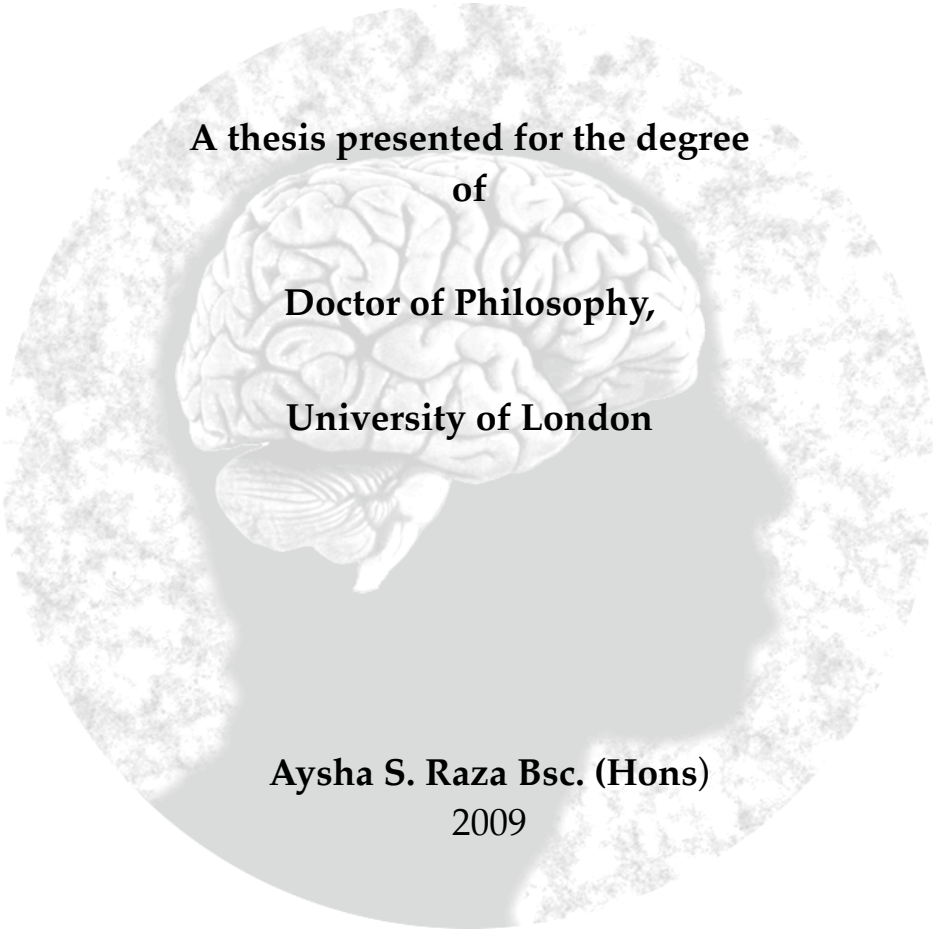
Neuropathological investigations of three murine models of Huntington's disease



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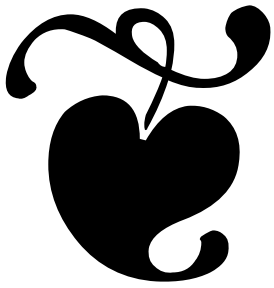
This thesis is dedicated to the memory of S.M.Jamil-ur Rahman, Who was the greatest advocate of a 'good English education'



I Aysha S Raza, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm this has been indicated in this thesis.

"I am just beginning to discover the difficulty of expressing one's ideas on paper. As long as it consists solely of descriptions it's pretty easy; but when reasoning comes into play, to make a proper connection, a clearness and a moderate fluency, is to me as I have said, a difficulty of which I had no idea."

Charles Darwin near the end of the voyage of HMS Beagle

Abstract

Huntington's disease (HD) is a purely genetic neurodegenerative disorder affecting approximately 1 in 10,000 people. It is most commonly associated with excessive involuntary movement, or chorea, combined with varying degrees of other motor, psychiatric and cognitive disturbances. Identification of the mutation in the HD gene prompted the generation of several transgenic mouse models. HD is but one of a family of at least 9 triplet repeat disorders, all of which exhibit protein aggregation by a similar mechanism. The understanding of one disease is therefore of importance to the understanding of them all. This thesis aims to be a comprehensive comparative study of three very different mouse models of HD elucidating the pathological changes that precede and accompany the disease process.

The work described in this thesis presents a detailed account of a longitudinal study of the pathological changes that occur within the brains of founder generations of mice transgenic for exon 1 of the HD gene, containing a highly expanded CAG repeat, the R6 lines. I have determined the intracellular sites for deposition and accumulation of the mutant protein huntingtin (*htt*), within both the neurons and glia of the central nervous system. The progressive accumulation of additional proteins within these aggregates has been described. The temporal evolution and spatial distribution of the neuronal intranuclear inclusion (NII) was determined using both immunohistochemical and morphometric analyses. The cellular consequences resulting from the aggregation of mutant *htt* were also investigated. I have conducted a detailed morphometric analysis of neurones within the *cerebral cortex*, *striatum* and *cerebellum* throughout the period of protein deposition, until the eventual degeneration of these cells. The dendritic and somal changes resulting from the cellular disruption associated with these NII are also described.

In a further series of experiments I have investigated the changes that occur in a novel model of HD, namely the conditional, doxycycline inducible double transgenic mouse, HD94 model. It was interesting to find that the same construct when differently manipulated in two mouse lines can produce such contrasting symptoms and pathology. This was highlighted by the comparison of immunohistochemical and morphometric analyses between the HD94 and the R6 lines, where the pattern of mutant protein deposition was found to vary significantly.

Lastly I have studied a more genetically accurate murine model of HD, the HD80 'knock-in model'. These mice develop a pathology broadly similar to that of the R6 lines but markedly different to that of the HD94, and over a much longer time frame

This detailed comparative analysis of the molecular and cellular pathology of three transgenic mouse models of HD provides new insights identifying novel and unique neuropathology and suggests new approaches for therapeutic treatments for this disease.

Acknowledgements

I must thank a lot of people whose roles have been vital in the long gestation of this thesis, and who are far too many to name individually. However I am enormously indebted to my supervisor Stephen Davies who has seen me through the pain barrier and I am grateful for his advice and friendship and it has been a real honour to know and work with such a gifted scientist. Grateful thanks go also to Jon Clarke for agreeing to be my second supervisor. Very special thanks to Mark Turmaine for advice on all things EM and for his friendship. And thanks are also due to Elizabeth Slavik-Smith[2] for advice on all things nuclear and shoes.

This PhD would not have been possible without the mouse models for which I am obliged to; Gillian Bates, Laura Mangiarini and Ben Woodman for the endless supply of the R6 mice, Peggy Shelbourne for her knock-in mice and Ai Yamamoto for her conditional mouse model and great times in New York. I would like also to thank the Huntington's Disease Society of America for funding this PhD, as well as the Hereditary Disease Foundation, The Wellcome Trust and The Bogue Fellowship for small sums of money over a very long time.

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I must also thank my Openlab elves Adil Raza and Jenny Parker who spent weeks measuring things instead of getting up to no good in their summer holidays! I am indebted to the late great Brian Ruth and 'Mac' Mike Corder for bailing me out of every misbehaving computer episode of which there have been many!

Finally I would like to thank the members of the lab, past, present and future, the good, bad and ugly all of whom have made the lab a colourful place to work in and who made it a character-building experience, hopefully one that has left me a better more patient person! I wish them all well in their own research projects and thank them for helping the evolution of mine.

And thanks inevitably to the fluffy people without whom there would be no thesis to quote Walt Disney: 'I only hope that we never lose sight of one thing- that it was all started by a mouse'.

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Abbreviations

A

ABC-Avidin-biotin complex

AD-Alzheimer's disease

ALS-Amyotrophic lateral sclerosis

B

BMI-Body mass index

β-Gal-Beta galactosidase, reporter protein from *E.coli.*

C

CAG-Codon coding for glutamine

CBP-Creb binding protein

CJD-Creutzfeld-Jakob disease

CNS-Central nervous system

C57 Black 6-Background strain of mice

D

DAB-Diaminobenzidine

DCD-Dark cell degeneration

DNI-Dystrophic neurite inclusion

DRPLA-dentato-rubra-palado luisian atrophy

E

EM-Electron microscopy / micrograph

ER-Endoplasmic reticulum

F

FL-Full length *Hdh* gene integrated into the HD80 knock-in mouse model

Fr-Fragment of the *Hdh* gene integrated into the HD80 knock-in model

FVB-Background strain of mice sensitive to B strain of Friend Leukaemia virus

G

GABA-γ-Aminobutyric acid

GAD-Glutamic acid decarboxylase

GFAP-Glial fibrillary acidic protein, astrocyte marker.

H

HD-Huntington's disease

HDCRG-Huntington's Disease Collaborative Research Group

HDF-Hereditary Disease Foundation

Hdh-Huntington's disease gene locus

HDSA-Huntington's Disease Society of America

HSP-Heat shock proteins

Htt-Huntingtin protein

L

LM-Light microscopy

LMC-Litter-mate control

LTP-Long-term potentiation

M

MRI-Magnetic resonance imaging

mRNA-Messenger ribonucleic acid

mTOR-Mammalian Target of Rapamycin protein

N

NG2-Integral membrane proteoglycan, marker for immature glia.

NII-Neuronal intranuclear inclusion

P

PD-Parkinson's disease

PDGF αR-Alpha receptor for platelet derived growth factor.

PML-Promyelocytic leukemia

R

RFLP-Restriction fragment linked polymorphism

S

SAHA-suberoylanilide hydroxamic acid

SBMA-Spinobulbar muscular atrophy

SCA-Spinocerebellar ataxia

SOD-Superoxide dismutase

T

TAU-Microtubule associated protein-Tau

TBP-TATA-binding protein

TEM-Transmission electron microscopy / micrograph

TUNEL-Terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labeling

U

Ubq-Ubiquitin

UPS-Ubiquitin proteasome system

Y

YAC-Yeast artificial chromosome