell colonization that were poorly correlated with behavioral abnormalities						
Hdh–/– cells in hippocampus	+	+++	++	++	+++	+
Hdh–/– cells in hypothalamus	++++	+++	++	+++	++++	++++
Hdh–/– cells in cerebellar GCL	++++	++	+++	++++	++	+
Structures with low *Hdh–/–* ES cell colonization that were slightly correlated with behavioral abnormalities						
Hdh–/– cells in globus pallidus	–	–	–	–	+	++
Hdh–/– cells in striatum	+	+	++	++	++	++
Structures with high *Hdh–/–* ES cell colonization that were highly correlated with behavioral abnormalities						
Hdh–/– cells in hindbrain	++	++	++	++	+++	+++
Hdh–/– cells in motoneurons	+++	++	+++	+++	++++	++++

Note that colonization of some brain areas by *Hdh–/–* cells is poor while for others it is ample. Note also that colonization of some brain regions by the *Hdh–/–* cells appears correlated with the behavioral deficit. Behavioral score code: – = no symptoms; + = slight clasping; severe neurological impairment by 20 wk = ++; severe neurological impairment by 4 wk = +++. Regional colonization by *Hdh–/–* cells codes: – = <1% *Hdh–/–* cells; + = 1–10% *Hdh–/–* cells; ++ = 11–25% *Hdh–/–* cells; +++ = 26–50% *Hdh–/–* cells; ++++ ≥50% *Hdh–/–* cells. Abbreviations: GCL = granule cell layer; PCL = Purkinje cell layer.

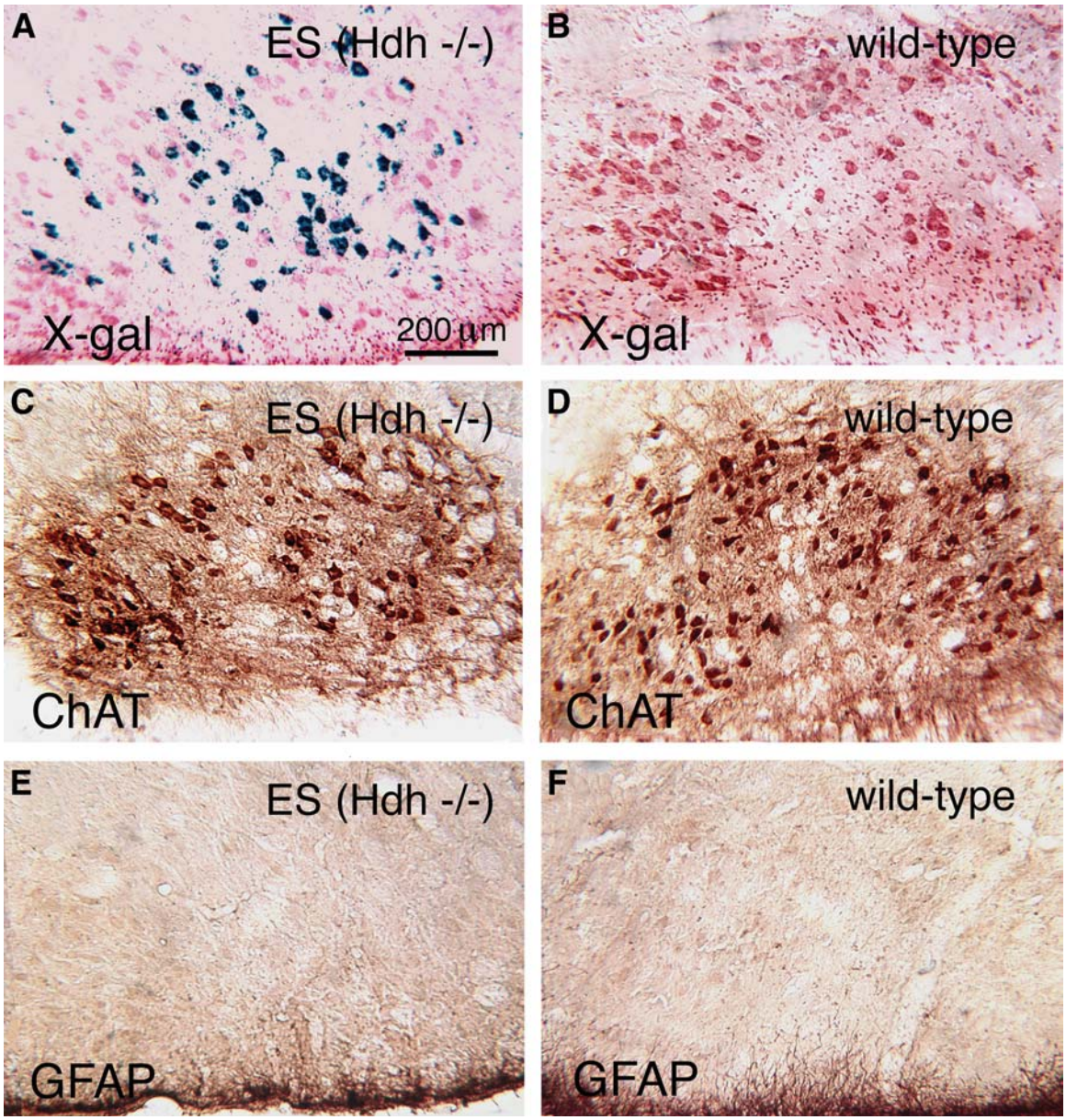

Fig. 3. Images of transverse sections through pons showing the distribution of *Hdh –/–* cells in the facial nucleus in a chimeric mouse created by blastocyst injection of *Hdh –/–* ES cells that showed no ill health up to 1 yr of age **(A),** compared to facial nucleus of a wild-type mouse in which no *Hdh –/–* cells are present **(B).** The *Hdh –/–* cells in A are labeled blue by X-gal histochemistry and neuronal cytoarchitecture in both A and B is visualized by neutral red counterstaining. Images **(C)** and **(D)** demonstrate that the presence of *Hdh –/–* cells shown in A has not produced any evident abnormality in the facial motoneurons (visualized by DAB immunolabeling for choline acetyltransferase) of the chimeric mouse. High magnification images of transverse sections through facial nucleus of the same chimeric and wild-type animals as shown in A and B reveal that the presence of *Hdh –/–* cells in the facial nucleus of the chimeric mouse has not produced any upregulation of GFAP in the facial nucleus **(E)** and **(F).** Medial is to the *left* and dorsal to the *top* in all images.

with no evident signs of brain pathology (Reiner et al., 2001). These results expand on recent data showing that *Hdh–/–* ES cells transformed into a neuronal phenotype in vitro can survive for several weeks and show typical neuronal electrophysiological traits (Metzler et al., 1999). Thus, for at least some neuron types, Htt is not required for establishing and maintaining a neuronal identity. The authors did, however, find in the blastocyst-injection chimeras that *Hdh–/–* cells do not effectively colonize and/or thrive in all brain areas. In particular, it was observed that neurons and/or glia derived from the *Hdh–/–* ES cells (Fig. 2; Table 1) showed only a sparse occupancy of the cerebral cortex, the basal ganglia, the thalamus, and the Purkinje cell layer of the cerebellum (Reiner et al., 2001). These findings suggest that Htt may be needed for cells to migrate to, proliferate in, and/or survive extensively within the cerebral cortex, basal ganglia, thalamus, and Purkinje cell layer of the cerebellum.

Thus, individual cells in many brain regions may not need Htt to differentiate into neurons that survive and function normally, while in other brain regions many cells may need to express normal levels of Htt if development is to proceed normally for that region. For example, brain development is seemingly normal even in the hypothalamus and brainstem of chimeras in which ≥50% of the resident neurons are *Hdh–/–*, and this may be the case because Htt plays no major role in the development and/or functioning of these regions (Reiner et al., 2001). By contrast, Htt clearly seems critical for cortical and striatal development. This preferential need for Htt in forebrain development is consistent with the occurrence of profound malformations of cortex and striatum, lateral ventricular enlargement, and agenesis of forebrain fiber tracts in mouse mutants wherein Htt was expressed at one-third of wild-type levels (White et al., 1997). Consistent with a regionally differential role of Htt in neural development, neurons in cortex and striatum commonly express higher levels of Htt than do neurons of hypothalamus and brainstem (Li et al., 1993; Landwehrmeyer et al., 1995; Sharp et al., 1995; Bhide et al., 1996; Sapp et al., 1997; Fusco et al., 1999). The absence of evident forebrain developmental abnormalities in chimeras with *Hdh–/–* cells may be a consequence of the relatively low colonization of cortex and striatum by *Hdh–/–* cells. This low colonization could stem from impaired proliferation of *Hdh–/–* cells during development or from death of these cells. Note, however, that we did not see signs that cell loss had recently occurred in cortex, basal ganglia, thalamus, or Purkinje cell layer in the chimeras (i.e., cell abundance appeared normal). Thus, if the paucity of *Hdh–/–* neurons in the telencephalon, thalamus, and Purkinje cell layer reflects their failure to survive in these regions, this loss had to occur at an early enough point in development for the *Hdh–/–* neuroblasts to have been replaced by wild-type neuroblasts.

To address the means by which *Hdh–/–* cells come to be underrepresented in forebrain in the blastocyst-injection chimeras, the authors performed a preliminary analysis of two chimeras sacrificed at E12.5 (Fig. 4). In these chimeric embryos, they found that the entire brain, including cortex and striatum, was colonized by *Hdh–/–* cells, with no evident underrepresentation in any brain region. There appeared to be, however, an ongoing degeneration of *Hdh–/–* cells that was specific to the cortex, striatum, and thalamus. This would suggest that *Hdh–/–* cells are not actively repulsed or segregated from telencephalic territories during development, as occurs for example, in the *valentine* mutation in zebrafish (Moens et al., 1998). To further confirm this conclusion, no piling up of *Hdh–/–* neurons in regions adjacent to the boundary between telencephalon and diencephalon, or between thalamus and hypothalamus, was observed. Rather, these preliminary findings support the view that neuroblasts in the cortex, striatum, and thalamus need to synthesize Htt if they are to progress in their development and differentiation.

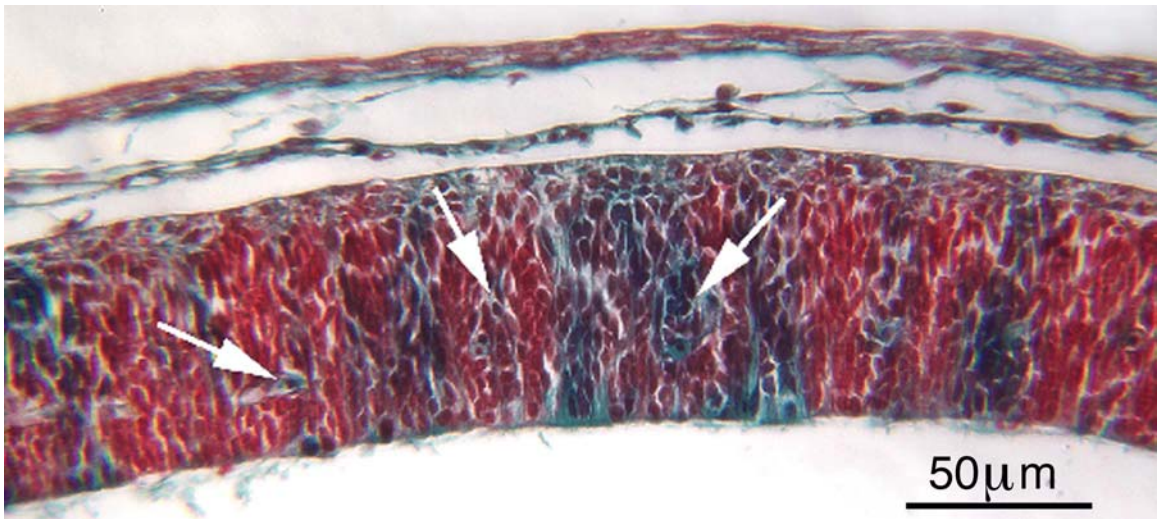

Fig. 4. Image of E12.5 *Hdh–/–* <–> +/+ chimera showing the neocortex in sagittal section. The blue cells are *Hdh–/–* and, in some cases, are arranged into the radial clones typical of cortical development. However, in several instances there are pyknotic *Hdh–/–* cells *(arrows),* suggesting that the *Hdh–/–* are degenerating.

Role of Huntingtin in Neuronal Survival

The results of these studies on chimeras created by the blastocyst-injection method showing that *Hdh–/–* cells largely fail to colonize cerebral cortex and striatum during development raise the possibility that Htt plays a region-specific role in neuronal survival. While this role might, in principle, be limited to the period of forebrain neurogenesis, recent studies using conditional knockout of *Hdh* in mouse forebrain, late during embryonic development, indicate that Htt may also play a role in survival of some populations of forebrain neurons beyond birth of the animal (Dragatsis et al., 2000). The conditional deletion of *Hdh* was achieved in two separate lines of mice using the *Cre/loxP* site-specific recombination system to generate a null mutation of *Hdh* in the mouse forebrain. The promoter for the alpha subunit of calcium-dependent calmodulin kinase-2 *(Camk2a)* was used to drive cre expression, with *Hdh* deletion starting after E15 in one line and after postnatal d 5 in the other (Dragatsis and Zeitlin, 2000). Note that since the forebrain in rodents is still undergoing developmental changes during the first postnatal week (which roughly corresponds to the third trimester in humans), the *Hdh* deletion in both lines of mice occurs during late stages of forebrain development.

Inactivation of *Hdh* in the brain was found to result in neurological abnormalities and a progressive degenerative phenotype in forebrain, leading to death by 1 yr of age. *Hdh* deletion beginning around E15 resulted in an earlier onset of behavioral abnormality and the common occurrence of forebrain degeneration, while the later occurring deletion of *Hdh* yielded neuronal degeneration in the forebrain only in some mutants. In both lines, hindlimb clasping upon tail suspension—an atypical behavior diagnostic of neurological abnormality in mice—was first detected after weaning, and reactive astrocytosis and axonal degeneration were observed as early as 3–4 mo of age throughout the forebrain. Coronal sections

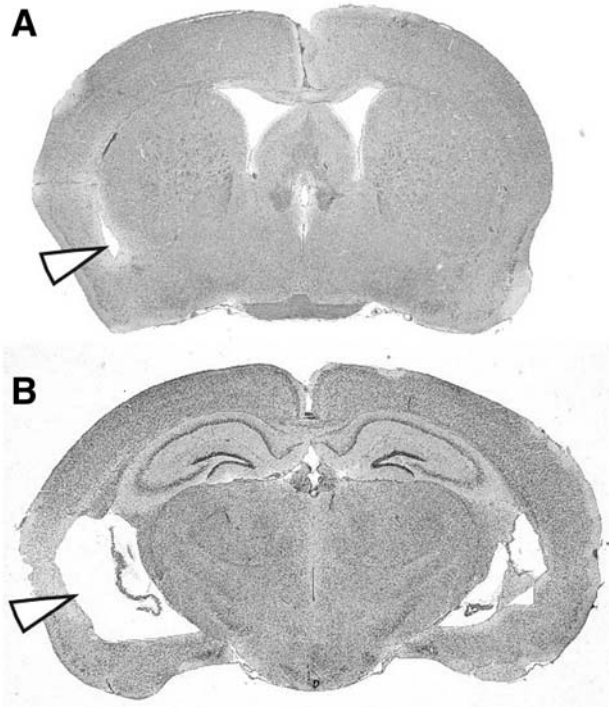

Fig. 5. Images of a successive pair of H&E-stained coronal sections from an 8-mo-old mouse from the line in which *Hdh* deletion occurred beginning around E15. Note the tissue loss in the caudolateral cortex and the amygdala *(arrowheads).*

through the brains of some 8-mo-old early *Hdh*-inactivation mutant brains revealed tissue loss adjacent to the external capsule in the forebrain (Fig. 5), notably in the caudal and lateral portion of the cerebral cortex, as well as in the claustrum and amygdala (Dragatsis et al., 2000). The tissue loss in the caudal/lateral pallium was so severe that it was grossly visible through the meninges of dissected mutant brains. Degeneration was also observed in the entorhinal cortex. MAP2 immunostaining, fluorojade labeling, and labeling of DNA-strand nicks confirmed cortical and amygdalar degeneration, and also revealed degeneration in striatum and hippocampus.

In this light, it is of interest that the behavioral abnormalities on a series of simple tasks (hindlimb clasping upon tail suspension, and gait and balance assessments) tend to be evident in those *Hdh–/–* <–> wild-type chimeras created by blastocyst injection of *Hdh–/–* ES cells that were most heavily colonized by *Hdh*-null cells (Reiner et al., 2001). Extensive colonization of midbrain and hindbrain by *Hdh–/–* cells appeared to be most predictive of these abnormalities (Table 1). Since no neuron death was evident, it seems likely that the underpinning of the behavioral abnormality is dysfunction of *Hdh–/–* cells in these brain regions. The dysfunction of these neurons may not cause overt behavioral abnormalities if a sufficient abundance of wild-type neurons is present in these same-cell groups.

Thus, in addition to its previously known critical role in mouse embryogenesis and forebrain development, Htt is also required for normal neuronal function and survival in at least some parts of the adult forebrain. It is uncertain whether late embryonic Htt deletion impairs function and survival of some neurons by altering their development and maturation, or whether the deletion has a progressive adverse effect on adult neuronal health. In any event, it seems unlikely that the beneficial effects of Htt are mediated via an essential and general role in brain metabolism, as widespread neuronal death in forebrain does not occur immediately after the elimination of forebrain *Hdh* expression, nor does widespread neuronal death occur in the brains of *Hdh–/–* <–> wild-type chimeras created by blastocyst injection of *Hdh–/–* ES cells (Fig. 3) (Reiner et al., 2001). A prosurvival role of wild-type Htt is also suggested by studies examining the effects of wild-type or mutant Htt alone or in combination at 10% of normal levels in mice. In particular, hypomorphic (i.e., underexpressing) CAG111/CAG20 mice, but not hypomorphic CAG20/CAG20 or CAG20/null mice, show a progressive lethal neurological phenotype that does not appear to involve the striatum and thus seems to be unlike HD (Auerbach et al., 2001). The occurrence of this progressive neurological phenotype when mutant huntingtin is expressed on a background of low wild-type

Htt levels, but not when mutant huntingtin is expressed on a background of normal wild-type Htt levels, is further evidence that wild-type Htt is protective. Additional evidence for the prosurvival effects of wild-type Htt, and the possible means by which it may exert its prosurvival influence, has been the subject of several recent studies by both Cattaneo and colleagues, and Hayden and colleagues, as discussed below.

BDNF and the Prosurvival Role of Huntingtin

Huntingtin appears to exert a neuroprotective effect in cultured striatal neurons subjected to such lethal stresses as serum deprivation or exposure to the mitochondrial toxin 3-nitropropionic acid (Rigamonti et al., 2000). Wild-type Htt appeared to exert this influence, at least in part, by an anti-apoptotic effect downstream of bcl2 but upstream of caspase-3 activation. Subsequent studies reveal that the neuroprotective action of Htt derived from its ability to block processing of procaspase-9, an effector of apoptosis that is itself activated by cleavage of the proenzyme form of the molecule (Rigamonti et al., 2001). Studies by Hayden and colleagues indicate that wild-type Htt may also have anti-apoptotic effects via its tendency to bind to and sequester HIP1, a pro-apoptotic molecule that appears to act by forming a heterodimer (with a polypeptide termed Hippi) that recruits and activates caspase-8 (Kalchman et al., 1997; Hackam et al., 2000; Gervais et al., 2002). Huntingtin also appears to promote neuronal survival via a stimulatory effect on production of brain-derived neurotrophic factor (BDNF) (Zuccato et al., 2001). BDNF itself is known to be critical for the viability of some brain neuron types (Schuman, 1999). As is true of trophic factors in general, BDNF is produced and secreted by specific populations of neurons and then taken up by the neurons synaptically connected with the BDNF-releasing neurons (Schuman, 1999). For example, corticostriatal neurons are known to produce BDNF and transport it to their axon terminals in the striatum, where it is released with depolarization and taken up by striatal neurons, upon which it exerts an important receptor-mediated, prosurvival effect (Altar et al., 1997; Ivkovic and Ehrlich, 1999; Schuman, 1999). Wild-type Htt appears to promote BDNF synthesis and release, since overexpression of Htt in cultured CNS cells increases BDNF production by these cells, and reduced levels of wild-type Htt in transgenic mice are associated with diminished production of BDNF (Zuccato et al., 2001). Wild-type Htt may have this effect on BDNF production by means of an interaction with the transcriptional regulator Sp1. The BDNF gene is known to possess an Sp1 response element in its proximal promoter region, and Sp1-dependent transcription, including that of BDNF, is diminished by mutant Htt (Luthi-Carter et al., 2002).

Is Wild-Type Huntingtin Function Relevant to HD Pathogenesis?

It is intriguing that the role of wild-type Htt in brain development and neuronal survival is particularly manifest in the cortex and striatum, the very structures most conspicuously affected in HD. It may be that wild-type Htt produces its effects on development via an action on neuron or neuroblast survival (as opposed to such other developmental phenomena as proliferation or migration), and this may explain the similar regional specificities of the role of Htt in brain development and neuron survival. Irrespective of whether this is the case or not, the specific roles of wild-type Htt in cortical and striatal development and survival unavoidably raise the issue of whether this is related somehow to the relative selectivity of HD for these very same brain regions. One possibility is that mutant Htt, while not obviously impairing brain and organism development, may lead to subtle developmen-

tal defects that have consequences that become insidious later in life and eventually manifest themselves as HD. The notion that developmental defects could set the conditions for subsequent degenerative disease during adult life, such as Alzheimer's disease (AD), has gained credence recently with the discovery that genes implicated in familial forms of AD interact with key developmental signaling pathways (Mehler and Gokhan, 2000, 2001). Similarly, mutant Htt and other mutant proteins with expanded polyglutamine tracts have been found to have impaired interactions with transcription factors and modulators that regulate neuronal specification and development (Mehler and Gokhan, 2001). Two findings in mice with mutations in their endogenous HD gene taken together directly support the notion that mutation of HD protein interferes with its role in development (Auerbach et al., 2001). First, mice with two 111 CAG repeat-bearing *Hdh* alleles expressed at 10% of normal levels do not survive to birth. Second, mice with one 111 CAG and one 20 CAG repeat *Hdh* allele (with both expressed at 10% of normal levels) resemble mice with one 20 CAG repeat *Hdh* allele and one *Hdh*-null allele in that both show stunted forebrain development, while mice with two hypomorphic alleles with 20 CAG repeats are normal in their forebrain development.

It is also possible that the HD mutation acts, at least in part, as a loss-of-function mutation, and this effect for some unknown reason is not manifest until well after birth. For example, it could be that the mutated version of Htt in HD heterozygotes comes to neutralize the function of the normal protein due to unknown age-related changes in the behavior of either the mutant or normal protein (Cattaneo et al., 2001). In this case, the gain-of-function acquired by mutant Htt would be, at least in part, a disabling of wild-type Htt (i.e., a dominant negative effect). A known gain-of-function associated with the HD mutation is the aggregation of the N-terminal fragment of mutated Htt within neuronal nuclei and cytoplasm (DiFiglia et al., 1997; Li and Li, 1998; Martindale et al., 1998; Gutekunst et al., 1999; Maat-Schieman et al., 1999). While considerable attention has been given to the possibility that these aggregates are themselves a key pathogenic event in HD (Davies et al., 1997; DiFiglia et al., 1997; Kim and Tanzi, 1998; Saudou et al., 1998; Sisodia, 1998), the means by which they might lead to neuronal death remains uncertain (Cha et al., 1998; Hackam et al., 1998; Sisodia, 1998). The possibility that the aggregates may, at least in part, act by inactivating both mutant and normal Htt has been raised by recent evidence showing that the aggregates which form in HD and in transgenic animal models of HD can sequester normal-length polyglutamine-containing proteins, including Htt and CREB-binding protein, both of which promote BDNF production (Tao et al., 1998; Shieh et al., 1998; Narain et al., 1999; Ona et al., 1999; Preisinger, 1999; Wheeler et al., 2000; Cattaneo et al., 2001; Nucifora et al., 2001). Moreover, since the polyglutamine expansion of mutant Htt diminishes its inhibitory binding to the pro-apoptotic protein Hip-1 (Hackham et al., 2000; Gervais et al., 2002), both the presence of mutant Htt and the sequestration of wild-type Htt could enhance neuronal injury by diminishing inhibition of the Hip-1-mediated apoptotic pathway. These findings have led to the recent suggestion that the HD mechanism of action might receive a contribution from a late-onset inactivation of normal Htt (Cattaneo et al., 2001), thereby leading to diminution of the anti-apoptotic and/or prosurvival actions of the wild-type protein. Consistent with this interpretation, cortical BDNF production is diminished in mice bearing a mutant HD transgene and in human HD victims (Zuccato et al., 2001), which could explain the selectivity of the disease for cortex and striatum. In this view, HD pathogenesis might involve multiple mechanisms (Aronin et al., 1999), including the widely accepted gain-of-function, a possible dominant–negative effect or even a loss-of-function contribution. The idea that HD pathogenesis might receive a contribution from diminished huntingtin function caused by the

polyglutamine expansion is bolstered by the recent finding that HD homozygotes do appear to have a more severe clinical disease course than do heterozygotes (Squitieri et al., 2003).

Nonetheless, the possibility that wild-type and mutant Htt function are both diminished with mutation of the HD gene leaves unexplained why striatal neurons are so much more vulnerable and cortical neurons somewhat more vulnerable than thalamic neurons and Purkinje cells in HD (Roos, 1986), the latter of which also appear to require Htt for normal development (Reiner et al., 2001). It may be that striatal and, less so, cortical neurons more critically depend on Htt for survival than do thalamic neurons and Purkinje cells; this may relate to the particular role of Htt in corticostriatal BDNF production noted above. Such a possibility is consistent with the preferential morbidity of *Hdh–/–* neurons in cortex and striatum in postweaning mice where the *Hdh* knockout is expressed beginning late in embryonic development (Dragatsis et al., 2000). On the other hand, the notion that inactivation of normal Htt plays a role in HD pathogenesis remains a hypothesis largely inferred from its apparent role in neuronal survival. Nonetheless, even if wild-type Htt inactivation plays no role in HD pathogenesis, its demonstrated role in neuron survival raises a very important point about therapeutic interventions in HD. One possible avenue for HD treatment involves pharmacological or gene-therapy strategies to prevent production of the mutant protein. If these approaches disrupt production of the wild-type protein as well, they may prove more harmful than beneficial.

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