Altered Adult Hippocampal Structural and Functional Plasticity in the YAC128 Transgenic Mouse Model of Huntington Disease

by

Jessica M. Simpson
BSc, University of Victoria, 2009

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Supervisory Committee

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Abstract

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Alterations in both structural and synaptic plasticity in the adult brain have been implicated in impaired learning and memory. In the present study, we investigated if hippocampal plasticity is affected in the transgenic YAC128 mouse model of Huntington disease (HD). Reductions in adult hippocampal neurogenesis were observed in the dentate gyrus (DG) of early symptomatic to end-stage mice compared with wild-type (WT) controls, however there were no changes in cell proliferation and differentiation in the subventricular zone. Early symptomatic mice also displayed attenuated paired-pulse plasticity and long-term depression in the DG, while long-term potentiation was found to be normal in YAC128 mice. The changes in hippocampal plasticity may contribute to the cognitive abnormalities observed in these animals.

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Acknowledgements

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List of Abbreviations

ACTH	adreno-corticotrophic hormone
AMPA	*a*-amino-3-hydroxy-5-methyl-4-isoxazolepropanoic acid
ANOVA	analysis of variance
BCM	Beinenstock-Cooper-Munro
BDNF	brain-derived neurotrophic factor
BrdU	bromodeoxyuridine
CA	cornu ammonis
CaMKII	calcium/calmodulin-dependent protein kinase II
CaMKIV	calcium/calmodulin-dependent protein kinase IV
cAMP	cyclic-adenosine monophosphate
CBP	CREB binding protein
CNS	central nervous system
CRE	cAMP reponse element
CREB	CRE binding protein
CS	conditioning stimulus
DA	dopamine
DAB	diaminobenzidine
D-AP5	2-amino-5-phosphonovalerate
DCX	doublecortin
DG	dentate gyrus
EC	entorhinal cortex
EPSPs	excitatory post-synaptic potentials
GABA	gamma-aminobutiric acid
GCL	granule cell layer
HAP1	huntingtin-associated protein 1
HD	Huntington disease
HEAT	huntingtin, elongation factor 3, protein phosphatise 2A, target of rapamycin 1
HFS	high-frequency stimulation
HIP1	huntingtin interacting protein 1
HPA	hypothalamic-pituitary-axis
i.p.	intraperitoneally
IGF1	insulin-like growth factor 1
IO	input-output
KGDHC	α-ketoglutarate-dehydrogenase complex
LFS	low-frequency stimulation
LTD	long-term depression
LTP	long-term potentiation
MAPK	mitogen-activated protein kinase
mGluR	metabotropic glutamate receptor
ML	molecular layer
MLK2	mixed-lineage kinase 2
MSNs	medium spiny neurons
mTOR	mammalian target of rapamycin
nACSF	normal artificial cerebrospinal fluid
NeuN	neuronal nuclei
NeuroD	neurogenic differentiation protein
NGS	normal goat serum
NHS	normal horse serum
NIIs	neuronal intranuclear inclusions
NMDA	N-methyl-D-aspartate
NRSE	neuron-restrictive silencer element
NRSF	neuron-restrictive silencer factor
NSF	N-ethylmaleimide-senstivie factor
PACSIN 1	protein kinase C and casein kinase substrate in neurons protein 1
PCNA	proliferating cell nuclear antigen
PFA	paraformaldehyde
PI3K	phosphoinositide 3-kinase
PKA	protein kinase A
PKC	protein kinase C
PP1	protein phosphatase 1
PSC	post-synaptic current
PSD95	postsynaptic density 95
PSP	post-synaptic potential
PTP	post-tetanic potentiation
RMS	rostral migratory stream
SEM	standard error of the mean
SGZ	subgranular zone
SNARE	soluble NSF attachment protein receptor
Sp1	specific protein-1
SSRIs	selective serotonin reuptake inhibitors
Sub	subiculum
SVZ	subventricular zone
VEGF	vascular endothelial growth factor
WT	wild-type
YAC	yeast artificial chromosome

I. General Introduction

1. Huntington Disease

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder that afflicts approximately 1 in 20 000 people, usually with mid-life onset of psychiatric, cognitive, and motor symptoms, with death occurring 12-15 years from the time of symptomatic onset (Folstein, 1989). Patients eventually become completely dependent and bedridden, with death usually resulting from disease related complications. Early symptoms include mood swings, depression, irritability, and trouble learning new things or remembering facts. As the disease progresses, concentration on intellectual tasks becomes increasingly difficult, as well as the manifestation of severe motor impairments with loss of voluntary movement coordination, dementia and weight loss. Motor deficits progress from awkward, clumsy and uncontrolled movements (referred to as chorea), to rigidity and subsequent loss of movement and coordination; slow movements (bradykinesia) may also occur early on (for review see Gil and Rego, 2008). In rare circumstances, the disease may manifest before the age of 20 (juvenile onset) with a more severe phenotype and a faster progression (Gil and Rego, 2008).

HD is a member of the trinucleotide-repeat disorder family, and is caused by an unstable expansion of CAG repeats within the coding region of the *huntingtin* gene, which resides on chromosome 4 (4p16.3). This gene encodes the approximately 350 kDa protein huntingtin, which is ubiquitously expressed throughout the body. However most of the changes in HD occur in the brain (Sathasivam et al., 1999). The disease is manifested when the number of CAG repeats in the polyglutamine tract near the N-terminus of the *huntingtin* gene is expanded beyond the critical threshold of 36-39 repeats, constituting a mutation that causes neuronal dysfunction and loss. The number of CAG repeats (i.e. glutamine residues) is the primary determinant of disease severity, accounting for 60% of the variance in the age of onset and severity of the first symptoms (Andrew et al., 1993).

The primary target of HD degeneration is the population of medium-sized gamma-aminobutiric acid (GABA)-ergic spiny neurons of the striatum (for review see

Vonsattel and DiFiglia, 1998), followed by other regions at later stages, including the cerebral cortex, hippocampus, hypothalamus, globus pallidus, substantia nigra, subthalamic nucleus, and cerebellum. Since these medium-sized spiny neurons (MSNs) exert an inhibitory effect, it is believed that the loss of their inhibitory input is the underlying cause of the uncontrolled movement's characteristic of HD (for review see Gusella and MacDonald, 1995). Striatal neurons receive excitatory glutamatergic inputs from the cerebral cortex, and it is thought that dysfunctional cortical glutamatergic neurons may lead to conditions of chronic excitotoxicity (for review see Vonsattel and DiFiglia, 1998). Indeed, MSNs express high levels of the N-methyl-D-aspartate (NMDA) receptor NR2B subunit, which increases the receptor channel permeability and determines its sensitivity to glycine, the Mg^{2+}-blockage, and the channel deactivation time (Davies and Ramsden, 2001). These conditions then lead to increases in intracellular calcium concentrations, leading to mitochondrial dysfunction, generation of reactive oxygen species, and activation of calcium-dependent proteases such as calpains (for review see Gil and Rego, 2008).

1.1 Huntingtin

Wild-type huntingtin is known to be essential for embryonic development, since knock-out mutations that inactivate the mouse HD gene homolog *Hdh* result in embryonic lethality (Duyao et al., 1995; Nasir et al., 1995). Within the cell, wild-type huntingtin is localized in the cytoplasm associated with organelles such as mitochondria, the Golgi apparatus, the endoplasmic reticulum, synaptic vesicles, and several components of the cytoskeleton. To a lesser extent, wild type huntingtin is also present in the nucleus. Wild-type huntingtin binds to several proteins, and may exert a variety of intracellular functions such as protein trafficking, vesicle transport and anchoring to the cytoskeleton, clathrin-mediated endocytosis, postsynaptic signaling, transcriptional regulation, and anti-apoptotic functions (for review see Gil and Rego, 2008). Therefore, wild-type huntingtin is believed to have a pro-survival role inside the cell.

The loss of neuroprotective functions that have been attributed to the wild-type gene are thought to contribute to neuronal dysfunction in HD (for review see Gil and Rego, 2008). In addition, neuropathological changes in HD result from a toxic gain of function of the mutant *HD* gene, causing aberrant proteolysis and the subsequent appearance of N-terminal mutant huntingtin fragments that precipitate and form neuronal intranuclear inclusions (NIIs) (DiFiglia et al., 1997). The mutant protein (in either its soluble or aggregate form) has been shown to disrupt several intracellular pathways by abnormally interacting and/or sequestering key components of these multiple pathways into the aggregates.

The aberrant proteolysis of the mutant protein by caspases and calpains has been suggested to produce N-terminal fragments that are more toxic and prone to aggregate (Kim et al., 2001) and that diffuse passively into the nucleus due to their smaller size (Sun et al., 2002). In turn, these fragments can recruit more proteases into the aggregates, favouring their activation that may eventually lead to cell death. Indeed, YAC128 mice resistant to cleavage by caspase-6 do not develop striatal neurodegeneration (Graham et al., 2006), suggesting that proteolysis of mutant huntingtin is an important event in HD.

Mutant huntingtin may also establish abnormal protein-protein interactions with several transcription factors, recruiting them into the aggregates and inhibiting their transcription activity. Examples include CREB [cyclic-adenosine monophosphate (cAMP) response element (CRE) binding protein]-binding protein (CBP) and specific protein-1 (Sp1), which interact with mutant huntingtin via its expanded polyglutamine tail (for review see Tobin and Signer, 2000). Mutant huntingtin may also fail to interact with other transcription factors, such as neuron-restrictive silencer element (NRSE)-binding transcription factors, leading to the suppression of NRSE-containing genes, including brain-derived neurotrophic factor (BDNF) (Zuccato et al., 2003), an important survival factor for neurons.

The mutant protein may also impair axonal transport, with aggregates physically blocking transport within narrow axonal terminals (for review see Gunawardena and Goldstein, 2005). Wild-type huntingtin is also thought to play a role during axonal transport via association with huntingtin-associated protein 1 (HAP1), promoting both retrograde and anterograde transport (for review see Gil and Rego, 2008). The expanded polyglutamine tract may inhibit this function, causing an impaired association between motor proteins and microtubules, and in one case, attenuating BDNF transport, resulting in the loss of neurotrophic support (Gauthier et al., 2004).

A direct consequence of impaired axonal transport is disruption of neuronal synaptic transmission. A number of studies suggest that mutant huntingtin can lead to synaptic dysfunction by altering the availability of various synaptic proteins, including complexin II, a protein that regulates the fusion of synaptic vesicles with the presynaptic membrane, and synaptobrevin-2, a protein involved in the formation of SNARE [soluble NSF (N-ethylmaleimide-sensitive factor) attachment protein receptor] complexes (Morton et al., 2001). Wild-type huntingtin also interacts with various vesicle proteins that are important for endocytosis, such as huntingtin interacting protein 1 (HIP1) (for review see Li and Li, 2004). At the postsynaptic level, the mutant protein can also disrupt the expression and activity of several postsynaptic neurotransmitter receptors (for review see Li et al., 2003). The expanded polyglutamine tract interferes with the ability of huntingtin to bind to postsynaptic density 95 (PSD95) and regulate the function of NMDA and kainate receptors (Sun et al., 2001), while also interfering with the recycling of membrane receptors through the interaction with several proteins that normally regulate this process (Modregger et al., 2002).

2. Genetic Mouse Models of HD

Since the expansion of the polyglutamine tract in the huntingtin protein was determined as the single cause of the disease (The Huntington's Disease Collaborative Research Group, 1993), several mouse models of HD have been created (**Appendix A**). Genetic mouse models include knock-in mice, in which an expanded CAG repeat is introduced into the endogenous mouse *huntingtin* gene, and transgenic mice, which express a portion of the human mutant *huntingtin* gene under the control of different promoters.

2.1 The R6 HD Transgenic Mice

The R6/2 transgenic mouse model was the first to be generated and due to the fast progression of the disease, it is still the most commonly used model in HD research. These mice express exon 1 of the human *huntingtin* gene (approximately 3% of the entire gene), with an expanded CAG repeat (approximately 150) under the control of the human *huntingtin* promoter (Mangiarini et al., 1996). An R6/1 transgenic mouse model was also created, which has less CAG repeats (approximately 115) and therefore has a slower disease progression (Mangiarini et al., 1996). Both lines of R6 mice develop behavioural and pathological changes that mimic those occurring in human HD, including progressive motor (Carter et al., 1999) and cognitive impairments (Murphy et al., 2000), weight loss, decreased striatal and brain size (Mangiarini et al., 1996), ubiquitinated nuclear and cytoplasmic inclusions of mutant huntingtin (Davies et al., 1997; Meade et al., 2002), and premature death (Mangiarini et al., 1996), although little neuronal death has been detected in the striatum and cortex (Turmaine et al., 2000; Iannicola et al., 2000; Stack et al., 2005). R6/1 mice live approximately 32-40 weeks, whereas R6/2 mice show an accelerated neurodegenerative phenotype and a shorter life span of 12-16 weeks, and are now considered to be more representative of early onset human HD (for review see Gil and Rego, 2009). It is also important to note that since these truncated models were generated by randomly inserting the transgene into the mouse genome, the mutation is not expressed under its natural genomic and protein context, which can possibly lead to altered gene regulation and to the loss of potential post-translational modifications and protein interactions that might occur in the human condition.

2.2 The YAC HD Transgenic Mice

2.2.1 The YAC46 and YAC72 HD Transgenic Mice

More recently, yeast artificial chromosome (YAC) mouse models expressing the full-length human mutant huntingtin gene carrying 46, 72 or 128 CAG repeats have been created (Hodgson et al., 1999; Slow et al., 2003), which faithfully recapitulate many features of the human condition. The first YAC mice to be generated expressed either normal huntingtin (with 18 glutamine residues; YAC18) or the mutant protein with either 46 (YAC46) or 72 (YAC72) glutamine residues in a developmental and tissue-specific manner identical to that observed in HD patients. Both mutant lines (YAC46 and YAC72) can live to at least 12 months of age, at which time they show a selective degeneration of medium-sized spiny striatal neurons and nuclear accummulation of N-terminal fragments of the mutant protein. However, neurodegeneration can be observed in the absence of huntingtin aggregates, suggesting that the formation of NIIs is not an essential step during the intracellular mechanisms that culminate in neuronal death in the HD brain (Hodgson et al., 1999). Interestingly, YAC72 medium-sized striatal spiny neurons were shown to be more vulnerable to excitotoxic cell death when compared with their wild-type littermate controls, suggesting that excitotoxicity may mediate neurodegeneration in HD (Zeron et al., 2002). Furthermore, YAC72 transgenic mice develop an abnormal behavior characterized by choreoathetoid movements of the head and neck, gait ataxia, and foot-clasping (Hodgson et al., 1999).

2.2.2 The YAC128 HD Transgenic Mice

In order to create a YAC mouse with both an accelerated and quantifiable phenotype, Michael R. Hayden and collaborators extended their previous research and generated a YAC transgenic mouse model expressing the full-length human HD gene with 128 CAG repeats (Slow et al., 2003).

2.2.2.1 Behavioural Deficits

In agreement with the previous knowledge that an increase in the number of CAG repeats is associated with an earlier onset of the disease (Brinkman et al., 1997), these mice develop behavioral abnormalities that follow a byphasic pattern with an initial phase of hyperactivity that can be detected as early as 3 months of age followed by the onset of motor deficits at 6 months of age (as determined by a decrease in their rotarod performance) and finally by hypokinesis at 8-12 months. Importantly, there is a positive correlation between the onset of motor deficits and the extent of striatal neuronal loss in these mice, which provides a neuropathological cause for the observed behavioral deficits (Slow et al., 2003). Furthermore, YAC128 transgenic HD mice also develop mild cognitive deficits, which precede the onset of motor abnormalities, can be detected as early as 2 months of age (as an impairment in motor learning on the rotarod test), and progressively deteriorate with age (Van Raamsdonk et al., 2005). Indeed, by 8 months of age, YAC128 mice show deficits in the swiming T-maze test as well as in open-field habituation (Van Raamsdonk et al., 2005). At 12 months, YAC128 transgenic mice show decreased prepulse inhibition and habituation to acoustic startle (Van Raamsdonk et al., 2005). Moreover, Pouladi and colleagues (2009) have recently reported that YAC128 transgenic mice also mimic the early depressive phenotype that is observed in HD patients. Indeed, a depressive-like behaviour (as assessed by the Porsolt forced swim test and the sucrose intake test as a measure of anhedonia) can be detected in these mice at the early stage of 3 months of age and the development of this phenotype does not seem to be correlated with CAG repeat length or disease duration (Pouladi et al., 2009).

2.2.2.2 Neuropathology

At 12 months of age these mice show a significant atrophy of the striatum, globus pallidus and cortex with relative sparing of the hippocampus and cerebellum (Van Raamsdonk et al., 2005). However, the first evidence of striatal degeneration can be detected at 8 months of age as a significant reduction in striatal volume (Lerch et al., 2008b), a feature that is accompained by an increase in cortical thickness, which might be indicative of a potential compensatory response (Lerch et al., 2008a).

In agreement with the gross atrophy of these brain structures, neuronal loss can be detected in the striatum and cortex, the two brain regions most affected in HD patients, but not in the hippocampus, of 12 month-old YAC128 mice (Van Raamsdonk et al., 2005). Importantly, YAC128 mice also display enhanced striatal sensitivity to multiple excitotoxins in the early phase of the disease, prior to development of motor abnormalities (Slow et al., 2005; Graham et al., 2009). However, by 10 months of age these mice become resistant to excitotoxic stress (Graham et al., 2009). This biphasic response to excitotoxins as well as an abnormal and selective increase in striatal glutamate receptor subunit expression and binding (Benn et al., 2007), strongly suggests that striatal cell death in HD is mediated, at least in part, through an excitotoxic mechanism that might be caused by a dysfunction of the glutamatergic corticostriatal pathway (for review see Gil and Rego, 2008).

2.2.2.3 Aggregation of Mutant Huntingtin and Inclusion Formation

Aggregation of mutant huntingtin and the consequent formation of NIIs has been considered as an hallmark of HD (for review see Gil and Rego, 2008) and a widespread distribution of these inclusions has been consistently observed in the striatum and cortex of both human HD patients (DiFiglia et al., 1997) and R6 mice (Davies et al., 1997; Meade et al., 2002). In the YAC128 HD mouse model it is possible to observe an increase in nuclear huntingtin immunoreactivity at 12 months of age (Slow et al., 2003; Van Raamsdonk et al., 2005). However, no intranuclear inclusions (i.e., nuclear aggregates of mutant huntingtin that are easily indentifiable by light microscopy) are present in these mice at this time point, although micro-agreggates can be vizualised by electron microscopy. Nevertheless, inclusions of mutant huntingtin can be clearly detected in striatal cells in older end-stage animals (i.e., at 18 months of age) (Slow et al., 2003). Importantly, the fact that YAC128 HD mice present significant neuronal dysfunction and loss prior to the appearance of clear intranuclear inclusions of mutant huntingtin strongly supports the idea that inclusions may represent a side effect of the ongoing cellular dysfunction, or may even exert a protective role during the early stages of the disease (for review see Gil and Rego, 2008). In further agreement with this hypothesis, “shortstop” YAC transgenic mice (which were serendipitously generated and express a truncated fragment of the human *HD* gene with approximately 120 CAG repeats) show widespread NIIs at a very early age. However, these mice display no features of neuronal dysfunction and/or degeneration, as determined by brain weight, striatal volume, and striatal neuronal count, further indicating that inclusions of mutant huntingtin may not be pathogenic *in vivo* (Slow et al., 2005). **Figure 1** illustrates the time course of behavioural and neuropathological deficits observed in HD YAC128 mice.

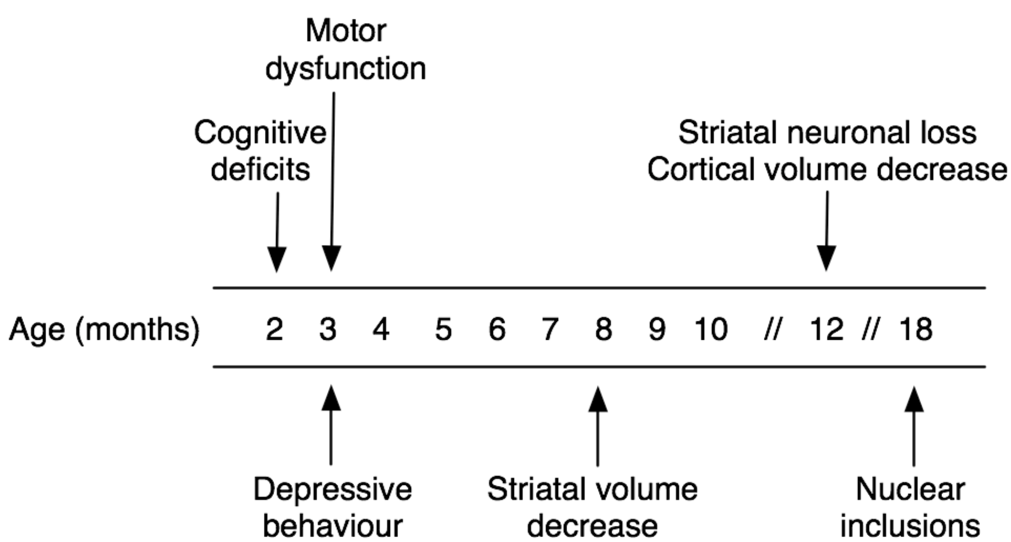

Figure 1. Time-course of behavioural and neuropathological deficits in HD YAC128 mice. Cognitive deficits begin at 2 months of age, motor dysfunction and depressive behaviour by 3 months of age, followed by selective striatal atrophy evident by 8 months of age and cortical volume loss by 12 months of age. Nuclear inclusions appear by 18 months of age. (Adapted from Pouladi et al., 2009).

3. The Hippocampal Formation

The relatively simple organization of the principle cell layers of the hippocampal formation has contributed to its use as a model system for studying neurobiology. In turn, this basic layout is similar across mammals, contributing to its use in comparative studies. The hippocampus is part of the limbic system and is a bilateral structure located within the medial temporal lobes of the cerebrum in humans. In rodents, it is an elongated, banana-shaped structure with its long axis extending in a C-shaped manner from the midline of the brain near the septal nuclei into the temporal lobe (Andersen et al., 2006). The hippocampus proper has three subdivisions: CA3, CA2, and CA1 (CA comes from cornu ammonis). The other regions of the hippocampal formation include the dentate gyrus (DG), subicular complex, and entorhinal cortex.

The hippocampus is organized such that the main excitatory projections follow a unidirectional pathway. Axons from the entorhinal cortex project to superficial layers of the DG via the perforant pathway, which includes medial and lateral subdivisions. These perforant path axons converge on dentate granule cells, which in turn send axonal projections called mossy fibres to synapse with pyramidal cells in CA3. These neurons then transmit information to via Schaffer collateral axons to pyramidal cells located in area CA1, which in turn send axonal projections to the subiculum and entorhinal cortex (reviewed in Andersen et al., 2006) (**Figure 2**).

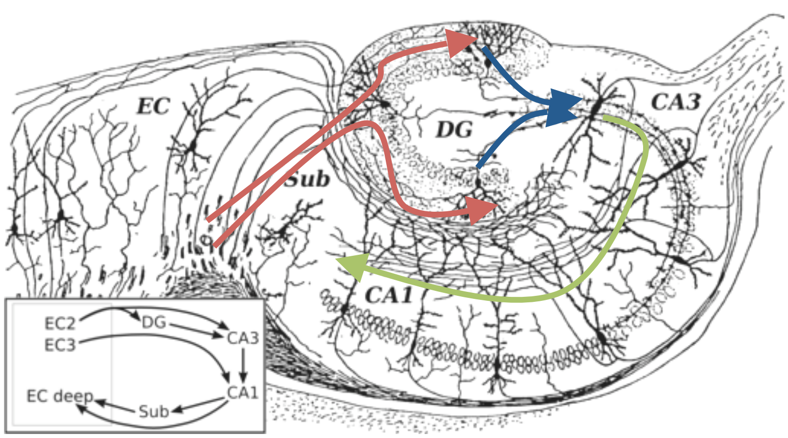

Figure 2. Coronal section of the rodent hippocampus.
The entorhinal cortex sends projections to the DG via the perforant path (red). Granule cells in the DG connect to CA3 via mossy fiber projections (blue), which in turn projects to CA1 through Schaffer collaterals (green). CA: corno ammonis, DG: dentate gyrus, EC: entorhinal cortex, Sub: subiculum (modified from y Cajal, 1909).

This thesis will focus on the DG, which consists of three layers, including the granule cell layer, which contains the cell bodies of the granule cells; the molecular layer, which contains the dendrites of granule cells and afferent projections from the entorhinal cortex; and the hilus, which contains axons from the DG granule cells that project to CA3. The principle cells (granule cells) have their soma densely packed in a thin layer. Their somas have a small diameter (14-18 μm) with an ovoid shape, while their dendritic trees emerge from the apical poles of the soma to form the molecular layer. Several types of inhibitory GABA-ergic interneurons innervate granule cells, while granule cells emit axon collaterals that locally innervate interneurons, providing feedback and feedforward inhibition within the network.

It is now well established that the hippocampus plays an integral role in the consolidation of declarative memory, spatial learning and context-dependent learning processes (reviewed in Squire, 1992; Burgess, 2002). Functional forms of neural plasticity were first discovered to occur in the hippocampus, and bidirectional synaptic plasticity is a candidate mechanism for memory formation in the brain (for review see Bliss and Collingridge, 1993). In addition, the DG is one of two regions in the brain that sustains neurogenesis throughout adulthood (Cameron and McKay, 2001). The hippocampus is also known to be susceptible to pathological conditions, including neurodegenerative disorders such as Alzheimer's disease (Braak and Braak, 1991), Parkinson's disease (Höglinger et al., 2004; Crews et al., 2008) and HD (Spargo et al., 1993; Rosas et al., 2003). Therefore, a disruption in structural and functional plasticity in the hippocampus may contribute to the cognitive deficits observed in both patients and mouse models of HD.

This thesis investigates the effects of mutant huntingtin on hippocampal DG neural plasticity in the YAC128 mouse model of HD. Specifically, the objectives of this thesis are 1) to observe how adult neurogenesis is affected throughout the disease in the DG and subventricular zone (SVZ) of the YAC128 mice; and 2) how DG synaptic plasticity is affected early on in the disease progression of these mice.

II. Altered adult hippocampal neurogenesis in the YAC128 mouse model of Huntington disease

1. Introduction

1.1 Adult Neurogenesis

Throughout adulthood, the mammalian brain retains the ability to generate new neurons, a process called neurogenesis. Adult neurogenesis has been demonstrated in two regions of the adult brain: the subventricular zone (SVZ) of the lateral ventricles, and the subgranular zone (SGZ) of the hippocampal DG. New neurons in the SVZ migrate along the rostral migratory stream to become granule neurons and periglomerular neurons in the olfactory bulb, while neurons born in the SGZ only migrate a short distance into the granule cell layer of the DG to become dentate granule cells, where they extend projections into the CA3 region of the hippocampus. It has been hypothesized that the microenvironments provided by the SVZ and SGZ, known as the neurogenic niche, may have specific factors that allow the proliferation and differentiation of new neurons (for review see Cameron and McKay, 1998).

1.1.1 Stages of Adult Hippocampal Neurogenesis

Adult neurogenesis is a complex process that can be subdivided into several distinct phases (**Figure 3**), each one of them being subjected to a complex system of intrinsic and extrinsic regulators (for review see Kempermann et al., 2004). During the proliferation stage, stem cells divide and give rise to three consecutive stages of putative transiently amplifying progenitor cells, which differ in their self-renewal potential and increasing neuronal differentiation. However, the hippocampal precursor has been reported to be capable of proliferation and differentiation but unable to self-renew, thus the stem cell may reside outside the hippocampus (possibly within the SVZ, which has been shown to contain self-renewing cells) and produce progenitors that migrate into the SGZ (Bull and Bartlett, 2005). Alternatively, the stem cell may only exist shortly after birth to create a progenitor pool (von Bohlen und Halbach, 2007). Once these progenitors exit the cell cycle, they enter a transient postmitotic stage, at which point network connections are established and selection for long-term survival occurs. The immature neurons send their dendrites towards the molecular layer of the DG and extend their axonal projections toward the hippocampal CA3 pyramidal cell layer (for review see Lie et al., 2004). Finally, the cells are terminally differentiated into new granule cells and express markers of mature neurons. Within four to seven weeks, the new neurons become integrated into the local circuits (Jessberger and Kempermann, 2003), and once mature, newborn granule cells receive similar glutamatergic and GABAergic inputs as existing neurons in the DG (Laplagne et al., 2007). Many of the newly generated cells die within four weeks after birth, and their survival is subject to regulation by diverse mechanisms, such as the animals' experiences, a few examples being spatial learning and exposure to an enriched environment, situations that increase the survival of new neurons (Kee et al., 2007; Kempermann et al., 1998).

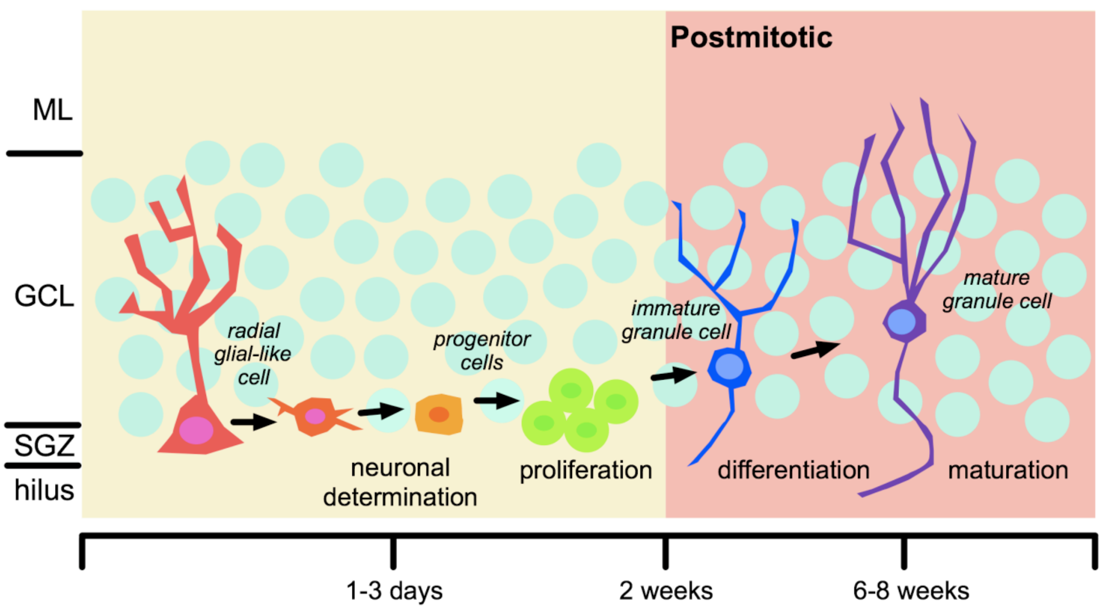

Figure 3. Stages of adult hippocampal neurogenesis.
During the proliferation stage, radial-like glial cells give rise to three consecutive stages of putative transiently amplifying progenitor cells, which differ in their self-renewal potential and increasing neuronal differentiation. Once these progenitors exit the cell cycle, they enter a transient postmitotic stage, at which point network connections are established and selection for long-term survival occurs. The immature neurons send their dendrites towards the molecular layer of the DG and extend their axonal projections toward the hippocampal CA3 pyramidal cell layer. Finally, the cells are terminally differentiated into new granule cells and within 6-8 weeks become integrated into the local circuits. ML: molecular layer; GCL: granule cell layer; SVZ: subventricular zone.

1.1.2 Regulation of Adult Hippocampal Neurogenesis

The hippocampus is involved in cognitive processes; therefore adult neurogenesis is thought to be required, at least in part, for these processes. Correlative studies have shown that the environment has an impact on SGZ neurogenesis, and may also have some effect on hippocampal-specific cognitive abilities. Voluntary exercise increases SGZ proliferation while exposure to an enriched environment promotes the survival of immature neurons, and both voluntary exercise and enriched environment improve the performance of young and aged mice in the Morris water maze (Kempermann et al., 1997; Kempermann et al., 1998; van Praag et al., 1999; van Praag et al., 2005). Environmental enrichment also leads to better recognition memory (Bruel-Jungerman et al., 2007). However, it is possible that other factors, such as neurotrophin and hormone levels also contribute to the changes in hippocampus-dependent learning and memory that are induced by the environment (Olson et al., 2006). In addition, associative learning tasks that require the hippocampus result in an enhancement of adult SGZ neurogenesis in rodents, while tasks that do not require the hippocampus had no effect on the number of new cells born (Gould et al., 1999). The regulation of SGZ neurogenesis by hippocampal-dependent learning is complex and can be affected by the age of the newborn neurons (Dupret et al., 2007), the stage of learning (Döbrössy et al., 2003), and specific learning protocols (Leuner et al., 2006). Due to the different connections that are formed along the dorsal-ventral axis of the hippocampus, the dorsal hippocampus may have a preferential role in learning and memory, while the ventral hippocampus is involved in affective behaviors (Bannerman et al., 2004).

Hippocampal neurogenesis has also been implicated in mood regulation, and SGZ cell division is suppressed by corticosteroids, which are often elevated in depressive patients and stressed animals (Gould et al., 1992; reviewed by Mirescu and Gould, 2006). In contrast to stress, administration of antidepressants increases neurogenesis in the DG (Malberg et al., 2000; Manev et al., 2001), and antidepressants prevent or reverse the stress-induced decrease in hippocampal neurogenesis (Malberg and Duman, 2003). BDNF, vascular endothelial growth factor (VEGF) and insulin-like growth factor 1 (IGF1) have all been found to play a role in the modulation of SGZ neurogenesis by antidepressants. In fact, the expression levels of BDNF and SGZ neurogenesis are both regulated by stress and antidepressants (Duman and Monteggia, 2006). Furthermore, infusion of BDNF into the DG mimics the effect of antidepressants in several behavioural tests and also increases DG neurogenesis, whereas infusion of VEGF or IGF1 stimulates SGZ cell proliferation and mimics the action of antidepressants in behavioural tests (Warner-Schmidt and Duman, 2007; Malberg et al., 2007). Thus these trophic factors are likely candidates to mediate both the behavioural and neurogenic effects of antidepressants. Importantly, inhibition of SGZ cell proliferation by low levels of irradiation eliminates the behavioural effects of antidepressants (Santarelli et al., 2003), strongly suggesting that DG neurogenesis mediates the effects of these drugs. However, irradiation does not specifically target the hippocampus; therefore additional inflammatory effects may confound these results. It still remains unclear to what extent SGZ neurogenesis contributes to depression, and better models of depression are needed to further test this hypothesis. Nevertheless, clinical studies have shown reduced hippocampal volumes in patients with depression (in Warner-Schmidt and Duman, 2006).

1.2 Neurogenesis and the HD Brain

Adult neurogenesis occurs in the human SGZ (Eriksson et al., 1998), and these adult neuronal stem cells have been proposed as an endogenous source of healthy cells for the treatment of neurodegenerative diseases (for reviews see Lie et al., 2004; Mohapel and Brundin, 2004; Gil-Mohapel et al., 2010). The process of adult neurogenesis appears vital for some forms of normal cognitive functioning, such as spatial learning and memory, and recent studies have demonstrated that neurogenesis is disrupted in a number of neurodegenerative disorders that include: Alzheimer's disease (Jin et al., 2004; Li et al., 2008); Parkinson's disease (Höglinger et al., 2004; Crews et al., 2008); and HD (Curtis et al., 2003; Gil et al., 2004; Lazic et al., 2004). In post-mortem HD brains, an increase in proliferation in the SVZ has been documented (Curtis et al., 2003). However, these results are in contrast with the findings obtained in R6/1 (Lazic et al., 2004) and R6/2 (Gil et al., 2004) HD transgenic mouse models, which show no differences in SVZ cell proliferation but do show a dramatic decrease in neurogenesis in the DG. In the R6/2 mice, this reduction in the DG can be detected as early as two-weeks of age, when no other behavioral symptoms are present (Gil et al., 2005). However, the fast progression of the disease in the R6/1 and R6/2 transgenic mouse lines, and the severity of their phenotype, have been suggested to be more representative of the juvenile onset form of HD (for review see Gil and Rego, 2009). On the other hand, the YAC128 mice seem to display a disease progression more akin to the human condition and have also been shown to display cognitive (Slow et al., 2003) and depressive-like symptoms (Pouladi et al., 2009) that precede the onset of motor deficits.

Importantly, both a decrease in spatial learning and memory and an increase in depression have been correlated with a reduction in adult hippocampal neurogenesis (Gould et al., 1999; Malberg et al., 2000; Manev et al., 2001; reviewed by Mirescu and Gould, 2006), suggesting a link between the development of these phenotypes and an altered neurogenic process in the HD brain. Thus, in the present study we investigated whether adult neurogenesis is compromised by the progression of the disease in the YAC128 HD transgenic mouse model.

2. Materials and Methods

2.1 Transgenic Mice

Transgenic HD mice expressing human *huntingtin* with approximately 128 CAG repeats (YAC128) and their wild-type (WT) littermates were used for these experiments. The colony was maintained at the Department of Medical Genetics, University of British Columbia (Vancouver, BC, Canada). Briefly, a well-characterized YAC (353G6) spanning the entire *HD* gene including its promoter region was used to create these mice (Slow et al., 2003). Homologous recombination was used to incorporate 128 CAG repeats obtained from the DNA of a juvenile-onset HD patient into the YAC following a previously described method (Duff et al., 1994). Mice were maintained on the FVB/N background strain (Charles River, Wilmington, MA, USA). These experiments used 3- (12 WT, 12 YAC128), 9- (20 WT, 22 YAC128), 12- (22 WT, 21 YAC128), and 18 month-old (9 WT, 8 YAC128) mice (equal numbers of males and females were used for each group). The mice were housed in groups with *ad libitum* access to food and water with a normal 12 hours light/dark cycle with ambient temperature and humidity. All experimental procedures were conducted in accordance with the University of Victoria, the University of British Columbia, and the Canadian Council for Animal Care policies.

2.2 Treatments and Tissue Processing

Generation of newborn cells was assessed by injecting 200 mg/kg of bromodeoxyuridine (BrdU, in 0.1 M TBS, pH 7.2; Sigma-Aldrich, St. Louis, MO, USA) intraperitoneally (i.p.) every 12 hours for 3 consecutive days. BrdU is a thymidine analogue that is incorporated into the DNA helix during the S-phase of the cell cycle and is commonly used as an exogenously administered marker for cell proliferation (Cooper-Kuhn and Kuhn, 2002). To examine cell proliferation, mice were sacrificed at 3, 9, 12, and 18 months of age, 12 hours after the last BrdU injection. To analyze both the differentiation and survival of the newborn cells that incorporated BrdU, a separate set of 8 months (9 WT, 11 YAC128) and 11 months (10 WT, 9 YAC128) old mice were sacrificed 4 weeks after receiving the last BrdU injection (i.e., at 9 and 12 months of age, respectively). Mice were deeply anaesthetized with avertin (2.5%, i.p.) and transcardially perfused with 0.9% saline followed by 4% paraformaldehyde (PFA). The brains were removed and left in 4% PFA overnight at 4°C and then transferred to 30% sucrose. Following saturation in sucrose, serial coronal sections were cut on a vibratome (Leica VT1000S, Nussloch, Germany) at a 30 μm thickness. Sections were collected in a 1/6 section sampling fraction and stored in a cryoprotectant solution (0.04 M TBS, 30% ethylene glycerol, 30% glycerol) at -20°C.

2.3 Immunohistochemistry

One series of free-floating sections was processed for BrdU immunohistochemistry as previously described (Gil et al., 2005). Briefly, after thorough rinsing, the sections were incubated in 2N HCl at 65°C for 30 minutes in order to denature the DNA. The sections were then pre-incubated for 1 hour in 5% normal horse serum (NHS) and 0.25% Triton X-100 in 0.1 M TBS and then incubated for 48 hours at 4°C with a mouse monoclonal antibody against BrdU (1:50; Dako, Glostrup, Denmark) in TBS containing 5% NHS. After incubation with a biotinylated horse anti-mouse IgG secondary antibody (1:200; Vector Laboratories, Burlingame, CA, USA) for 2 hours, the bound antibodies were visualized using an avidin-biotin-peroxidase complex system (Vectastain ABC Elite Kit, Vector Laboratories) with diaminobenzidine (DAB; Vector Laboratories) as a chromogen. The sections were mounted onto 2% gelatin-coated microscope slides, dehydrated in a series of ethanol solutions of increasing concentrations, and coverslipped with Permount mounting medium (Fisher Scientific, Fair Lawn, NJ, USA).

Adjacent series of sections were also processed for detection of the endogenous proliferative markers Ki-67, a nuclear protein that is expressed during all active phases of the cell cycle, but is absent from cells at rest (Kee et al., 2002; for review see Christie and Cameron, 2006), and proliferating cell nuclear antigen (PCNA), which is expressed during all active phases of the cell cycle and for a short period of time after cells become post-mitotic (for review see Christie and Cameron, 2006). Briefly, after thorough rinsing, the sections were incubated in 10 mM sodium citrate buffer (in 0.1 M TBS, pH 6.0) at 95°C for 5 minutes. This step was repeated twice in order to completely unmask the antigens. After quenching with 3% H_20_2/10% methanol in 0.1 M TBS for 15 minutes and pre-incubating with 5% normal goat serum (NGS) for 1 hour, the sections were incubated for 48 hours at 4°C with a rabbit polyclonal anti-Ki-67 primary antibody (1:500; Vector Laboratories) or a rabbit polyclonal antibody against PCNA (1:100; Santa Cruz Biotechnology, Santa Cruz, CA, USA). After thorough rinsing the sections were then incubated for 2 hours with the secondary antibody (biotin-conjugated goat anti-rabbit IgG, 1:200; Vector Laboratories), and visualized as described above.

Finally, two additional series of sections were processed for doublecortin (DCX) immunohistochemistry, a marker for newly differentiated and migrating neuroblasts (Brown et al., 2003), or the neurogenic differentiation protein (NeuroD), a basic helix-loop-helix transcription factor involved in neuronal differentiation (Miyata et al., 1999). Briefly, after quenching and pre-incubation with NHS, the sections were incubated for 48 hours at 4°C with a goat anti-DCX primary antibody (1:400; c-18, Santa Cruz Biotechnology) or a goat anti-NeuroD primary antibody (1:200; Santa Cruz Biotechnology), respectively. The sections were then incubated for 2 hours with the secondary antibody (biotin-conjugated horse anti-goat IgG, 1:200; Vector Laboratories), and visualized as described above.

Neuronal differentiation was also assessed by BrdU/neuronal nuclei (NeuN) double-labelling. Briefly, after DNA denaturation in 2N HCl at 65°C for 30 minutes and pre-incubation with the proper sera, the sections were incubated for 48 hours at 4°C in rat anti-BrdU (1:100; Harlan Sera-Lab, Belton, UK) and mouse anti-NeuN (1:100;

Chemicon, Billerica, MA, USA) primary antibodies. The sections were then incubated with the secondary antibodies Cy3-conjugated donkey anti-rat IgG (1:200; Jackson ImmunoResearch, West Grove, PA, USA) and biotinylated horse anti-mouse (1:200; Vector Laboratories) for 2 hours, followed by incubation with Alexa-488-conjugated streptavidin (1:200; Molecular Probes, Leiden, The Netherlands) for 2 hours. The sections were mounted onto 2% gelatin-coated microscope slides, coverslipped with PVA mounting medium with DABCO antifading (Sigma-Aldrich), and stored in the dark at 4°C.

2.4 Morphological Quantification

All morphological analyses were performed on coded slides, with the experimenter blinded, using an Olympus microscope (Olympus BX51, Center Valley, PA, USA) with 10x, 40x and 100x objectives. Image Pro-Plus software (version 5.0 for WindowsTM, Media Cybermetic Inc., Silver Spring, MD, USA) and a Cool Snap HQ camera (Photometrics, Tucson, AZ, USA) were used for image capture. A profile analysis was performed to estimate the total numbers of BrdU-, Ki-67-, PCNA-, DCX- and NeuroD-immunopositive cells in the SGZ of the DG of the hippocampus by manually counting all positive cells present within two-three nuclear diameters below the granule cell layer (GCL). All sections containing the DG region of the hippocampus (from 1.34 mm posterior to Bregma to 3.52 mm posterior to Bregma; Paxinos and Franklin, 2001) were used for the analysis. To estimate any volumetric discrepancy between the DG of WT and YAC128 mice, we compared the volume of the GCL between 18 month-old WT and YAC128 mice, using adjacent coronal sections throughout the entire rostral/caudal extent of the DG region of the hippocampus. Every sixth 30 µm-thick section from the NeuroD immunostained brains was imaged. Using Image Pro-Plus software (Media Cybermetic Inc.), the GCL was outlined and the area obtained. Volume was estimated using the formula $\sum A \times T \times I$, where $\sum A$ is the sum of the area measured on each section, T is the section thickness (30 µm), and I is the number of section intervals (6). There were no significant volumetric differences found between the GCL of WT (0.251 ± 0.013 mm^3, n = 9) and YAC128 (0.227 ± 0.011 mm^3, n = 8; Student's *t*-test $p = 0.16$) mice at 18 months of age, a time-point when YAC128 are severely diseased and show pronounced striatal atrophy and cell loss (Slow et al., 2003).

Thus, the results were expressed as the total number of labelled cells in the DG sub-region of the hippocampus by multiplying the average number of labelled cells/DG section by the total number of 30 µm thick-sections that contain the DG (estimated as 73 sections). The total numbers of Ki67-, PCNA- and DCX-positive cells in the SVZ were also assessed by manually counting all positive cells in all sections containing this region (from 1.42 mm anterior to Bregma to 0.58 mm posterior to Bregma; Paxinos and Franklin, 2001). The total number of labelled cells in the SVZ was calculated by multiplying the average number of labelled cells/SVZ section by the total number of 30 µm thick-sections that contain the SVZ (estimated as 48 sections). As we did not observe significant differences between sexes (data not shown), cell counts from both males and females were pooled within genotypes.

For sections that were processed for BrdU/NeuN immunohistochemistry, a maximum of 50 BrdU-labelled cells per mouse were randomly selected for analysis of co-labelling with NeuN, using a confocal laser-scanning microscope (Olympus BX61WI) connected to a PC running the Olympus FluoView FV10-ASW 1.7c Software, at 1 µm optical thickness. BrdU and NeuN were considered to be co-localized if nuclear co-localization was observed over the extent of the nucleus in consecutive 1 µm z-stacks, if profiles of green (NeuN) and red (BrdU) fluorescence coincided, and when co-localization was confirmed in *x-y, x-z* and *y-z* cross-sections produced by orthogonal reconstructions from z-series.

2.5 Statistical Analysis

Differences between mean values of experimental groups were compared using Student's *t*-test or two-factor analysis of variance (ANOVA), followed by Tukey's post-hoc tests as appropriate, using R 2.10.1 (R Project for Statistical Computing, Free Software Foundation, Boston, MA, USA). Data are presented as mean ± standard error of the mean (SEM). A p value of < 0.05 was considered statistically significant.

3. Results

3.1 YAC128 mice exhibit altered hippocampal cell proliferation

We used BrdU to label cells in the S-phase of the cell cycle by injecting YAC128 and WT mice with this exogenous proliferation marker every 12 hours during three consecutive days and sacrificing the animals 12 hours after the last injection. We observed an age related decline in the number of proliferating cells in mice of both genotypes after 3 months of age. This decrease reached a plateau at 9 months of age, which is consistent with previous literature (Drapeau et al., 2007; Ben Abdallah et al., 2010). We also observed a small but significant decrease in the number of BrdU-positive cells between YAC128 mice and their WT controls (two-factor ANOVA: genotype: $p = 0.04$, $F(1,77) = 4.32$; age: $p < 0.0001$, $F(3,77) = 171.67$; age x genotype: $p = 0.14$, $F(3,77) = 1.89$). Thus, at 18 months of age YAC128 mice exhibit 26% less BrdU-positive cells (i.e., proliferating cells in the S-phase) when compared with their WT littermate controls (**Figure 4**).

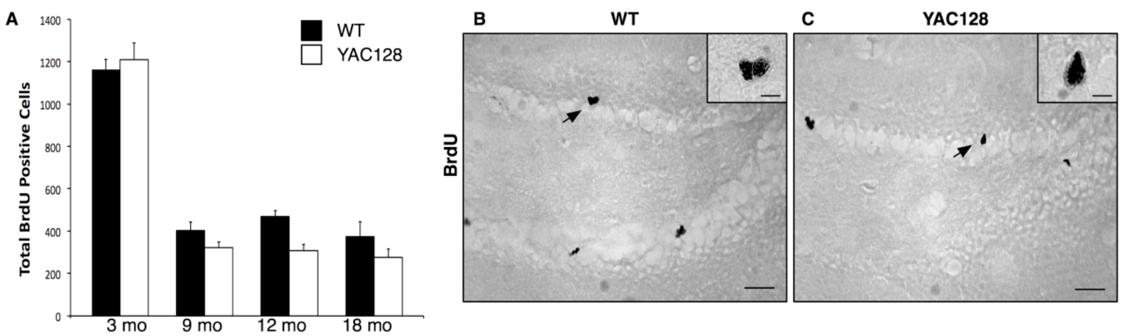

Figure 4. Decrease in the number of BrdU-labeled cells in the dentate gyrus of YAC128 mice.
(A) Cells in the S-phase of the cell cycle were assessed by immunohistochemistry for the exogenous marker BrdU. An age-related decline in the number of BrdU-labeled cells was observed in the DG after 3 months of age. A small but significant overall decrease in the number of BrdU-positive cells in the DG of YAC128 mice as compared to their WT controls was also observed (two-factor ANOVA: genotype: $p = 0.04$, $F(1,77) = 4.32$; age: $p < 0.0001$, $F(3,77) = 171.67$; age x genotype: $p = 0.14$, $F(3,77) = 1.89$). Representative sections of the DG processed for BrdU immunohistochemistry in WT (B) and YAC128 (C) mice at 3 months of age. Arrows indicate BrdU-positive cells in inserts. Data are presented as means ± SEM. Scale bars = 20 µm, scale bars (inserts) = 5 µm.

In order to further analyze DG cell proliferation in the YAC128 HD mouse model we used the endogenous proliferation marker Ki-67, which is expressed during all active phases of the cell cycle (Kee et al., 2002; for review see Christie and Cameron, 2006). Again, we observed an age-related decline in the number of proliferating cells in mice of both genotypes, as well as a small but significant decrease in the number of Ki-67-positive cells between YAC128 mice and WT controls (two-factor ANOVA: genotype: $p = 0.04$, $F(1,71) = 4.57$; age: $p < 0.0001$, $F(3,71) = 52.16$; age x genotype: $p = 0.88$, $F(3,71) = 0.22$). Moreover, similarly to the results obtained with BrdU, at 18 months of age YAC128 mice exhibit 26% less Ki-67-positive cells when compared with their WT littermate controls (**Figures 5A-C**). Since the cell cycle marker PCNA, which is expressed during all phases of the cell cycle (for review see Christie and Cameron, 2006), has been used in previous studies assessing cell proliferation in both human HD tissue (Curtis et al., 2003) and R6/2 mice (Gil et al., 2005), we also used this marker to evaluate cell proliferation in YAC128 mice. Again, we found an age related decline in the number of PCNA-positive cells. However, no significant differences in the number of mitotic cells between genotypes was detected with this marker (two-factor ANOVA: genotype: $p = 0.28$, $F(1,76) = 1.19$; age: $p < 0.0001$, $F(3,76) = 49.35$; age x genotype: $p = 0.91$, $F(3,76) = 0.17$) (**Figures 5D-F**). The fact that more cells were labelled with PCNA than with Ki-67 is a common finding; levels of Ki-67 expression appear to change across the cell cycle, which may cause Ki-67 to be undetectable during the early portion of the G1 phase. In contrast, PCNA appears to continue being expressed for a short time just after cells have become post-mitotic (for review see Christie and Cameron, 2006). Moreover, it has been suggested that PCNA is expressed in cells undergoing DNA repair (Tomasevic et al., 1998), which might also account for the discrepant results obtained with these two cell cycle markers. Regarding the fact that a significant reduction in the number of proliferating cells between YAC128 mice and their WT littermate controls was observed with Ki-67 and BrdU but not with PCNA, these results may reflect differences in cell cycle kinetics between WT and YAC128 mice.

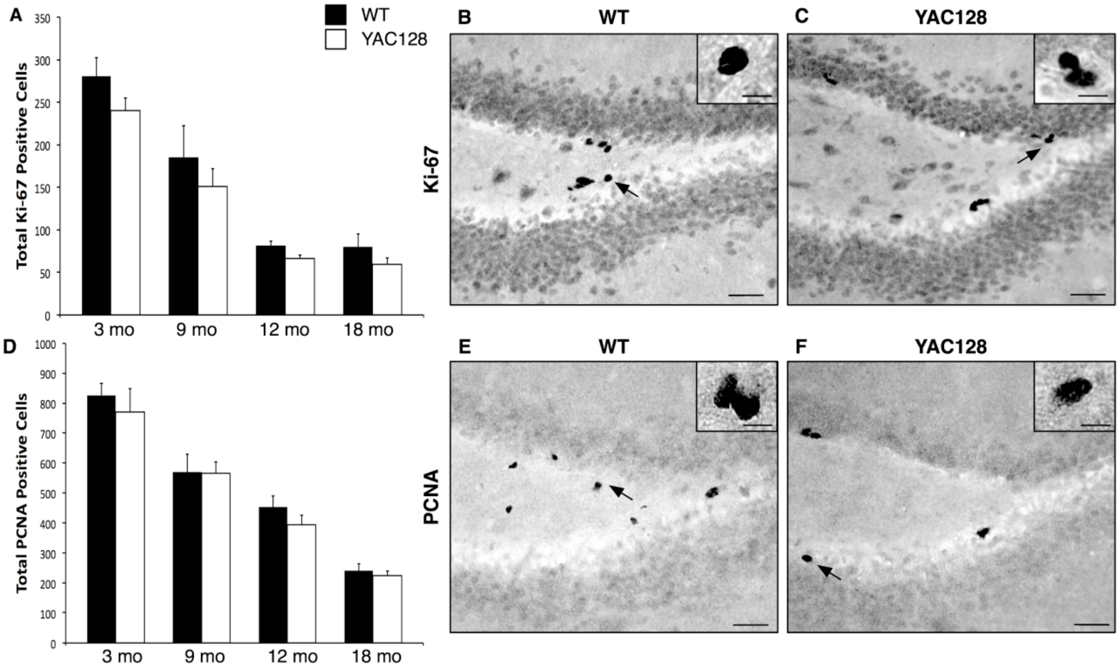

Figure 5. Evaluation of cell proliferation in the dentate gryus of YAC128 and control mice using endogenous cell cycle markers.
Cell proliferation was examined by immunohistochemistry for the endogenous cell cycle markers Ki-67 (A-C) and PCNA (D-F) at 3-, 9-, 12- and 18-months of age. There was a progressive decline in the number of proliferating cells with increasing age in the DG of both YAC128 and WT mice (A,D). (A) There was a small but significant overall decrease in the number of Ki67-positive cells in YAC128 mice compared to their controls (two-factor ANOVA: genotype: $p = 0.04$, $F(1,71) = 4.57$; age: $p < 0.0001$, $F(3,71) = 52.16$; age x genotype: $p = 0.88$, $F(3,71) = 0.22$), but no significant differences were found between the genotypes with PCNA immunohistochemistry (D; two-factor ANOVA: genotype: $p = 0.28$, $F(1,76) = 1.19$; age: $p < 0.0001$, $F(3,76) = 49.35$; age x genotype: $p = 0.91$, $F(3,76) = 0.17$). Representative sections of the DG processed for Ki-67 (B,C) and PCNA (E,F) immunohistochemistry in WT (B,E) and YAC128 (C,F) mice at 3 months of age. Arrows indicate immunopositive cells in inserts. Data are presented as means ± SEM. Scale bars = 20 µm, scale bars (inserts) = 5 µm.

3.2 YAC128 mice exhibit decreased hippocampal neuronal differentiation

The endogenous marker DCX, a microtubule-binding protein that is expressed in newly differentiated and migrating neuroblasts (Brown et al., 2003), was used to determine if there was an overall difference in the number of immature neurons between the two genotypes (**Figures 6A-C**). Similar to the results obtained using BrdU, there was an age-related decline in DCX-positive neuroblasts after 3 months of age, at which time a plateau was reached. There was also a striking significant decrease in the number of cells immunopositive for DCX in YAC128 mice compared to their WT controls (two-factor ANOVA: genotype: $p < 0.0001$, $F(1,78) = 33.80$; age: $p < 0.0001$, $F(3,78) = 367.55$; age x genotype: $p = 0.10$, $F(3,78) = 2.17$). Importantly, at 18 months of age, YAC128 mice exhibit 63% less DCX-positive neuroblasts when compared to their WT littermate controls, a more dramatic decrease than the observed reduction in the proliferation markers BrdU and Ki-67. Although not quantitatively assessed, it is of interest to note that there seemed to be differences in the morphology of immature neurons in the DG of YAC128 mice. In the YAC128 mice we typically observed a reduction in dendritic arborization, with DCX staining predominately confined to the cell bodies (**Figures 6B,C**), further suggesting that neuronal maturation is compromised in the DG of YAC128 HD transgenic mouse model.

In addition, the endogenous marker NeuroD, a helix-loop-helix transcription factor which has been shown to be sufficient to promote neuronal differentiation in adult hippocampal neural progenitors (Hsieh et al., 2004) and is involved in granule cell development (Liu et al., 2000; Pleasure et al., 2000), was utilized in this study. Interestingly, NeuroD has also been shown to interact with both normal and mutant huntingtin (Marcora et al., 2003). NeuroD has an expression pattern very similar to DCX (Steiner et al., 2006; Breunig et al., 2007; Borgs et al., 2009; Attardo et al., 2010) and comparable to the results obtained with DCX, there was a dramatic and significant reduction in the number of NeuroD-positive cells in the DG of YAC128 compared to their WT controls along with an age related decline in the number of NeuroD-positive cells (two-factor ANOVA: genotype: $p < 0.0001$, $F(1,80) = 116.71$; age: $p < 0.0001$, $F(3,80) = 506.77$; age x genotype: $p = 0.05$, $F(3,80) = 2.70$). Indeed, at 18 months of age YAC128 mice show 71% less NeuroD-positive neuroblasts when compared with their WT littermate controls (**Figures 6D-F**).

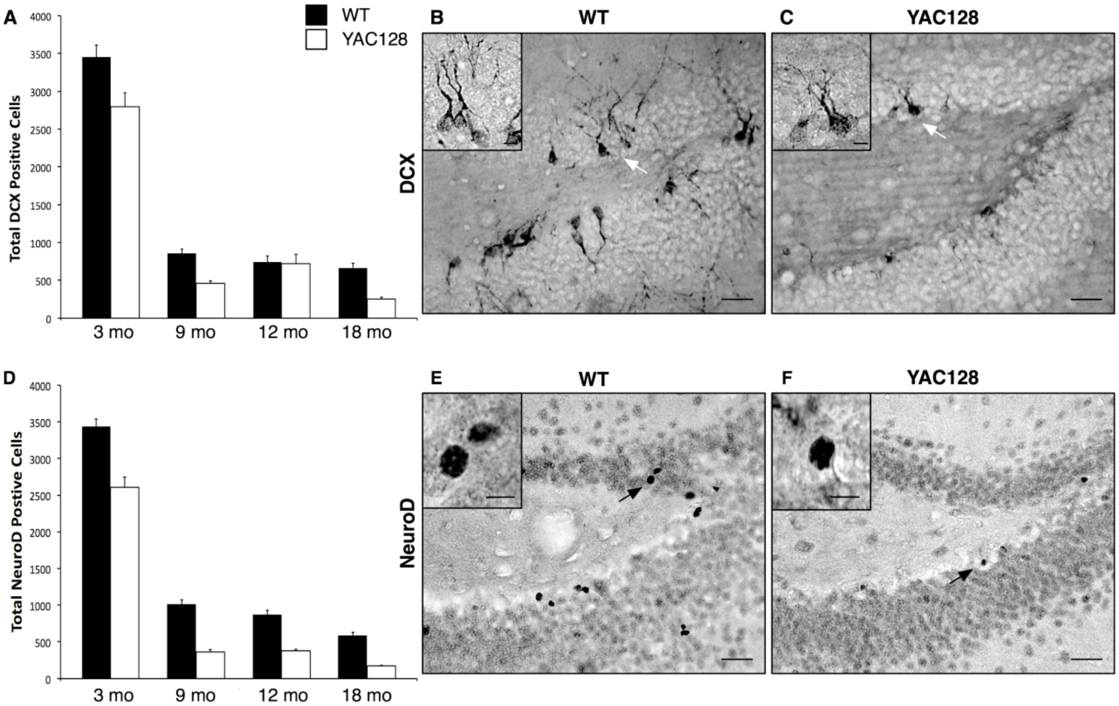

Figure 6. Decreased number of neuroblasts in the dentate gyrus of YAC128 mice.
(A) A significant decrease in the number of DCX-immunopositive immature neurons was observed in the DG of YAC128 mice as compared to their WT controls, as well as an age-related decline after 3 months of age (two-factor ANOVA: genotype: $p < 0.0001$, $F(1,78) = 33.80$; age: $p < 0.0001$, $F(3,78) = 367.55$; age x genotype: $p = 0.10$, $F(3,78) = 2.17$). Representative sections of the DG processed for DCX immunohistochemistry in WT (B) and YAC128 (C) mice at 3 months of age. (D) A significant decrease in the number of NeuroD-immunopositive immature neurons was also observed in the DG of YAC128 mice as compared to their WT controls, as well as an age-related decline from 3 to 18 months of age (two-factor ANOVA: genotype: $p < 0.0001$, $F(1,80) = 116.71$; age: $p < 0.0001$, $F(3,80) = 506.77$; age x genotype: $p = 0.05$, $F(3,80) = 2.70$). Representative sections of the DG processed for NeuroD imunohistochemistry in WT (E) and YAC128 (F) mice at 3 months of age. Arrows indicate immunopositive cells in inserts. Data presented as means ± SEM. Scale bars = 20 µm, scale bars (inserts) = 5 µm.

3.3 YAC128 mice exhibit a reduction in overall adult hippocampal neurogenesis

To examine the rate of survival of the newly born cells in the hippocampal DG of YAC128 and WT mice, eight month-old and eleven month-old mice were injected with BrdU (every 12 h, during 3 days) and allowed to survive for an additional 4 weeks before being sacrificed (i.e., at nine and twelve months respectively). Although with this procedure we cannot exclude the possibility that some dilution of the BrdU could have occurred, such factor is more of a concern when low doses of BrdU are used. Indeed, low doses of BrdU have been found not to be saturating in the adult brain, while higher doses such as the one used in the present study label cells more uniformly and in large enough numbers to enable quantitative analyses such as this (Christie and Cameron, 2006). We observed an age-related decline in the total number of BrdU-labelled cells that were present 4 weeks after the BrdU pulse (**Figure 7A**), which is consistent with previous literature (Kempermann et al., 1998). Importantly, this number was significantly smaller in YAC128 compared to WT mice at both 9 and 12 months of age (two-factor ANOVA: genotype: $p = 0.0008$, $F(1,34) = 13.63$; age: $p = 0.0004$, $F(1,34) = 15.32$; age x genotype: $p = 0.67$, $F(1,34) = 0.19$).

By comparing the number of BrdU-positive cells that were proliferating in the DG 12 hours after the last BrdU injection (**Figure 4A**) with the number of BrdU-positive cells that were still present 4 weeks after the last BrdU injection (**Figure 7A**), we observed a drastic reduction in the number of BrdU-positive cells in both genotypes (e.g., at 9 months of age, WT: 12 hours post-injection = 401.3 cells versus 4 weeks post-injection = 137.3 cells; YAC128: 12 hours post-injection = 322.2 cells versus 4 weeks post-injection = 110.7 cells). Such dramatic reduction is thought to be mainly due to cell death. Thus, by calculating the ratio between the number of BrdU-labelled cells 12 hours post-injection and the number of BrdU-labelled cells 4 weeks post-injection, it was possible to estimate an approximate rate of cell survival for both the 9- and 12-month time-points (assuming that factors such as cell cycle length and proliferation rate of the cells that incorporated BrdU are relatively constant among mice of the same genotype). Identical survival rates were present in both WT (34%) and YAC128 (34%) mice at 9 months of age, as well as at 12 months of age (WT = 23%; YAC128 = 23%). Thus, although the total number of BrdU-positive cells present in YAC128 mice both at 12 hours and 4 weeks after the BrdU pulse was significantly smaller than that of their age-matched WT controls (**Figures 4A and 7A**), the ratio between these two measurements was the same between the two genotypes. This result indicates that although the pool of proliferating cells is significantly smaller in YAC128 mice, the rate of survival of the existing proliferating cells is approximately the same in both WT and YAC128 mice.

By waiting 4 weeks after the last BrdU injection to perform our histological analysis, we were also able to determine the phenotypes of the new cells that incorporated BrdU. Using confocal analysis, we found that NeuN, a widely used marker of terminally differentiated neurons (for review see Kempermann et al., 2004), and BrdU were co-expressed in 18% of the cells from WT animals and 13% of cells from YAC128 mice at 9 months of age, while about 15% in WT and 7% in YAC128 mice co-expressed NeuN at 12 months of age. A two-factor ANOVA revealed that the differences between the percentages of BrdU/NeuN co-labelled neurons in YAC128 mice and their WT controls were statistically significant (two-factor ANOVA: genotype: $p = 0.02$, $F(1,36) = 5.83$; age: $p = 0.11$, $F(1,36) = 2.61$; age x genotype: $p = 0.68$, $F(1,36) = 0.18$) (**Figures 7B,D,E**). Overall neurogenesis, calculated by multiplying the numbers of BrdU-positive cells that survived the 4-week period by the proportion of BrdU-positive cells that co-expressed NeuN, was significantly reduced with age and also between genotypes (two-factor ANOVA: genotype: $p = 0.003$, $F(1,32) = 10.45$; age: $p = 0.016$, $F(1,32) = 6.47$; age x genotype: $p = 0.90$, $F(1,32) = 0.02$) (**Figure 7C**). Therefore, although the rate of survival was similar between genotypes, the reduction in the number of proliferating cells (**Figures 4 and 5A-C**) and immature neurons (**Figure 6**) in the YAC128 mice translated into an overall decrease in the number of new neurons reaching a mature stage.

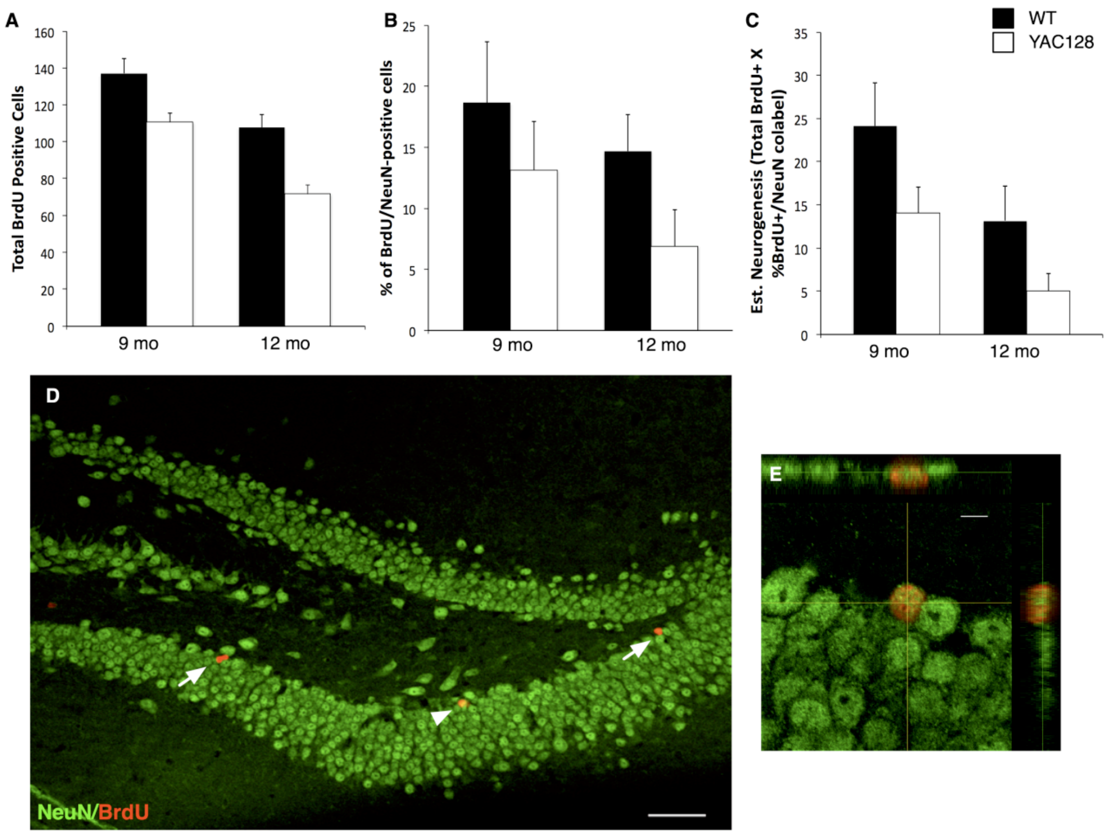

Figure 7. Decreased hippocampal neurogenesis in YAC128 mice.
8- and 11-month old mice were sacrificed 4 weeks after the last BrdU injection (i.e., at 9- and 12- months of age respectively) to allow for differentiation of the proliferating cells that incorporated BrdU. (A) Significant effects of age (9 versus 12 months of age) and genotype (WT versus YAC128) were found regarding the number of BrdU-labelled cells present in the DG 4 weeks after the last BrdU administration (two-factor ANOVA: genotype: $p = 0.0008$, $F(1,34) = 13.63$; age: $p = 0.0004$, $F(1,34) = 15.32$; age x genotype: $p = 0.67$, $F(1,34) = 0.19$). (B) The percentage of newly born cells (that incorporated BrdU) that co-express the mature neuronal marker NeuN is significantly lower in YAC128 mice when compared to their WT littermates (two-factor ANOVA: genotype: $p = 0.02$, $F(1,36) = 5.83$; age: $p = 0.11$, $F(1,36) = 2.61$; age x genotype: $p = 0.68$, $F(1,36) = 0.18$). (C) An estimation of overall neurogenesis based on the total number of BrdU-positive cells that survived the 4-week period multiplied by the proportion of BrdU-positive cells that acquired a mature neuronal phenotype indicates a significant decrease in the overall number of new neurons produced at both 9 and 12 months of age in YAC128 mice (two-factor ANOVA: genotype: $p = 0.003$, $F(1,32) = 10.45$; age: $p = 0.016$, $F(1,32) = 6.47$; age x genotype: $p = 0.90$, $F(1,32) = 0.02$). (D) Representative confocal image of the YAC128 DG showing mature granule neurons labelled with NeuN (green) and BrdU-positive cells (red) that survived over 4 weeks. Arrows indicate BrdU+/NeuN- immunopositive cells; arrowhead indicates a BrdU+/NeuN+ immunopositive cell. (E) Representative example of a cell immunopositive for both BrdU (red) and NeuN (green) present in the DG of a YAC128 mouse at 9 months of age. Data presented as means ± SEM. Scale bars = 50 μm (D), 5 μm (E).

3.4 YAC128 mice exhibit normal proliferation in the subventricular zone

In order to address if there are any significant differences in cell proliferation in the SVZ (the second neurogenic region in the adult brain), immunohistochemistry for the endogenous cell proliferation markers Ki-67 **(Figure 8A)** and PCNA (**Figure 8D**) was performed in both 9- and 12-month old YAC128 mice and their WT controls. Consistent with previous literature, there was an age-related decline in the total number of proliferating cells in the SVZ of both YAC128 and WT mice (for review see Zhao et al., 2008). However, no significant differences in cell proliferation were observed between genotypes at either 9 or 12 months of age with both Ki-67 (two-factor ANOVA: genotype: $p = 0.73$, $F(1,41) = 0.13$; age: $p < 0.0001$, $F(1,41) = 109.52$; age x genotype: $p = 0.92$, $F(1,41) = 0.01$) and PCNA (two-factor ANOVA: genotype: $p = 0.25$, $F(1,41) = 1.39$; age: $p < 0.0001$, $F(1,41) = 19.36$; age x genotype: $p = 0.67$, $F(1,41) = 0.18$). The number of neuroblasts in the SVZ was also quantified by immunohistochemistry for the immature neuronal marker DCX, and at 12 months of age YAC128 mice displayed similar numbers of neuroblasts within the SVZ as their WT controls (WT: 7879.4 ± 831.2 DCX-positive cells, YAC128: 8515.3 ± 441.9 DCX-positive cells, Student's *t* test $p = 0.51$). Therefore, both proliferation and differentiation appear unaltered in the SVZ of YAC128 HD mice compared to WT littermates.

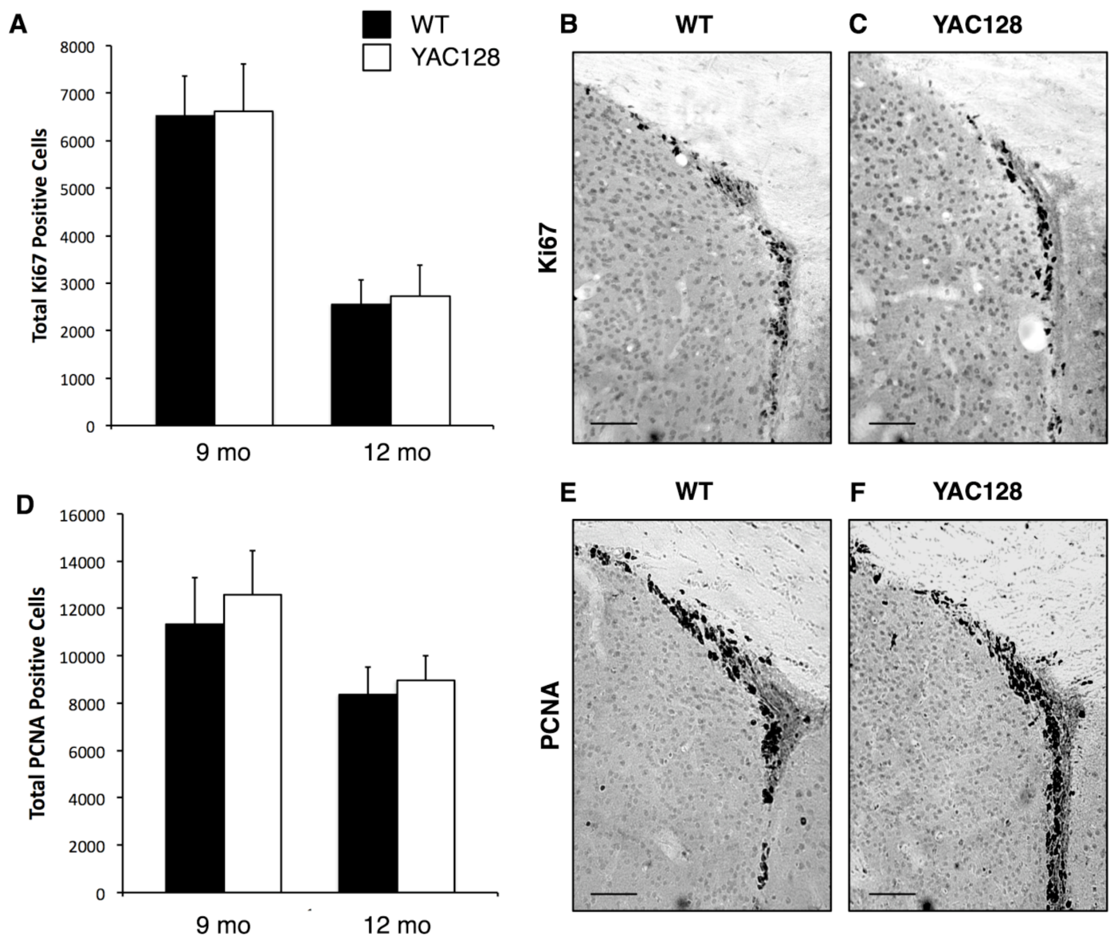

Figure 8. No differences in cell proliferation in the subventricular zone between YAC128 mice and WT controls.
Cell proliferation in the SVZ of 9- and 12-month old YAC128 mice and their WT littermates was assessed by immunohistochemistry for the endogenous cell cycle markers Ki67 (A-C) and PCNA (D-F). There was a progressive decline in the number of proliferating cells with increasing age in the SVZ of both YAC128 and WT mice (A,D). However, no significant differences between the genotypes in the number of both (A) Ki67- (two-factor ANOVA: genotype: $p = 0.73$, $F(1,41) = 0.13$; age: $p < 0.0001$, $F(1,41) = 109.52$; age x genotype: $p = 0.92$, $F(1,41) = 0.01$) and (D) PCNA-positive cells (two-factor ANOVA: genotype: $p = 0.25$, $F(1,41) = 1.39$; age: $p < 0.0001$, $F(1,41) = 19.36$; age x genotype: $p = 0.67$, $F(1,41) = 0.18$) were observed. Representative sections of the SVZ processed for Ki-67 (B,C) and PCNA (E,F) immunohistochemistry in WT (B,E) and YAC128 mice (C,F) at 9 months of age. Data presented as means ± SEM. Scale bars = 50 μm.

4. Discussion

Neurogenesis continues to occur robustly in the adult brain primarily in two specific regions, the SVZ and the SGZ of the DG in the hippocampus (for review see Zhao et al., 2008). Adult neurogenesis in the hippocampus has been implicated in various cognitive processes such as learning and memory (for review see Kempermann, 2008), and it is possible that alterations in the neurogenic process may contribute to some of the cognitive deficits that characterize several neurodegenerative disorders (for reviews see Lie et al., 2004; Mohapel and Brundin, 2004; Gil-Mohapel et al., 2010). Thus, the possibility of restoring brain function by promoting neurogenesis in HD brains has emerged as a potential therapeutic option (for reviews see Mohapel and Brundin, 2004; Gil and Rego, 2009; Gil-Mohapel et al., 2010).

In this study, we report an age-related decline in hippocampal cell proliferation, both in YAC128 mice and in WT littermates. The progressive reduction in hippocampal cell proliferation in WT mice is in agreement with previous reports showing reduced cell proliferation in the rodent DG with ageing (Bizon et al., 2004; Drapeau et al., 2007; Ahlenius et al., 2009; Ben Abdallah et al., 2010). However, YAC128 mice exhibited a small but significant reduction in hippocampal proliferation when compared to WT mice as assessed with BrdU and Ki-67. The fact that this significant reduction in the number of proliferating cells was observed with these two markers but not with PCNA (e.g., at 18 months of age we observed a 26% significant reduction in the number of BrdU-positive cells, a 26% significant reduction in the number of Ki-67-positive cells, and a 6% non-significant trend towards a reduction in the number of PCNA-positive cells) might reflect differences in cell cycle kinetics between WT and YAC128 mice. Indeed, it is possible that the ratio between the number of cells in G0 and the number of cells in all phases of the cell cycle is higher in YAC128 mice when compared with their WT controls. Thus, even though YAC128 mice have significantly less cells in the active phases of the cell cycle (as assessed by Ki-67 and BrdU), an increase in the number of cells in the G0 phase might counterbalance this reduction and account for the non-significant differences in the number of PCNA-positive cells that were observed between YAC128 and WT mice. In support of this hypothesis, wild-type huntingtin is known to interact with the cell cycle machinery (for review see Li and Li, 2004), as well as with the centrosome and microtubules (Gutekunst et al., 1995; Hoffner et al., 2002), the latter through interactions with β-tubulin. Therefore, it is possible that mutant huntingtin might induce a similar dysregulation of the mitotic machinery thus underlying the proposed alteration in cell cycle kinetics observed in YAC128 mice. Indeed, a recent study in a transgenic rat model of HD found a progressive decline in hippocampal progenitor cell proliferation, accompanied by an expansion of the pool of quiescent stem cells in the DG (Kandasamy et al., 2010). Importantly, a reduction in cell proliferation has also been observed in the R6 lines of HD transgenic mice (Gil et al., 2004; Lazic et al., 2004), with a 66% reduction in the number of BrdU-labelled cells being reported in end-stage R6/2 mice (Gil et al., 2005), which might reflect the severity of the phenotype characteristic of this truncated model.

In the present study we found a dramatic reduction in the number of immature neurons in the YAC128 DG (e.g., at 18 months of age we observed a 63% significant reduction in the number of DCX-positive cells and a 71% significant reduction in the number of NeuroD-positive cells). Similar results were observed in the R6/2 mouse model of HD, in which a 65% decrease in the number of DCX-positive cells was observed in the DG of 10 week-old mice (Gil et al., 2005). In the present study, the survival rate of BrdU-labelled cells was similar between YAC128 and WT mice (34% at 9 months and 23% at 12 months of age). These low survival rates are in agreement with the massive reduction in the number of BrdU-labelled cells commonly reported after the first days up to more than 4 weeks post-injection, and is thought to be due to cell death (Dayer et al., 2003; Gould et al., 1999). Nevertheless, we found a significant reduction in the total number of surviving BrdU-labelled cells in YAC128 mice when compared to their WT littermates and this resulted in a decrease in the proportion of surviving newly born cells that became neurons. The study by Gil et al. (2005) found no significant differences between R6/2 and WT mice in the proportion of surviving BrdU-labelled cells that developed a neuronal phenotype, and Lazic and colleagues (2004) failed to detect any cells co-labelled for BrdU and NeuN in R6/1 mice. Interestingly, in agreement with the observations made by Gil et al. (2005), we also noted morphological differences between YAC128 and WT immature neurons in the DG of the hippocampus. Indeed, YAC128 neuroblasts appear atrophied and show a decrease in dendritic arborization when compared with those present in WT mice. Striatal and cortical morphological changes have also been reported in other mouse models of HD, including loss of spines and reduction of somatic areas and dendritic fields (Spires et al., 2004; Laforet et al., 2001), and similar features have also been observed in human HD brains (Vonsattel and DiFiglia, 1998).

Taken together, our results strongly suggest that although a decrease in the pool of proliferating cells may contribute to the overall reduction in hippocampal neurogenesis, an intrinsic impairment in the actual differentiation stage is probably a major contributor to the observed changes in neurogenesis in the YAC128 mice. Indeed, the relatively small decrease in cell proliferation (observed with BrdU and Ki-67) alone cannot account for the much greater reduction in cell differentiation (assessed with DCX and NeuroD) that was observed in YAC128 mice as compared with their WT controls. It can be speculated that this deficit would correspond to an abnormal transition from Type-2a to Type-2b progenitor cells, when the first markers of the neuronal lineage (such as NeuroD and DCX) start being expressed (reviewed in Kempermann et al., 2004). However, we cannot exclude the possibility that the transitions from Type-2b to Type-3 progenitor cells and from Type-3 progenitor cells to early post-mitotic immature neurons are also compromised, as NeuroD and DCX continue being expressed throughout these stages of the neurogenic process (reviewed in Kempermann et al., 2004). A proper quantification of the percentages of Types-2a, -2b, and -3 progenitor cells and of post-mitotic immature neurons will provide further insight on the stage(s) of the neurogenic process that are directly affected by mutant huntingtin in the YAC128 transgenic mice.

4.1 Differences in adult hippocampal neurogenesis between truncated and full-length HD transgenic mouse models

The discrepancies in the changes in hippocampal neurogenesis observed in the R6 lines (Gil et al., 2004; Lazic et al., 2004) and the YAC128 mice are likely due to differences between the models used. The R6 lines are truncated HD models that only express exon 1 of the *HD* gene (i.e, approximately 3% of the entire gene) with a long tail of CAG repeats (approximately 115 and 150 repeats in the R6/1 and R6/2 lines respectively) (Mangiarini et al., 1996). As a result of their truncated *HD* construct, the R6 mice only express N-terminal huntingtin fragments, which have been suggested to be the toxic component of the mutant protein (for review see Gil and Rego, 2008). Consequently, these mice display an accelerated phenotype (Mangiarini et al., 1996) with limited striatal cell loss (Iannicola et al., 2000; Turmaine et al., 2000; Yu et al., 2003; Stack et al., 2005). Therefore, the R6 lines may not reflect the actual adult-onset human condition, being more representative of the juvenile and infantile cases of HD (for review see Gil and Rego, 2009). On the other hand, the YAC128 model expresses the full-length human *HD* gene under the control of its endogenous promoter and regulatory elements and presents a slower progression of the HD phenotype with marked striatal cell loss in the end-stages of the disease (Slow et al., 2003). Interestingly, a recent study has shown that YAC mice expressing full-length mutant huntingtin that is resistant to cleavage by caspase-6 do not develop motor deficits and striatal neurodegeneration and are resistant to exitotoxicity induced by quinolinic acid (Graham et al., 2006), further suggesting that the generation of the N-terminal fragments likely represents the initial step in the degeneration process. Furthermore, the depressive-like phenotype of the YAC128 mice was also completely rescued in these caspase-6 resistant YAC128 mice (Pouladi et al., 2009). On the other hand, R6 mice only express the truncated N-terminal fragments, therefore bypassing the caspase- and calpain-mediated proteolysis of full-length mutant huntingtin, a factor that might contribute to the faster disease progression observed in these mice (Mangiarini et al., 1996). Thus, the above differences between the truncated R6 and the full-length YAC128 models may account for the observed discrepancies in hippocampal cell proliferation between these two HD transgenic mouse models (i.e., a marked reduction in the case of R6 mice and a less severe decrease in the case of the YAC128 mice). However, the fact that both transgenic models show a severe reduction in the number of immature neurons in the DG strongly suggests that an impairment of the differentiation stage of the neurogenic process might be a common feature in HD. Studies in the hippocampal DG of human HD post-mortem tissue are thus warranted in order to confirm this hypothesis.

Given the neurodegenerative nature of HD, there is concern that the observed decrease in the number of immature neurons could be due to an overall loss of DG granule cells. Volume loss in the hippocampus of HD patients has been reported in a few cases (Rosas et al., 2003), while no significant differences in hippocampal neuronal numerical densities between HD patients and controls was observed by Spargo and colleagues (1993). In addition, no neuronal loss in the DG of R6/2 HD mice was observed as assessed by counting the total number of mature neurons by stereology (Gil et al., 2004). In the YAC models, there is no obvious difference in gross organization (Hodgson et al., 1999) or change in hippocampal volume or the number of estimated hippocampal neurons compared to WT mice (Van Raamsdonk et al., 2005). Nonetheless, there is strong electrophysiological and behavioural evidence for hippocampal dysfunction in the transgenic truncated HD mouse models (Murphy et al., 2000). Disturbed neurogenesis could thus contribute, at least in part, to this dysfunction.

4.2 Possible mechanisms underlying the deficits in adult hippocampal neurogenesis observed in HD transgenic mouse models

4.2.1 Transcriptional dysregulation

Given that the expanded CAG repeats in *huntingtin* can lead to the dysregulation of transcription, intracellular trafficking and metabolism (for review see Gil and Rego, 2008), it is plausible that they may also interfere with the proliferative capacity of progenitor cells. Indeed, huntingtin is known to interact with the cell cycle machinery (for review see Li and Li, 2004). Huntingtin can also become associated with the centrosome and microtubules (Gutekunst et al., 1995; Hoffner et al., 2002; Godin et al., 2010), the latter through interactions with β-tubulin. Indeed, huntingtin has been found to be essential in controlling mitosis, and loss of huntingtin alters spindle orientation and cell fate of cortical progenitors in murine embryos (Godin et al., 2010). Interestingly, the HEAT (huntingtin, elongation factor 3, protein phosphatase 2A, target of rapamycin 1) repeat motifs present in the huntingtin sequence (Andrade and Bork, 1995) are also found in a number of proteins that are involved in mitotic progression, chromosomal dynamics and segregation (Neuwald and Hirano, 2000) and several of these proteins exert their functions via microtubules. Dysfunctional microtubule dynamics caused by mutant huntingtin may be a broader phenomenon in HD, since a reduced proliferative capacity is not only found in the brain but also in the pancreas of end-stage R6/2 mice (Björkqvist et al., 2005). Whether a reduction in pancreatic proliferation is also observed in other models of HD and in human HD patients is not currently known.

An alternative mechanism that might contribute to the observed reduction in hippocampal neurogenesis in HD transgenic mice involves the regulation of the helix-loop-helix transcription factor NeuroD by huntingtin. Normal huntingtin and the huntingtin-associated proteins HAP1 and mixed-lineage kinase 2 (MLK2) modulate and stimulate the activity of NeuroD (Marcora et al., 2003), which is crucial for the development of a functional DG (Liu et al., 2000). Interestingly, a drastic decrease in the number of NeuroD-positive cells has been found in the DG of both R6/2 (Gil et al., 2005; Fedele et al., 2010) and YAC128 mice in the present study. Whether this decrease is a cause or a consequence of the observed reduction in neurogenesis remains to be elucidated. Further experiments are needed in order to conclude if a compromised NeuroD pathway (due, for instance, to its abnormal interaction with mutant huntingtin) is contributing to a reduction in the generation of new neurons. In agreement with this hypothesis, the reduction in NeuroD expression that was observed in the R6/2 DG was not accompanied by a decrease in the number of amplifying neuronal progenitors, as assessed by Pax6 and Tbr2 expression (Fedele et al., 2010) . These results strongly suggest that a compromised transition to the neuroblast stage is indeed underlying the reduction in hippocampal neurogenesis observed in these HD transgenic mice.

Nevertheless, a disturbed NeuroD function would also explain the down-regulation of certain genes that are regulated by this transcription factor. One example is the neurotrophin BDNF, whose expression is regulated by wild-type huntingtin and NeuroD, and that had been shown to be down regulated in HD (Zuccato et al., 2001; Luthi-Carter et al., 2002; Canals et al., 2004; Spires et al., 2004). Mutant huntingtin fails to interact with the complex neuron-restrictive silencer factor (NRSF) in the cytoplasm, and consequently leads to its nuclear accumulation. In the nucleus this complex binds to NRSE sequences and promotes histone deacetylation, leading to the remodelling of the chromatin into a closed structure. As a result, there is a suppression of NRSE-containing genes, including the *bdnf* gene (Zuccato et al., 2003). In addition, mutant huntingtin causes an impaired association between motor proteins and microtubules, attenuating BDNF transport along axons, which results in the loss of neurotrophic support (Gauthier et al., 2004). HAP1 has recently been shown to interact with the prodomain of BDNF, and this interaction is reduced in the presence of mutant huntingtin (Wu et al., 2010).

Given the fact that BDNF has been shown to up-regulate adult hippocampal neurogenesis (Zigova et al., 1998; Benraiss et al., 2001), it is possible that the down-regulation of BDNF observed in HD can also contribute to a compromised neurogenic process in this disorder.

Given that adult neurogenesis is highly regulated by the environment in which it occurs, the impact of mutant huntingtin on the local microenvironment may be more detrimental than the sole intrinsic presence of this mutant protein in adult neural stem cells. Indeed, proliferation and differentiation of SVZ-derived adult neural precursor cells in R6/2 mice were not affected *in vitro* (Chu-LaGraff et al., 2001; Phillips et al., 2005; Batista et al., 2006). On the other hand, disturbances in key positive regulators of adult neural progenitors, such as BDNF, may result in an environment not conducive to the production of new neurons. Furthermore, impairments in neurotrophic support may also affect neuroblast migration along the rostral migratory stream (RMS). Indeed, viral-induced BDNF over-expression or infusion of this neurotrophic factor into the lateral ventricles can increase the number of newly generated neurons in the olfactory bulb and striatum of adult rats (Zigova et al., 1998; Benraiss et al., 2001). Thus, the reduced migration of neuroblasts to the olfactory bulb that has been observed in R6/2 mice (Batista et al., 2006) might also be a consequence of a decrease in the levels of BDNF in these mice (Strand et al., 2007; Peng et al., 2008; Conforti et al., 2008)

4.2.2 Neurotransmission deficits

The dopaminergic system has been implicated in the regulation of adult neurogenesis. Indeed, it has been shown in rodent models of Parkinson's disease that the experimental depletion of dopamine (DA) decreases precursor cell proliferation in both the DG and the SVZ (Höglinger et al., 2004) and that a selective agonist of D2-like DA receptors is enough to completely restore proliferation in these models (Höglinger et al., 2004). However, other studies have also shown that D2 DA receptor stimulation can inhibit the proliferation of neural stem cells *in vitro* (Kippin et al., 2005), and that a decrease in dopaminergic signalling in the hippocampus leads to a transient activation of stem and progenitor cells in the DG (Park and Enikolopov, 2010). However, the specific cell populations and the stages of neuronal differentiation affected by dopamine are not known; thus, it is possible that the role of DA on adult neurogenesis might be dosedependent and differences in the levels of dopamine may influence varying responses of its receptors and transporters, activation of compensatory mechanisms, as well as stages of the disease progression. Nevertheless, several studies have shown that a dopaminergic dysfunction may also be implicated in HD (for review see Petersén et al., 2001; Gil and Rego, 2008). In fact, striatal DA levels are decreased in symptomatic R6/2 (Hickey et al., 2002) and R6/1 mice (Petersén and Brundin, 2002), and both transgenic HD mice and HD patients express reduced levels of tyrosine hydroxylase (the rate-limiting enzyme in the biosynthesis of DA) (Yohrling et al., 2003) and a loss of D2 DA receptors (Cha et al., 1998; Weeks et al., 1996). Mutant huntingtin can also induce the down-regulation of D1, D2 and D3 DA receptors by interfering with Sp1-mediated transcription (Dunah et al., 2002). Therefore, a dysregulation of the dopaminergic system might also contribute, at least in part, to the observed deficits in the neurogenic process that are observed in the truncated HD transgenic models. On the other hand, a loss of dopaminergic inhibition on stem cell proliferation could mediate the observed increase in neural stem cells in the subependyma of R6/2 mice (Batista et al., 2006) and human HD brains (Curtis et al., 2005). However, no abnormalities in DA receptor binding were observed in the YAC128 transgenic mouse model (Benn et al., 2007), suggesting that at least in this full-length transgenic mouse model other factors are likely to play a major role in the observed decrease in hippocampal neurogenesis.

YAC128 mice have been found to display cognitive deficits and a depressive-like behaviour as early as 3 months of age (Pouladi et al., 2009). Importantly, both cognitive disturbances (specifically learning and memory) and depression have been correlated with a reduction in hippocampal neurogenesis (Gould et al., 1999; reviewed by Mirescu and Gould, 2006). Selective serotonin reuptake inhibitors (SSRIs), antidepressants that regulate the reuptake of serotonin, are known to stimulate adult neurogenesis (Malberg et al., 2000; Manev et al., 2001; Boldrini et al., 2009; reviewed by Marlatt et al., 2010). The use of SSRIs has been shown to ameliorate depression-like symptoms and the deficits in hippocampal neurogenesis in some transgenic models of HD (Grote et al., 2005; Duan et al., 2008; Peng et al., 2008; Pang et al., 2009) therefore a dysregulation of the serotonergic system may also play a role in these processes. In fact, reduced levels of serotonin have been shown in R6/2 mice (Reynolds et al., 1999) and decreased serotonin receptor binding sites have been reported in patients with HD (Steward et al., 1993). However, antidepressant treatment had no effect on the development of the YAC128 depressive-like behaviour (Pouladi et al., 2009). In a recent study in R6/1 mice, the analysis of serotonin receptor expression in the hippocampus and cortex revealed a general reduction in mRNA levels of serotonin 1A, 1B, and 2A receptors (Pang et al., 2009). In addition, female R6/1 mice had a further reduction in hippocampal serotonin 1A receptor and cortical serotonin transporter mRNA levels compare to male R6/1 mice (Pang et al., 2009). Also of interest, recent meta-analysis data on antidepressant medication indicates that the magnitude of benefit increases with the severity of depression, and may be minimal in patients with mild or moderate symptoms (Fournier et al., 2010). Therefore, this therapeutic approach could still have beneficial results in HD patients showing a severe depressive phenotype.

4.2.3 Dysregulation of the hypothalamic-pituitary-adrenal axis

Still another factor that can account for the observed reductions in hippocampal neurogenesis in HD mouse models is an increase in the levels of glucocorticoids, which are known to down-regulate neurogenesis (Cameron and McKay, 1999). Indeed, a progressive increase in the serum and urine levels of corticosterone in R6/2 mice has been shown (Björkqvist et al., 2006). This increase appears to be related to a dysfunction of the hypothalamic-pituitary-adrenal (HPA) axis since a hypertrophy of the adrenal cortex, an enlargement of the intermediate pituitary lobe, and an increase in the levels of circulating adreno-corticotrophic hormone (ACTH) have also been observed in these mice (Björkqvist et al., 2006). Thus, it is possible that alterations in the HPA axis and a consequent increase in the levels of circulating corticosterone may contribute to the progressive decrease in hippocampal cell proliferation and neurogenesis observed in HD mice.

4.3 Differences in adult SVZ neurogenesis between HD transgenic mouse models and human HD

In contrast to the decrease in neurogenesis observed in the DG, we noted no significant differences in both SVZ proliferation and differentiation in YAC128 mice, which is consistent with previous studies in R6/2 (Gil et al., 2005; Phillips et al., 2005) and R6/1 mice (Lazic et al., 2006). The reasons for this specific impairment in hippocampal neurogenesis are currently not clear. An interesting explanation involves a reduction in the mitochondrial enzyme α-ketoglutarate-dehydrogenase complex (KGDHC), which has been implicated in the pathology of HD in humans (Klivenyi et al., 2004). Indeed, a recent study found that mice deficient in various subunits of KGDHC show increased levels of lipid peroxidation as well as reduced numbers of neuroblasts and proliferating cells in the adult DG, despite a normal SVZ proliferation (Calingasan et al., 2008), closely resembling the neurogenesis deficits observed in both the R6/2 (Gil et al., 2005) and the YAC128 transgenic mice. In agreement with these findings, it was recently shown that the deficiencies in KGDHC observed in the malonate lesion model of HD, did not alter the lesion-induced increase in migration of DCX-positive cells from the SVZ into the ipsilateral striatum (Calingasan et al., 2008). Therefore, while mitochondrial dysfunction may influence the number of neural progenitor cells in the adult hippocampus, it does not necessarily inhibit lesion-induced migration of SVZ neuroblasts (Calingasan et al., 2008). These findings might explain, at least in part, why a deficit in neurogenesis is specifically observed in the DG (and not in the SVZ) of transgenic mouse models of HD. Further studies are thus warranted in order to determine whether this mitochondrial enzyme is also compromised in R6 and YAC mice.

However, the absence of alterations in SVZ neurogenesis that has been consistently reported in HD transgenic mouse models is in contrast to findings in human HD post-mortem tissue, where an increase in cell proliferation estimated by PCNA immunohistochemistry has been reported (Curtis et al., 2003). This discrepancy could be due to factors such as higher numbers of CAG repeats and frequency of inclusions in the transgenic mouse models as compared to those in HD patients. Indeed, the human HD striatum is characterized by a lower frequency of inclusions than that observed in R6/2 mice (DiFiglia et al., 1997). This difference might suggest that the amount of free N-terminal toxic huntingtin fragments present in the human striatum is higher than in the R6/2 mice (where the N-terminal fragments are primarily recruited into inclusions). This may underlie the striatal cell loss that is characteristically observed in the human HD brain. Thus, the increase in cell proliferation that was observed in the human SVZ might occur in response to the greater number of degenerating neurons that is present in the adjacent striatum (Curtis et al., 2003). In agreement, no overt cell loss is found in the striatum of R6 mice (Iannicola et al., 2000; Turmaine et al., 2000; Yu et al., 2003; Stack et al., 2005), which might account for the absence of increased SVZ proliferation in these mice. However, YAC128 mice replicate the striatal loss that occurs in the HD brain, and yet no significant differences in SVZ proliferation were observed in these HD mice. Although the reasons for this discrepancy are currently unclear, the increase in SVZ proliferation that was observed in the human HD brain was inferred based on an increase in the number of PCNA-positive cells present in this region. While PCNA is indeed a marker of cell proliferation (for review see Christie and Cameron, 2006), it has also been suggested that cells undergoing DNA repair will express this protein (Tomasevic et al., 1998). Therefore caution should be taken when interpreting cell proliferation results that are exclusively based on PCNA immunohistochemistry and the use of alternative markers of cell proliferation is recommended to further validate these results.

4.4 Conclusions

In conclusion, the progressive decline in hippocampal neurogenesis observed in the YAC128 mice is a novel neuropathological feature that occurs early in the progression of the disease, before the appearance of overt motor and cognitive symptoms. Since neurogenesis may play a role in hippocampal function, it is possible that therapies aimed at restoring hippocampal neurogenesis may be beneficial in alleviating the cognitive deficits characteristic of these mice and the human condition.

III. Altered synaptic plasticity in the dentate gyrus of the YAC128 transgenic mouse model of Huntington disease

1. Introduction

1.1 Hippocampal Synaptic Plasticity

Early theories of learning postulated that memories might be formed by strengthening the connections between existing neurons, thereby improving the effectiveness of their communication (Hebb, 1949). In the early 1970's, the first reports emerged that synapses in the hippocampus could sustain long-lasting changes in synaptic efficacy (Bliss and Gardner-Medwin, 1973; Bliss and Lomo, 1973), and was eventually termed long-term potentiation (LTP). LTP refers to a long-lasting increase in the size of an evoked post-synaptic potential (PSP) or current (PSC). Typically, a single pulse of electrical stimulation to presynaptic fibres causes excitatory post-synaptic potentials (EPSPs) in the postsynaptic cells. The response of the postsynaptic cells to these single-pulse stimuli can be enhanced for a long period of time if a high-frequency train of stimuli is first delivered to the presynaptic fibres. To truly be considered LTP, this change in response size should be sustained for at least one hour. While several mechanisms of LTP have been elucidated, one of the best characterized is mediated by the NMDA receptor.

NMDA receptor-dependent LTP exhibits several properties that make it an attractive candidate for a memory mechanism (for review see Bliss and Collingridge, 1993): 1) LTP is input specific, therefore once induced, LTP at one synapse does not spread to other synapses; 2) LTP is associative in the sense that a weak input can be potentiated if it is active at the same time as a strong stimulus to a separate but convergent input; 3) LTP can also be induced cooperatively by weaker stimulation to many pathways to a synapse; 4) LTP is persistent, lasting from several minutes up to a year in animals whose life span is approximately 2 years (Abraham et al., 2002). In addition, LTP has also been postulated to be the neural equivalent of an arousal or attention device (Shors and Matzel, 1997), and plays an incidental role in memory formation, rather than a mechanism for memory storage and retrieval per se.

LTP induction occurs when repetitive synaptic activation releases the neurotransmitter glutamate, while at the same time depolarizes the postsynaptic cell, mediated by the opening of glutamatergic *a*-amino-3-hydroxy-5-methyl-4-isoxazolepropanoic acid (AMPA) receptor channels. The combined effect of glutamate binding to synaptic NMDA receptors and depolarization relieves the magnesium blockade of these receptors and allows the entry of calcium ions. The rapid rise in intracellular calcium concentration triggers the activation of protein kinases, including calcium/calmodulin-dependent protein kinase II (CaMKII) and protein kinase C (PKC). The induction of LTP has been associated with phosphorylation of ser-831 on the C-tail of the GluR1 subunit of AMPA receptors, a substrate of CaMKII and PKC, without a change in ser-845, a protein kinase A (PKA) substrate (Barria et al., 1997; Lee et al., 2000). The phosphorylation of existing AMPA receptors increases their activity, as well as mediates the insertion of additional AMPA receptors into the postsynaptic membrane from an adjacent nonsynaptic pool (Malenka and Bear, 2004). In addition to these postsynaptic changes, there is some evidence that presynaptic mechanisms may also play a role (Pavlidis et al., 2000; Emptage et al., 2003), possibly due to the presence of a retrograde messenger. Nitric oxide and arachidonic acid have been proposed as candidates, while cell adhesion proteins may also be involved (Malenka and Bear, 2004).

While early phases of LTP are dependent on protein phosphorylation, late LTP requires gene transcription and protein synthesis in the postsynaptic cell (Abraham and Williams, 2003; Lynch, 2004; Pittenger and Kandel, 2003). The persistent activation of protein kinases, including PKA, calcium/calmodulin-dependent protein kinase IV (CaMKIV) and mitogen-activated protein kinase (MAPK), activate the key transcription factor CREB and CRE-mediated gene expression (Silva et al., 1998; Abraham and Williams, 2003; Lynch, 2004). Changes in transcription factor activity may trigger the synthesis of proteins that underlie the maintenance of LTP. Such proteins may contribute to the increase in dendritic spine number, spine surface area and postsynaptic sensitivity to glutamate associated with LTP expression (Lynch, 2004). The actin cytoskeleton is thought to play a key role in structural changes in dendritic spines, and LTP is accompanied by a long-lasting increase in F-actin content within spines (Fukazawa et al., 2003).

In contrast, long-term depression (LTD) is a long-lasting reduction in the efficacy of neuronal synapses, and is thought to exist at most excitatory synapses in the central nervous system (CNS). LTD is also thought to participate in some forms of learning and memory, and allows a sort of neural homeostasis to be established, referred to as bidirectional synaptic plasticity (for review see Bear, 2003). In the hippocampus, LTD is typically induced by repetitive stimuli at low frequencies (0.5-5 Hz) for extended periods of time (10-15 minutes) (Dudek and Bear, 1992). The induction of LTD can also require the activation of NMDA receptors; however a small, slow rise in postsynaptic calcium levels is thought to induce LTD, as opposed to a sharp rise that induces LTP (Mulkey and Malenka, 1992). Calcium release from intracellular stores has also been proposed to contribute to LTD (Nishiyama et al., 2000).

While the activation of protein kinases is essential during the early phase of LTP, the activation of calcium-dependent phosphatases are involved in the early phases of LTD, such as calcineurin and protein phosphatase 1 (PP1) (Kirkwood and Bear, 1994; Mulkey et al., 1993). These protein phosphatases dephosphorylate postsynaptic PKC and PKA, while CaMKII remains relatively unaffected (for review see Malenka and Bear, 2004). The induction of LTP has been associated with phosphorylation of ser-831 on the C-tail of the GluR1 subunit of AMPA receptors, a substrate of CaMKII and PKC, without a change in ser-845, a PKA substrate (Barria et al., 1997; Lee et al., 2000). In contrast, LTD is associated with dephosphorylation of ser-845, without any change in ser-831 (Lee et al., 1998). Dephosphorylation of ser-845 decreases the AMPA receptor open channel probability (Banke et al., 2000) and their internalization via dynamin- and clathrin-dependent mechanisms (for review see Malenka and Bear, 2004).

1.2 Hippocampal Synaptic Plasticity and the HD Brain

The earliest symptoms of HD include mood swings, depression, irritability, and trouble learning new things or remembering facts. As the disease progresses, concentration on intellectual tasks becomes increasingly difficult, eventually leading to dementia (Harper, 1996). These symptoms appear in patients before the onset of the classical motor deficits, and post-mortem studies suggest that the first symptoms appear in the absence of overt neurodegeneration and cell death (Vonsattel et al., 1985), with the possibility that impaired cognition is likely caused by a cellular dysfunction rather than neuronal cell loss.

Cognitive processes such as learning and memory are thought to depend on changes in synaptic efficacy in regions such as the hippocampus. Therefore, several studies have investigated the synaptic function and plasticity of hippocampal slices from mouse models of HD at early stages of the disease. Most studies have reported impaired LTP in the CA1 region of the hippocampus (Usdin et al., 1999; Murphy et al., 2000; Lynch et al., 2007; Simmons et al., 2009), with only one study reporting an impairment in DG LTP in the R6/2 mouse model of HD only at a late stage in the disease progression (Murphy et al., 2000). In addition, Gibson and colleagues (2005) found impaired mossy fibre potentiation in R6/2 mice. Enhanced CA1 LTD has been reported in 5-18-week old R6/2 mice compared to WT (Murphy et al., 2000), while in the R6/1 mouse model of HD, a re-emergence of LTD that was lost with age was observed in the CA1 region of the hippocampus at 12 weeks of age (Milnerwood et al., 2006).

While these studies indicate that LTP deficits are present in HD mouse models, they disagree as to whether they are due to a presynaptic or postsynaptic problem. Assays for paired-pulse plasticity (which are commonly used to assess presynaptic function) were found to be reduced in HD knock-in (72/80 CAG) mice, suggesting that mutant huntingtin alters release kinetics (Usdin et al., 1999), however further studies in both knock-in and truncated models found no differences in paired-pulse plasticity in the CA1 region (Usdin et al., 1999; Murphy et al., 2000; Gibson et al., 2005; Milnerwood et al., 2006; Lynch et al., 2007; Simmons et al., 2009). Another study found that R6/2 and WT mice are comparable in their basic synaptic physiology of CA1 pyramidal cells, including normal resting potential, input resistance, firing threshold and presynaptic neurotransmitter mobilization and release, suggesting a postsynaptic locus for the LTP deficit (Murphy et al., 2000). Similar results were found in the *Hdh*^{Q111} knock-in mice (Lynch et al., 2007).

However, a study characterizing the newly developed YAC46 and YAC72 mice, which express the full length human mutant *huntingtin* gene including all regulatory elements, found that 6 month-old YAC72 mice had enhanced induction and maintenance of CA1 LTP (Usdin et al., 1999), a time-point prior to the development of a behavioural phenotype. This enhanced LTP was deemed NMDA receptor dependent, since the NMDA receptor antagonist 2-amino-5-phosphonovalerate (D-AP5) blocked the somatic spiking and reduced the area under the field EPSP (fEPSP) curve. YAC72 mice also displayed greater short-term potentiation. However, by 10 months of age, LTP could not be induced in either YAC46 or YAC72 mice, and even produced LTD in the YAC72 mice. In addition, resting levels of calcium were higher in mutant neurons from 10 month-old YAC46 mice. This evidence of NMDA receptor hyperactivity is consistent with the theory of excitotoxicity in HD (for review see Gil and Rego, 2008).

To assess the importance of NMDA receptor-dependent forms of hippocampal synaptic plasticity in HD, we examined the ability of hippocampal synapses to support both LTP and LTD in YAC128 transgenic mice, which express the full-length human mutant *HD* gene with 128 CAG repeats (Slow et al., 2003) and faithfully recapitulates many features of the human condition. Specifically, synaptic plasticity was examined in the DG, a region known to support structural forms of plasticity, which have also been correlated with spatial learning and memory.

2. Materials and Methods

2.1 Transgenic Mice

Transgenic HD mice expressing human *huntingtin* with approximately 128 CAG repeats (YAC128) and their wild-type (WT) littermates were used for these experiments. The colony was maintained at the University of Victoria (Victoria, BC, Canada). Briefly, a well-characterized YAC (353G6) spanning the entire *HD* gene including its promoter region was used to create these mice (Slow et al., 2003). Homologous recombination was used to incorporate 128 CAG repeats obtained from the DNA of a juvenile-onset HD patient into the YAC following a previously described method (Duff et al., 1994). Mice were maintained on the FVB/N background strain (Charles River, Wilmington, MA, USA). These experiments used 3- [4 WT (3 males; 1 female), 6 YAC128 (4 males, 2 females)] and 6-month old [7 WT (4 males; 3 females), 7 YAC128 (3 males; 4 females)] mice. All animals were weaned on postnatal day 21, and sexed and ear-punched at postnatal days 21-25. Animals were group-housed with *ad libitum* access to food and water with a normal 12 h light/dark cycle with ambient temperature and humidity. All experimental procedures were conducted in accordance with the University of Victoria and the Canadian Council for Animal Care policies.

2.2 Genotyping

2.2.1 DNA extraction

For DNA extraction, tissue from each animal was placed in 100 µL Lysis Solution (40 µL Tris pH 9, 50 µL KCl, 20 µL Proteinase K, 20 µL Tween 20, 870 µL Milli-Q H_2O) in a nuclease-free 1.5 mL Eppendorf tube and incubated at 50-55°C for 3 hours, followed by 15 min at 95°C. Samples were then centrifuges at 13 000 RPM for 12 min and the supernatant transferred to a new tube.

2.2.2 PCR analysis

The reaction was performed by mixing 10.1 µL nuclease-free H_2O, 5 µL PCR reaction buffer, 1 µL (50 mM) $MgCl_2$, 4 µL (2.5 mM) dNTP, 0.5 µL of each forward and reverse primer, 2 µL DNA, and 0.2 µL Taq DNA polymerase (Invitrogen; Burlington, Ontario, Canada). The cycling parameters employed were: first cycle of 3 min at 94°C, then 35 cycles of 30 s at 94°C, 30 s at 63°C, and 30 s at 72°C. The following primers were used to test for genotype: LYA1 = 5' CCTGCTCGCTTCGCTACTTGGAGC 3', LYA2 = 5' GTCTTGCGCCTTAAACCAACTTGG 3', RYA1 = 5' CTTGAGATCGGGCGTTCGACTCGC 3', RYA2 = 5' CCGCACCTGTGGCGCCGGTGATGC 3'. The following positive control primers were used: Actin R = 5' AGCCTCAGGGCATCGGAACC 3' and Actin F = 5' GGAGACGGGGTCACCCACAC 3'. PCR products were run on a 1.5% agarose gel with 10 000x SYBR-safe and visualized under a BioRad trans-illuminator.

2.3 Electrophysiology

2.3.1 Slice preparation

YAC128 (n=13) and WT littermate (n=11) mice were used for the electrophysiology experiments. Mice were anaesthetized with isoflurane, rapidly decapitated, and their brains removed in oxygenated (95% O_2/5% CO_2), ice-cold normal artificial cerebrospinal fluid (nACSF) [(in mM) 125 NaCl, 2.5 KCl, 1.25 $NaHPO_4$, 25 $NaHCO_3$, 2 $CaCl_2$, 1.3 $MgCl_2$, and 10 dextrose, pH 7.3]. Transverse hippocampal slices (350 µm) were sectioned using a Vibratome 1500 (Ted Pella, CA). Sections were kept in order using a modified 24-well plate and incubated in continuously oxygenated nACSF at 35°C. Sections were allowed to rest for a minimum of 1 h before recordings commenced.

2.3.2 Field electrophysiology recordings

Field recordings were collected in nACSF using an Axon MultiClamp 700B amplifier connected to a PC running Clampex 10.2 software (Molecular Devices, CA). Electrodes were placed under visual guidance using an Olympus BX51 microscope and motorized micromanipulators (Siskyou Design, OR). fEPSPs were elicited by delivering a 120 μs (10-40 μA) current pulse to the medial perforant path (MPP) of the DG using a digital stimulus amplifier (Getting Instruments, CA) and a single, concentric bipolar stimulating electrode (FHC, Bowdoin, ME). fEPSPs were recorded using a single glass recording electrode (0.5-1.5 MΩ) filled with nACSF, and placed in the MPP about 200 μm from the stimulating electrode. Stimulation magnitude was set to elicit a response of ~50% and paired-pulse experiment was conducted using an interpulse interval of 50 ms (5X; 20 s between pairings). The $GABA_A$ receptor antagonist bicuculline methiodide (5 μM; Sigma-Aldrich, Oakville, ON, Canada) was included to isolate the excitatory component of synaptic transmission for all high-frequency stimulations. A stable baseline (minimum 20 min) of the slope of fEPSPs elicited every 15 s was then achieved before a conditioning stimulus (CS) was delivered to induce synaptic plasticity. Baseline stimulation parameters were returned to following the CS (minimum 60 min). An input-output experiment was subsequently performed with increasing stimulation magnitude (30- to 300-μs pulse width; 15 s intervals). All analyses were conducted with Axon ClampFit 10.2 software (Molecular Devices, CA).

2.3.3 Conditioning stimulation protocols

LTP of fEPSPs was induced using a CS consisting of four trains of 50 pulses at 100 Hz, 30 s apart (high-frequency stimulation; HFS); whereas, LTD of fEPSPs was induced using a CS of 900 pulses delivered at 1 Hz over 15 min (low-frequency stimulation; LFS).

2.4 Statistical Analyses

Group data are presented as mean ± standard error of the mean (SEM) Differences between mean values of experimental groups were compared using Student's *t* test or analysis of variance (ANOVA), followed by Fisher LSD post-hoc tests as appropriate, using Statistica 7.0 software (StatSoft, Tulsa, OK). A p value of < 0.05 was considered statistically significant.

3. Results

3.1 Normal basal synaptic transmission but altered paired-pulse plasticity in YAC128 mice

Stimulating and recording electrodes were placed in the medial aspect of the DG molecular layer to elicit fEPSPs in transverse hippocampal sections obtained from 3- and 6-month old WT and YAC128 littermate mice. Input-output (IO) functions and paired-pulse measures of presynaptic transmitter release were recorded from WT and YAC128 mice (**Figure 9**). In 3-month old mice, the slope of the fEPSP significantly increased with increasing stimulation (repeated measures ANOVA: WT n = 9, YAC128 n = 14; $F(8,168) = 232.26$, $p = 0.000$) (**Figure 9A**). There was no significant interaction between stimulation strength and genotype ($F(8,168) = 0.906$, $p = 0.513$) or a significant main effect of genotype ($F(1,21) = 0.008$, $p = 0.93$). Similarly, in 6-month old mice, the slope of the fEPSP significantly increased with increasing stimulation (repeated measures ANOVA: WT n = 12, YAC128 n = 16; $F(8,208) = 105.37$, $p = 0.000$) (**Figure 9B**). There was no significant interaction between stimulation strength and genotype ($F(8,208) = 0.157$, $p = 0.996$) or a significant main effect of genotype ($F(1,26) = 0.277$, $p = 0.603$). These data suggest that basal synaptic transmission is not significantly altered in the DG of 3- and 6-month old YAC128 mice.

Short-term synaptic plasticity was then assayed using a paired-pulse protocol with a 50-ms interpulse interval, a timescale traditionally used to characterize paired-pulse plasticity (McNaughton, 1980). YAC128 HD mice displayed a reduction in paired-pulse depression in the medial perforant path at 3- (WT: 83.00 % ± 3.94 %, n = 20; YAC128: 97.49 % ± 2.93 %, n = 27; one-factor ANOVA: $F(1,45) = 9.13$, $p = 0.004$) (**Figure 9C**) and 6-months of age (WT: 84.27 % ± 2.01 %, n = 50; YAC128: 95.05 % ± 2.16 %, n = 43; one-factor ANOVA: $F(1,91) = 13.28$, $p = 0.001$) (**Figure 9D**), indicating that there is a modification of either the probability of release or the number of vesicles available at the presynaptic membrane in YAC128 mice.

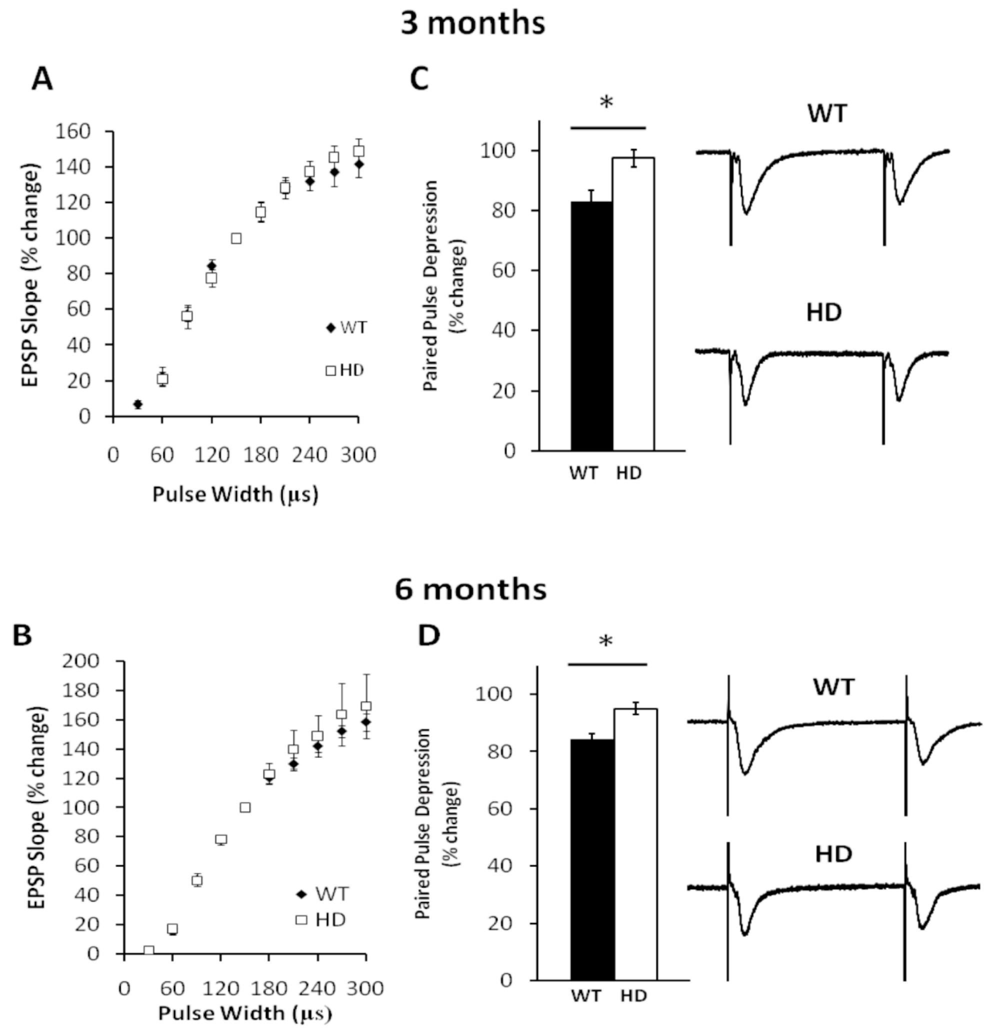

Figure 9. Normal basal synaptic transmission but altered paired-pulse plasticity in 3- and 6-month old YAC128 mice.
fEPSPs approximately 50% of maximum were evoked using increasing pulse widths from 30 to 300 μs to obtain input-output (IO) curves. No differences between WT and YAC128 mice were observed at 3- (A) and 6-months of age (B). Paired-pulse plasticity was assessed by delivering two stimuli to the medial perforant path 50 μs apart to induce consecutive fEPSPs. (C) 3- and (D) 6-month old YAC128 mice displayed a reduced paired-pulse depression (* denotes $p < 0.05$ between genotypes). Sample traces are shown for both WT and YAC128 HD mice. Data presented as means ± SEM.

3.2 Normal LTP in the DG of 3- and 6-month old YAC128 mice

The application of a conditioning stimulus, consisting of four trains of HFS (50 pulses at 100 Hz), produced LTP in the DG of both WT (55-60 min post-HFS: 21.48% ± 12.6 %, n = 7) and YAC128 mice (55-60 min post-HFS: 34.20 % ± 7.53 %, n = 10) at 3 months of age (**Figure 10A,C**). Similarly, in 6-month old mice, HFS produced LTP in the DG of both WT (55-60 min post-HFS: 25.70 % ± 6.82 %, n = 13) and YAC128 mice (55-60 min post-HFS: 24.34 % ± 7.11 %, n = 18) (**Figure 10B,D**). However, there was no significant difference between genotypes (2-factor ANOVA: genotype: $F(1,43) = 0.493$, $p = 0.486$; age: $F(1,43) = 0.088$, $p = 0.769$; genotype x age: $F(1,43) = 0.517$, $p = 0.476$) at 3 and 6 months of age (**Figure 10F**). While 3- and 6-month old YAC128 mice did not display any differences in long-term forms of plasticity, there was a significant main effect of genotype within the first minute post-HFS (WT: 69.11 % ± 11.09 %, n = 13; YAC128: 104.10 % ± 14.21 %, n = 18; 2-factor ANOVA: genotype: $F(1,44) = 7.26$, $p = 0.01$; age: $F(1,44) = 0.120$, $p = 0.731$; genotype x age: $F(1,44) = 0.0001$, $p = 0.991$). Post hoc analysis revealed that YAC128 mice had enhanced post-tetanic potentiation (PTP; up to 3 min post-CS) at 6-months of age ($p = 0.029$) (**Figure 10E**).

In order to further elucidate the short-term plasticity results, a paired pulse protocol was run both pre- and post-conditioning stimulus in 6-month old WT and YAC128 slices (**Figure 10G**). There was a significant effect of genotype, similar to the previous paired-pulse results, however no significant effect due to HFS or an interaction effect was observed (2-factor ANOVA: genotype: $F(1,36) = 8.890$, $p = 0.005$; HFS: $F(1,36) = 0.309$, $p = 0.582$; genotype x HFS: $F(1,36) = 0.107$, $p = 0.745$).

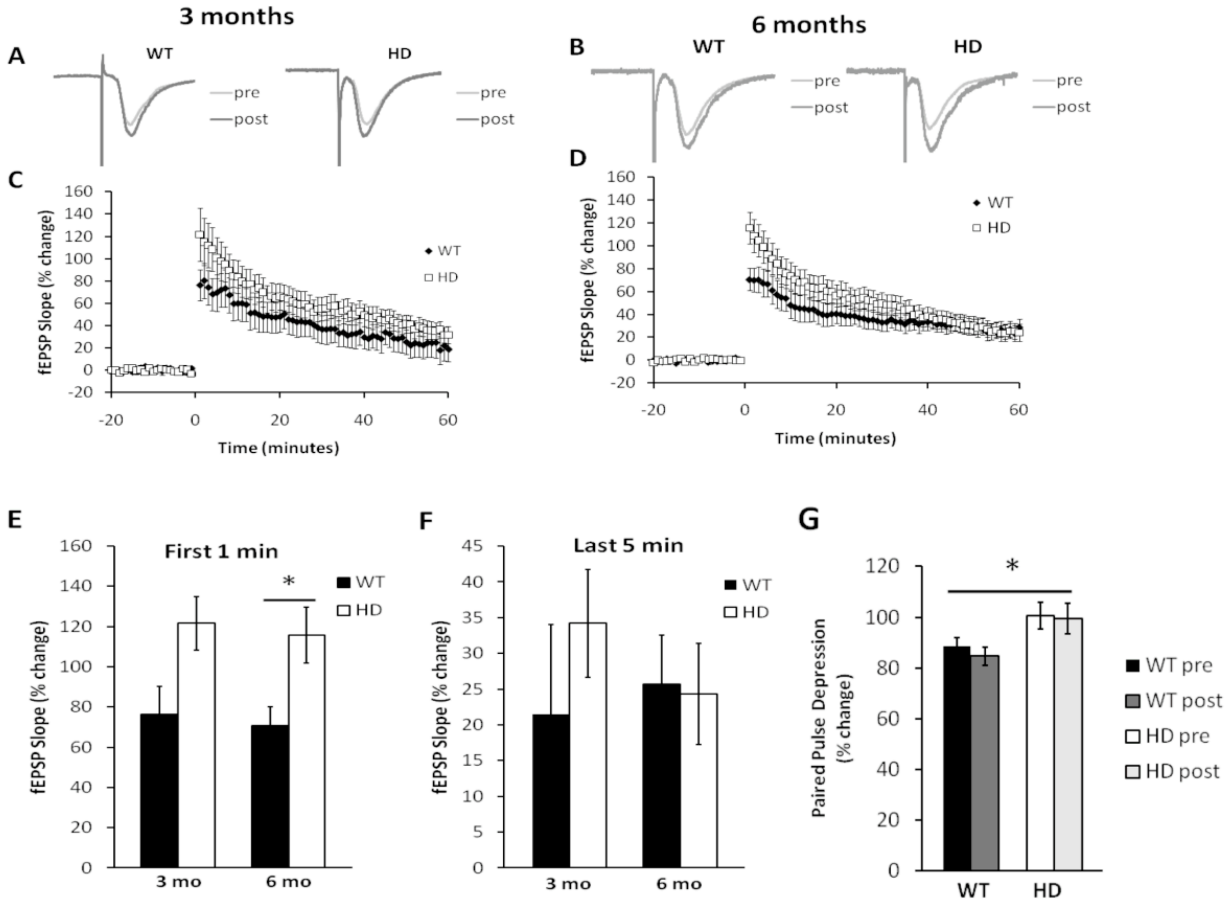

Figure 10. Long-term potentiation (LTP) is not significantly different in the dentate gyrus of 3- and 6-month old YAC128 mice.
(A-B) Field excitatory postsynaptic potentials (fEPSPs) were elicited every 15 s for 20 min in wild-type (WT) and YAC128 HD mice prior to high frequency stimulation (HFS). fEPSPs were obtained for 60 min following HFS. Traces were averaged from 20 min immediately prior to HFS ("pre") or 55-60 min after HFS ("post"). The absolute slope of fEPSP (expressed as percent change from baseline) was not significantly different between genotypes at (C) 3- or (D) 6-months of age. Bar graphs showing the change in slope at (E) 1 min and (F) 55-60 min post conditioning for each group. (G) Paired-pulse plasticity was assessed both pre- and post-conditioning stimulus. There was a significant effect of genotype, however, no effect due to HFS was observed (* denotes $p < 0.05$ between genotypes). Data presented as means ± SEM.

3.3 Decreased LTD in the DG of 3-month old but not 6-month old YAC128 mice

The application of LFS induced a significant LTD in the DG of both WT (55-60 min post-LFS: -35.4 % ± 3.99 %, n = 6) and YAC128 mice (55-60 min post-LFS: -20.67 % ± 4.02 %, n = 8) at 3 months of age (**Figure 11A,C**). Similarly, in 6-month old mice, LFS induced a significant LTD in the DG of both WT (55-60 min post-LFS: -30.95 % ± 3.69 %, n = 14) and YAC128 mice (55-60 min post-LFS: -24.60 % ± 3.86 %, n = 15) (**Figure 11B,D**). However, there was a significant main effect of genotype (2-factor ANOVA: genotype: $F(1,39) = 0.6.415$, $p = 0.015$; age: $F(1,39) = 0.003$, $p = 0.950$; genotype x age: $F(1,39) = 1.01$, $p = 0.321$) at 3 and 6 months of age (**Figure 11E**). Post hoc analysis revealed that YAC128 mice had reduced LTD at 3 months of age ($p = 0.038$). However, WT mice displayed a trend towards reduced LTD with age, while YAC128 mice displayed a trend towards increased LTD with age, possibly contributing to the non-significant difference between WT and YAC128 mice at 6-months.

Once again, a paired pulse protocol was run both pre- and post-conditioning stimulus in 6-month old WT and YAC128 slices. There was a significant effect of genotype, similar to the previous paired-pulse results, however no significant effect due to either LFS or interaction was observed (2-factor ANOVA: genotype: $F(1,32) = 8.029$, $p = 0.008$; LFS: $F(1,32) = 0.017$, $p = 0.898$; genotype x LFS: $F(1,32) = 0.047$, $p = 0.829$).

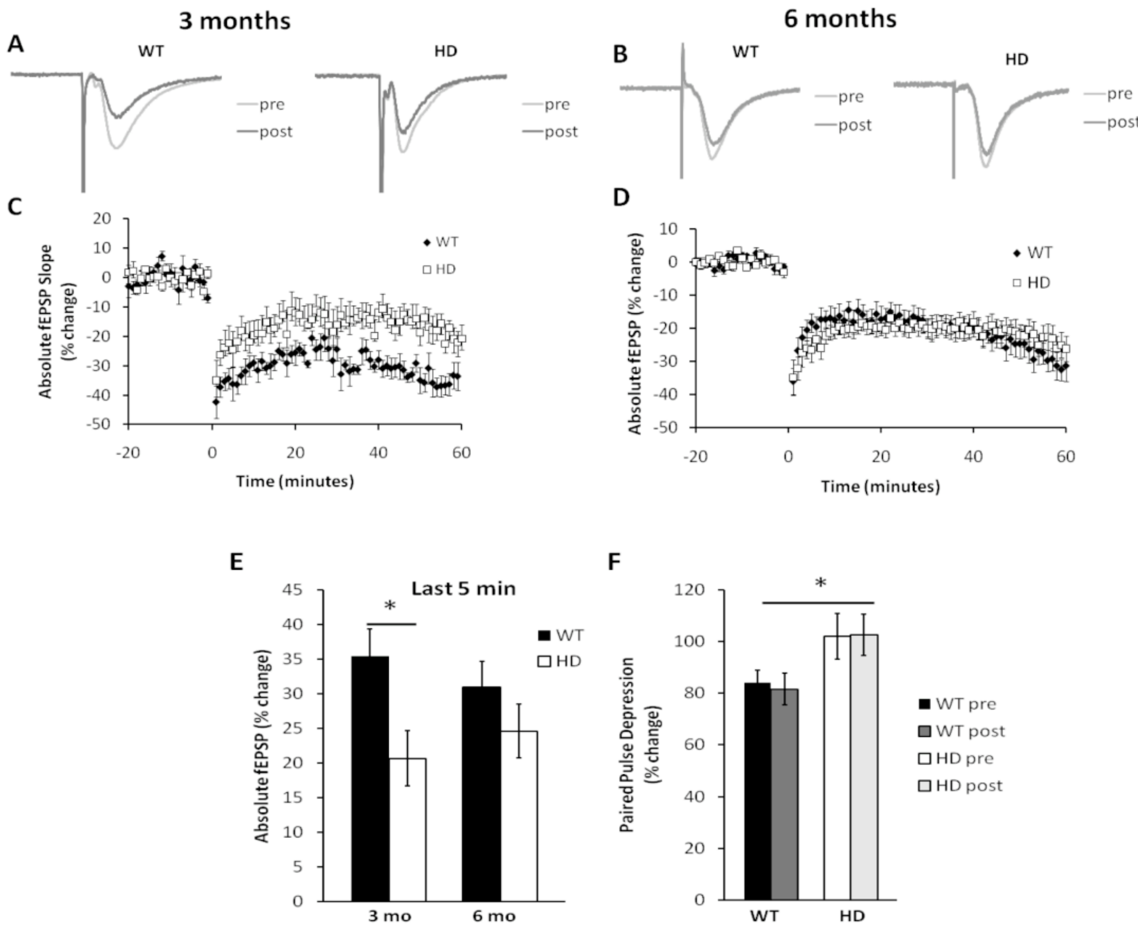

Figure 11. Long-term depression (LTD) is significantly attenuated in the dentate gyrus of 3-month but not 6-month old YAC128 mice.

(A-B) fEPSPs were elicited every 15 s for 20 min in WT and HD mice prior to low frequency stimulation (LFS). fEPSPs were obtained for 60 min following LFS. Traces were averaged from 20 min immediately prior to LFS ("pre") or 55-60 min after LFS ("post"). The absolute slope of fEPSP (expressed as percent change from baseline) was significantly increased in both genotypes at (C) 3- or (D) 6-months of age. However, the magnitude of LTD was significantly less in YAC128 mice at 3-months of age. (E) Bar graphs showing the change in fEPSP slope at 55-60 min post conditioning for each group. (F) Paired-pulse plasticity was assessed both pre- and post-conditioning stimulus. There was a significant effect of genotype, however, no effect due to LFS was observed (* denotes $p< 0.05$ between genotypes). Data presented as means ± SEM.

4. Discussion

Bidirectional hippocampal synaptic plasticity has been postulated as a candidate mechanism for memory formation in the brain, and cognitive processes such as learning and memory are thought to depend on changes in synaptic efficacy in regions such as the DG (reviewed in Bliss and Collingridge, 1993). Therefore, it is possible that alterations in neural synaptic plasticity may contribute to some of the cognitive deficits that are observed in HD (Harper, 1996).

In this study, we report alterations in paired-pulse plasticity in YAC128 HD mice at 3 and 6 months of age compared to their WT littermates. Specifically, YAC128 mice displayed a reduction in paired-pulse depression in response to a pair of stimuli to the medial perforant pathway. Paired-pulse depression is thought to occur at the medial perforant path (as opposed to the lateral perforant path, which generally displays paired-pulse facilitation), and is believed to reflect high intrinsic release probabilities (McNaughton, 1980). Transient depression of synaptic efficacy that occurs to the second of a pair of stimuli is thought to be due to a process of vesicle depletion and a decrease in the probability of release (reviewed in Zucker and Regehr, 2002). Systems which display facilitation have lower intrinsic probabilities of release, and show facilitation in response to a second stimuli due to residual calcium from the first stimuli, and hence an increase in the probability of release. It appears that YAC128 mice display an increased probability of release in the DG compared to WT mice. It has previously been found that the basal calcium concentration is elevated in hippocampal neurons from symptomatic YAC46 mice (Hodgson et al., 1999), striatal neurons from R6/2 mice (Hansson et al., 2001) and immortalized striatal cells from knock-in mice (Seong et al., 2005). Higher levels of intracellular calcium in the presynaptic terminal could account for the differences in paired-pulse plasticity observed between the YAC128 and WT mice.

In agreement with an increased probability of release from the presynaptic terminal, post-tetanic potentiation (PTP) was enhanced in 6-month old YAC128 HD mice in this study. PTP is the sum of two exponential processes known as augmentation (lasting up to 7 seconds) and potentiation (lasting up to 3 minutes), and an increase in calcium concentration has been shown to be increased in terminal boutons during PTP

(Wu and Saggau, 1994; Tang and Zucker, 1997), suggesting that, like facilitation, it is a presynaptic process. PTP was also increased in YAC72 mice at 6 months of age in the CA1 region (Hodgson et al., 1999).

While YAC128 mice were found to have enhanced short-term forms of plasticity, they did not display changes in LTP at 3 or 6 months of age compared to their WT littermates, time-points before overt motor symptoms characteristic of HD manifest (Slow et al., 2003). These results are in contrast to those found in another YAC mouse model, in which enhancements in LTP were found in the CA1 region of YAC72 mice at 6 months of age, although their experiments were performed in the absence of extracellular magnesium (Hodgson et al., 1999). However, direct comparisons between different models, as well as different hippocampal regions are difficult to make. The CA1 and DG are structurally and functionally different, containing different principle cells types with dissimilar resting membrane properties (Spruston and Johnston, 1992; Staley et al., 1992) and spontaneous firing frequencies (Penttonen et al., 1997). In fact, LTP was found to be normal in the DG of R6/2 mice at 4-7 weeks, before the appearance of an overt disease phenotype, and LTP was only impaired in the DG of R6/2 mice older than 12 weeks of age (these mice then expire suddenly by 16-18 weeks) (Murphy et al., 2000). In comparison, CA1 LTP was decreased by 5 weeks of age in the same study. The authors speculate that these regional differences in plasticity may be related to the appearance and distribution of intranuclear inclusions, which are present in the CA1 region by 3 weeks of age in the R6/2 mice; however have a low expression in the DG until 12 weeks of age (Murphy et al., 2000). In contrast, intranuclear inclusions are not present in the YAC128 striatum until 18 months of age (Van Raamsdonk et al., 2007). However, the fact that several mouse models of HD present significant neuronal dysfunction and loss prior to the appearance of clear intranuclear inclusions of mutant huntingtin suggests that inclusions may represent a side effect of the ongoing cellular dysfunction, or may even exert a protective role during the early stages of the disease (for review see Gil and Rego, 2008). Therefore, the timing of the appearance of intranuclear inclusions and synaptic plasticity dysfunction in the R6/2 DG could be a coincidence, or the fact that YAC128 mice do not have intranuclear inclusions at 3 and 6 months could play a role in the normal LTP they display. Further work elucidating the role of intranuclear inclusions may aid in explaining these results.

We also report a reduction of LTD in the DG of YAC128 mice compared to their WT littermates at 3 months of age. However, this significant difference is not present at 6 months of age. LTD is known to be regulated by aging, and aged rodents appear to have a lower threshold for LTD than adult rodents (reviewed in Rosenzweig and Barnes, 2003). Standard LFS protocols (900 stimlus pulses at 1 Hz), which generally fail to induce LTD in adult slices (Milner et al., 2004) induce a durable, input-specific NMDA receptor-dependent depression of synaptic strength in aged animals (Norris et al., 1996). Hence, LTD presents a unique age-dependent pattern of expression, with the ability of synapses to support LTD being lost in young adulthood, then once again appearing in aged animals. Interestingly, Milnerwood and colleauges (2006) found that both WT and R6/1 mice aged less than 1 month expressed LTD, however, by 3-4 months of age, R6/1 mice displayed a re-emergence of NMDA-dependent LTD, while their WT littermates failed to support LTD. Since this study failed to induce LTD in WT slices at both 3 and 6 months of age, it is difficult to directly compare to the results of the present study, where LTD was reliably induced in both WT and HD transgenic slices at these same time-points. However, there remains the possibility that a trend towards an enhancement in LTD with age in HD mice (Murphy et al., 2000; Milnerwood et al., 2006) could account for the non-significant differences observed in the YAC128 mice at 6 months of age. It will be necessary to investigate LTD in a cohort of older WT and YAC128 mice, in order to see if there are further changes in DG LTD, and the subsequent effects of aging.

4.1 Differences in hippocampal synaptic plasticity between truncated and full-length HD transgenic mouse models

The discrepancies in the changes in hippocampal synaptic plasticity observed in the R6 lines (Murphy et al., 2000; Gibson et al., 2005; Milnerwood et al., 2006) and the YAC128 mice are possibly due to the differences between the models used. The R6 mice express N-terminal toxic huntingtin fragments early on in the disease progression, and hence have an accelerated phenotype (for review see Gil and Rego, 2008). Ultrastructural studies have identified many aggregates of mutant huntingtin in axon terminals of R6/2 mice, which are co-localized with synaptic vesicles and may therefore disrupt transmission and vesicular trafficking (Li et al., 2001). Early impairments of proteins involved in the control of neurotransmitter release have also been noted in R6/2 mice (Morton and Edwardson, 2001), and the abnormal association of mutant huntingtin with synaptic vesicles may impair neurotransmitter release (Li et al., 2003). Alterations in synaptic transmission can change synaptic plasticity, and may explain the impairments in LTP observed in the R6 mice (Murphy et al., 2000; Gibson et al., 2005; Milnerwood et al., 2006). In contrast, YAC72 and YAC128 mice do not display macroaggregates of mutant huntingtin until 12 months of age (Van Raamsdonk et al., 2007); therefore, before 6 months of age, mutant huntingtin may not yet impair synaptic vesicle release in this model. However, a causal relationship between the appearance of aggregates and alterations in synaptic plasticity has not been established, and it should be noted that the *Hdh* mouse, which exhibits a form of impaired LTP, does not develop inclusions (Shelbourne et al., 1999).

In addition, the fast progression of the disease in R6 mice confers a confounding factor of age on the changes in synaptic plasticity, since bi-directional plasticity is differentially expressed depending on the age of the animal (for review see Rosenzweig and Barnes, 2003). For example, the threshold for LTP increases with age, but only under certain conditions (Lynch and Voss, 1994; Rosenzweig and Barnes, 2003), while the threshold for LTD follows a biphasic pattern, being easier to induce in young animals and then again in aged animals, compared to young adults (Norris et al., 1996; Foster and Norris, 1997; Milner et al., 2004). Therefore it is difficult to compare results from mice aged 5 weeks to those between 3- to 6-months old.

4.2 Possible mechanisms underlying the alterations in hippocampal synaptic plasticity in pre-symptomatic HD transgenic mouse models

4.2.1 Receptor recycling

The impairments in LTD observed at 3 months in the YAC128 mice may indicate altered postsynaptic properties. LTD is thought to be due to the internalization of AMPA receptors via dynamin- and clathrin-dependent mechanisms (for review see Malenka and Bear, 2004). In turn, wild-type huntingtin is known to interact with various vesicle proteins important for endocytosis, such as HIP1, a protein that binds to α-adaptin and clathrin (for review see Li and Li, 2004). These interactions may be impaired in the presence of the mutation, thus compromising the synaptic process. Mutant huntingtin also interferes with the recycling of membrane receptors through interactions with proteins such as PACSIN 1 (protein kinase C and casein kinase substrate in neurons protein 1) in the synapse, and may lead to their sequestration in huntingtin inclusions (Modregger et al., 2002).

4.2.2 Metabotropic glutamate receptors

In addition to NMDA receptor-mediated LTD, a mechanistically distinct form of LTD is present at the synapse, mediated by metabotropic glutamate receptors (mGluRs) (Bashir et al., 1993; Bashir and Collingridge, 1994). It is possible to obtain either form of LTD by manipulating the induction protocol, thus confirming the existence of two independent forms of LTD (Oliet et al., 1997). In both areas CA1 and the DG, mGluR-dependent LTD is reliably induced at all ages by exposure to the group 1 agonist DHPG (dihydroxyphenylglycine) (Palmer et al., 1997) or by low-frequency trains of pulses (Kemp et al., 2000). While the molecular mechanisms responsible for the expression of NMDA receptor dependent LTD have been extensively studied, the full mechanism behind mGluR-mediated LTD still remains to be elucidated. However, activation of mGluRs, in particular mGlu5, leads to the removal of AMPA receptors from the synapses. The signalling mechanisms involve protein tyrosine phosphatases, mammalian target of rapamycin (mTOR), phosphoinositide 3-kinase (PI3K) and MAPKs; however, how these enzymes interact is not yet known (reviewed in Bashir, 2003).

Furthermore, the mRNA and protein levels of the mGluRs 1, 2 and 3 were found to be reduced in R6/2 mice (Cha et al., 1998). Importantly, mGluR2 is a presynaptic receptor that regulates glutamate release. Therefore, a reduction in this receptor can lead to an increased release of glutamate due to a decreased feedback control, thus contributing to excitotoxicity. Alterations in mGluRs may be involved in the changes in synaptic plasticity observed in the DG of the YAC128 mice, resulting impairments in LTD as well as the increased neurotransmitter release during the paired-pulse experiments. However, in the YAC128 mouse model, mutant huntingtin was found to cause increases in NMDA, AMPA and mGluR binding in the striatum, whereas no changes in DA, GABA or adenosine receptor binding were observed (Benn et al., 2007).

It still remains to be seen if there are mGluR binding abnormalities within the DG of YAC128 mice.

4.2.3 NMDA receptors

There is a large body of work characterizing NMDA receptor-mediated excitotoxicity in MSN's of the striatum in both cell lines and mouse models of HD (for review see Fan and Raymond, 2007; Milnerwood and Raymond, 2010). Many studies have observed early augmentation of NMDA receptor activity in HD mouse MSNs in brain slices (Milnerwood and Raymond, 2007; Cepeda et al., 2001; Milnerwood et al., 2010) and altered NMDA receptor trafficking and signalling is observed in cultured striatal neurons derived from neonatal HD mice (Fan et al., 2007; Fernandes et al., 2007; Zhang et al., 2008). In addition, the NR2B subunit of the NMDA receptor is specifically enriched in the striatum, and mutant huntingtin has been found to have a selective role in promoting excitotoxicity in the striatum mediated specifically by the NR2B subunit. As well, several studies suggest that NR2B-containing NMDA receptors predominate at extrasynaptic sites (Köhr, 2006), although this differential localization is not absolute (Thomas et al., 2006; Petralia et al., 2010). Recent studies have linked the activation of extra-synaptic NMDA receptors with cell death pathways, and synaptic NMDA receptors with signalling cell survival (Hardingham et al., 2002; Hardingham and Bading, 2003; Soriano et al., 2006). It has been found that the balance between synaptic and extra-synaptic NMDA receptors is altered in HD, such that basal synaptic NMDA receptor signalling is less effective in mutant huntingtin expressing cells (Okamoto et al., 2009), and that extra-synaptic NMDA receptor currents are greatly elevated in MSNs of young HD mice (Milnerwood et al., 2010). These observations have been speculated to help explain the specific degeneration and cell death of striatal MSNs, compared to other regions of the forebrain (for reviews see Fan and Raymond, 2007; Milnerwood and Raymond, 2010).

In contrast, no neuronal loss in the DG of R6/2 HD mice was observed as assessed by counting the total number of mature neurons by stereology (Gil et al., 2004), and in the YAC models, there is no obvious difference in gross organization (Hodgson et al., 1999) or change in hippocampal volume or the number of estimated hippocampal neurons compared to WT mice (Van Raamsdonk et al., 2005). Whereas it is difficult to reconcile our findings with other mouse models, as well as other brain regions, alterations in NMDA receptors may still account for the alterations in synaptic plasticity observed in the YAC128 DG at early stages of the disease. While purely speculative, the possibility remains that the distribution of extra-synaptic and synaptic NMDA receptors, as well as its NR2B subunit, is altered in the DG of early symptomaticYAC128 mice. NR2B-containing NMDA receptors mediate maximal increases in intracellular calcium in response to low frequency trains, while NR2A-type receptors activate maximally in response to high frequency trains (Erreger et al., 2005). Other studies suggest that activation of NR2B-containing NMDA receptors mediates the induction of LTD, whereas NR2A-containing receptors are involved in LTP induction (Liu et al., 2004; Massey et al., 2004), although more recent studies have reported contrary results (Berberich et al., 2005; Morishita et al., 2007). Perhaps in YAC128 mice there is a reduction in the proportion of NR2B-containing NMDA receptors in the DG, as opposed their specific up-regulation in the striatum, contributing to the impairments in LTD observed in the present study, as well as the absence of cell loss in this region compared to the striatum. In addition, NR2B-containing NMDA receptors contribute to AMPA receptor internalization (Tigaret et al., 2006). Further characterization of NMDA receptor subunit synaptic localization and subunit composition may aid in elucidating the region-specific changes in synaptic plasticity and cellular dysfunction observed in HD mice.

4.2.4 Metaplasticity

Metaplasticity is a term used to describe when synaptic activity affects the ability of future synaptic events to elicit either LTP or LTD (for review see Abraham and Bear, 1996). Metaplasticity is closely related to the sliding-threshold plasticity mechanisms employed in the BCM (Bienenstock-Cooper-Munro) model (Bienenstock et al., 1982). In essence, synapses must be kept within a dynamic functional range, and not allowed to reach maximal levels of potentiation or depression. Therefore, relatively low levels of synaptic activity should facilitate future LTP induction, and relatively high levels of synaptic activity should facilitate future LTD induction, and there is evidence of such processes in the rodent hippocampus (Abraham et al., 2001). Of relevance to the present results, granule cells in the DG have low spontaneous firing rates (Penttonen et al., 1997), and one may speculate that it is easier to induce LTP in the YAC128 DG than area CA1 (as investigated in previous studies), while more difficult to induce LTD, due to a shift in the BCM curve. Experiments addressing CA1 bi-directional synaptic plasticity in YAC128 mice will thus be informative.

4.3 Implications for cognition

Traditionally, LTP has been thought of as the main mediator of spatial memory storage in the hippocampus, with LTD being assigned a secondary role of signal-to-noise regulation (Dayan and Willshaw, 1991) or in forgetting (Tsumoto, 1993). However, more recently, LTD has been found to contribute directly to hippocampal information storage, and could dominate processing of precise spatial characteristics (reviewed in Kemp and Manahan-Vaughan, 2007). Indeed, the role of the hippocampus in declarative memory processing may take place in a subregion-specific and plasticity-specific manner. Kemp and Manahan-Vaughan (2007) propose that the encoding of new space is prioritized and mediated by LTP, regardless of subregion, while conveying information about orientation in space could be mediated by LTD in the DG and finer features and details of space could be mediated by LTD in the CA1. Therefore, while YAC128 mice did not display alterations in DG LTP, deficits in DG LTD could still account for cognitive deficits observed in these mice, in particular deficits in the swimming T-maze tests and open-field habituation (Van Raamsdonk et al., 2005).

4.4 Conclusions

In conclusion, the alterations in DG synaptic plasticity observed in the YAC128 mice is a novel neuropathological feature that occurs early in the progression of the disease, before the appearance of overt motor symptoms. Since synaptic plasticity may play a role in hippocampal function, it is possible that therapies aimed at hippocampal plasticity (Lynch et al., 2007; Simmons et al., 2009) may be beneficial in alleviating the cognitive deficits characteristic of these mice and HD patients.

IV. General Conclusion

In these studies, we report deficits in hippocampal structural plasticity with the progression of HD, as well as more subtle alterations in DG synaptic plasticity of YAC128 transgenic mice early on in the disease. While a direct link between alterations in adult neurogenesis and synaptic plasticity has not been elucidated, some studies indicate that NMDA-mediated activity in the hippocampus may play a role in the regulation of proliferation and neuronal differentiation of neural precursor cells (Gould et al., 1994; Cameron et al., 1995). Excitation of NMDA receptors is thought to limit neurogenesis by inhibiting cell proliferation in the DG (Gould et al., 1994; Cameron et al., 1995; Bernabeu and Sharp, 2000; Arvidsson et al., 2001; Nacher et al., 2001; Okuyama et al., 2004), however an *in vitro* study found that NMDA receptor-mediated activity increased neurogenesis in hippocampal precursor cells via L-type calcium channels and changed transcription factor patterns to a neuronal program (Deisseroth et al., 2004). Therefore, it remains to be seen if alterations in synaptic plasticity can account for changes in adult neurogenesis in the hippocampus, or vice versa; or if mutant huntingtin plays a separate role on each of these processes.

While the mechanisms behind the changes in plasticity remain to be discovered, the results of these studies will hopefully further the search for the effects of mutant huntingtin on hippocampal function. In turn, the disruptions observed in structural and functional plasticity in the hippocampus may contribute to the cognitive deficits observed in mouse models as well as patients of HD. Therapies aimed at restoring plasticity in the hippocampus may therefore be beneficial for the cognitive decline in HD mice and patients.

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Appendix A
Characteristics of Select HD Genetic Mouse Models

Transgenic Model	*Promoter*	*CAG Repeats*	*Expression Level*	*Background Strain*	*Lifespan*	*Abnormal Behavior*	*Neuropathology*	*References*
Knock-in Mice								
Hdh^{Q92}	*Hdh*	90	n.d.	CD1 x 129Sv	Normal life span	Not observed	NIIs; cell dysfunction; no cell loss or gliosis	(Wheeler et al., 2000)
Hdh^{Q111}	*Hdh*	109	n.d.	CD1 x 129Sv	Normal life span	n.d.	NIIs	(Wheeler et al., 2000)
Hdh72	*Hdh*	72	55%	C57BL6, FVB/N	Normal life span	Aggression	NIIs; no cell loss or gliosis	(Shelbourne et al., 1999)
CAG140	*Hdh*	140	50%	C57BL6	>12 months	Hyperkinesia followed by hypokinesia	NIIs	(Menalled et al., 2003)
YAC Transgenic Mice								
YAC46	*IT15*	46	30-50%	FVB/N	>12 months	Not observed	Selective striatal cell death; no NIIs	(Hodgson et al., 1999)
YAC72	*IT15*	72	30-50%	FVB/N	>12 months	Hyperkinesia followed by hypokinesia	Selective striatal cell death; no NIIs	(Hodgson et al., 1999)
YAC128	*IT15*	128	75%	FVB/N	> 12 months	Hyperkinesia followed by hypokinesia; cognitive deficits	NIIs; selective striatal and cortical cell death and atrophy	(Slow et al., 2003)
Truncated Transgenic Mice								
R6/1	*IT15*	~115	31%	CBA x C57BL/6J	32-40 weeks	Behavioral and motor deficits	NIIs; limited cell loss; brain atrophy; cell dysfunction	(Mangiarini et al., 1996)
R6/2	*IT15*	~145	75%	CBA x C57BL/6J	10-16 weeks	Rapid deterioration of cognitive and motor functions	NIIs; limited cell loss; brain atrophy; cell dysfunction	(Mangiarini et al., 1996)
N171-82Q	*Mouse prion protein*	82	10-20%	C3H/HEJ x C57BL/6J	24-30 weeks	Motor deficits	NIIs; striatal neuronal degeneration	(Schilling et al., 1999)

NIIs: neuronal intranuclear inclusions; n.d.: not determined