Therapeutic effects of ganglioside GM1 and gangliomimetic compounds in multiple models of
Huntington disease

by

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Abstract

Huntington disease (HD) is a neurodegenerative disorder that results in motor, cognitive and psychiatric deficits. The disease is caused by the expansion of a polyglutamine stretch in huntingtin (HTT), a ubiquitous protein with unclear functions.

The molecular mechanisms underlying neurodegeneration in HD are complex and include transcriptional dysregulation, mitochondrial dysfunction, impaired intracellular and axonal transport, as well as aberrant cell signaling and neurotransmission.

Previous work showed that the synthesis of ganglioside GM1, a lipid highly enriched in the brain, is also impaired in HD models. Decreased levels of GM1 contribute to heightened HD cells susceptibility to apoptosis, and chronic intra-ventricular infusion of GM1 reverts the pathological motor phenotype in already symptomatic transgenic models of HD. The dramatic therapeutic effects of GM1 are accompanied by phosphorylation of mutant huntingtin (mHTT) at Ser13 and Ser16, a post-translational modification that has been shown to decrease mHTT toxicity. This suggests that GM1 might be able to modify the course of HD and to provide therapeutic benefits that go beyond the treatment of motor symptoms.

To test this hypothesis we investigated whether administration of GM1 affects neurodegeneration and disease progression in R6/2 mice, an established model of HD that displays profound motor deficits, early-onset neurodegeneration and premature death. In these mice, treatment with GM1 resulted in improved motor performance, accompanied by a significant attenuation of the neurodegenerative process. GM1 reduced the loss of striatal neurons and increased overall brain and striatal volume. In addition, GM1 displayed trophic

effects on the brain white matter and restored the volume of the corpus callosum to normal levels.

Next, we determined whether GM1 improves cognitive and psychiatric-like symptoms in YAC128, a second transgenic model of HD, and Q140 mice, a knock-in mouse model. Both models display a slower disease progression, with respect to R6/2 mice, allowing for a more accurate analysis of non-motor behaviour. Significant attenuation of cognitive and psychiatric-like symptoms was observed in YAC128 mice after 14 days of treatment and in Q140 mice after 28 days of treatment with GM1. Both models returned to WT performance levels on tests of anxiety, cognition, and depression, when treated with GM1.

One issue that could limit the therapeutic use of GM1 is its poor ability to cross the blood-brain barrier (BBB). Although intra-ventricular or intra-thecal administration of GM1 is feasible, development of "gangliomimetic" therapies that can be administered peripherally is highly desirable and would greatly accelerate the development of a therapy for HD.

As a first step towards the identification or design of gangliomimetic compounds with improved pharmacokinetic profile, we have screened natural gangliosides and strategically designed gangliomimetic compounds to identify the key chemical structure/s required to recapitulate the neuroprotective effects of GM1 in models of HD.

Using an *in vitro* assay to measure cell apoptosis, we have identified compounds with similar or increased potency with respect to GM1. In preliminary studies, one of these compounds provided therapeutic benefits in an HD mouse model after intraperitoneal injection for 28 days. Overall, my thesis shows that the therapeutic effects of GM1 extend beyond amelioration of motor symptoms and support the hypothesis that GM1 has disease-modifying properties in HD

models. Furthermore, my thesis supports the idea that second generation compounds can be

developed with improved administration profiles while maintaining similar efficacy.

Preface

This thesis is an original work by Melanie Alpaugh.

A modified version of Chapter 2 of this thesis is a collaborative project between myself and D. Galleguillos that will be submitted as M. Alpaugh, D. Galleguillos, J. Forero, L.C. Morales, S. Lackey, K. Paw, K. Todd, K. Fouad, and S. Sipione, "GM1 is a potential disease modifying therapy in Huntington Disease". I was responsible for data collection and analysis for figures 1, 3, 4, 6, 9 and 10 and contributed to data collection and analysis for figures 5, 7 and 8. I was additionally responsible for the composition of the manuscript. D. Galleguillos was responsible for data collection and analysis for figures 5, 7, 8, and 11. He also contributed to composition of the manuscript. J. Forero was responsible for data collection and analysis for figure 2 and contributed to the composition of the manuscript. L.C. Morales contributed to data collection and analysis for figure 4. S. Lackey contributed to data collection and analysis for figures 5 and 8. K. Paw contributed to data collection and analysis of figure 4. K. Todd assisted in experimental design and contributed to editing the manuscript. K. Fouad contributed to experimental design and to editing the manuscript. S. Sipione was the supervisory author and was involved with project conception, experimental design and manuscript composition and editing.

A modified version of Chapter 3 of this thesis has been submitted to the Journal of Neuroscience as M. Alpaugh, D. Galleguillos, P. Kar, S. Lackey, A. Holt, B. Kerr, G. Baker, and S. Sipione, "Ganglioside GM1 restores normal non-motor behaviour in Huntington's disease mouse models". I was responsible for the data collection and analysis and contributed to the manuscript composition. D. Galleguillos performed all surgical procedures. P. Kar assisted with data collection. S. Lackey assisted with data collection. A. Holt assisted with data collection. B. Kerr, assisted in experimental design and contributed to editing the manuscript. G. Baker, assisted with data collection, analysis, and contributed to editing the manuscript. S. Sipione, was the supervisory author and was involved with project conception, experimental design and manuscript composition.

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responsible for data collection, analysis, and composition of the manuscript. D. Bundle contributed to data collection. S. Sipione, was the supervisory author and was involved with project conception, experimental design and manuscript composition.

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List of Abbreviations

5HIAA	5-Hydroxindoleacetic acid
5HT	5-Hydroxytryptamine (Serotonin)
ABB	Annexin binding buffer
AD	Alzheimer's disease
ALS	Amyotrophic lateral sclerosis
ANOVA	Analysis of variance
ASOs	Antisense oligonucleotides
Aβ	Amyloid β
BAC	Bacterial artificial chromosome
BCA	Bichinchonic acid
BBB	Blood-brain barrier
BDNF	Brain-derived neurotrophic factor
BSA	Bovine serum albumin
CK2	Casein kinase 2
CNS	Central nervous system
COMT	Catechol-o-methyltransferase
CREB	Cyclic AMP response element-binding protein
CSF	Cerebral spinal fluid
DA	Dopamine
DAB	3,3′-Diaminobenzidine
DAPI	4',6-Diamidino-2-phenylindole
DARPP32	Dopamine- and cyclic adenosine 3′-5′-monophosphate-regulated phosphoprotein, 32 kDa

DKO	Double knock-out
DMEM	Dulbecco modified Eagle's minimal essential medium
DNA	Deoxyribonucleic acid
DOPAC	3,4-Dihydroxyphenylacetic acid
D-PBS	Dulbecco phosphate-buffered saline
DTI	Diffusion tensor imaging
EDTA	Ethylenediaminetetraacetic acid
EGTA	Ethylene glycol-bis(β-aminoethylether)-N,N,N',N'-tetraacetic acid
ER	Endoplasmic reticulum
ERK	Extracellular signal-regulated kinases
FAN1	Fanconi anemia FANC1/FANCD2-associated [endo] nuclease
GABA	Gamma-aminobutyric acid
GalNAc	N-acetylgalactosamine
GFAP	Glial fibrillary acidic protein
GWAS	Genome wide associated study
HAP1	Huntingtin associated protein 1
HD	Huntington disease
HMGCoA	Hydroxyl-methylglutaryl-coenzyme A
HPA	Hypothalamic-pituitary-adrenal
HPLC	High performance liquid chromatography
HTT	Huntingtin
Htt	Mouse huntingtin gene
HVA	Homovanicllic acid

IgF-1	Insulin-like growth factor 1
IKK	IκB kinase
Iba1	Ionized calcium-binding adapter molecule 1
Ibc	N-isobutyryl-L-cysteine
Il-6	Interleukin-6
IL-8	Interleukin-8
KO	Knock-out
LH	Left hemisphere
LTP	Long-term potentiation
MAG	Myelin associated glycoprotein
MAO	Monoamine oxidase
MFI	Mean fluorescence intensity
mHTT	Mutant huntingtin
MS	Multiple sclerosis
MSN	Medium spiny neurons
MTMR	Myotubularin related protein 10
NeuN	Neuronal Nuclei
NF-κB	Nuclear factor-κ B
NIH	National institute of health
NRSE	Neuron restrictive silencer element
NMDA	N-methyl D-aspartate
OPA	O-pthaldialdehyde
PBS	Phosphate buffered saline

PD	Parkinson disease
PET	Positron emission tomography
PGC1-α	Proliferator-activated gamma coactivator 1-alpha
PI3K	Phosphatidylinositol 3-kinase
PINK1	PTEN-induced putative kinase 1
polyQ	Polyglutamine
PSD95	Post synaptic density 95
PVDF	Polyvinylidene fluoride
REST/NRSF	Repressor element-1 silencing transcription/neuron restrictive silencer factors
RH	Right hemisphere
ROS	Reactive oxygen species
RPM	Revolutions per minute
RT	Room temperature
SDS	Sodium dodecyl sulfate
Ser	Serine
SiRNA	Small interfering ribonucleic acid
SNP	Single nucleotide polymorphisms
SOD1	Superoxide dismutase 1
SREBP2	Sterol regulatory element binding protein 2
SSRI	Selective serotonin reuptake inhibitors
TBS	Tris-buffered saline
TBS-T	Tris-buffered saline with Tween
Trk	Tyrosine receptor kinase

TRPC5	Transient receptor potential channel 5
UHDRS	Unified Huntingtin disease rating scale
UPDRS	Unified Parkinson disease rating scale
VMAT-2	Vesicular monoamine transporter
WT	Wild-type
YAC	Yeast artificial chromosome

CHAPTER 1

INTRODUCTION

1.1 Clinical Huntington disease

1.1.1 Inheritance and prevalence

Huntington disease (HD) is a devastating genetic neurodegenerative disease that is dominantly inherited. HD is caused by an expansion in the number of CAG repeats in the 5′ coding region of the Huntingtin (*HTT*) gene (1). The normal CAG repeat size in healthy individuals commonly ranges from 16-20 repeats; 27-35 repeats are considered to be intermediate alleles and are prone to expansion during meiosis (2). Expansions above 36 are disease causing, but expansions containing 36-40 repeats have more limited penetrance than those above 40 repeats, which are 100% penetrant (3, 4). In rare cases late onset and very late onset HD have been detected in individuals with the intermediate alleles (5, 6). Due to the relative instability of the HTT intermediate allele new mutations may arise from the latter during meiosis, accounting for up to 10% of new HD cases (7). Paternally transmitted intermediate alleles demonstrate the greatest predisposition to expansion and likely account for a large proportion of newly emerging cases. The prevalence of HD is variable depending on geographical region and ethnic background (2). Particularly high incidence rates of HD have been documented in the region surrounding Maracaibo Lake in Venezuela (700 in 100,000), Cañate in Peru (40 in 100,000), and Feira Grande in Brazil (72 in 100,000) (8). In general the highest disease prevalence is found in western European countries and countries where a large degree of immigration from Western Europe has occurred, including the United States, Canada, and Australia (9, 10). Conversely, lower prevalence rates have been documented in Finnish (11), Asian (2) and black South African populations (12).

1.1.2 Genetic modifiers

Regardless of geographical region there is an inverse correlation between age of onset of HD and size of the CAG expansion. However, the size of the expansion does not account for all of the variability in age of onset. Recent studies have attempted to find other genetic modifiers to explain the remaining variability in the age of onset. Associations between age of onset and pathways expected to be involved in HD pathology include polymorphisms in the brain-derived neurotrophic factor (BDNF) gene, N-methyl D-aspartate (NMDA) receptors, and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1α) (13-15). At least some of these associations, however, are still controversial as many studies have failed to replicate the link between BDNF polymorphisms and age of onset (16). Recently an unbiased genome wide association study (GWAS) was performed and investigators found three loci that significantly altered the age of onset from what would have been predicted based on the length of the CAG expansion (17). The two loci on chromosome 15 are predicted to correspond to Fanconi anemia FANC1/FANCD2-associated [endo] nuclease (FAN1) and myotubularin related protein 10 (MTMR) while the third locus contained multiple candidate genes. None of the predicted genes matched previously implicated modifiers. These new genes may represent novel pathways that can be targeted to design therapeutic interventions. Currently, the only factor that has been consistently found to alter age of onset is the length of the CAG expansion which accounts for up to 70% of the variability in age of onset (4).

1.1.3 Symptoms and disease course

While HD is characterized by a wide array of symptoms, including cognitive impairments and psychiatric disturbances, onset is typically defined by the presence of motor symptoms as determined using the unified HD rating scale (UHDRS) (18), in the presence of a positive family

history or a positive genetic test (19). Non-motor symptoms frequently occur prior to onset of motor symptoms (20) and are more strongly associated with disease burden and impaired functioning (21). Chorea is the primary motor symptom early in disease progression, however, as the disease progresses motor dysfunction, bradykinesia, and dystonia become more pronounced and chorea declines (20). Motor dysfunction in HD progresses from predominantly hyperkinetic to predominantly hypokinetic and is characterized by bradykinesia and severe gait abnormalities. Gait abnormalities are frequently severe enough to lead to an increased risk of falls and consequent requirement of wheelchairs to avoid injury (20). Motor dysfunction leads to a significantly decreased functional capacity and quality of life (21). Chorea, conversely, may contribute less to disease burden as patients frequently display poor insight into the presence of choreatic movements (22). Motor dysfunction closely mirrors neurodegeneration, and is consequently a good marker of disease progression (23).

In addition to functional capacity and depressed mood, cognitive deficits have also been shown to be associated with higher disease burden and decreased quality of life (24). Cognitive dysfunction frequently presents prior to disease onset, with recent studies detecting changes up to a decade prior to predicted diagnosis (25). Cognitive problems in HD include executive dysfunction, memory, visuospatial perception, language, and emotional recognition impairments (25, 26). Of these deficits executive dysfunction is among the earliest and most prevalent (27). Executive dysfunction refers to a frontal syndrome that includes problems in task switching, planning, and problem solving (27). Cognitive disturbances are among the most difficult symptoms to treat. Therapies that are typically successful in improving cognitive deficits in other neurodegenerative diseases, such as acetylcholine esterase inhibitors and memantine, unfortunately show little or no benefit when used in HD patients (28-30). Memantine, a NMDA

receptor antagonist used in Alzheimer disease (AD) to improve cognition, was reported to potentially slow disease progression in a case study and in a small clinical trial but no control groups were included in these studies (31, 32). Cognitive changes in HD patients correlate well with motor dysfunction and neuronal atrophy and have been proposed as relevant markers of disease progression in clinical trials where disease-modifying therapies are assessed (33, 34). Psychiatric disturbances are another common feature of pre-manifest HD. Patients frequently present with aggression, apathy, anxiety, depression, suicidal ideation, irritability, and more rarely obsessive compulsive symptoms and psychosis (20). Apathy, aggression and irritability are particularly common. Apathy in particular is inversely related to quality of life (21). Currently, traditional anti-depressant therapies are used to treat apathy, depression and anxiety. However, the efficacy of these therapies in the HD population has not yet been firmly established (35). The only symptomatic therapy that has been specifically approved for HD is tetrabenazine for chorea. Tetrabenazine improves chorea by depleting dopamine (DA) levels, particularly in the striatum, through inhibition of the vesicular monoamine transporter (VMAT-2) (36, 37). While tetrabenazine has shown some beneficial effects on functional mobility (38, 39) it is contraindicated in patients that have depression or aggression as these symptoms can be exacerbated (40). The wide spectrum of behavioural and personality changes that occur during disease progression, in addition to the heritable nature, makes HD a particularly devastating disease. Frequently, family members will look after a manifest HD patient prior to developing the disease themselves. The predictability of who will develop HD does have some major advantages when designing trials to test disease-modifying therapies. Since it can be accurately predicted who will develop the disease it is possible to test the ability of treatment to delay onset of symptoms.

1.2 Neuropathology of HD

1.2.1 Regional atrophy and behavioural correlates

In association with the behavioural symptoms of HD there is significant neuropathology, particularly in the striatum. The marked striatal pathology resulted in neuropathological progression of the disease being defined by the degree of striatal atrophy (41). More recent studies have focused on relating behavioural changes to structural deficits in order to develop clinically relevant end-points to be used in clinical trials. Striatal volume loss has been detected prior to the onset of motor symptoms and can be tracked for progression even in the prodromal phase of disease (34, 42, 43). Volume loss in the putamen and striatum correlate better with motor dysfunction than neuronal loss, although both measures are significantly correlated (23). Striatal atrophy is not the only early neuropathological marker of disease. Total brain volume and white matter integrity have also been shown to progressively decrease starting several years prior to disease onset (42, 43). Brain atrophy in HD has been shown to follow a defined progressive pattern with loss proceeding from posterior to anterior and from dorsal to ventral regions. This pattern of tissue dysfunction and loss is particularly evident in the striatum (41, 44). Regional brain atrophy has also been correlated to clinical deficits. UHDRS motor score correlates well with striatal atrophy as well as CAG repeat length (42). Cognitive dysfunction and apathy have also been shown to correlate well with brain atrophy in the prodromal stage of disease (44, 45). Specifically caudate and whole brain atrophy correlates with total functional capacity and total brain volume loss correlates with cognitive and motor impairment (34). Other studies have found correlations between cognitive performance and the degree of cortical thinning (44, 46). Correlations between symptom progression and neuropathology are not restricted to grey matter atrophy. A detailed analysis of changes in white matter integrity, as

measured by changes detected using diffusion tensor imaging (DTI), indicate that regional changes in white matter integrity relate to apathy, deficits in odor recognition, and inconsistent speed in a self-paced tapping task (45). Regardless of the type of atrophy or behavioural measure tested, the aforementioned studies all indicate there is a strong relationship between brain atrophy and behavioural abnormalities in HD with cortical, striatal, and white matter structures showing the strongest relationships.

While atrophy of white matter as well as subcortical (striatum, palladium, subthalamic nucleus), and cortical gray matter is detected in prodromal phases and progress with disease symptomatology, additional brain areas previously believed to be spared become affected in manifest disease. These include the hippocampus (47, 48) and the nucleus accumbens. Interestingly, the amygdala and cerebellum are still believed to be relatively spared, while the involvement of the thalamus is controversial (48, 49).

1.2.2 Immunohistochemical changes and gliosis

Structural changes are not the only differences that have been documented between brains of patients with HD and healthy controls. Dysfunction in multiple cellular populations including subsets of neurons, endothelial cells and glia have been documented (41, 50). Within neurons, the GABAergic (Gamma-aminobutyric acid) medium spiny neurons of the striatum are particularly sensitive to mHTT toxicity, with 95% of this group of neurons dying by end stages of disease (41).

Recent evidence from patients and mouse models indicates that the endothelial cells of the BBB express fewer tight junction proteins and allow increased leakage of substances from the blood into the brain (50). Correlation of disease state and BBB leakage indicates that dysfunction of the BBB progresses with disease course. While endothelial cells certainly contribute to pathology of

the BBB, astrocytes are also important for the maintenance of tight junctions and likely contribute to the impairments. Progressive gliosis develops in HD brains, particularly in the striatum and to a lesser extent the cortex (41, 51, 52).

Gliosis includes both microglia and astrocytic dysregulation, which are already detectable in the brains of pre-manifest individuals up to ten years prior to disease onset, as measured by positron emission tomography (PET) imaging (52-54). Microglial activation in particular has been shown to be associated with striatal dysfunction, thus implicating microglia in cellular pathology in the striatum (53). In, parallel, innate immune mediators such complement, interleukin-6 (Il-6), and interleukin-8 (Il-8) have been shown to be increased in manifest and pre-manifest disease states (55, 56). Il-6 and Il-8 have both been shown to correlate with disease state (56).

1.3 Animal Models of HD

In an attempt to increase the understanding of disease pathology and progression many different animal models have been developed to study HD. The first models of HD were based on the use of chemical toxins such as quinolinic acid (57), an NMDA receptor agonist, and 3-nitroproprionic acid (58), a mitochondrial toxin, to induce striatal degeneration. The discovery of the causative gene in 1993 (1), has allowed for the development of precise genetic models of HD in a wide array of species including mouse, rat, sheep, mini pig, and monkey (59, 60).

1.3.1 Fragment mouse models

The first genetic models of HD were the R6 series of mice, of which the R6/1 and the R6/2 lines are still commonly used (61). These transgenic mice incorporate and overexpress exon 1 of the human *HTT* gene with ~115 to 150 CAG repeats under the control of the human *HTT* promoter. Of all transgenic mice developed over the years, the R6/2 mouse model remains the one with the

most severe behavioural deficits and neurodegeneration. It is characterized by rapid onset of symptoms by 6-8 weeks of age and early detection of neuropathological changes. R6/2 mice also display decreased body weight and lifespan (61). The early onset and rapid disease progression of the R6/2 mice mirrors more closely the juvenile form of HD than the typical adult form (62). The rapid onset makes R6/2 mice a very useful model for analyzing the effects of therapeutic compounds on neuropathology as they can be analyzed (12 weeks of age) much younger than other models which do not display overt neurodegeneration or pathology until after 1 year of age. R6/1 mice have a less severe phenotype with onset of motor symptoms absent prior to 5-6 months of age (61). Both R6/1 and R6/2 lines of transgenic mice have been shown to accumulate intranuclear aggregates of HTT in the striatum (62). A second common transgenic fragment model of HD is the N171-82Q mouse, which was developed in 1999 (63). This model presents with a very similar phenotype to R6/1 mice, including intranuclear mutant HTT aggregates and weight loss, but behavioural abnormalities appear later (11 months) (63).

1.3.2 Full-length HTT transgenic mouse models

A few years after the development of the R6 mouse lines, transgenic models incorporating the entire human *HTT* gene and expressing mutant full-length HTT within yeast artificial chromosome (YAC) (YAC128 mice) or bacterial artificial chromosome (BAC) (BACHD mice) vector system were developed (64, 65). The most commonly used model from the YAC vector system contains ~125 repeats while the mice derived from the BAC vector system contains 97 repeats (66). Both the YAC and the BAC full length transgenic models of HD have slower disease progression than the R6/2 model. Onset of motor symptoms is typically around 2-3 months of age with overt neuropathology not developing until 9 to 12 months of age (64, 65). Both BACHD and YAC128 present with weight gain which is not a symptom that is associated

with the clinical manifestation of HD, where weight loss is a common problem (67, 68). It has been shown that this weight gain is caused by increased expression of insulin-like growth factor 1 (IGF-1) in both models. Both models also have similar symptomatology to the clinical condition, presenting with motor and cognitive deficits as well as psychiatric disturbances which mirror the typical triad of symptoms in HD patients (66). Important differences between the two models do, however, exist. YAC128 mice show biochemical changes in the striatum, including reduction of the expression of DA- and cyclic adenosine 3′-5′-monophosphate-regulated phosphoprotein, 32 kDa (DARRP32), that are common to other models and to human HD, but which are not present in BACHD mice (66). Conversely, BACHD mice demonstrate little somatic expansion of the CAG repeat. Expansion is very common in other models, particularly knock-in models (65). Despite replicating many features of the human disease, transgenic models have significant drawbacks and have been criticized for the random, and potentially confounding, insertion of the transgene into the genome as well as for the expression of mutant HTT above endogenous physiological levels.

1.3.3 Knock-in mouse models

A number of these concerns are addressed by knock-in mice. While these are theoretically the most faithful genetic model, they tend to have very mild symptoms and require larger expansions to observe symptoms at the same age as transgenic mice (59). Two groups of knock-in models currently exist. In one group, which include the Q140 and zQ175 mouse models, exon 1 of the human *mHTT* gene was knocked-in within the mouse gene, resulting in the expression of a chimeric mHTT protein where the polyglutamine (polyQ) and polyproline repeats derive from the human gene, while the remainder of the protein has the mouse sequence (69-71). Similar to the full-length transgenic models, onset of motor symptoms occurs between 5 and 6 months of

age and cognitive dysfunctions and psychiatric disturbances have also been documented (69) in Q140 mice. Homozygous Q140 mice are typically used in order to observe a more readily distinguishable phenotype.

The zQ175 mice were generated by spontaneous germline expansion of the CAG repeat in the Q140 colony. These mice were developed as a model with a stronger phenotype to facilitate the use of heterozygous animals, as they are a more faithful genetic model of the human condition (70). ZQ175 mice do have a more severe phenotype than the original Q140 model, with decreased body weight in both heterozygous and homozygous animals and decreased lifespan in homozygous mice (70).

The second group of knock-in mouse models include the Q111 and Q150 models, which include ~109 and ~150 CAG repeats respectively within the endogenous mouse gene (72, 73). Q111 and Q150 homozygous mice have only a very mild motor phenotype even at late stages of disease, with detectable deficits appearing only at 24 and 18 months of age respectively (72, 74). In Q150 mice motor deficits were assessed using the rotarod and were specific to homozygous mice. In Q111 mice motor deficits were limited to subtle gait deficits which were present in both homozygous and heterozygous mice. However, these mice do display biochemical similarities to the human condition, with both models developing mHTT aggregates throughout the brain (72, 74).

While all of the aforementioned mouse models display many similarities to the clinical condition, no one model perfectly recapitulates HD. Consequently, guidelines for pre-clinical trials for HD specify the need to replicate mouse studies in at least two genetically distinct mouse models in different background strains (75, 76).

1.3.4 Transgenic rat model

It is worth noting that none of the above described mouse models display chorea, a hallmark symptom of the human pathology. The only rodent model of HD to display chorea is the transgenic rat model derived from the insertion of a complementary deoxyribonucleic acid (cDNA) transgene containing 51 CAG repeats under the control of the endogenous rat *HTT* promoter (77). These rats display many of the characteristics of the R6/2 mouse model but on a slower time scale. Cognitive and motor deficits first appear around 10 months of age and the transgenic rats show a decreased lifespan compared to WT rats and weigh significantly less. Mutant HTT aggregates and striatal volume loss are also present in this transgenic model (78).

1.3.5 Large animal models

In order to facilitate the translation of treatments from the lab to the clinic it is frequently necessary to test potential therapies in large animal models to ensure safety and support efficacy. In recent years a number of large animal models for HD have been developed. Large animal models present more challenges as they are harder to maintain and require longer to develop symptoms since they have longer lifespans than the commonly used rodents. A sheep model of HD was developed in New Zealand in 2010 that incorporates full-length HTT (79). This model has very mild symptoms and does not display detectable motor deficits although mHTT aggregates and DARRP32 reduction has been found in the striatum of at least one animal (60). A mini pig model of HD incorporating full-length human mHTT revealed a similar phenotype to that described in the sheep model. No overt motor phenotype has been detected to date, though a decrease in DARRP32 levels as compared to WT littermates has been observed (80). A second line of transgenic mini pigs were developed to express a toxic N-terminal fragment of the mHTT protein. This is the first HD model to show detectable apoptosis, particularly in striatal neurons,

which is an integral feature of the clinical disease phenotype, as 95% of medium spiny

GABAergic neurons are lost by end stages of disease (41, 81). Stronger motor phenotypes have

been observed in monkey models expressing transgenes contacting either exon 1 or the first 11

exons of the human *mHTT* gene (82, 83). The transgene containing only exon 1 resulted in the

early death (around 1 month of age) of three of the five monkeys (81). The surviving monkeys

and those derived from the longer transgene displayed dystonia and motor deficits by 36 months

of age (83). In association with these behavioural changes a reduction in neurons was also

detected in monkeys expressing both transgenes, although overt striatal atrophy was only present

in the monkey with the transgene expressing exon 1 at 36 months of age (83). The presence of

both behavioural and neuropathological stages suggests that these transgenic monkeys closely

replicate the human condition. This finding will, however, require further confirmation due to the

small sample sizes used in the experiment. Interestingly, the development of large animal models

to date has confirmed the finding from rodent models that an N-terminal fragment of the mHTT

protein results in higher levels of toxicity than does the full-length protein (84).

1.4 Pathogenic mechanisms in HD

1.4.1 Functions of wild-type HTT

HD is a complicated disorder with several biochemical pathways implicated in neuronal

dysfunction and death. The main pathways include impaired axonal transport, altered gene

transcription, mitochondrial dysfunction, and dysfunctions in lipid metabolism. Most deficits are

imputed to a toxic gain of function of the mHTT protein with an expanded polyQ stretch,

however, some dysfunctions are likely caused by a loss of function of the wild-type (WT) protein

(85, 86). WT huntingtin (wtHtt) is a large (348 KD) protein that is present primarily in the

cytoplasm but is also found in the cell nucleus (85). The overall importance of wtHTT is

underscored by the finding that knock-out of the *HTT* gene prior to gastrulation is embryonic

lethal and the adult knock-out of *HTT* is sufficient to result in a neurodegenerative phenotype

similar to HD (87). Currently, the exact function of the WT protein is unclear, however, roles for

wtHTT in axonal and vesicle transport, transcriptional regulation, cell division, cilia formation,

and autophagy have been postulated (86). WtHTT facilitates axonal transport through an

interaction with HTT associated protein 1 (HAP1), which associates with the motor complexes

dynein/dynactin and kinesin (88). The polyQ expansion in HTT results in increased binding of

HTT to HAP1, resulting in a lessening of the association between HAP1 and the motor complex.

This consequently leads to decreased efficiency of transport along the microtubules (88).

Post-translational modification of HTT also influences axonal transport. More specifically, the

phosphorylation of HTT at serine (Ser) residue 421 acts as a molecular switch to regulate the

direction of transport. Phosphorylation at Ser421 favours anterograde transport, whereas

dephosphorylation favours retrograde transport (89). Transcriptional regulation of various genes

has been shown to depend on HTT, directly or indirectly. Direct effects include the binding of

HTT to cytoplasmic transcription repressor element-1 transcription/neuron restrictive silencer

factors (REST/NRSF) (90). This binding results in sequestration of REST/NRSF and inhibition

of the neuron restrictive silencer element (NRSE) in the nucleus. Overall this results in increased

transcription of target genes, including BDNF and other neuronal-specific genes (90). It has also

been suggested that HTT can indirectly modulate gene expression by acting as shuttle between

the nucleus and the cytoplasm (91). Specifically, it has been shown that HTT interacts with

nuclear factor-κB (NF-κB) and facilitates retrograde transport from the synapse to the nucleus in

a dynein/dynactin dependent manner and additionally interacts with active NF-κB and importin-

α (91). In support of a role for HTT in transportation and translocation of NF-κB, loss of wtHTT or the presence of mHTT result in decreased levels in the nucleus (91).

1.4.2 HTT aggregation

While loss of the WT protein is likely a contributing factor to dysfunction in HD, the toxicity of the expanded glutamine stretch certainly plays a major role in disease pathogenesis. This is highlighted by the identification of nine diseases that occur as a result of polyQ expansions in completely unrelated proteins and that are all late-onset neurodegenerative disorders (92). One common feature of all polyQ expansion diseases is the presence of aggregates of the mutant proteins which follow similar patterns of aggregation (93).

The role of mHTT aggregated in HD has been a source of controversy for many years due to the presence of experimental evidence supporting both a toxic and protective role of aggregates (94-96). Advocates for aggregates having beneficial effects, have hypothesized that aggregate formation is a compensatory mechanism of cells to sequester toxic oligomers. Evidence for a protective role includes experiments demonstrating that cells with large aggregates tend to survive better than cells without aggregates, and that interventions aimed at reducing the oligomeric form of HTT by favouring aggregate formation are protective (94, 97). Additionally, the "short-stop" mouse model, where the mHTT protein is cleaved after exon 2, has a large number of aggregates but no other behavioural or neuropathological phenotype (95). This indicates that aggregates are not sufficient to induce the disease pathogenesis. On the other hand, evidence supporting the toxicity of aggregates suggest that many normal proteins are sequestered within aggregates of mutant HTT, thus, preventing them from performing their normal functions. This has been demonstrated both with nuclear and cytoplasmic aggregates. Nuclear aggregates can interfere with the transcriptional machinery while cytoplasmic aggregates may interfere with

axonal transport (98). Recent experiments analyzing the formation of aggregates also suggest that not all aggregates are the same and potentially different forms of aggregates may have differing toxicity (99, 100).

1.4.3 Impairment of axonal transport and BDNF

Impairments in axonal transport have been suggested to be one of the mechanisms by which HTT aggregates can contribute to neurotoxicity. Aggregates can both sequester transport machinery and/or act as a physical barricade (56). One of the major proteins impacted by the impairment of axonal transport in HD is BDNF (88). Striatal neurons are almost entirely dependent on BDNF produced by cortical neurons and delivered through the cortical-striatal afferents, thus proper transport is critical (101). Both cortical levels of BDNF and activation of its receptor, tyrosine receptor kinase (trk B), in the striatum have been observed to be reduced in HD. Loss of BDNF has also been implicated in disease progression and restoration of BDNF levels, or downstream signaling through the activation of trk B receptors, improve the symptoms of HD (102). In addition to the impaired trafficking of BDNF, mutant HTT also reduces BDNF levels through effects on transcriptional regulators (90). The importance of BDNF to the proper functioning of striatal neurons has been demonstrated in mice where BDNF has been specifically knocked out in cortical cells. These animals demonstrated reduced dendritic branching, soma area, and spine density in the striatum (103). Additionally, loss of BDNF is not the only consequence of mutant HTT-mediated changes in transport; endosomes and other organelles, mitochondria in particular, also show impaired trafficking (104, 105). However, whether this is secondary to impaired HTT/Hap 1 interaction or mHTT aggregation is not completely clear (105).

1.4.4 Mitochondrial dysfunction

Mitochondria are critical organelles, not only for energy production, but also for buffering calcium, reducing reactive oxygen species (ROS), and initiating apoptotic processes. Any change in mitochondria function, therefore, has large repercussions on overall cell functioning. Mitochondria are highly dynamic organelles for which biogenesis, fission, fusion, trafficking and degradation need to be finely regulated for optimal functioning (106). Many of these processes are known to be affected in HD. Mitochondria biogenesis is thought to be altered in HD due to a reduction in the expression and activity of the master mitochondrial regulator PGC1α which, in turn, is caused by an aberrant interaction between the transcriptional co-activator cAMP response element-binding protein (CREB) and mHTT (107). Mitochondrial fission and fusion are normally balanced in healthy cells, but this balance is progressively disrupted in HD, leading to fragmentation and swelling of mitochondria (108, 109). Dysfunctions in mitochondrial dynamics in HD are also associated with disease progression and regions of increased disease severity (cortex and striatum) (108). Additionally, pharmacological reversal of increased fission improves mitochondrial function as indicated by reduced ROS production and normalization of mitochondrial membrane potential (110). Disruptions in the electron transport chain- particularly complexes II and III-and energy production have also been described in patients with HD (111). Impairment of the electron transport chain, and particularly of complex III, can result in increased production of ROS (112). This is also the case in HD, where increased markers of oxidative stress have been detected in the striatum and cortex (108).

Calcium buffering is another critical function of mitochondria, dysregulation of which can result the opening of the mitochondrial transition pore and release of pro-apoptotic factors into the cytoplasm, with consequent cell death (113). This dysfunction in calcium buffering likely

contributes to excitotoxicity and the sensitivity of some HD cells and animal models to NMDA receptor agonists (114). Mitochondrial dyshomeostasis is not the only contributing factor to excitotoxicity in HD. Changes to the localization and sensitization of NMDA receptors have been implicated in increased excitotoxicity in HD models (115, 116). Specifically, the expanded polyQ in mutant HTT has been shown to impede the normal association between HTT and postsynaptic density 95 (PSD95) resulting in less sequestration of PSD95 and concomitant sensitization of NMDA receptors due to increased association between PSD95 and NMDA receptors (115). Additionally, a higher percentage of NMDA receptors are localized extrasynaptically in HD models than under physiological conditions. Localization of NMDA receptors determines if pro-survival or apoptotic pathways are activated, with synaptic localization associated with pro-survival and extrasynaptic associated with pro-apoptotic. Consequently increased extrasynaptic NMDA receptors contributes to excitotoxicity and cell death (116).

1.4.5 Iron dysregulation

In addition to increased ROS produced by mitochondrial dysfunction, in HD there is also an increased load of ROS due to dysfunctions in iron metabolism (117). Imaging studies in patients using magnetic resonance imaging (MRI), as well as direct staining of tissue, have demonstrated increased levels of iron and ferritin, an iron binding protein, early in the disease course (118, 119). Increased iron levels may have many detrimental effects including production of ROS and activation of microglia (120). Iron homeostasis is altered by inflammatory mediators, such that an increased inflammatory phenotype has been frequently linked with dysregulation of iron metabolism and iron accumulation in microglia (121, 122).

1.4.6 Impairment of cholesterol metabolism

Transcriptional down-regulation of cholesterol biosynthetic enzymes in HD was initially

reported in an inducible cell model following induction of mHTT expression (123). In recent

years dysfunction of cholesterol metabolism in HD has received much attention due to the

importance of cholesterol in many normal neuronal functions (124, 125) and because of the

established connection between cholesterol and other neurodegenerative disorders including AD

(126, 127). The brain has a high demand for cholesterol, but is separated from peripheral

cholesterol homeostasis by the BBB (128). As a result of the isolation of the central nervous

system cholesterol pool, neurons are particularly sensitive to deficits in cholesterol synthesis.

In HD models, cholesterol biosynthetic precursors are decreased at early disease stages, while

overall cholesterol levels are decreased at later stages (129, 130). This decrease is associated

with loss of activity in enzymes of the cholesterol biosynthetic pathway, including the rate-

limiting enzyme, hydroxyl-methylglutaryl-coenzyme A (HMGCoA) reductase (123, 130, 131),

and is due to impairment of the sterol regulatory element binding protein 2 (SREPB2), the master

regulator of cholesterol synthesis (131). Recently, changes in cholesterol metabolism were

detected in post-mortem brains of patient (132) and plasma levels of 24S-hydroxycholesterol, a

product of cholesterol metabolism that is released from the brain into circulation, were found to

correlate with disease stage and progression in HD patients (133).

1.4.7 Impairment of ganglioside biosynthesis

 Another important class of lipids that are known to be altered in HD are gangliosides.

Gangliosides are sialic acid-containing glycosphingolipids that are ubiquitously expressed in all

cell membranes, but are found in relatively higher concentrations in the brain. Gangliosides are

known to be important mediators of signal transduction cell-cell recognition and cell adhesion

(134). The first evidence of ganglioside disruption in HD was obtained over thirty years ago when total ganglioside levels were found to be decreased by approximately 40% in both the caudate and putamen of HD patients (135). This decrease was not consistent across all species of gangliosides, as ganglioside GD3 was increased. Ganglioside GD3 has frequently been linked to apoptosis and astrogliosis, suggesting a detrimental effect of high concentrations of this ganglioside in the brain (136). Because these early studies were performed in post-mortem tissue where a significant amount of neuronal mass (rich in complex gangliosides) was lost while reactive glia (enriched in GD3) were present, changes in ganglioside levels could have reflected the underlying neurodegenerative process. More recently, decreased ganglioside synthesis and levels were reported in multiple models of HD including R6/1 mice, YAC128 mice, Q111/111 cells, and human fibroblasts (137-139). Although different gangliosides were found to be affected to various extents depending on brain region or model analyzed, ganglioside GM1 was the most consistently decreased across models (137-139). In many HD models changes in ganglioside levels occurred independently and/or prior to neuronal loss (137), suggesting that dysfunction of ganglioside biosynthesis might occur at early disease stages and could contribute to disease pathogenesis

1.5 Gangliosides in brain pathologies

1.5.1 Ganglioside nomenclature and biosynthesis

The biosynthetic pathway for gangliosides is shown in Fig 1.1. The standard nomenclature for gangliosides is that first described by Svennerholm (140). According to the Svennerholm system the first letter in the name indicates which series the glycosphingolipid belongs to. The different series are based on the predominant sugar group and are the ganglio-, globo-, lacto/neolacto-, and asialo-series. Therefore, ganglio-series gangliosides are indicated by the letter G. The second

letter indicates the number of sialic acid residues contained in the molecule, M for 1, D for 2, T for 3, etc. The number in the ganglioside name is derived from the formula: *5-number of neutral sugar groups present in the molecule.* Thus, GM1 has four sugar groups in addition to 1 sialic acid residue, while GM3 has two. Small letters in this nomenclature indicate the position of the sialic acid residue and the number of sialic acids in that location (140).

The most common gangliosides are the a-series and b-series gangliosides. Glycosphingolipid synthesis begins with the conversion of ceramide to glucosyl-ceramide in the Golgi apparatus (141). Glucosyl-ceramide is then converted to lactosylceramide, which, in turn, gives rise to the first and simplest of gangliosides, GM3, after addition of one sialic acid residue. All of the a-series and b-series gangliosides are produced from GM3 by the addition of a second sialic acid residue (GD3) or N-acetylgalactosamine (GalNac) (GM2). Further complexity comes from the addition of either galactose or glucose and additional sialic acid residues to produce progressively larger sugar head groups (141).

1.5.2 Gangliosides in the nervous system

Gangliosides are important for development, neural transmission, neuronal repair, and inflammation (134, 142). While all cells contain gangliosides, the levels and relative abundance of different ganglioside species vary depending on cell type, tissue, differentiation state and aging (143-146). In peripheral tissues, the ganglioside present in highest concentrations is GM3. In the brain, more complex gangliosides are found at higher concentrations, with GM1, GD1a, GD1b and GT1b representing the largest percentage of brain gangliosides. Functionally, gangliosides have many important roles in intracellular signaling (147, 148). In the plasma membrane gangliosides influence signaling in four main ways, by direct interactions with receptors, formation of lipid rafts, direct binding with glycan receptors and by acting as co-

receptors (147-150). GM1 and GM3 have been shown to directly associate with and activate Trk A (151) and insulin receptor respectively (152). In addition to interactions with receptors, gangliosides can regulate the activity of receptors by inclusion or exclusion from lipid raft domains, as in activation of C-terminal Src kinase-binding protein phosphorylation (153). An example of gangliosides binding with glycan receptors is the interaction between myelin associated glycoprotein (MAG) and both GD1a and GT1b. This interaction stabilizes the connection between glia and neurons (154). Loss of GD1a and GT1b can lead to delayed myelination as well as slowed neural conduction (155). The action of gangliosides as co-receptors is important for facilitating the action of certain neurotransmitters (156). Serotonin (5HT) in particular has been shown to more effectively bind 5HT receptors in the presence of GM1 (157).

While the majority of gangliosides are located within the plasma membrane, GM1 and GD1a are also present in the nuclear envelope where they contribute to the regulation of calcium homeostasis. GM1 in particular appears to be important for this, while GD1a acts as a reserve pool that is converted to GM1 by neuraminidase 3 (158). In addition to the aforementioned functions, gangliosides have also been implicated in regulation of the immune system (159, 160) and the proper functioning of cholinergic synapses (161).

1.5.3 Mouse models of impaired ganglioside synthesis

The importance of complex gangliosides in the nervous system has been demonstrated by a series of mouse models where individual ganglioside biosynthetic enzymes were knocked-out. GM2/GD2 synthase knock-out mice (KO) mice lack all complex gangliosides and express only GM3 and GD3 (162). These mice were found to develop normally, but had moderate decreases in the speed of neuronal conduction (162). A second study using older mice found more severe

deficits including delayed myelination, central axonal degeneration, and peripheral

demyelination (155). Further work showed progressive motor and cognitive impairment in these

mice (163, 164) and, more recently, the presence of remarkable degeneration in the substantia

nigra, which suggests a potential link between gangliosides and Parkinson disease (PD) (165).

GD3 synthase KO mice and GM2/GD2 synthase-GD3 synthase double KO (DKO) mice have

also been generated. They express a-series gangliosides or GM3 only, respectively (166). The

DKO mice present with a much more severe phenotype than the single knock-out mice. DKO

mice have decreased survival with sudden death occurring by 4 weeks of age (166). In addition

to a decreased life span, these mice also have motor impairment decreased anxiety, impaired

cholinergic transmission (both centrally and peripherally) and increased complement signaling,

which leads to a pro-inflammatory phenotype (159, 161, 166).

The most dramatic phenotype is shown by mice that lack both GM2/GD2 synthase and GM3

synthase and cannot synthesize any ganglio-series gangliosides (167). These DKO mice are

viable but have reduced life-span, with less than 50% of mice surviving to 1 month of age (167).

In addition to decreased life-span mice lacking all ganglio-series gangliosides have severe

neurodegeneration with a loss of total brain volume observed in two and three month old mice,

which is accompanied by increased activation of astrocytes (167).

1.5.4 Gangliosides and neurological disorders

Several neurodegenerative disorders are caused by impairment of ganglioside metabolism. The

most common are the disorders associated with impaired ganglioside degradation and their

accumulation in the lysosomes.

Both GM1 and GM2 (Tay-Sachs and Sandhoff diseases) gangliosidosis are associated with

decreased catabolism and consequent accumulation of gangliosides. GM1 gangliosidosis is an

inherited disease caused by loss of function of the lysosomal enzyme β-galactosidase (168) and characterized by severe neurological deterioration (168). Tay-Sachs and Sandhoff disease are autosomal recessive diseases resulting from decreased activity of either β-hexosaminidase A or B respectively, and lysosomal accumulation of ganglioside GM2. Similar to GM1 gangliosidosis, both infantile and juvenile onset forms of Tay-Sachs and Sandhoffs diseases as well as a late onset disease have been described, depending on the amount of residual activity of β-hexosaminidase (169). Similar to GM1 gangliosidosis, GM2 gangliosidoses are characterized by severe neurological deficits and decreased life expectancy (169, 170).

At the opposite end of the spectrum are diseases caused by impaired ganglioside synthesis. Loss-of-function mutations of GM3 synthase cause the infantile-onset symptomatic epilepsy syndrome, a rare disorder characterized by progressive brain atrophy, epilepsy and chorea (171). Interestingly, many of the clinical signs are also shared by the juvenile form of HD (172). Deficiency of GM2/GD2 synthase causes a complex from of hereditary spastic paraplegia (173, 174).

In addition to the rare disease associated with loss-of-function mutations in ganglioside biosynthetic enzymes (171, 173, 174), changes in the ganglioside profile (i.e. in the relative abundance of specific gangliosides) were reported in AD (175, 176), PD (177), multiple sclerosis (MS) (178), amyotrophic lateral sclerosis (ALS) (179), and in children with white matter abnormalities (180). The relevance of these changes to pathogenesis awaits clarification.

In AD, ganglioside GM1 was proposed to have a pathogenic role by acting as an endogenous seed for the formation of toxic amyloid β (Aβ) plaques (181). The conformation acquired by the ganglioside within cellular membranes seems to be an important factor in this process. Additionally, cholesterol, which has been shown to be an independent risk factor for the

development of AD (127), influences the conformation of GM1 in the membrane and influences

seeding of Aβ. The interaction between Aβ and GM1 also results in a reduction of GM1

movement within the membrane, thereby interfering with lipid raft formation and GM1-

modulated cell signaling (182). These findings may explain the somewhat contradictory finding

that treatment of an AD mouse model and patients with exogenous GM1 has beneficial effects

(183, 184). In mice where toxic Aβ was injected into the lateral ventricle of the brain, exogenous

GM1 was able to reverse cognitive deficits as measured by novel object recognition (183).

Exogenous GM1 also prevented the progression of AD symptoms in 5 patients treated with

centrally administered GM1 for 1 year. GM1 additionally reversed motor symptoms in the subset

of patients presenting motor deficits. No adverse reactions were reported. It is important to note

that this trial was not placebo controlled and patients also underwent cognitive training.

However, these results still suggest that GM1 is safe and has potential therapeutic value in AD.

Abnormal production of auto-antibodies against specific gangliosides can also result in

neurological problems (185, 186), due to interference with ganglioside functions in the nervous

system (150). This is the case for Guillain-Barre syndrome, a disease frequently associated with

functional loss of gangliosides and peripheral neuropathy (187). Anti-ganglioside antibodies

have been detected in patients with both relapsing remitting and primary progressive MS, leading

to the hypothesis that loss of ganglioside functions may contribute to the disease course in MS

(188).

Antibodies against GM1 have also been detected in a subset of patients (approximately 27%)

with PD. Interestingly, the presence of GM1 auto-antibodies correlates with the development of

dementia in these patients, suggesting that loss of GM1 may be a contributing factor in the

development of PD-associated dementia (189).

1.6 Gangliosides as neuroprotective agents

The number and severity of the neurological diseases resulting from alterations of ganglioside levels demonstrate the importance of gangliosides in the nervous system. In line with this evidence, exogenous gangliosides have shown neuroprotective effect in various *in vitro* and *in vivo* models of neurodegeneration (149), including models of, excitotoxicity (190), stroke (191), ethanol toxicity (192), brain injury and chemically-induced PD (193). In these models, the protective effects of GM1 occurred concomitantly with increased neurotrophin signaling, stimulation of neurogenesis and/or regulation of neuronal calcium homeostasis (149, 194). More recently, chronic administration of GM3 (the precursor of GM1 and other brain gangliosides) was shown to significantly delay onset of paralysis and death in the superoxide dismutase (SOD)1^{G93A} mouse model of ALS, while an inhibitor of ganglioside synthesis had the opposite effects (179). Furthermore, safety and sustained benefits of GM1 administration were shown in a randomized double blind placebo controlled trial, where PD patients receiving GM1 by daily subcutaneous injections improved on the unified PD rating scale (UPDRS) (195) and showed a decreased loss of DA transporter in striatal regions (196). The beneficial effects of gangliosides on such a wide array of conditions suggests that gangliosides must have general and pleiotropic neuroprotective effects. These include, but are not limited to, increased neurotrophin signaling, regulation of calcium homeostasis, and reduction of ROS and oxidative stress.

1.6.1 Gangliosides and neurotrophins

One established neuroprotective action of gangliosides is facilitation of neurotrophin signaling. Neurotrophins are a class of proteins which are essential for the development, function, and survival of neurons (197). Neurotrophins can also improve survival after exposure of the central nervous system (CNS) to stressors such as ischemia and excitotoxicity (149). Ganglioside

interaction with neurotrophins is complex and seems to be highly variable depending on the concentration of ganglioside tested and on the cell type (149). GM1 is the main ganglioside that has been implicated in neurotrophin signaling although a few reports also document effects of GT1b (198). In general GM1 facilitates neurotrophin signaling and exogenous GM1 has been shown to induce the release of BDNF (198-200). GM1 has also been shown to modulate the activity of the trk family of neurotrophin receptors by inducing phosphorylation, dimerization, and activation. (201-203).

1.6.2 Gangliosides, calcium homeostasis and excitotoxicity

In addition to their effects on neurotrophin signaling, gangliosides modulate intracellular calcium signaling. Calcium is a very important second messenger in cell signaling and is generally tightly regulated. Gangliosides have been shown to increase both calcium influx and efflux in a cell-type dependent manner (204). In particular, GM1 present at the plasma membrane was shown to regulate calcium influx through the transient receptor potential channel 5 (TRPC5) (205), while GM1 in the nuclear envelope modulates the activity of the nuclear Na+/CA++ exchanger and contributes to nuclear Ca++ homeostasis (158). These modulatory effects might explain the ability of gangliotetraose-gangliosides (including GM1, GD1a, GT1b and GQ1b) to protect neurons from calcium accumulation after administration of glutamate (206, 207).

1.6.3 Gangliosides, iron and oxidative stress

Gangliosides, particularly GM1 and GT1b but also GD1b and GD1a, have been shown to sequester iron and to reduce the production of reactive oxygen species (ROS) (208). Iron is an important signaling molecule and a co-factor for many enzymatic complexes and proteins in the cell, but when present in its free form, unbound by ferritin or other iron binding proteins, it can

contribute to the production of ROS and corresponding oxidative stress (209). Sequestration of iron by exogenously administered GM1 and GT1b was shown to be due to binding of ferrous iron to the negatively-charged ganglioside micelles that form in solution when gangliosides are present at high concentrations. As a direct consequence of their iron-binding properties, gangliosides have also been shown to limit iron-induced peroxidation of membrane lipids (208). In addition, ganglioside GM1 was shown to alter iron transport through a modulatory effect on the activity and/or membrane localization of the transferrin receptor (210). Since dysregulation of iron metabolism is a common side effect of many neurological diseases and gangliosides can reduce iron induced ROS, the interactions between gangliosides and iron are likely to contribute to the neuroprotective actions of gangliosides.

1.7 Beneficial effects of exogenous ganglioside GM1 in HD

Previous work in our lab suggested that ganglioside GM1 has therapeutic potential in HD. Evidence for this comes from experiments in the HdhQ111/111 cell model of HD and in the YAC128 mouse model. Decreased levels of ganglioside GM1 are found in both models, as described above (137) . In HdhQ111/111 cells, administration of exogenous GM1 restored normal membrane levels of the ganglioside and decreased cell death under serum-deprivation conditions. *Vice versa,* reducing ganglioside levels in wild-type cells with a pharmacological inhibitor of the ganglioside biosynthetic pathway resulted in increased cell death. These experiments provided the first evidence that ganglioside deficiency may contribute to disease phenotypes in cellular and potentially also animal models of HD. The neuroprotective activity of GM1 on HD cell models was in part due to the activation of the pro-survival kinase Akt (137). The phosphatidylinositol 3-kinase (PI3K)/Akt pathway is impaired in HD (211), but GM1 treatment abolished the difference in the phospho-Akt/Akt ratio between wild-type and HD cells.

However, GM1-treated cells were still protected from apoptosis, at least in part, even in the presence of an inhibitor of the PI3K/Akt pathway, suggesting the occurrence of additional neuroprotective mechanisms activated by GM1 (137). As a matter of fact, administration of exogenous GM1 to HdhQ111/111 cells and to fibroblasts isolated from HD patients caused an increase in the amount of HTT that was phosphorylated at Ser13 and Ser16 (212). Phosphorylation at these two amino acids has previously been shown to reduce the toxicity of mHTT (213). GM1 was also able to trigger phosphorylation of HTT *in vivo,* upon chronic intraventricular infusion in YAC128 mice (212). Furthermore, after 14 days of GM1 infusion, 6 month-old symptomatic YAC128 mice demonstrated a complete reversal of motor dysfunction as measured by the rotarod, horizontal ladder, and narrow beam tests. This reversal of phenotype was associated with increased levels of the signaling protein DARPP32 and its phosphorylated form (212), DARPP32 is a signaling protein that integrates dopaminergic and glutamatergic signaling in the striatum and modulates striatum output pathways (214). Decreased levels of DARPP32 and phospho-DARPP32 are early signs of neuronal dysfunction in HD mouse models (215-217). Altogether, these findings suggest that GM1 could be a potential disease modifying therapy for HD.

1.8 Hypothesis and objectives of the thesis

The experiments presented in this thesis test the hypothesis that ganglioside GM1 is a disease-modifying treatment for HD.

The first objective of my thesis was to determine whether GM1 can attenuate motor as well as non-motor dysfunctions and correct underlying molecular and neuropathological correlates in HD mouse models.

In **Chapter 2** I present data that demonstrate that GM1 corrects motor symptoms in various models of HD and across different genetic backgrounds. Furthermore, GM1 decreases mHTT aggregates and slows down neurodegeneration in the R6/2 model.

In **Chapter 3** I demonstrate that GM1 abrogates psychiatric-like behaviour and improves cognitive functions in various HD mouse models. These behavioural effects correlate with neurochemical changes and restoration of normal levels of specific neurotransmitters.

The second objective of this thesis was to determine the minimal chemical structure of the GM1 molecule required and sufficient to provide neuroprotection in HD models and to test the potential neuroprotective activity of other gangliosides and synthetic gangliomimetic compounds.

In **Chapter 4** I present data demonstrating that the sphingosine arm in the GM1 molecule is necessary for the neuroprotective activity of the ganglioside. Furthermore, I provide evidence that synthetic gangliomimetic compounds with increased solubility compared to GM1 retain neuroprotective activity and show increased potency *in vitro.* One of these compounds also improves motor and non-motor symptoms in YAC128 mice.

Overall, the work present in my thesis demonstrates that GM1, and structurally related compounds, have disease-modifying properties in HD models.

Figure 1.1: Ganglioside biosynthetic pathway
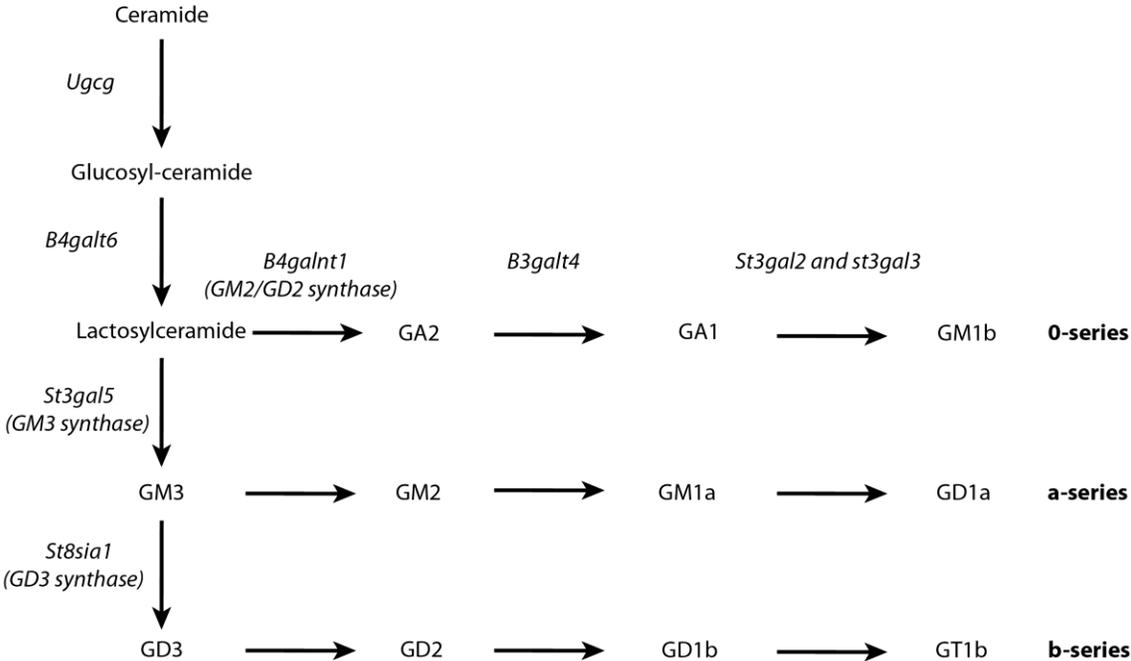
Figure 1.1 ganglioside biosynthetic pathway. Summary of ganglioside biosynthetic pathway with products denoted in regular font and enzymes indicated in italics. Ganglioside naming system as described by Svennerholm (140).

Figure 1.2: GM1 restores normal motor behavior in YAC128 mice
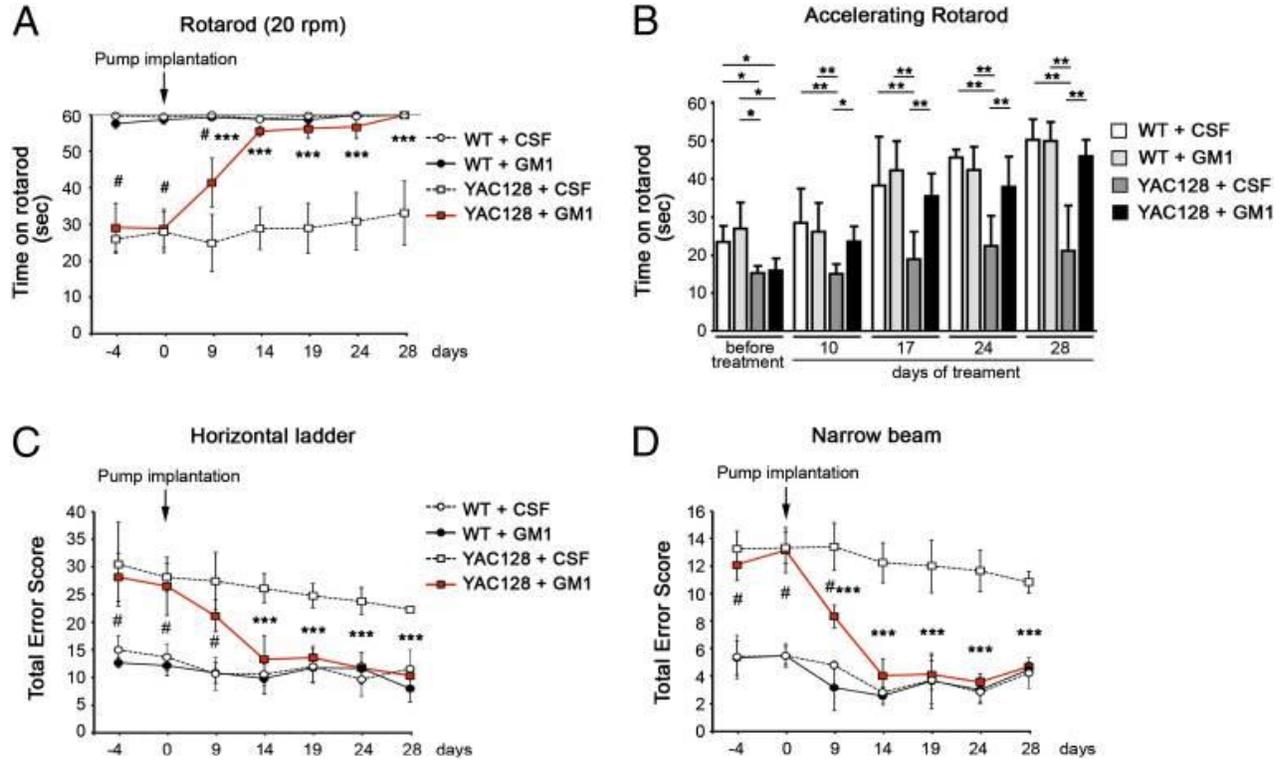
Figure 1.2 GM1 restores normal motor behavior in YAC128 mice. Behavioral tests were conducted at the indicated time, on 5-month-old YAC128 and WT mice, before and during GM1 chronic brain infusion. Each data point represents the average performance ±SD of six mice. (A) Rotarod test at fixed speed (20 rpm for 60 s). YAC128 mice treated with GM1 showed progressive improvement and, by the end of the treatment, were able to finish the test like most WT mice. The horizontal gray line in the graph marks the test endpoint. (B) Accelerating rotarod (4–40 rpm in 1 min). In this challenging test, YAC128 mice treated with GM1 performed as well as WT mice (differences between WT and GM1-treated YAC128 mice were not statistically significant). (C) Horizontal ladder test. The ability of mice to cross a horizontal ladder with irregular rung pattern was analyzed. A score was assigned to each type of footfall and other mistakes made by the mice according to ref. (218). (D) Narrow beam test. Motor performance was scored as the mice walked along a narrow beam (100 cm long, 0.75 cm wide). [#]$P < 0.01$ (YAC128 vs. WT); *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ (GM1-treated vs. CSF-treated YAC128). Figure and figure legend reproduced from ref. (212).

Figure 1.3: GM1 infusion induces HTT phosphorylation *in vivo*
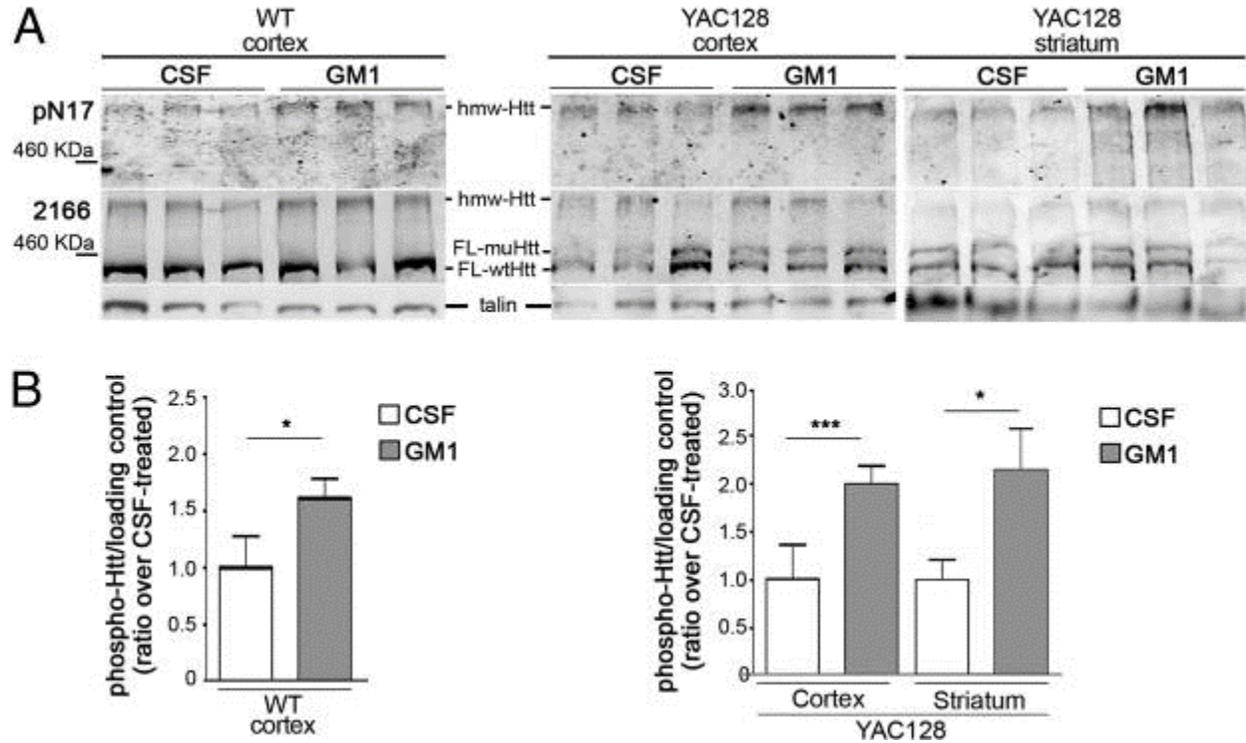
Figure 1.3 GM1 infusion induces HTT phosphorylation *in vivo*. Total protein lysates were prepared from the cortex and the striatum of YAC128 mice and WT littermates chronically infused with artificial cerebrospinal fluid (CSF) or GM1 in CSF for 28 days (six mice per group). (A) Representative immunoblots showing increased phosphorylation of HTT at Ser13 and Ser16 in GM1-infused brains, as detected using pN17 antibody. Each lane corresponds to the lysate from one mouse. (B) Li-Cor Odyssey infrared densitometric analysis of pHTT normalized over talin (loading control). Bars represent the mean ± SD of six mice per experimental group. *$P <$ 0.05; ***$P< 0.001$. Figure and figure legend reproduced from ref. (212).

1.9 References

1.   A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. The Huntington's Disease Collaborative Research Group. Cell. 1993;72(6):971-83.
2.   Pringsheim T, Wiltshire K, Day L, Dykeman J, Steeves T, Jette N. The incidence and prevalence of Huntington's disease: a systematic review and meta-analysis. Movement disorders : official journal of the Movement Disorder Society. 2012;27(9):1083-91.
3.   Langbehn DR, Brinkman RR, Falush D, Paulsen JS, Hayden MR, International Huntington's Disease Collaborative G. A new model for prediction of the age of onset and penetrance for Huntington's disease based on CAG length. Clinical genetics. 2004;65(4):267-77.
4.   Brinkman RR, Mezei MM, Theilmann J, Almqvist E, Hayden MR. The likelihood of being affected with Huntington disease by a particular age, for a specific CAG size. American journal of human genetics. 1997;60(5):1202-10.
5.   Garcia-Ruiz PJ, Garcia-Caldentey J, Feliz C, Del Val J, Herranz A, Martinez-Castrillo JC. Late onset Huntington's disease with 29 CAG repeat expansion. Journal of the neurological sciences. 2016;363:114-5.
6.   Groen JL, de Bie RM, Foncke EM, Roos RA, Leenders KL, Tijssen MA. Late-onset Huntington disease with intermediate CAG repeats: true or false? Journal of neurology, neurosurgery, and psychiatry. 2010;81(2):228-30.
7.   Falush D, Almqvist EW, Brinkmann RR, Iwasa Y, Hayden MR. Measurement of mutational flow implies both a high new-mutation rate for Huntington disease and substantial underascertainment of late-onset cases. American journal of human genetics. 2001;68(2):373-85.
8.   Castilhos RM, Augustin MC, Santos JA, Perandones C, Saraiva-Pereira ML, Jardim LB, et al. Genetic aspects of Huntington's disease in Latin America. A systematic review. Clinical genetics. 2016;89(3):295-303.
9.   Squitieri F, Andrew SE, Goldberg YP, Kremer B, Spence N, Zeisler J, et al. DNA haplotype analysis of Huntington disease reveals clues to the origins and mechanisms of CAG expansion and reasons for geographic variations of prevalence. Human molecular genetics. 1994;3(12):2103-14.
10.   Warby SC, Visscher H, Collins JA, Doty CN, Carter C, Butland SL, et al. HTT haplotypes contribute to differences in Huntington disease prevalence between Europe and East Asia. European journal of human genetics : EJHG. 2011;19(5):561-6.
11.   Sipila JO, Hietala M, Siitonen A, Paivarinta M, Majamaa K. Epidemiology of Huntington's disease in Finland. Parkinsonism & related disorders. 2015;21(1):46-9.
12.   Baine FK, Krause A, Greenberg LJ. The Frequency of Huntington Disease and Huntington Disease-Like 2 in the South African Population. Neuroepidemiology. 2016;46(3):198-202.
13.   Weydt P, Soyal SM, Gellera C, Didonato S, Weidinger C, Oberkofler H, et al. The gene coding for PGC-1alpha modifies age at onset in Huntington's Disease. Molecular neurodegeneration. 2009;4:3.
14.   Alberch J, Lopez M, Badenas C, Carrasco JL, Mila M, Munoz E, et al. Association between BDNF Val66Met polymorphism and age at onset in Huntington disease. Neurology. 2005;65(6):964-5.
15.   Arning L, Kraus PH, Valentin S, Saft C, Andrich J, Epplen JT. NR2A and NR2B receptor gene variations modify age at onset in Huntington disease. Neurogenetics. 2005;6(1):25-8.

16.   Arning L, Epplen JT. Genetic modifiers in Huntington's disease: fiction or fact? Neurogenetics. 2013;14(3-4):171-2.

17.   Genetic Modifiers of Huntington's Disease C. Identification of Genetic Factors that Modify Clinical Onset of Huntington's Disease. Cell. 2015;162(3):516-26.

18.   Unified Huntington's Disease Rating Scale: reliability and consistency. Huntington Study Group. Movement disorders : official journal of the Movement Disorder Society. 1996;11(2):136-42.

19.   Reilmann R, Leavitt BR, Ross CA. Diagnostic criteria for Huntington's disease based on natural history. Movement disorders : official journal of the Movement Disorder Society. 2014;29(11):1335-41.

20.   Novak MJ, Tabrizi SJ. Huntington's disease: clinical presentation and treatment. International review of neurobiology. 2011;98:297-323.

21.   Ho AK, Gilbert AS, Mason SL, Goodman AO, Barker RA. Health-related quality of life in Huntington's disease: Which factors matter most? Movement disorders : official journal of the Movement Disorder Society. 2009;24(4):574-8.

22.   Sitek EJ, Thompson JC, Craufurd D, Snowden JS. Unawareness of deficits in Huntington's disease. Journal of Huntington's disease. 2014;3(2):125-35.

23.   Guo Z, Rudow G, Pletnikova O, Codispoti KE, Orr BA, Crain BJ, et al. Striatal neuronal loss correlates with clinical motor impairment in Huntington's disease. Movement disorders : official journal of the Movement Disorder Society. 2012;27(11):1379-86.

24.   Rothlind JC, Bylsma FW, Peyser C, Folstein SE, Brandt J. Cognitive and motor correlates of everyday functioning in early Huntington's disease. The Journal of nervous and mental disease. 1993;181(3):194-9.

25.   Williams JK, Kim JI, Downing N, Farias S, Harrington DL, Long JD, et al. Everyday cognition in prodromal Huntington disease. Neuropsychology. 2015;29(2):255-67.

26.   Dumas EM, van den Bogaard SJ, Middelkoop HA, Roos RA. A review of cognition in Huntington's disease. Frontiers in bioscience. 2013;5:1-18.

27.   Duff K, Paulsen JS, Beglinger LJ, Langbehn DR, Wang C, Stout JC, et al. "Frontal" behaviors before the diagnosis of Huntington's disease and their relationship to markers of disease progression: evidence of early lack of awareness. The Journal of neuropsychiatry and clinical neurosciences. 2010;22(2):196-207.

28.   Cubo E, Shannon KM, Tracy D, Jaglin JA, Bernard BA, Wuu J, et al. Effect of donepezil on motor and cognitive function in Huntington disease. Neurology. 2006;67(7):1268-71.

29.   Ondo WG, Mejia NI, Hunter CB. A pilot study of the clinical efficacy and safety of memantine for Huntington's disease. Parkinsonism & related disorders. 2007;13(7):453-4.

30.   Vattakatuchery JJ, Kurien R. Acetylcholinesterase inhibitors in cognitive impairment in Huntington's disease: A brief review. World journal of psychiatry. 2013;3(3):62-4.

31.   Cankurtaran ES, Ozalp E, Soygur H, Cakir A. Clinical experience with risperidone and memantine in the treatment of Huntington's disease. Journal of the National Medical Association. 2006;98(8):1353-5.

32.   Beister A, Kraus P, Kuhn W, Dose M, Weindl A, Gerlach M. The N-methyl-D-aspartate antagonist memantine retards progression of Huntington's disease. Journal of neural transmission Supplementum. 2004(68):117-22.

33.   Stout JC, Queller S, Baker KN, Cowlishaw S, Sampaio C, Fitzer-Attas C, et al. HD-CAB: a cognitive assessment battery for clinical trials in Huntington's disease 1,2,3. Movement disorders : official journal of the Movement Disorder Society. 2014;29(10):1281-8.

34.   Tabrizi SJ, Scahill RI, Durr A, Roos RA, Leavitt BR, Jones R, et al. Biological and clinical changes in premanifest and early stage Huntington's disease in the TRACK-HD study: the 12-month longitudinal analysis. The Lancet Neurology. 2011;10(1):31-42.

35.   Moulton CD, Hopkins CW, Bevan-Jones WR. Systematic review of pharmacological treatments for depressive symptoms in Huntington's disease. Movement disorders : official journal of the Movement Disorder Society. 2014;29(12):1556-61.

36.   Pettibone DJ, Totaro JA, Pflueger AB. Tetrabenazine-induced depletion of brain monoamines: characterization and interaction with selected antidepressants. European journal of pharmacology. 1984;102(3-4):425-30.

37.   Podurgiel SJ, Yohn SE, Dortche K, Correa M, Salamone JD. The MAO-B inhibitor deprenyl reduces the oral tremor and the dopamine depletion induced by the VMAT-2 inhibitor tetrabenazine. Behavioural brain research. 2016;298(Pt B):188-91.

38.   Kegelmeyer DA, Kloos AD, Fritz NE, Fiumedora MM, White SE, Kostyk SK. Impact of tetrabenazine on gait and functional mobility in individuals with Huntington's disease. Journal of the neurological sciences. 2014;347(1-2):219-23.

39.   Fekete R, Davidson A, Jankovic J. Clinical assessment of the effect of tetrabenazine on functional scales in huntington disease: a pilot open label study. Tremor and other hyperkinetic movements. 2012;2.

40.   Burgunder JM, Guttman M, Perlman S, Goodman N, van Kammen DP, Goodman L. An International Survey-based Algorithm for the Pharmacologic Treatment of Chorea in Huntington's Disease. PLoS currents. 2011;3:RRN1260.

41.   Vonsattel JP, Myers RH, Stevens TJ, Ferrante RJ, Bird ED, Richardson EP, Jr. Neuropathological classification of Huntington's disease. Journal of neuropathology and experimental neurology. 1985;44(6):559-77.

42.   Jech R, Klempir J, Vymazal J, Zidovska J, Klempirova O, Ruzicka E, et al. Variation of selective gray and white matter atrophy in Huntington's disease. Movement disorders : official journal of the Movement Disorder Society. 2007;22(12):1783-9.

43.   Aylward EH, Liu D, Nopoulos PC, Ross CA, Pierson RK, Mills JA, et al. Striatal volume contributes to the prediction of onset of Huntington disease in incident cases. Biological psychiatry. 2012;71(9):822-8.

44.   Rosas HD, Salat DH, Lee SY, Zaleta AK, Pappu V, Fischl B, et al. Cerebral cortex and the clinical expression of Huntington's disease: complexity and heterogeneity. Brain : a journal of neurology. 2008;131(Pt 4):1057-68.

45.   Delmaire C, Dumas EM, Sharman MA, van den Bogaard SJ, Valabregue R, Jauffret C, et al. The structural correlates of functional deficits in early huntington's disease. Human brain mapping. 2013;34(9):2141-53.

46.   Rosas HD, Hevelone ND, Zaleta AK, Greve DN, Salat DH, Fischl B. Regional cortical thinning in preclinical Huntington disease and its relationship to cognition. Neurology. 2005;65(5):745-7.

47.   van den Bogaard SJ, Dumas EM, Ferrarini L, Milles J, van Buchem MA, van der Grond J, et al. Shape analysis of subcortical nuclei in Huntington's disease, global versus local atrophy--results from the TRACK-HD study. Journal of the neurological sciences. 2011;307(1-2):60-8.

48.   van den Bogaard SJ, Dumas EM, Acharya TP, Johnson H, Langbehn DR, Scahill RI, et al. Early atrophy of pallidum and accumbens nucleus in Huntington's disease. Journal of neurology. 2011;258(3):412-20.

49.   Thieben MJ, Duggins AJ, Good CD, Gomes L, Mahant N, Richards F, et al. The distribution of structural neuropathology in pre-clinical Huntington's disease. Brain : a journal of neurology. 2002;125(Pt 8):1815-28.
50.   Drouin-Ouellet J, Sawiak SJ, Cisbani G, Lagace M, Kuan WL, Saint-Pierre M, et al. Cerebrovascular and blood-brain barrier impairments in Huntington's disease: Potential implications for its pathophysiology. Annals of neurology. 2015;78(2):160-77.
51.   Guncova I, Latr I, Mazurova Y. The neurodegenerative process in a neurotoxic rat model and in patients with Huntington's disease: histopathological parallels and differences. Acta histochemica. 2011;113(8):783-92.
52.   Singhrao SK, Neal JW, Morgan BP, Gasque P. Increased complement biosynthesis by microglia and complement activation on neurons in Huntington's disease. Experimental neurology. 1999;159(2):362-76.
53.   Tai YF, Pavese N, Gerhard A, Tabrizi SJ, Barker RA, Brooks DJ, et al. Microglial activation in presymptomatic Huntington's disease gene carriers. Brain : a journal of neurology. 2007;130(Pt 7):1759-66.
54.   Sapp E, Kegel KB, Aronin N, Hashikawa T, Uchiyama Y, Tohyama K, et al. Early and progressive accumulation of reactive microglia in the Huntington disease brain. Journal of neuropathology and experimental neurology. 2001;60(2):161-72.
55.   Dalrymple A, Wild EJ, Joubert R, Sathasivam K, Bjorkqvist M, Petersen A, et al. Proteomic profiling of plasma in Huntington's disease reveals neuroinflammatory activation and biomarker candidates. Journal of proteome research. 2007;6(7):2833-40.
56.   Bjorkqvist M, Wild EJ, Thiele J, Silvestroni A, Andre R, Lahiri N, et al. A novel pathogenic pathway of immune activation detectable before clinical onset in Huntington's disease. The Journal of experimental medicine. 2008;205(8):1869-77.
57.   Beal MF, Kowall NW, Ellison DW, Mazurek MF, Swartz KJ, Martin JB. Replication of the neurochemical characteristics of Huntington's disease by quinolinic acid. Nature. 1986;321(6066):168-71.
58.   Borlongan CV, Koutouzis TK, Randall TS, Freeman TB, Cahill DW, Sanberg PR. Systemic 3-nitropropionic acid: behavioral deficits and striatal damage in adult rats. Brain research bulletin. 1995;36(6):549-56.
59.   Wang LH, Qin ZH. Animal models of Huntington's disease: implications in uncovering pathogenic mechanisms and developing therapies. Acta pharmacologica Sinica. 2006;27(10):1287-302.
60.   Morton AJ, Howland DS. Large genetic animal models of Huntington's Disease. Journal of Huntington's disease. 2013;2(1):3-19.
61.   Mangiarini L, Sathasivam K, Seller M, Cozens B, Harper A, Hetherington C, et al. Exon 1 of the HD gene with an expanded CAG repeat is sufficient to cause a progressive neurological phenotype in transgenic mice. Cell. 1996;87(3):493-506.
62.   Davies SW, Turmaine M, Cozens BA, DiFiglia M, Sharp AH, Ross CA, et al. Formation of neuronal intranuclear inclusions underlies the neurological dysfunction in mice transgenic for the HD mutation. Cell. 1997;90(3):537-48.
63.   Schilling G, Becher MW, Sharp AH, Jinnah HA, Duan K, Kotzuk JA, et al. Intranuclear inclusions and neuritic aggregates in transgenic mice expressing a mutant N-terminal fragment of huntingtin. Human molecular genetics. 1999;8(3):397-407.

64.   Slow EJ, van Raamsdonk J, Rogers D, Coleman SH, Graham RK, Deng Y, et al. Selective striatal neuronal loss in a YAC128 mouse model of Huntington disease. Human molecular genetics. 2003;12(13):1555-67.

65.   Gray M, Shirasaki DI, Cepeda C, Andre VM, Wilburn B, Lu XH, et al. Full-length human mutant huntingtin with a stable polyglutamine repeat can elicit progressive and selective neuropathogenesis in BACHD mice. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2008;28(24):6182-95.

66.   Pouladi MA, Stanek LM, Xie Y, Franciosi S, Southwell AL, Deng Y, et al. Marked differences in neurochemistry and aggregates despite similar behavioural and neuropathological features of Huntington disease in the full-length BACHD and YAC128 mice. Human molecular genetics. 2012;21(10):2219-32.

67.   Pouladi MA, Xie Y, Skotte NH, Ehrnhoefer DE, Graham RK, Kim JE, et al. Full-length huntingtin levels modulate body weight by influencing insulin-like growth factor 1 expression. Human molecular genetics. 2010;19(8):1528-38.

68.   Sanberg PR, Fibiger HC, Mark RF. Body weight and dietary factors in Huntington's disease patients compared with matched controls. The Medical journal of Australia. 1981;1(8):407-9.

69.   Menalled LB, Sison JD, Dragatsis I, Zeitlin S, Chesselet MF. Time course of early motor and neuropathological anomalies in a knock-in mouse model of Huntington's disease with 140 CAG repeats. The Journal of comparative neurology. 2003;465(1):11-26.

70.   Menalled LB, Kudwa AE, Miller S, Fitzpatrick J, Watson-Johnson J, Keating N, et al. Comprehensive behavioral and molecular characterization of a new knock-in mouse model of Huntington's disease: zQ175. PloS one. 2012;7(12):e49838.

71.   Levine MS, Klapstein GJ, Koppel A, Gruen E, Cepeda C, Vargas ME, et al. Enhanced sensitivity to N-methyl-D-aspartate receptor activation in transgenic and knockin mouse models of Huntington's disease. Journal of neuroscience research. 1999;58(4):515-32.

72.   Woodman B, Butler R, Landles C, Lupton MK, Tse J, Hockly E, et al. The Hdh(Q150/Q150) knock-in mouse model of HD and the R6/2 exon 1 model develop comparable and widespread molecular phenotypes. Brain research bulletin. 2007;72(2-3):83-97.

73.   Wheeler VC, Auerbach W, White JK, Srinidhi J, Auerbach A, Ryan A, et al. Length-dependent gametic CAG repeat instability in the Huntington's disease knock-in mouse. Human molecular genetics. 1999;8(1):115-22.

74.   Wheeler VC, Gutekunst CA, Vrbanac V, Lebel LA, Schilling G, Hersch S, et al. Early phenotypes that presage late-onset neurodegenerative disease allow testing of modifiers in Hdh CAG knock-in mice. Human molecular genetics. 2002;11(6):633-40.

75.   Bates GP, Hockly E. Experimental therapeutics in Huntington's disease: are models useful for therapeutic trials? Current opinion in neurology. 2003;16(4):465-70.

76.   Menalled L, Brunner D. Animal models of Huntington's disease for translation to the clinic: best practices. Movement disorders : official journal of the Movement Disorder Society. 2014;29(11):1375-90.

77.   Zeef DH, Jahanshahi A, Vlamings R, Casaca-Carreira J, Santegoeds RG, Janssen ML, et al. An experimental model for Huntington's chorea? Behavioural brain research. 2014;262:31-4.

78.   von Horsten S, Schmitt I, Nguyen HP, Holzmann C, Schmidt T, Walther T, et al. Transgenic rat model of Huntington's disease. Human molecular genetics. 2003;12(6):617-24.

79.   Jacobsen JC, Bawden CS, Rudiger SR, McLaughlan CJ, Reid SJ, Waldvogel HJ, et al. An ovine transgenic Huntington's disease model. Human molecular genetics. 2010;19(10):1873-82.

80.   Baxa M, Hruska-Plochan M, Juhas S, Vodicka P, Pavlok A, Juhasova J, et al. A transgenic minipig model of Huntington's Disease. Journal of Huntington's disease. 2013;2(1):47-68.

81.   Yang D, Wang CE, Zhao B, Li W, Ouyang Z, Liu Z, et al. Expression of Huntington's disease protein results in apoptotic neurons in the brains of cloned transgenic pigs. Human molecular genetics. 2010;19(20):3983-94.

82.   Yang SH, Cheng PH, Banta H, Piotrowska-Nitsche K, Yang JJ, Cheng EC, et al. Towards a transgenic model of Huntington's disease in a non-human primate. Nature. 2008;453(7197):921-4.

83.   Chan AW, Jiang J, Chen Y, Li C, Prucha MS, Hu Y, et al. Progressive cognitive deficit, motor impairment and striatal pathology in a transgenic Huntington disease monkey model from infancy to adulthood. PloS one. 2015;10(5):e0122335.

84.   Li XJ, Li S. Large Animal Models of Huntington's Disease. Current topics in behavioral neurosciences. 2015;22:149-60.

85.   Zuccato C, Valenza M, Cattaneo E. Molecular mechanisms and potential therapeutical targets in Huntington's disease. Physiological reviews. 2010;90(3):905-81.

86.   Saudou F, Humbert S. The Biology of Huntingtin. Neuron. 2016;89(5):910-26.

87.   Dragatsis I, Levine MS, Zeitlin S. Inactivation of Hdh in the brain and testis results in progressive neurodegeneration and sterility in mice. Nature genetics. 2000;26(3):300-6.

88.   Gauthier LR, Charrin BC, Borrell-Pages M, Dompierre JP, Rangone H, Cordelieres FP, et al. Huntingtin controls neurotrophic support and survival of neurons by enhancing BDNF vesicular transport along microtubules. Cell. 2004;118(1):127-38.

89.   Zala D, Colin E, Rangone H, Liot G, Humbert S, Saudou F. Phosphorylation of mutant huntingtin at S421 restores anterograde and retrograde transport in neurons. Human molecular genetics. 2008;17(24):3837-46.

90.   Zuccato C, Tartari M, Crotti A, Goffredo D, Valenza M, Conti L, et al. Huntingtin interacts with REST/NRSF to modulate the transcription of NRSE-controlled neuronal genes. Nature genetics. 2003;35(1):76-83.

91.   Marcora E, Kennedy MB. The Huntington's disease mutation impairs Huntingtin's role in the transport of NF-kappaB from the synapse to the nucleus. Human molecular genetics. 2010;19(22):4373-84.

92.   Hoffner G, Djian P. Monomeric, oligomeric and polymeric proteins in huntington disease and other diseases of polyglutamine expansion. Brain sciences. 2014;4(1):91-122.

93.   Seidel K, Siswanto S, Fredrich M, Bouzrou M, Brunt ER, van Leeuwen FW, et al. Polyglutamine aggregation in Huntington's disease and spinocerebellar ataxia type 3: similar mechanisms in aggregate formation. Neuropathology and applied neurobiology. 2016;42(2):153-66.

94.   Arrasate M, Mitra S, Schweitzer ES, Segal MR, Finkbeiner S. Inclusion body formation reduces levels of mutant huntingtin and the risk of neuronal death. Nature. 2004;431(7010):805-10.

95.   Slow EJ, Graham RK, Osmand AP, Devon RS, Lu G, Deng Y, et al. Absence of behavioral abnormalities and neurodegeneration in vivo despite widespread neuronal huntingtin

inclusions. Proceedings of the National Academy of Sciences of the United States of America. 2005;102(32):11402-7.

96.   Hackam AS, Singaraja R, Wellington CL, Metzler M, McCutcheon K, Zhang T, et al. The influence of huntingtin protein size on nuclear localization and cellular toxicity. The Journal of cell biology. 1998;141(5):1097-105.

97.   Sun CS, Lee CC, Li YN, Yao-Chen Yang S, Lin CH, Chang YC, et al. Conformational switch of polyglutamine-expanded huntingtin into benign aggregates leads to neuroprotective effect. Scientific reports. 2015;5:14992.

98.   Lee WC, Yoshihara M, Littleton JT. Cytoplasmic aggregates trap polyglutamine-containing proteins and block axonal transport in a Drosophila model of Huntington's disease. Proceedings of the National Academy of Sciences of the United States of America. 2004;101(9):3224-9.

99.   Duim WC, Jiang Y, Shen K, Frydman J, Moerner WE. Super-resolution fluorescence of huntingtin reveals growth of globular species into short fibers and coexistence of distinct aggregates. ACS chemical biology. 2014;9(12):2767-78.

100.   Chen S, Berthelier V, Yang W, Wetzel R. Polyglutamine aggregation behavior in vitro supports a recruitment mechanism of cytotoxicity. Journal of molecular biology. 2001;311(1):173-82.

101.   Altar CA, Cai N, Bliven T, Juhasz M, Conner JM, Acheson AL, et al. Anterograde transport of brain-derived neurotrophic factor and its role in the brain. Nature. 1997;389(6653):856-60.

102.   Kells AP, Fong DM, Dragunow M, During MJ, Young D, Connor B. AAV-mediated gene delivery of BDNF or GDNF is neuroprotective in a model of Huntington disease. Molecular therapy : the journal of the American Society of Gene Therapy. 2004;9(5):682-8.

103.   Baquet ZC, Gorski JA, Jones KR. Early striatal dendrite deficits followed by neuron loss with advanced age in the absence of anterograde cortical brain-derived neurotrophic factor. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2004;24(17):4250-8.

104.   Martin EJ, Kim M, Velier J, Sapp E, Lee HS, Laforet G, et al. Analysis of Huntingtin-associated protein 1 in mouse brain and immortalized striatal neurons. The Journal of comparative neurology. 1999;403(4):421-30.

105.   Trushina E, Dyer RB, Badger JD, 2nd, Ure D, Eide L, Tran DD, et al. Mutant huntingtin impairs axonal trafficking in mammalian neurons in vivo and in vitro. Molecular and cellular biology. 2004;24(18):8195-209.

106.   Barnhart EL. Mechanics of mitochondrial motility in neurons. Current opinion in cell biology. 2016;38:90-9.

107.   Cui L, Jeong H, Borovecki F, Parkhurst CN, Tanese N, Krainc D. Transcriptional repression of PGC-1alpha by mutant huntingtin leads to mitochondrial dysfunction and neurodegeneration. Cell. 2006;127(1):59-69.

108.   Shirendeb U, Reddy AP, Manczak M, Calkins MJ, Mao P, Tagle DA, et al. Abnormal mitochondrial dynamics, mitochondrial loss and mutant huntingtin oligomers in Huntington's disease: implications for selective neuronal damage. Human molecular genetics. 2011;20(7):1438-55.

109.   Wang H, Lim PJ, Karbowski M, Monteiro MJ. Effects of overexpression of huntingtin proteins on mitochondrial integrity. Human molecular genetics. 2009;18(4):737-52.

110.   Guo X, Disatnik MH, Monbureau M, Shamloo M, Mochly-Rosen D, Qi X. Inhibition of mitochondrial fragmentation diminishes Huntington's disease-associated neurodegeneration. The Journal of clinical investigation. 2013;123(12):5371-88.

111.   Gu M, Gash MT, Mann VM, Javoy-Agid F, Cooper JM, Schapira AH. Mitochondrial defect in Huntington's disease caudate nucleus. Annals of neurology. 1996;39(3):385-9.

112.   El-Hattab AW, Scaglia F. Mitochondrial cytopathies. Cell calcium. 2016.

113.   Baumgartner HK, Gerasimenko JV, Thorne C, Ferdek P, Pozzan T, Tepikin AV, et al. Calcium elevation in mitochondria is the main Ca2+ requirement for mitochondrial permeability transition pore (mPTP) opening. The Journal of biological chemistry. 2009;284(31):20796-803.

114.   Graham RK, Pouladi MA, Joshi P, Lu G, Deng Y, Wu NP, et al. Differential susceptibility to excitotoxic stress in YAC128 mouse models of Huntington disease between initiation and progression of disease. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2009;29(7):2193-204.

115.   Sun Y, Savanenin A, Reddy PH, Liu YF. Polyglutamine-expanded huntingtin promotes sensitization of N-methyl-D-aspartate receptors via post-synaptic density 95. The Journal of biological chemistry. 2001;276(27):24713-8.

116.   Milnerwood AJ, Gladding CM, Pouladi MA, Kaufman AM, Hines RM, Boyd JD, et al. Early increase in extrasynaptic NMDA receptor signaling and expression contributes to phenotype onset in Huntington's disease mice. Neuron. 2010;65(2):178-90.

117.   Muller M, Leavitt BR. Iron dysregulation in Huntington's disease. Journal of neurochemistry. 2014;130(3):328-50.

118.   Jurgens CK, Jasinschi R, Ekin A, Witjes-Ane MN, Middelkoop H, van der Grond J, et al. MRI T2 Hypointensities in basal ganglia of premanifest Huntington's disease. PLoS currents. 2010;2.

119.   Bartzokis G, Tishler TA. MRI evaluation of basal ganglia ferritin iron and neurotoxicity in Alzheimer's and Huntingon's disease. Cellular and molecular biology. 2000;46(4):821-33.

120.   Shoham S, Youdim MB. Iron involvement in neural damage and microgliosis in models of neurodegenerative diseases. Cellular and molecular biology. 2000;46(4):743-60.

121.   Rathore KI, Redensek A, David S. Iron homeostasis in astrocytes and microglia is differentially regulated by TNF-alpha and TGF-beta1. Glia. 2012;60(5):738-50.

122.   Neher JJ, Neniskyte U, Brown GC. Primary phagocytosis of neurons by inflamed microglia: potential roles in neurodegeneration. Frontiers in pharmacology. 2012;3:27.

123.   Sipione S, Rigamonti D, Valenza M, Zuccato C, Conti L, Pritchard J, et al. Early transcriptional profiles in huntingtin-inducible striatal cells by microarray analyses. Human molecular genetics. 2002;11(17):1953-65.

124.   Allen JA, Halverson-Tamboli RA, Rasenick MM. Lipid raft microdomains and neurotransmitter signalling. Nature reviews Neuroscience. 2007;8(2):128-40.

125.   Valenza M, Cattaneo E. Emerging roles for cholesterol in Huntington's disease. Trends in neurosciences. 2011;34(9):474-86.

126.   Goedeke L, Fernandez-Hernando C. MicroRNAs: a connection between cholesterol metabolism and neurodegeneration. Neurobiology of disease. 2014;72 Pt A:48-53.

127.   Xue-Shan Z, Juan P, Qi W, Zhong R, Li-Hong P, Zhi-Han T, et al. Imbalanced cholesterol metabolism in Alzheimer's disease. Clinica chimica acta; international journal of clinical chemistry. 2016;456:107-14.

128.   Leoni V, Caccia C. The impairment of cholesterol metabolism in Huntington disease. Biochimica et biophysica acta. 2015;1851(8):1095-105.

129.	Leoni V, Mariotti C, Tabrizi SJ, Valenza M, Wild EJ, Henley SM, et al. Plasma 24S-hydroxycholesterol and caudate MRI in pre-manifest and early Huntington's disease. Brain : a journal of neurology. 2008;131(Pt 11):2851-9.
130.	Valenza M, Leoni V, Tarditi A, Mariotti C, Bjorkhem I, Di Donato S, et al. Progressive dysfunction of the cholesterol biosynthesis pathway in the R6/2 mouse model of Huntington's disease. Neurobiology of disease. 2007;28(1):133-42.
131.	Valenza M, Rigamonti D, Goffredo D, Zuccato C, Fenu S, Jamot L, et al. Dysfunction of the cholesterol biosynthetic pathway in Huntington's disease. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2005;25(43):9932-9.
132.	Kreilaus F, Spiro AS, McLean CA, Garner B, Jenner AM. Evidence for altered cholesterol metabolism in Huntington's disease post mortem brain tissue. Neuropathology and applied neurobiology. 2015.
133.	Leoni V, Long JD, Mills JA, Di Donato S, Paulsen JS, group P-Hs. Plasma 24S-hydroxycholesterol correlation with markers of Huntington disease progression. Neurobiology of disease. 2013;55:37-43.
134.	Yu RK, Tsai YT, Ariga T, Yanagisawa M. Structures, biosynthesis, and functions of gangliosides--an overview. Journal of oleo science. 2011;60(10):537-44.
135.	Higatsberger MR, Sperk G, Bernheimer H, Shannak KS, Hornykiewicz O. Striatal ganglioside levels in the rat following kainic acid lesions: comparison with Huntington's disease. Experimental brain research. 1981;44(1):93-6.
136.	Scorrano L, Petronilli V, Di Lisa F, Bernardi P. Commitment to apoptosis by GD3 ganglioside depends on opening of the mitochondrial permeability transition pore. The Journal of biological chemistry. 1999;274(32):22581-5.
137.	Maglione V, Marchi P, Di Pardo A, Lingrell S, Horkey M, Tidmarsh E, et al. Impaired ganglioside metabolism in Huntington's disease and neuroprotective role of GM1. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2010;30(11):4072-80.
138.	Denny CA, Desplats PA, Thomas EA, Seyfried TN. Cerebellar lipid differences between R6/1 transgenic mice and humans with Huntington's disease. Journal of neurochemistry. 2010;115(3):748-58.
139.	Desplats PA, Denny CA, Kass KE, Gilmartin T, Head SR, Sutcliffe JG, et al. Glycolipid and ganglioside metabolism imbalances in Huntington's disease. Neurobiology of disease. 2007;27(3):265-77.
140.	Svennerholm L. Designation and schematic structure of gangliosides and allied glycosphingolipids. Progress in brain research. 1994;101:XI-XIV.
141.	Kolter T. Ganglioside biochemistry. ISRN biochemistry. 2012;2012:506160.
142.	Wang H, Wang A, Wang D, Bright A, Sency V, Zhou A, et al. Early growth and development impairment in patients with ganglioside GM3 synthase deficiency. Clinical genetics. 2015.
143.	Sonnino S, Chigorno V. Ganglioside molecular species containing C18- and C20-sphingosine in mammalian nervous tissues and neuronal cell cultures. Biochimica et biophysica acta. 2000;1469(2):63-77.
144.	Kracun I, Rosner H, Drnovsek V, Vukelic Z, Cosovic C, Trbojevic-Cepe M, et al. Gangliosides in the human brain development and aging. Neurochemistry international. 1992;20(3):421-31.
145.	Rosner H. Developmental expression and possible roles of gangliosides in brain development. Progress in molecular and subcellular biology. 2003;32:49-73.

146.   Weishaupt N, Caughlin S, Yeung KK, Whitehead SN. Differential Anatomical Expression of Ganglioside GM1 Species Containing d18:1 or d20:1 Sphingosine Detected by MALDI Imaging Mass Spectrometry in Mature Rat Brain. Frontiers in neuroanatomy. 2015;9:155.

147.   Schnaar RL. Gangliosides of the Vertebrate Nervous System. Journal of molecular biology. 2016.

148.   Posse de Chaves E, Sipione S. Sphingolipids and gangliosides of the nervous system in membrane function and dysfunction. FEBS letters. 2010;584(9):1748-59.

149.   Mocchetti I. Exogenous gangliosides, neuronal plasticity and repair, and the neurotrophins. Cell Mol Life Sci. 2005;62(19-20):2283-94.

150.   Schengrund CL. Gangliosides: glycosphingolipids essential for normal neural development and function. Trends in biochemical sciences. 2015;40(7):397-406.

151.   Ferrari G, Anderson BL, Stephens RM, Kaplan DR, Greene LA. Prevention of apoptotic neuronal death by GM1 ganglioside. Involvement of Trk neurotrophin receptors. The Journal of biological chemistry. 1995;270(7):3074-80.

152.   Kabayama K, Sato T, Saito K, Loberto N, Prinetti A, Sonnino S, et al. Dissociation of the insulin receptor and caveolin-1 complex by ganglioside GM3 in the state of insulin resistance. Proceedings of the National Academy of Sciences of the United States of America. 2007;104(34):13678-83.

153.   Sekino-Suzuki N, Yuyama K, Miki T, Kaneda M, Suzuki H, Yamamoto N, et al. Involvement of gangliosides in the process of Cbp/PAG phosphorylation by Lyn in developing cerebellar growth cones. Journal of neurochemistry. 2013;124(4):514-22.

154.   Yang LJ, Zeller CB, Shaper NL, Kiso M, Hasegawa A, Shapiro RE, et al. Gangliosides are neuronal ligands for myelin-associated glycoprotein. Proceedings of the National Academy of Sciences of the United States of America. 1996;93(2):814-8.

155.   Sheikh KA, Sun J, Liu Y, Kawai H, Crawford TO, Proia RL, et al. Mice lacking complex gangliosides develop Wallerian degeneration and myelination defects. Proceedings of the National Academy of Sciences of the United States of America. 1999;96(13):7532-7.

156.   Fantini J, Barrantes FJ. Sphingolipid/cholesterol regulation of neurotransmitter receptor conformation and function. Biochimica et biophysica acta. 2009;1788(11):2345-61.

157.   Krishnan KS, Balaram P. A nuclear magnetic resonance study of the interaction of serotonin with gangliosides. FEBS letters. 1976;63(2):313-5.

158.   Wu G, Xie X, Lu ZH, Ledeen RW. Sodium-calcium exchanger complexed with GM1 ganglioside in nuclear membrane transfers calcium from nucleoplasm to endoplasmic reticulum. Proceedings of the National Academy of Sciences of the United States of America. 2009;106(26):10829-34.

159.   Ohmi Y, Tajima O, Ohkawa Y, Mori A, Sugiura Y, Furukawa K, et al. Gangliosides play pivotal roles in the regulation of complement systems and in the maintenance of integrity in nerve tissues. Proceedings of the National Academy of Sciences of the United States of America. 2009;106(52):22405-10.

160.   Ledeen RW, Wu G. The multi-tasked life of GM1 ganglioside, a true factotum of nature. Trends in biochemical sciences. 2015;40(7):407-18.

161.   Tajima O, Egashira N, Ohmi Y, Fukue Y, Mishima K, Iwasaki K, et al. Dysfunction of muscarinic acetylcholine receptors as a substantial basis for progressive neurological deterioration in GM3-only mice. Behavioural brain research. 2010;206(1):101-8.

162.   Takamiya K, Yamamoto A, Furukawa K, Yamashiro S, Shin M, Okada M, et al. Mice with disrupted GM2/GD2 synthase gene lack complex gangliosides but exhibit only subtle defects in their nervous system. Proceedings of the National Academy of Sciences of the United States of America. 1996;93(20):10662-7.

163.   Chiavegatto S, Sun J, Nelson RJ, Schnaar RL. A functional role for complex gangliosides: motor deficits in GM2/GD2 synthase knockout mice. Experimental neurology. 2000;166(2):227-34.

164.   Sha S, Zhou L, Yin J, Takamiya K, Furukawa K, Furukawa K, et al. Deficits in cognitive function and hippocampal plasticity in GM2/GD2 synthase knockout mice. Hippocampus. 2014;24(4):369-82.

165.   Wu G, Lu ZH, Kulkarni N, Amin R, Ledeen RW. Mice lacking major brain gangliosides develop parkinsonism. Neurochemical research. 2011;36(9):1706-14.

166.   Tajima O, Egashira N, Ohmi Y, Fukue Y, Mishima K, Iwasaki K, et al. Reduced motor and sensory functions and emotional response in GM3-only mice: emergence from early stage of life and exacerbation with aging. Behavioural brain research. 2009;198(1):74-82.

167.   Yamashita T, Wu YP, Sandhoff R, Werth N, Mizukami H, Ellis JM, et al. Interruption of ganglioside synthesis produces central nervous system degeneration and altered axon-glial interactions. Proceedings of the National Academy of Sciences of the United States of America. 2005;102(8):2725-30.

168.   Goldman JE, Katz D, Rapin I, Purpura DP, Suzuki K. Chronic GM1 gangliosidosis presenting as dystonia: I. Clinical and pathological features. Annals of neurology. 1981;9(5):465-75.

169.   Fernandes Filho JA, Shapiro BE. Tay-Sachs disease. Archives of neurology. 2004;61(9):1466-8.

170.   Bley AE, Giannikopoulos OA, Hayden D, Kubilus K, Tifft CJ, Eichler FS. Natural history of infantile G(M2) gangliosidosis. Pediatrics. 2011;128(5):e1233-41.

171.   Simpson MA, Cross H, Proukakis C, Priestman DA, Neville DC, Reinkensmeier G, et al. Infantile-onset symptomatic epilepsy syndrome caused by a homozygous loss-of-function mutation of GM3 synthase. Nature genetics. 2004;36(11):1225-9.

172.   Arber C, Li A, Houlden H, Wray S. Insights into molecular mechanisms of disease in Neurodegeneration with Brain Iron Accumulation; unifying theories. Neuropathology and applied neurobiology. 2015.

173.   Harlalka GV, Lehman A, Chioza B, Baple EL, Maroofian R, Cross H, et al. Mutations in B4GALNT1 (GM2 synthase) underlie a new disorder of ganglioside biosynthesis. Brain : a journal of neurology. 2013;136(Pt 12):3618-24.

174.   Boukhris A, Schule R, Loureiro JL, Lourenco CM, Mundwiller E, Gonzalez MA, et al. Alteration of ganglioside biosynthesis responsible for complex hereditary spastic paraplegia. American journal of human genetics. 2013;93(1):118-23.

175.   Taki T. An approach to glycobiology from glycolipidomics: ganglioside molecular scanning in the brains of patients with Alzheimer's disease by TLC-blot/matrix assisted laser desorption/ionization-time of flight MS. Biological & pharmaceutical bulletin. 2012;35(10):1642-7.

176.   Gizaw ST, Ohashi T, Tanaka M, Hinou H, Nishimura S. Glycoblotting method allows for rapid and efficient glycome profiling of human Alzheimer's disease brain, serum and cerebrospinal fluid towards potential biomarker discovery. Biochimica et biophysica acta. 2016;1860(8):1716-27.

177.   Wu G, Lu ZH, Kulkarni N, Ledeen RW. Deficiency of ganglioside GM1 correlates with Parkinson's disease in mice and humans. Journal of neuroscience research. 2012;90(10):1997-2008.
178.   Miyatani N, Saito M, Ariga T, Yoshino H, Yu RK. Glycosphingolipids in the cerebrospinal fluid of patients with multiple sclerosis. Molecular and chemical neuropathology / sponsored by the International Society for Neurochemistry and the World Federation of Neurology and research groups on neurochemistry and cerebrospinal fluid. 1990;13(3):205-16.
179.   Dodge JC, Treleaven CM, Pacheco J, Cooper S, Bao C, Abraham M, et al. Glycosphingolipids are modulators of disease pathogenesis in amyotrophic lateral sclerosis. Proceedings of the National Academy of Sciences of the United States of America. 2015;112(26):8100-5.
180.   Kristjansdottir R, Uvebrant P, Lekman A, Mansson JE. Cerebrospinal fluid markers in children with cerebral white matter abnormalities. Neuropediatrics. 2001;32(4):176-82.
181.   Yanagisawa K. GM1 ganglioside and Alzheimer's disease. Glycoconjugate journal. 2015;32(3-4):87-91.
182.   Calamai M, Pavone FS. Partitioning and confinement of GM1 ganglioside induced by amyloid aggregates. FEBS letters. 2013;587(9):1385-91.
183.   Kreutz F, Scherer EB, Ferreira AG, Petry Fdos S, Pereira CL, Santana F, et al. Alterations on Na(+),K(+)-ATPase and acetylcholinesterase activities induced by amyloid-beta peptide in rat brain and GM1 ganglioside neuroprotective action. Neurochemical research. 2013;38(11):2342-50.
184.   Svennerholm L, Brane G, Karlsson I, Lekman A, Ramstrom I, Wikkelso C. Alzheimer disease - effect of continuous intracerebroventricular treatment with GM1 ganglioside and a systematic activation programme. Dementia and geriatric cognitive disorders. 2002;14(3):128-36.
185.   Ariga T. Pathogenic role of ganglioside metabolism in neurodegenerative diseases. J Neurosci Res. 2014;92(10):1227-42.
186.   Sadatipour BT, Greer JM, Pender MP. Increased circulating antiganglioside antibodies in primary and secondary progressive multiple sclerosis. Annals of neurology. 1998;44(6):980-3.
187.   Head VA, Wakerley BR. Guillain-Barre syndrome in general practice: clinical features suggestive of early diagnosis. The British journal of general practice : the journal of the Royal College of General Practitioners. 2016;66(645):218-9.
188.   Pender MP, Greer JM. Immunology of multiple sclerosis. Current allergy and asthma reports. 2007;7(4):285-92.
189.   Chapman J, Sela BA, Wertman E, Michaelson DM. Antibodies to ganglioside GM1 in patients with Alzheimer's disease. Neuroscience letters. 1988;86(2):235-40.
190.   Bachis A, Rabin SJ, Del Fiacco M, Mocchetti I. Gangliosides prevent excitotoxicity through activation of TrkB receptor. Neurotoxicity research. 2002;4(3):225-34.
191.   Lazzaro A, Seren MS, Koga T, Zanoni R, Schiavo N, Manev H. GM1 reduces infarct volume after focal cerebral ischemia. Experimental neurology. 1994;125(2):278-85.
192.   Saito M, Mao RF, Wang R, Vadasz C, Saito M. Effects of gangliosides on ethanol-induced neurodegeneration in the developing mouse brain. Alcoholism, clinical and experimental research. 2007;31(4):665-74.
193.   Rothblat DS, Schneider JS. Effects of GM1 ganglioside treatment on dopamine innervation of the striatum of MPTP-treated mice. Annals of the New York Academy of Sciences. 1998;845:274-7.

194.	Wu G, Lu ZH, Xie X, Ledeen RW. Susceptibility of cerebellar granule neurons from GM2/GD2 synthase-null mice to apoptosis induced by glutamate excitotoxicity and elevated KCl: rescue by GM1 and LIGA20. Glycoconjugate journal. 2004;21(6):305-13.

195.	Schneider JS, Roeltgen DP, Mancall EL, Chapas-Crilly J, Rothblat DS, Tatarian GT. Parkinson's disease: improved function with GM1 ganglioside treatment in a randomized placebo-controlled study. Neurology. 1998;50(6):1630-6.

196.	Schneider JS, Cambi F, Gollomp SM, Kuwabara H, Brasic JR, Leiby B, et al. GM1 ganglioside in Parkinson's disease: Pilot study of effects on dopamine transporter binding. Journal of the neurological sciences. 2015;356(1-2):118-23.

197.	Ceni C, Unsain N, Zeinieh MP, Barker PA. Neurotrophins in the regulation of cellular survival and death. Handbook of experimental pharmacology. 2014;220:193-221.

198.	Lim ST, Esfahani K, Avdoshina V, Mocchetti I. Exogenous gangliosides increase the release of brain-derived neurotrophic factor. Neuropharmacology. 2011;60(7-8):1160-7.

199.	Rabin SJ, Bachis A, Mocchetti I. Gangliosides activate Trk receptors by inducing the release of neurotrophins. The Journal of biological chemistry. 2002;277(51):49466-72.

200.	Valdomero A, Perondi MC, Orsingher OA, Cuadra GR. Exogenous GM1 ganglioside increases accumbal BDNF levels in rats. Behavioural brain research. 2015;278:303-6.

201.	Rabin SJ, Mocchetti I. GM1 ganglioside activates the high-affinity nerve growth factor receptor trkA. Journal of neurochemistry. 1995;65(1):347-54.

202.	Farooqui T, Yates AJ. Effect of GM1 on TrkA dimerization. Annals of the New York Academy of Sciences. 1998;845:407.

203.	Duchemin AM, Ren Q, Mo L, Neff NH, Hadjiconstantinou M. GM1 ganglioside induces phosphorylation and activation of Trk and Erk in brain. Journal of neurochemistry. 2002;81(4):696-707.

204.	Ledeen RW, Wu G. Ganglioside function in calcium homeostasis and signaling. Neurochemical research. 2002;27(7-8):637-47.

205.	Wu G, Lu ZH, Obukhov AG, Nowycky MC, Ledeen RW. Induction of calcium influx through TRPC5 channels by cross-linking of GM1 ganglioside associated with alpha5beta1 integrin initiates neurite outgrowth. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2007;27(28):7447-58.

206.	Favaron M, Manev H, Alho H, Bertolino M, Ferret B, Guidotti A, et al. Gangliosides prevent glutamate and kainate neurotoxicity in primary neuronal cultures of neonatal rat cerebellum and cortex. Proceedings of the National Academy of Sciences of the United States of America. 1988;85(19):7351-5.

207.	Nakamura K, Wu G, Ledeen RW. Protection of neuro-2a cells against calcium ionophore cytotoxicity by gangliosides. J Neurosci Res. 1992;31(2):245-53.

208.	Gavella M, Garaj-Vrhovac V, Lipovac V, Antica M, Gajski G, Car N. Ganglioside GT1b protects human spermatozoa from hydrogen peroxide-induced DNA and membrane damage. International journal of andrology. 2010;33(3):536-44.

209.	Kruszewski M. Labile iron pool: the main determinant of cellular response to oxidative stress. Mutation research. 2003;531(1-2):81-92.

210.	Gorria M, Huc L, Sergent O, Rebillard A, Gaboriau F, Dimanche-Boitrel MT, et al. Protective effect of monosialoganglioside GM1 against chemically induced apoptosis through targeting of mitochondrial function and iron transport. Biochemical pharmacology. 2006;72(10):1343-53.

211. Brito V, Puigdellivol M, Giralt A, del Toro D, Alberch J, Gines S. Imbalance of p75(NTR)/TrkB protein expression in Huntington's disease: implication for neuroprotective therapies. Cell death & disease. 2013;4:e595.

212. Di Pardo A, Maglione V, Alpaugh M, Horkey M, Atwal RS, Sassone J, et al. Ganglioside GM1 induces phosphorylation of mutant huntingtin and restores normal motor behavior in Huntington disease mice. Proceedings of the National Academy of Sciences of the United States of America. 2012;109(9):3528-33.

213. Gu X, Greiner ER, Mishra R, Kodali R, Osmand A, Finkbeiner S, et al. Serines 13 and 16 are critical determinants of full-length human mutant huntingtin induced disease pathogenesis in HD mice. Neuron. 2009;64(6):828-40.

214. Fienberg AA, Hiroi N, Mermelstein PG, Song W, Snyder GL, Nishi A, et al. DARPP-32: regulator of the efficacy of dopaminergic neurotransmission. Science. 1998;281(5378):838-42.

215. Young D, Mayer F, Vidotto N, Schweizer T, Berth R, Abramowski D, et al. Mutant huntingtin gene-dose impacts on aggregate deposition, DARPP32 expression and neuroinflammation in HdhQ150 mice. PloS one. 2013;8(9):e75108.

216. Valencia A, Sapp E, Kimm JS, McClory H, Ansong KA, Yohrling G, et al. Striatal synaptosomes from Hdh140Q/140Q knock-in mice have altered protein levels, novel sites of methionine oxidation, and excess glutamate release after stimulation. Journal of Huntington's disease. 2013;2(4):459-75.

217. Van Raamsdonk JM, Pearson J, Rogers DA, Lu G, Barakauskas VE, Barr AM, et al. Ethyl-EPA treatment improves motor dysfunction, but not neurodegeneration in the YAC128 mouse model of Huntington disease. Experimental neurology. 2005;196(2):266-72.

218. Metz GA, Whishaw IQ. Cortical and subcortical lesions impair skilled walking in the ladder rung walking test: a new task to evaluate fore- and hindlimb stepping, placing, and co-ordination. Journal of neuroscience methods. 2002;115(2):169-79.

CHAPTER 2

GM1 IS A POTENTIAL DISEASE MODIFYING THERAPY IN HUNTINGTON DISEASE

2.1 Introduction

HD is a dominantly inherited neurodegenerative disease that is characterized by a triad of motor, cognitive and psychiatric symptoms (219). The characteristic combination of motor and non-motor symptoms in HD results, at least in part, from the progressive dysfunction of cortico-striatal circuits (220) and the degeneration of large pyramidal projection neurons in the cortex and medium spiny neurons (MSN) in the striatum (41, 62, 221).

These underlying genetic cause consists in expansion in the number of CAG trinucleotide repeats in the first exon of the gene that codes for HTT (1). This mutation results in the expression of a mHTT protein with an abnormally long polyQ stretch, which is prone to misfold into toxic species and to aggregate (222). Expression of mHTT affects a plethora of cellular and molecular pathways prior to causing cell death (62) . Among these is the synthesis of gangliosides (137-139), sialic acid-containing glycosphingolipids that are found in relatively high concentrations in the normal brain (148). One ganglioside in particular, GM1, was found to be consistently decreased across HD cell and animal models (137-139), as well as in fibroblasts from patients (137).

Gangliosides play important functions in the nervous system, ranging from modulation of cell signaling and neurotransmission, to regulation of calcium homeostasis, cell adhesion and myelin-axon interaction (148, 150). This suggests that impaired synthesis of gangliosides might contribute to disease pathogenesis and progression in HD, and that restoring normal brain ganglioside levels might have beneficial effects.

In previous studies we showed that administration of exogenous GM1 improves HD cell survival *in vitro* (137), and corrects motor dysfunction in YAC128 mice (212). These therapeutic effects

are accompanied by phosphorylation of HTT at amino acid residues Ser13 and Ser16 (212, 223), a post-translational modification that decreases mHTT toxicity in cell and animal models (213, 224, 225). These data suggest that GM1 might have disease-modifying activity in HD and might be able not only to reduce motor symptoms, but also to correct the underlying dysfunctions and to slow down neurodegeneration (226). Our previous studies in YAC128 mice did not address potential effects of GM1 on neuropathology and HD disease course, in part because the YAC128 mouse model displays a subtle neurodegenerative phenotype that appears late in the disease course (227).

To investigate potential disease-modifying effects of GM1 on HD brain neuropathology, in this study we used R6/2 mice, an HD model with an accelerated phenotype and widespread neurodegeneration due to the overexpression of a toxic N-terminal fragment of mHTT (61). We also tested the effects of GM1 on knock-in Q140 mice (69), according to rigorous guidelines that recommend the use of at least two different genetic models of HD for preclinical testing of therapeutic compounds, in order to overcome potential limitations and artifacts specific to individual models and/or genetic background (75, 76).

In this study, we show that in addition to YAC128 mice (212), the beneficial effects of GM1 extend to R6/2 and Q140 mice. We further demonstrate that GM1 decreases neuropathology and slows down neurodegeneration in the R6/2 model. These effects are accompanied by a reduction in the levels of soluble mHTT and insoluble protein aggregates in both models. Altogether, our data support the hypothesis that GM1 has disease-modifying activity and is a novel potential treatment for HD.

2.2 Materials and Methods

2.2.1 Animal Models

Q140 knock-in mice expressing endogenous levels of full-length mHTT with approximately 140Q repeats (69) were kindly donated by Cure HD Initiative (CHDI) and maintained on C57Bl/6J background in our animal facility. Throughout the thesis I will use the term Q140 mice to refer to both Q7/Q140 heterozygous and Q140/Q140 homozygous mice, carrying one or two mutant *Htt* alleles, respectively, while WT littermates will be referred to as Q7/Q7 (or simply WT) mice. R6/2 mice overexpressing human HTT exon 1 (120Q ±5) (61) (B6CBA-Tg (HDExon1)62Gpb/3J) were obtained at 5 weeks of age from the Jackson Laboratory (stock number 006494) and used for experiments between 5 and 12 weeks of age. For practical reasons and to minimize the number of animals used we did not include WT Q7/Q7 mice treated with GM1 in our experiments. Previous experiments had shown that GM1 does not have any toxic effects and that it also does not affect motor performance in WT mice (C57Bl or FVB strain). All mice were maintained in our animal facility at the University of Alberta on a 14-10h light dark cycle (lights on at 0500h) in a temperature and humidity-controlled room. All procedures on animals were approved by the University of Alberta's Animal Care and Use Committee and were in accordance with the guidelines of the Canadian Council on Animal Care.

2.2.2 Antibodies

Primary antibodies used were: rabbit anti-horse ferritin (1:400; Sigma F6136), rabbit anti-GFAP (glial fibrillary acidic protein) (1:1000 for immunohistochemistry and 1:500 for immunobloting; Dako, Z0334), rabbit anti-Iba1 (ionized calcium binding adapter molecule 1 (1:250 for

immunohistochemistry and 1:500 for immunoblotting; Wako, 019-19741), biotin-conjugated mouse anti-Neuronal Nuclei (NeuN) (1:250; Sigma MAB377B), mouse anti-DARPP32 (1:2,000 611520 BD Biosciences), rabbit anti-pThr34-DARPP32 (1:2,000; 5393 Cell Signaling), mouse anti-alpha-tubulin (1:20,000; T5168 Sigma), rabbit anti-HTT N-18 (1:5,000; kindly provided by Dr. Truant, McMaster University), mouse anti-HTT MW8 (1:2,000; DSHB), mouse anti-HTT clone EM48 (1:1,000; mAB5374 Millipore), rabbit anti-HTT PW0595 (1:2,000; Enzo) and mouse anti-HTT (1:10,000; mAB2166 Millipore). Secondary antibodies used were donkey anti-rabbit A488 (1:200; Invitrogen A21206), goat anti-rabbit biotinylated (1:200; Vector labs BA-1000), goat anti-rabbit IRDye 680 (1:20,000 for immunoblotting and 1:40,000 filter trap assay; Li-Cor Biosciences 926-32221), goat anti-rabbit IRDye 800CW (1:20,000 for immunoblotting and 1:40,000 filter trap assay; Li-Cor Biosciences 926-32211), goat anti-mouse IRDye 680 (1:20,000 for immunoblotting and 1:40,000 filter trap assay; Li-Cor Biosciences 926-68070) and goat anti-mouse IRDye 800CW (1:20,000 for immunoblotting and 1:40,000 filter trap assay; Li-Cor Biosciences 926-32210).

2.2.3 Drugs

For surgery, Q140 and R6/2 mice were anaesthetized using 4% isoflurane in oxygen (Pharmaceutical partners of Canada, DIN 02237518) for induction and 1-2% isoflurane in oxygen for maintenance of surgical plane. 5 min prior to induction both mouse strains were administered butorphanol (0.1 mg/kg) diluted in isotonic saline solution to decrease anxiety and to provide analgesia during surgery. Immediately after surgery mice received an injection of meloxicam (2 mg/kg) diluted in isotonic saline. Mice were monitored for signs of pain for the three days following surgery and given a daily injection of meloxicam if required. Butorphanol

and meloxicam were administered by subcutaneous injection using a 26-gauge needle in a volume of 10 ml/kg.

2.2.4 Chronic intraventricular administration of GM1

Administration of GM1 was performed as previously published (212), Briefly, a microcannula (Alzet brain infusion kit 3, 1-3 mm) was stereotaxically implanted into the right lateral ventricle of anaesthetized mice (stereotaxic coordinates:1.2 mm right lateral and 0.6 mm posterior to bregma, 3 mm deep). The microcannula was connected to an osmotic pump (Alzet mini-osmotic pump model 2004, or 2006) implanted subcutaneously on the back of the mouse. Animals were infused with a solution of 3.6 mM semi-synthetic GM1 provided by Seneb BioSciences INC. (Holliston, MA) or artificial cerebrospinal fluid (aCSF, Harvard Apparatus 59-7316) into the right cerebral ventricle at constant rate (0.25 µL/h) for 28 or 42 days.

2.2.5 Motor Behaviour Test

Treatment with GM1 or aCSF (vehicle) started between 6 and 8 months of age for Q140 mice, and between 6 and 8 weeks of age for R6/2 mice. All mice were housed individually through the 28 (for R6/2 mice) or 42 days (for Q140 mice) of treatment. R6/2 mice and WT littermates used in our studies were all males. Both male and female Q140 mice (heterozygous Q7/Q140 and homozygous Q140/Q140 combined) were used, in three separate experiments. Data from heterozygous and homozygous Q140 mice were combined for all behaviour tests where the two genotypes showed comparable performance. The only exception was the ladder test, where female heterozygous mice showed no impairment compared to Q7/7 littermates and were therefore excluded from the analysis. Data from male and female Q140 mice were combined for all behaviour tests where the two genders showed comparable performance. The only exception

was the rotarod test, where male mice showed no impairment compared to Q7/7 littermates and were therefore excluded from the analysis.

Behavioural testing was conducted in the light phase of the light cycle between 0800 h and 1800 h. In all behavioural training and testing sessions, mice were allowed to acclimate to the testing room for 1 h. For each experimental cohort, each behavioural test was performed on the same day post-surgery. All experiments were performed by experimenters who were blind to animal genotype and treatment.

All equipment was cleaned with 70% ethanol after each test and prior to testing with the next animal.

Rotarod:

Mice were tested in three consecutive trials of 3 min each, with 1 min rest in between trials, at fixed speed (12 revolutions per minute (RPM)). The time spent on the rotarod in each of the three trials was averaged to give the overall latency to fall time for each mouse. A similar training protocol was used to test mice on an accelerating rotarod (4-40 RPM in 2 min).

Narrow beam:

Mice were placed at the extremity of a 100-cm-long wooden narrow beam (0.75 cm wide, suspended 30 cm above the floor) and allowed to traverse the beam from one extremity to the other three times. Animals' performance was recorded with a video camera and footfalls, body balance, and motor coordination were analyzed using a footfall scoring system (212).

Horizontal ladder:

Mice were scored as they spontaneously walked along a horizontal ladder with variable and irregular spacing between rungs. In each test session, mouse performance was evaluated using an established footfall scoring system (218).

Open field:

Distance travelled in an open field arena was used as an additional measure of motor dysfunction. R6/2 and Q140 mice were placed in the open field apparatus, a clear 90 cm X 90 cm Plexiglas box for 5 min. The distance travelled by R6/2 mice in the last 3 min was determined from video-recordings, by tracing the path travelled onto a grid. The distance travelled by Q140 mice in 5 min was analyzed using EthoVision XT tracking software.

Climbing test:

The climbing test was performed according to (228) with slight modifications. Briefly, mice were placed in perforated wire containers for 5 min and the number of climbs (four paws on the side of the container) and rears (two paws on the side of the container) were counted. Only rearing behavior was analyzed as climbing behavior was extremely rare in all groups.

Kinematics analysis:

Recordings from each animal were made 31 days after beginning of the treatment. Before recording, each animal was anaesthetized with isoflurane, and the location for the iliac crest on both hind legs was labeled with a spot of white paint. After full recovery from anesthesia, the mice where placed into a custom-made Plexiglas walkway (length: 90 cm, width: 5 cm, height: 13 cm) with a mirror underneath that enabled monitoring lateral and ventral views of their free walking across the walkway. Two video recordings were captured for each walking direction

using a high-speed video camera (200 frames per second) to monitor kinematic patterns. Video data were recorded directly to a computer hard drive, and analyzed offline using a customized LabVIEW program. Footfall diagrams were obtained from identifying paw contact and paw lift on the ventral view, and these data were used to calculate stride duration (time between two consecutive paw contact with the ground), stance-to-stride ratio, and coupling between limbs during locomotion for each mouse (229). Stick diagram representation of the hind limb based on the iliac crest marker and the paw captured from the lateral view were used to illustrate the step cycle of the mouse. The distance from the iliac crest marker to the ground was used to evaluate the ability of the mouse to support its weight.

2.2.6 Body weight and food intake

Body weight was measured prior to the start of treatment and three times after beginning of treatment, at days 10, 18 and 28. Food intake was monitored daily from day 7 of treatment until day 21. Each mouse was provided with 10 g of chow diet/day and food was replaced daily, so that food consumption was not restricted. Food remaining in the cage was weighted daily at 1700.

2.2.7 Tissue collection and processing

Tissue for histological analysis was collected after 28 days of treatment from 10 week old R6/2 mice and WT littermates. Mice were euthanized by cervical dislocation and their brains were immediately removed and flash frozen in isopentane (2-methylbutane, Acros Organics 12647-0010) pre-refrigerated at -80°C and kept on dry ice. Brains were then stored at -80°C until cryosectioning. Twenty μm-thick serial coronal sections were obtained using a cryostat and thaw mounted onto charged slides (Superfrost Plus, Fisher 12-550-15). Fifteen slides with 12-13

sections each were prepared for each brain. On each slide, serial sections represented brain areas that were approximately 260 μm apart. For each brain, less than 10 sections were lost during cryosectioning. Slides were left to dry at room temperature (RT) overnight, prior to be stored at -20°C. All sections were post-fixed in formalin (10% buffered formalin phosphate, Fisher) for 5 min at RT immediately prior to use.

Tissue lysates for immunoblotting were prepared at the end of the 28 day or 42 day treatment period 12 week old R6/2 mice and 7-9 month old Q140 mice were euthanized by cervical dislocation and their brains were immediately dissected to collect left and right hemisphere striatum and cortex. Dissected tissue was flash frozen in liquid nitrogen immediately after collection for biochemical analysis. Samples used for filter trap and western blots were immediately homogenized in ice-cold lysis buffer (20 mM Tris, pH 7.4, 1% Igepal CA-630, 1mM EDTA, 1 mM Ethylene glycol-bis(β-aminoethylether)-N,N,N',N'-tetraacetic acid (EGTA), 50 μM MG132, 1X cOmplete protease inhibitor cocktail and 1X PhosStop phosphatase inhibitor cocktail, Roche). Samples were sonicated two times for 10 seconds each at power 2 using a Sonic Dismembrator Model 100.

2.2.8 Cell count and volumetric analyses

Brain volume and corpus callosum areas were measured from photomicrographs taken with a 2X objective. Striatal volume and white matter tract analysis occurred from photomicrographs taken with a 4X objective. Photomicrographs taken using 2X and 4X objectives were taken using Olympus BX60 light microscope coupled to a Cool Snap Image pro colour camera. Striatal neuron counts, GFAP and Iba1 positive area were analyzed from the 20X objective taken using a Zeiss Observer.Z1 microscope coupled to a Zeiss AxioCam MRm camera. Images for ferritin

analysis were composite images from the entire striatum taken with the 20X objective on Leica microsystems CMS GmbH DM4000 B LED microscope using a Leica DFC 450 camera. All images were processed and analyzed using the NIH ImageJ software-based image processing package "Fiji". When multiple photographs were required for analysis, composite images were created using the "MosaicJ" plugin. White matter tract number and volume were analyzed using composite images transformed to 8-bit images.

Volumes were calculated using the Cavalieri principle (230). For brain volume, the outside of the brain was traced from 2X photomicrographs of eriochrome-stained sections to determine brain volume. Brain volume was calculated only from 1.98 mm to bregma up to -2.3 mm to bregma. The striatum (bregma 1.98 mm to -2.3 mm), was manually traced from 4X photomicrographs of NeuN-stained sections using the "Mouse brain in stereotaxic coordinates" by Franklin and Paxinos for reference. The globus pallidus was excluded from the analysis of striatal volume. For corpus callosum volume and white matter tracts, the corpus callosum, (bregma 0.02 mm up to -2.3 mm), was manually outlined from the 2X objective photomicrographs of sections stained with eriochrome cyanine, in order to calculate the area in each composite image. The striatum was manually outlined and set as a region of interest (ROI). A threshold was set for the images, then particle analysis was performed filtering by size (>80 μm^2). Values for the area of individual tracts and total white matter area per section were obtained.

Only volumes for the left brain hemisphere (LH) is shown, to avoid confounding factors such as inflammation and presence of scar tissue around the infusion kit implanted into the right hemisphere (RH). Therefore, the RH was excluded from all volumetric and cell count analyses.

For cell count and calculation of percent area stained, slides were photomicrographed using a 20X objective and transformed to 8-bit images. A threshold was set and images were transformed to binary images. For ferritin analysis the threshold was set at 126, and for GFAP analysis at 31, with slight fluctuations where background staining was higher or lower than average. Particles with an area < 25μM were excluded from the analysis. The area immunostained (% of total) was calculated by dividing the total area stained by the area of the photomicrograph analyzed. For NeuN the threshold was calculated based on the intensity of the background staining in the image and size filtering was set to 80 pixels, to exclude non-specific background staining. The number of NeuN-positive cells was counted in each section, and average number of NeuN-positive cells in the striatum was calculated by dividing the number of cells counted by the number of images analyzed. A minimum of 16 images was analyzed per brain. The total number neurons in the striatum was also estimated by multiplying the neuronal count by the total striatal volume. For both cell density and cell count the region between 1.98 mm from bregma and 0.02 mm from bregma was analyzed. The number of Iba1-positive cells was counted manually and, therefore, no threshold was used. Cell density was calculated by dividing the total number of Iba1-positive cells by the area of the image.

For standardization purposes, 1/10 of the images were scored by a second experimenter who was blind to genotype and treatment, in order to ensure inter-counter reliability

2.2.9 Immunofluorescence staining and immunohistochemistry

For immunofluorescence staining, brain sections were blocked with 0.2% Triton X-100 (Fluka) in Universal Blocker (DakoCytomation protein block X0909) for 1 h at RT. Blocking solution was tapped off and primary antibody was added (GFAP or Iba1) and incubated overnight in a humidity chamber at 4°C. Sections were washed 3 times for 4 min each with phosphate-buffered

saline (PBS), before incubation with secondary antibodies for 30 min at RT. Once incubation with the secondary antibody was complete sections were washed 3 times for 4 min each with PBS and then twice for 2 min each in tap water and twice for 2 min each in milliQ water. Sections were mounted using ProLong gold mounting media (P36934 life technologies).

For immunohistochemistry, sections were dehydrated by washing in a series of alcohol dilutions, 70% ethanol, 90% ethanol, 100% ethanol (2 min each) and cleared with xylene in order to remove excess fats. Slides were then rehydrated starting from 100% ethanol. After rehydration endogenous peroxidase activity was quenched in 1% hydrogen peroxide in 50% methanol for 10 min and then blocked in 0.3% Triton (for NeuN staining) or 0.2% Triton (for ferritin staining) in Universal Blocker (DakoCytomation protein block X0909) for 20 min (NeuN) or 1 h (ferritin) at RT, before incubating with the primary antibody overnight at 4°C. Primary antibodies were followed by biotinylated secondary antibodies for 30 min at RT, where the primary antibody was not conjugated to biotin. The signal was amplified by incubation with the avidin biotin complex (ABC) system for 30 min at RT and then detected with diaminobenzidine (DAB) (Sigma D 5905). Slides were mounted with the organic mounting agent, Permount (Fisher SP15-100).

2.2.10 Eriochrome staining

To assess the morphology and size of the corpus callosum and the white matter tracts in the striatum, brain sections were stained with Eriochrome Cyanine R as previously described (231) with minor modifications. Briefly, slices were air-dried for 30 min and stained with Eriochrome Cyanine R solution (0.4% $FeCl_3$ w/v, 0.16% Eriochrome Cyanine R w/v and 0.4% H_2SO_4 v/v) for 1h at RT. Sections were washed in ddH_2O for 1 min. Sections were differentiated in 0.5% NH_4OH (v/v) for 10 sec. Slides were submerged in a series of alcohol dilutions, 70% ethanol,

90% ethanol, 100% ethanol followed by isopropanol and xylene to dehydrate sections prior to mounting with Permount (Fisher Scientific. Waltham, MA).

2.2.11 Immunoblotting

After sonication, brain lysates were centrifuged at 20,000 x g for 10 min at 4°C. Protein concentration in the supernatants was measured using the bicinchoninic acid (BCA) assay. For standard protein analysis 30 μg of proteins were electrophoresed on a 4-20% SDS-polyacrylamide gel and transferred onto an Immobilon-FL polyvinylidene fluoride (PVDF) membrane (Millipore). For HTT immunoblotting, proteins were separated in 4-12% SDS-polyacrylamide gels and transferred overnight onto an Immobilon-FL PVDF membrane in transfer buffer containing 0.01% sodium dodecyl sulfate (SDS) and 16% methanol. All membranes were then blocked with 5% bovine serum albumin (BSA) in tris-buffered saline with tween (TBS-T) and incubated overnight at 4°C with primary antibodies. After 3 X 5 min washes, membranes were incubated with the appropriate IRDye secondary antibody (1:40,000, LI-COR Biotechnology) for 45 min at RT. Membranes were washed 3 X 10 min in TBS-T and 1 X 10 min in TBS. Infrared signal was acquired and quantified using the Odyssey Imaging System.

2.2.12 Filter retardation assay

Filter retardation assay was performed as described in (232), with slight modifications. Briefly, 30 μg of protein lysates were diluted in PBS, denatured and reduced by adding 2% SDS and 100 mM DTT to the lysis buffer, followed by heating at 98°C for 10 min. Samples were filtered through a cellulose acetate membrane (0.2 μm pore size, Sterlitech) in a Bio-Dot microfiltration unit (Bio-Rad). Wells were washed twice with 200 μl PBS. After drying for 30 min, membranes were washed twice with 2% SDS in PBS and then blocked with 5% BSA in TBS-T followed by

incubation with anti-HTT antibodies: N-18 (1:5,000; gift from Dr. Truant, McMaster University), MW8 (1:2,000; DSHB), EM48 (1:1,000; mAB5374 Millipore) or PW0595 (1:2,000; Enzo). IRDye secondary antibodies (LI-COR Biotechnology) were used at 1:20,000 for 1 h at RT. Infrared signal was acquired and quantified using the Odyssey Imaging System.

2.2.13 Statistical analysis

All statistical analyses for behaviour tests of R6/2 mice were performed using linear mixed effect regression model with 95% confidence intervals calculated at each time point. Analysis of food consumption was analyzed using a repeated measures two-way analysis of variance (ANOVA) followed by Bonferroni post-tests. One-way ANOVA followed by Bonferroni post-tests was used to compare treatment groups in behavioural experiments involving Q140 mice, except for the nesting test, for which a Kruskal-Wallis test with Dunn post-test was applied. For kinematics data, separate three-factor ANOVAs (GROUP [3] x SEX [2] x PUMP [2]) were used to determine any effects of the sex (SEX), the side at which the pump hang (PUMP) and the treatment group (GROUP) on the different kinematic measures. If a significant interaction between SEX (or PUMP) was found, Tukey's post-hoc comparisons were used for each SEX (or PUMP) separate case, to test the effect of the treatment separately. As indicated in the figure legends, the two-tail T-test was used when two groups were compared to each other. One-way ANOVA followed by Tukey's multiple comparison test was used when three groups were compared. Two-way ANOVA followed by Holm-Sidak post-test was used for comparisons including two genotypes and two treatments. All comparisons were performed using a statistical significance level of 0.05.

2.3 Results

2.3.1 Treatment with GM1 improves motor performance in R6/2 and Q140 mice

The effects of GM1 on motor dysfunctions induced by mHTT were analyzed in symptomatic R6/2 and Q140 mice. Different batteries of motor tests were used for the two models, to take into account the much more severe motor phenotype of R6/2 mice compared to Q140 mice.

In the horizontal ladder, a test of skilled motor control (218), R6/2 mice performed significantly worse than their WT littermates over four testing days (Fig. 2.1A). When individual time points were analyzed, GM1 significantly improved performance of R6/2 mice at every time point, measured ($p \leq 0.01$, day 7 95% CI: -19.4 to -0.1; day 11 95% CI: -19.9 to -1.6; day 15 95% CI: -21.1 to -2.5, day 21 95% CI: -23.9 to -2.7) as compared to cerebral spinal fluid (CSF)-treated R6/2 mice.

For Q140 mice, results are shown only for day 28 of treatment, as no effect of GM1 was observed prior to that. No Q7/7 mice were treated with GM1 as in previous experiments GM1 did not have any effect on motor performance in WT mice. Data for homozygous Q140/Q140 males and females and heterozygous Q7/Q140 males were combined, as these animals performed similarly in this and other tests. However, heterozygous Q7/140 female mice were excluded from the analysis, as in this test they showed similar performance as to Q7/7 littermates. At day 28 there was a main effect of treatment ($F_{2,73} = 21.19$, $p \leq 0.0001$). Vehicle-treated Q140 mice had a significantly higher error score than Q7/7 animals ($p < 0.0001$). GM1 significantly reduced the error score of Q140 mice ($p = 0.015$), although mice still made more errors than Q7/7 littermates ($p = 0.017$) (Fig. 2.1A).

To test the effects of GM1 on general exploratory behavior, we placed mice in an open field arena for either 5 (for R6/2 mice) or 30 minutes (for Q140 mice). R6/2 mice performed significantly worse than WT littermates (Fig. 2.1B). The average distance travelled by vehicle-treated R6/2 mice decreased at an estimated rate of 81.39 cm per day of testing (95% CI: 9.16 to 81.39) as compared to WT animals, mirroring progressive motor impairment. GM1 administration prevented this decline in performance and increased significantly the distance travelled by R6/2 mice at each time point tested compared to vehicle-treated R6/2 animals (day 7 95% CI: 79.1 to 673.2, $p \leq 0.001$; day 11 95% CI: 68.3 to 828.2, $p \leq 0.001$; day 15 95% CI: 23.8 to 1017.0 $p \leq 0.01$; day 21 95% CI: 85.6 to 1171.6, $p \leq 0.01$).

Similar effects of treatment were obtained in Q140 mice ($F_{2,76} = 15.58$, $p < 0.0001$). Vehicle-treated Q140 mice travelled less than Q7/7 littermates ($p < 0.0001$). GM1 restored performance to normal ($p < 0.01$ compared to vehicle-treated Q140; $p = 0.2$ compared to Q7/Q7) (Fig. 2.1B). In this test we did not detect any differences between male and female or homozygous and heterozygous animals for distance travelled (data not shown), therefore data for these groups were pooled and analyzed together.

To determine the effects of GM1 on motor coordination and strength, we tested mice on the fixed speed rotarod (12RPM). Vehicle- and GM1-treated R6/2 mice had severe deficits on this motor test. The R6/2 mice showed a decrease in latency to fall over time that was estimated at 3.07 seconds per day (95% CI: 1.13 to 5.02). GM1 did not improve the performance of R6/2 mice.

Vehicle-treated Q140 female mice also showed a significant impairment in performance on the fixed rotarod compared to Q7/7 ($p = 0.0006$). Male Q140 mice did not show a deficit on this test

and were excluded from this analysis. In females Q140 there was a main effect of treatment ($F_{2,36}$ = 8.992, p=0.0007) and Q140 mice treated with GM1 significantly improved motor performance as compared to vehicle-treated Q140 mice (p=0.0368). After treatment with GM1, Q140 mice performed as well as CSF-treated Q7/7 littermates (p>0.9).

To analyze motor coordination and spontaneous activity, Q140 mice were placed into mesh cylinders and the number of rearing and climbing events were scored over a 5 minute period (228). Climbing events were very rare and consequently results shown are only for rearing behaviour (Fig. 2.1D). A main effect of treatment ($F_{2,79}$ = 9.91, p=0.0001) was observed on the climbing test which coincided with a decrease in rearing behaviour in vehicle-treated Q140 mice as compared to Q7/7 littermates (p=0.0013). GM1 improved motor performance to WT levels (p=0.0005 compared to vehicle-treated Q140 mice; p>0.9 compared to Q7/7). R6/2 mice displayed very little spontaneous activity in the open field and consequently were not analyzed for climbing behaviour.

2.3.2 Differences in walking spatio-temporal parameters across groups

To study the effect of GM1 administration on walking in Q140 mice, we measured various spatio-temporal parameters of gait while mice walked freely across a walkway (Fig. 2.2). We found visible changes in walking patterns across mice from the three groups analyzed (i.e., Q7/7 CSF, Q140 CSF and Q140 GM1). This difference was not consistent between male and female mice and an effect of interaction of the sex of the animal (SEX) on the height of the iliac crest (GROUP x SEX, p=0.048) -a measurement of weight support - was detected (Fig 2.2B). CSF-treated Q140 male mice walked with a significantly reduced (p=0.007) height of the iliac crest compared to Q7/7 male mice. Decreased iliac crest height was reversed by the administration of

GM1 (p=0.007 compared to CSF-treated Q140; p=0.39 compared to Q7/7). Thus, weight support was restored to normal after administration of GM1 (Fig. 2.2A and B).

The average stride duration, a measure of gate speed, was also significantly different between groups (Fig. 2.2C). CSF-treated Q140 mice walked with a significantly longer (p=0.007) stride duration (i.e., slower gait) compared to CSF-treated Q7/7 littermates, while GM1-treated Q140 mice walked with a significantly shorter (p=0.014) stride duration (i.e., faster gait) compared to Q140 CSF mice. After treatment with GM1 stride duration was similar for GM1-treated Q140 mice and Q7/7 mice (p=0.719).

2.3.3 GM1 increases survival and body weight in R6/2 mice

Survival and body weight are frequently used as indices of treatment efficacy in R6/2 mice (233). CSF-treated R6/2 mice showed a decrease in body weight over time (Fig 2.3A) that was significantly attenuated by administration of GM1 for 21 days (interaction: $F_{1,65}$ = 6.43, p<0.01). The increase in body weight observed after GM1 treatment was not the result of increased food intake, as GM1 did not alter food consumption (Fig 2.3B). Furthermore, GM1 did not induce changes in the weight of WT mice, suggesting that the effect observed in R6/2 mice is the result of overall improved mouse health conditions.

GM1 administration also resulted in a trend towards increased lifespan of R6/2 mice, although results did not reach statistical significance (p=0.08) (Fig 2.4).

2.3.4 GM1 slows down the neurodegenerative process in R6/2 mice

To assess the effects of GM1 on brain neuropathology and neurodegeneration we focused on R6/2 mice, as Q140 mice do not present changes in striatal volume and striatal neuronal number at the age analyzed in our experiments (228).

We calculated the volume of the left brain hemisphere in the region where the striatum is present (bregma 1.98 to -2.3). A main effect of genotype ($F_{1,41} = 44.06$, $p<0.0001$) was present and the observed 16% decrease in volume in CSF-treated R6/2 mice as compared to WT mice ($p<0.0001$) is in line with the original description of the R6/2 mice (61). GM1-treated R6/2 mice had only an 11% decrease in brain volume as compared to WT mice. This reduction of volume loss between in R6/2 mice that received was significant ($p<0.05$) and a main effect of treatment was present ($F_{1,41}=14.33$, $p=0.0005$) (Fig. 2.5B). In addition to brain volume, we also measured brain weight in CSF- and GM1-treated R6/2 mice, as well as in a group of 6 and 8 week-old untreated R6/2 mice. Brain weight was reduced progressively from 6 to 10 weeks of age (effect of age $F_{3,33}=10.86$, $p<0.0001$; HD 6 weeks vs HD 8 weeks $p=0.0426$; HD 8 weeks vs HD CSF $p=0.0133$) and administration of GM1 maintained brain weight of 10 week mice at the level observed at 8 weeks of age without treatment (HD 8 week vs HD GM1 $p>0.9$) (Fig. 2.5A). In other words, treatment with GM1 significantly slowed down progressive neurodegeneration in the R6/2 mice.

The striatum is the earliest and most severely affected region of the brain in patients (34) as well as in R6/2 mice (228). R6/2 mice had a significant reduction in striatal volume, as compared to WT mice (effect of genotype: $F_{1,40} = 6.39$, $p=0.0155$) and striatal volume was increased by administration of GM1 (Fig. 2.6A) (effect of treatment: $F_{1,40} = 152.4$, $p<0.0001$). We also measured a decrease in NeuN$^+$ cells in the striatum of vehicle-treated R6/2 mice (Fig. 2.6B) as compared to WT littermates ($p=0.0001$). GM1 significantly increased the number of NeuN$^+$ cells in the striatum (effect of treatment: $F_{1,35} = 21.0$, $p<0.001$), and, interestingly, the neuronal count in GM1-treated R6/2 mice was not significantly different from GM1-treated WT mice ($p=0.6267$).

In recent years it has been described that in addition to the well-established grey matter changes in HD there is also a progressive decrease in white matter (42). The corpus callosum is the most important commissure in the brain and its volume is reduced in HD patients (42). Corpus callosum volume loss is also mirrored in various mouse models including YAC128 (227) and R6/2 mice (234). In 10 week old CSF-treated R6/2 mice we confirmed atrophy of the corpus callosum, which was completely reverted by the administration of GM1 (interaction: $F_{1,42} = 4.26$, $p=0.0451$) (Fig. 2.6C), such that GM1-treated R6/2 mice were not significantly different from WT mice ($p=0.9553$).

In HD, impaired communication between the cortex and striatum occurs due to degeneration in the cortical-striatal white matter tracts (235). A significant reduction in the volume of these white matter tracts was indeed observed in 10 week-old R6/2 mice as compared to WT mice (Genotype: $F_{1,43} = 54.01$, $p<0.0001$) (Fig. 2.6D). In GM1-treated R6/2 mice cortico-striatal white matter tracts in the left striatum were significantly larger as compared to CSF-treated R6/2 mice ($p=0.0473$), although their volume was still smaller than in WT littermates ($p=0.0001$).

2.3.5 GM1 increases striatal markers in HD mice

While volume of the striatum correlates well with disease progression, it does not provide information on the functionality of the striatum. As a surrogate measure of striatal function, we used expression and phosphorylation levels of DARPP32, a key regulator of DA signaling in the striatum (236) that is highly expressed by GABAergic medium spiny neurons, the most affected population of cells in HD (237). Loss of DARPP32 is indicative of disrupted DA signaling (237) and occurs in both R6/2 (61) and Q140 mice, in the latter even prior to changes in striatal volume or striatal cell number (228) (Fig. 2.7). In R6/2 mice we found that DARPP32 expression is greatly reduced in R6/2 mice as compared to WT mice (Genotype: $F_{1,21}=134.8$, $p<0.001$). GM1

decreased DARPP32 levels in WT (Interaction: $F_{1,21}=4.61$, $p=0.0435$). T-test analysis also shows

an increased amount of DARPP32 in GM1-treated R6/2 mice (Fig. 2.8) (T-test, $p=0.0009$), but

was not able to restore normal levels, which were significantly higher in WT mice. pDARPP32

T34 was not analyzed in R6/2 mice as the signal was too low, making quantitation unreliable. In

Q140 mice, as expected, both DARPP32 and pDARPP32 levels were lower than in Q7/7

littermates (DARPP32: heterozygous Q7/Q140, $p=0.0136$; homozygous Q140/140, $p=0.0014$;

pDARPP32: heterozygous Q7/Q140, $p=0.0390$, homozygous Q140/140, $p=0.0118$) (Fig. 2.7).

GM1 increased expression of DARPP32 and pDARPP32 to WT levels in heterozygous Q7/Q140

mice (DARPP32: $p=0.0419$, pDARPP32: $p=0.0118$), but not in homozygous Q140/140 mice

(DARPP32: $p=0.9149$, pDARPP32: $p=0.8902$).

2.3.6 GM1 reduces ferritin expression in R6/2 mice

Dysregulation of iron metabolism and ferritin expression have been implicated in many

neurodegenerative diseases including HD (172, 238). Dysregulation of iron homeostasis is

detrimental to cells as free iron can produce free radicals and contribute to oxidative stress (238).

In response to an increase in iron levels, particularly in the extracellular space, the expression of

the iron-binding protein ferritin is increased to scavenge unbound iron. Our studies confirmed

previous reports (239) that expression of ferritin is significantly increased in the brains of CSF-

treated R6/2 mice as compared to CSF-treated WT mice ($p<0.0001$). However, ferritin levels

were significantly decreased in R6/2 mice treated with GM1 (Interaction: $F_{1,14} = 14.49$,

$p=0.0019$) (Fig. 2.9) to levels similar to WT ($p=0.2930$).

2.3.7 Effects of GM1 administration on astrocytic and microglia markers

We next measured the effects of GM1 on astrocytic and microglial markers. Alterations in the number and activation state of astrocytes and microglia as well as in the expression of astrocytic and microglial markers are common among many neurodegenerative conditions, including HD, and are usually associated with neuroinflammation (240, 241). To characterize potential neuroinflammation in R6/2 mice, we assessed expression of the astrocytic marker GFAP and the microglial marker Iba1 by immunoblotting and immunohistochemical analyses. Striatal GFAP immunostaining (% immunopositive area) and expression levels were similar across groups (Fig. 2.10A). There was however a main effect of genotype on GFAP immunostaining in the cortex ($F_{1,22}$=4.32, *p*=0.0494), indicating a higher number and/or extension of GFAP$^+$ cells in both CSF- and GM1- treated R6/2 mice. Western blotting analysis, however, showed significantly decreased expression of GFAP in CSF-treated R6/2 mice compared to WT (Genotype: $F_{1,24}$=13, *p*=0.0014), which was reverted by administration of GM1 (*p*=0.0283) (Fig. 2.10B). This apparent discrepancy between immunohistochemistry and immunoblotting data might reflect quantitative (changes in the number of cortical astroglia) and qualitative (differential expression of GFAP) changes in R6/2 mice and upon GM1 treatment.

In the striatum a significant reduction in Iba1$^+$ cell density was observed in GM1- treated WT mice (*p*=0.0335) as well as in CSF-treated R6/2 mice as compared to CSF-treated WT mice (*p*=0.0326) (Interaction $F_{1,36}$ = 8.69, *p*=0.0056) (Fig. 2.10C). GM1-treated R6/2 mice were not significantly different from any other treatment group. Immunoblotting analysis of Iba1 expression showed results that were generally in line with the immunohistochemical analysis, with CSF-treated R6/2 mice and GM1-treated WT mice both showing decreased Iba1 expression as compared to CSF-treated WT mice, but also GM1-treated R6/2 mice (Interaction: $F_{1,23}$=20.62,

p=0.001). In the cortex, we observed an effect of genotype, with R6/2 mice (both GM1- and CSF-treated) displaying a higher number of Iba1$^+$ cells as compared to WT littermates (effect of genotype: $F_{1,36}$ = 5.658 p=0.0228). Post-test analysis revealed that the number of Iba1$^+$ cells was slightly, but significantly, increased in GM1-treated R6/2 mice compared to all other groups. However, protein levels of Iba1, as measured by immunoblotting, were significantly decreased in R6/2 mice regardless of treatment (Genotype: $F_{1,24}$=13.32, p=0.232), suggesting the presence, in these mice, of a slightly higher number of microglial cells, but with decreased expression of Iba1 (Fig. 2.10D).

2.3.8 HTT levels are reduced by administration of GM1

To determine if GM1 is able to affect the amount of HTT, we immunoblotted cortical and striatal lysates of Q140 mice to measure the amount of wtHTT and mHTT present in each area after CSF or GM1 treatment. In heterozygous Q7/140 mice, we detected a decrease in the protein levels of both wtHtt (p=0.0084) and mHTT (p=0.0284) in the cortex (Fig 2.11A) and a decrease in mHTT in the striatum (p=0.0454). The levels of wtHtt showed a trend towards a decrease (p=0.0802) that did not reach significance (Fig. 2.11B). No effect of GM1 on levels of mHTT was observed in either the striatum or the cortex of homozygous Q140 mice.

SDS-insoluble aggregates of mHTT were quantified in both Q140 and R6/2 mice by filter-trap assay. In the R6/2 mice, aggregates were detected in both the cortex and the striatum (Fig. 2.11C). The amount of aggregates was significantly decreased in the cortex after administration with GM1 (p=0.0437), although we did not see changes in the striatum. In the Q140 mouse model, heterozygous Q7/140 mice had little aggregates detectable by filter-trap, and these did not appear to be affected by GM1 (data not shown). In homozygous Q140/140 mice aggregates

were more abundant. Administration of GM1 decreased insoluble aggregates in the striatum (p=0.0033) (Fig 2.11D), but not in the cortex (data not shown).

2.4 Discussion

There are currently no disease modifying treatments for HD, pharmacological intervention extends only as far as symptomatic management of anxiety, depression, and chorea. In previous work we showed that GM1 improves motor performance in the YAC128 mouse model (212). In this study we confirmed the ability of GM1 to improve motor performance in two additional models of HD, the R6/2 and the Q140 mice. Since these mice have a very different genetic make-up compared to YAC128 mice and to each other, our studies demonstrate that the therapeutic effects of GM1 are independent from mouse strain and site of integration of the transgene (in YAC128 and R6/2 mice). We also demonstrated that GM1 decreases accumulation of toxic mHTT and protects R6/2 mice from neurodegeneration. These findings underscore the profound therapeutic actions of GM1 and demonstrate that GM1 is a potential disease-modifying therapy in HD.

In addition to standard motor tests, in this study we performed a kinematic analysis of the walking behaviour of Q140 mice, incorporating an analysis of the iliac crest in addition to measurements of stride determined from foot placement. Gait analysis has been used as a measure of motor impairment in many mouse models of HD including the BACHD and R6/2 models (242, 243). It has also been shown to be a highly sensitive measure of motor impairment in models of HD, demonstrating greater sensitivity than the rotarod test (244). Clinically, gait deficits are well documented in HD patients (38, 245-247), and studies analyzing gait after treatment with tetrabenazine suggest that gait abnormalities are resistant to treatment.

Our kinematic analysis of gait revealed gait abnormalities that have not previously been observed at this age in Q140 mice (69), a finding with significant implications since other clinical treatments for motor impairments do not improve gait deficits.

The motor improvement observed in the R6/2 mice was not as marked as that seen in Q140 and YAC128 mice, which is not surprising considering the severity of symptoms and the early onset widespread neurodegeneration that characterizes these mice. Nevertheless, the effects of the treatment in this model were consistent with a disease-modifying action of GM1. While a rapidly progressing motor dysfunction was observed in CSF-treated R6/2 mice throughout the course of the study (especially in the ladder and in the open field test), this was not true for GM1-treated R6/2 mice, which maintained stable performance throughout the study. GM1 treatment also prevented weight loss and stabilized body weight in R6/2 mice, in spite of similar food consumption between groups. These data are in line with the effects of GM1 on neuropathology (discussed later) and support a disease-modifying effect of GM1.

We also observed a 2 week increase in the lifespan of R6/2 mice, although statistical significance was not reached, most likely because of the limited number of mice included in this analysis. The strain of R6/2 mice (B6CBA-Tg (HDExon1)62Gpb/3J) we used in our study does not usually survive past 12-14 weeks of age, making 2 weeks nearly a 20% increase in lifespan. The improvement in motor performance and lifespan are comparable or better to studies in R6/2 mice where expression of mHTT has been indirectly targeted (248, 249).

Concomitant with the improvement in behaviour and overall health status, in R6/2 mice treated with GM1, we observed a reduced degree of neurodegeneration. Striatal volume loss is correlated with motor and cognitive deficits in HD patients (250) as well as with disease severity

and progression (34, 251). Therefore the reduction in striatal volume loss occurring during treatment with GM1 suggests a slowing of disease progression and correlates well with the effects of GM1 on behavior and survival. Interestingly, cross-sectional analysis of brain weight in mice from 6 to 10 weeks of age indicated that treatment with GM1 slowed down degeneration by 2 weeks, which match the observed increase in lifespan in GM1-treated mice.

When neuronal cells (NeuN$^+$ cells) were counted in the striatum, a restoration to WT was observed. Although our study did not address whether GM1 could stimulate neurogenesis, other considerations are more likely to explain the presence of nearly normal numbers of striatal neurons in GM1-treated R6/2 mice. Previous reports indicate there is no striatal loss in R6/2 mice at five weeks of age (252). Since we started treatment at 6 weeks of age, GM1 administration may have been initiated early enough to prevent neuronal loss during the period of GM1 administration.

Beside early stage grey matter loss, particularly in the striatum and cortex an important role for white matter loss early in disease has emerged in recent years. MRI studies in pre-manifest individuals have highlighted white matter volume loss prior to the onset of motor symptoms (34, 251, 253). In the PREDICT and TRACK HD studies, the presymptomatic loss of corpus callosum volume in HD patients has been related to cognitive deficits (254-256). Additionally degeneration of axons in subcortical white matter tracts has been observed early in disease progression (257) and is likely related to corticostriatal functional deficits (258). White matter and axonal loss that mirror the human HD pathology (254, 255) have also been described in R6/2 mice (259) and loss of cortico-striatal terminals occurs by 1 year of age in the Q140 model (260). In our study, treatment with GM1 significantly increased the volume of white matter tracts and corpus callosum in R6/2 mice. This is in line with the established role of gangliosides in the

maintenance of the myelin sheath (154). GM1 is found in high concentrations in white matter in the mouse brain and is particularly localized to the myelin membrane, (261) where GM1 has been shown to localize to lipid rafts and consequently stabilize nodal regions and improve overall myelin integrity (262). GM1 was also shown to prevent loss of myelin basic protein, a marker of oligodendrocyte health, after bilateral carotid artery occlusion (263).

In HD, striatal dysfunction precedes striatal atrophy and is associated with the disruption of several signaling pathways and regulatory proteins, including DARPP32 (264). DARPP32 is a pivotal signal integrator of DA signaling and also serves as a marker of striatal health and functionality (265).

As expected, expression and phosphorylation (Thr34) of DARPP32 were also decreased in Q140 mice, and GM1 restored both, but only in heterozygous Q7/140 mice. Female animals were excluded from this and other biochemical analyses to avoid potential confounding effects of the estrous cycle, as some of the markers of interest, including GFAP and DARRP32, are modulated by estrogen (266, 267). The lack of effect in the homozygous mice could be explained if the action of GM1 on DARPP32 required the presence of wtHTT. Similar considerations apply to the effects of GM1 on the levels of HTT (see discussion below).

In R6/2 mice the reduction in DARPP32 expression has been shown to precede behavioural changes (264). In our study, GM1 administration increased the expression of DARPP32 in a similar way as in an experiment model where BDNF was overexpressed in R6/2 (268). However, the effects of GM1 in our study are not likely due to BDNF expression, as the latter was similar across all genotypes and treatments in our study (data not shown), despite the described ability of GM1 to increase BDNF levels and signaling in other models (198). Thus, the effect of GM1 on

DARPP32 expression in R6/2 mice could be a consequence of the increased number of striatal neurons or a side effect of improved iron signaling. Previous studies have used DARPP32 as a marker to quantify damage in the striatum after intracerebral hemorrhage (269) and have demonstrated that its expression is influenced by an iron chelator. Whether this increase was secondary to reduced cell death, or a result of increased DARPP32 expression in the remaining cells has yet to be clarified. Regardless, these findings indicate that DARPP32 expressing cells are sensitive to damage mediated by excess iron.

Dysregulation of iron metabolism and ferritin expression has been detected as an early event in the pathological progression of HD. An increase in iron and ferritin in the striatum and decreased levels in the corpus callosum of HD patients have been detected prior to the onset of motor symptoms (270). Some data suggests a relationship between increased iron concentration and atrophy of the cortex and basal ganglia, with iron levels correlating with volume loss as well as CAG repeat length (271). Dysregulation of iron homeostasis is detrimental to cells as free iron can produce free radicals and contribute to oxidative stress (238). It was speculated that iron accumulation might be causally linked to grey matter loss in HD (272). The compensatory increase in ferritin expression observed in pre-symptomatic HD patients and animal models (R6/2 mice) mostly occurs within dystrophic microglia (239). It has been proposed that the presence of iron in microglia can affect the expression of inflammatory factors (273). Microglia activation and dysfunction, astrogliosis and changes in cytokine expression have indeed been reported in HD (52, 274).

Both iron and ferritin accumulate in R6/2 mouse brains (239). In our study we confirmed the presence of increased levels of ferritin in R6/2 mice, which was markedly reduced after treatment with GM1. This was one of the most striking molecular changes after GM1

administration in R6/2 mice. This finding is in line with previous reports that indicate that GM1, and related gangliosides, can alter iron metabolism in the brain. In models of GM1 and GM2 gangliosidosis, neurological disorders in which gangliosides accumulate, a detrimental reduction in iron content in the brain and other organs occurs, as a consequence of changes in iron transporters (275). One example of this, is the GM1-mediated alteration of iron uptake has also been implicated in the ability of GM1 to prevent benzo[a]pyrene-mediated apoptosis in rat hepatic epithelia cells (210). Benzo[a]pyrene is an environmental toxin that is known to increase apoptosis by increasing ROS in a manner that is at least partially mediated by increased intracellular iron. The reduction of ferritin expression in R6/2 mice by GM1, therefore, may be mediated through increasing transport of iron out of the brain and may be independent from any action of GM1 on microglia. Improved iron metabolism would decrease oxidative stress and potentially improve cell viability.

High levels of ferritin and ferric iron have previously been shown to co-localize with dystrophic microglia in R6/2 brains (239), however, we did not see any markers of increased inflammation in the brains of vehicle-treated R6/2 mice in our experiments. While astrogliosis and microgliosis are present in late stages of disease in HD patients, (52) these phenotypes have not been consistently recapitulated in R6/2 mice. Some studies have observed microgliosis (276, 277), while others have documented decreases in microglia density (278). In our study, microglial cell (Iba1$^{+)}$ density in R6/2 mice was either normal (in the cortex) or slightly decreased (in the striatum) as compared to WT mice, with GM1 restoring normal cell density in the striatum. It has previously been suggested that a decrease in microglia number could be associated with impaired ability of the microglia to provide normal support to neurons (278). Furthermore, in spite of normal cell density in the cortex, here Iba1 protein expression levels were significantly decreased

compared to WT, suggesting that microglia could be dysfunctional. Iba1 is a calcium binding protein that is important for microglia motility and phagocytosis (279). Decreased expression could be consistent with a change in the activity state of microglia (280) or with microglia that have decreased motility and phagocytic ability. As a matter of fact, HD microglia were shown to be impaired in both of these functions (281). GM1 showed a trend toward normalizing Iba1 expression and significantly increased the number of Iba1$^+$ cells in the cortex, perhaps compensating for the dysfunctional nature of microglia in this brain region in R6/2 mice.

In R6/2 mice we also observed an effect of GM1 on GFAP protein expression. This was lower than normal in the striatum and cortex in spite of normal GFAP$^+$ area, and was normalized by treatment with GM1. While neurodegenerative diseases are typically associated with increased GFAP expression, decreased expression has been previously described in a rat model of HD as well as in PINK1 KO mice (282, 283). Decreased GFAP expression has also been shown to be associated with depression (284). GFAP is an important class III intermediate filament with numerous functions in astrocytes (285). Mice that lack GFAP have been shown to have deficits in BBB and myelin integrity (284). When GFAP expression is suppressed in a human astrocytoma cell line the cells take on a less differentiated phenotype and are impaired in their ability to respond to neurons (286). The observed decrease in GFAP expression in R6/2 astrocytes could therefore be associated with a less mature astrocytic phenotype that does not fulfill some of the important functions of healthy astrocytes. Normalization of GFAP expression after the administration of GM1 suggests that GM1 might improve the functionality of the astrocytes in R6/2 brains.

The widespread beneficial effects of GM1 described in this study support a disease-modifying action of this ganglioside in HD models. This is further supported by its effects on mHTT itself.

We have previously shown that GM1 administration modifies levels of HTT phosphorylation at Ser13 and Ser16 (212), two sites that have previously been associated to a reduction in HTT aggregation and toxicity (213). As a follow up on those findings, in this study we showed that administration of GM1 decreases the amount of mHTT protein aggregates, as measured by a filter trap assay, in both R6/2 and Q140 models, and reduces soluble HTT (both WT and mutant forms) in Q140 mice. These effects were brain-region and genotype dependent. For example, no changes in aggregates were observed in heterozygous Q7/140 mice or in the cortex of homozygous Q140/140 mice, where very few aggregates were present to start with. Inter-animal variability and overall low aggregate levels might have made it more difficult to detect GM1 effects here. Genotype-related differences in the ability of GM1 to decrease the expression of mHTT suggest that, as in the case of changes in DARPP32 expression, one copy of the WT protein is necessary to mediate this specific action of GM1.

The finding that GM1 also decreased levels of wtHtt in the cortex of heterozygous Q140 mice was somewhat surprising. This decrease did not appear to be detrimental to mice in any way we could observe.

Due to the combination of beneficial effects observed in this study we propose that GM1 may have therapeutic value in HD. GM1 was previously tested in multiple human conditions including, stroke, AD, and PD; demonstrating its overall safety in patients. In PD, GM1 was shown to be safe and effective in a 5 year open study (287). Patients receiving GM1 showed improvements on the UPDRS. In a follow up randomized control study, GM1 administration resulted in an improvement on motor symptoms over placebo, and appeared to slow disease progression (288). In a more recent study, similar beneficial effects were observed using intraventricular administration of a sialidase to increase endogenous GM1 levels (289). These

studies all indicate that GM1 can be safely administered to people, and suggest that GM1 has

general neuroprotective effects which could also benefit HD patients. GM1 is, however, of

particular interest in HD as it has disease-specific effects (phosphorylation of HTT and decreased

protein levels) in addition to potential more general neuroprotective effects. Thus, GM1 could

have an even greater impact in HD than in PD where clinical benefits have already been shown.

Figure 2.1: GM1 improves motor behaviour in R6/2 and Q140 mice
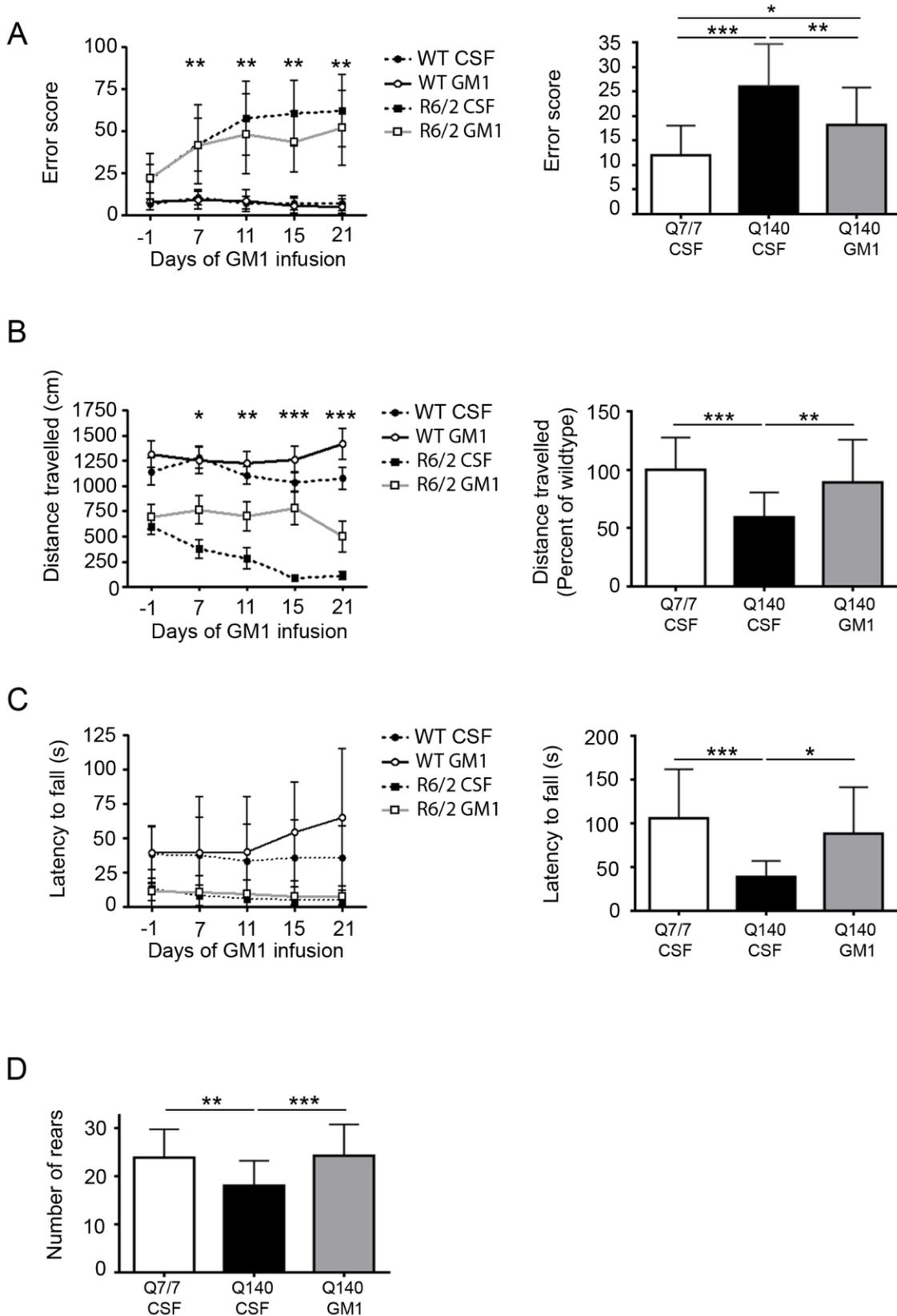
Figure 2.1 GM1 improves motor behaviour in R6/2 and Q140 mice. Motor performance was assessed in 7-week old R6/2 mice and 6-8 month old Q140 mice and WT littermates. (A) Horizontal ladder. (B) Open field activity. Distance travelled during 5 min session is reported. For Q140 mice, the distance travelled relative to Q7/7 is shown. The number of animals tested was: N=22-23 WT CSF, 21 WT GM1, 17-20 R6/2 CSF, 17-21 R6/2 GM1; and 27-28 Q7/7 CSF, 23-29 Q140 CSF, 22-26 Q140 GM1. (C) Fixed speed (12 RPM) rotarod test. Latency to fall is the average of three consecutive trials for each animal. N=22 WT CSF, 21 WT GM1, 17 R6/2 CSF, 12 R6/2 GM1 and 6-8 month old female Q140 mice and WT littermates N= 15 Q7/7 CSF, 15 Q140 CSF, 11 Q140 GM1) (D) Climbing test performed in 6-8 month Q140 mice. Mice were placed in a wire mesh container for 5 min and the number of rears was scored. N=26 Q7/7 CSF, 29 Q140 CSF, 27 Q140 GM1. All data are means ±SD. Statistical analysis in experiments involving R6/2 mice was performed using a linear mixed effect regression model with 95% confidence intervals calculated at each time point. Asterisks on R6/2 graphs indicate statistically significant differences between HD CSF and HD GM1. In experiments involving Q140 mice, one-way ANOVA followed by Bonferroni correction was used. *$p<0.05$, **$p<0.01$, ***$p<0.001$.

Figure 2.2: GM1 corrects gait deficits in Q140 mice
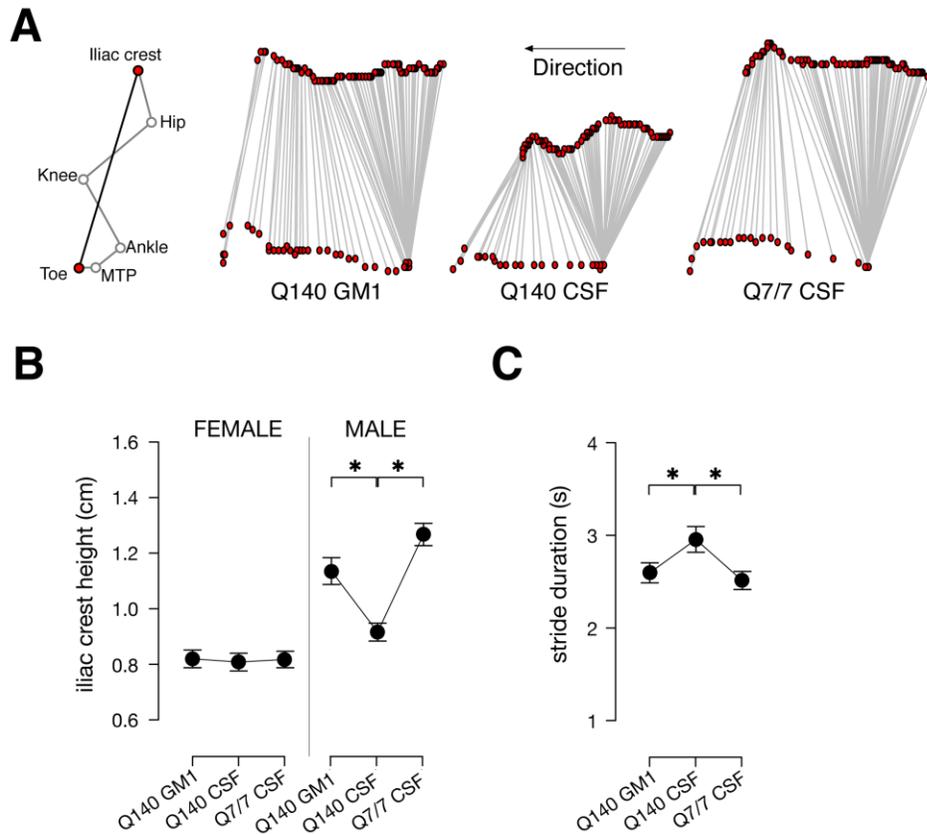
Figure 2.2 GM1 corrects gait deficits in Q140 mice. Spatiotemporal parameters of gait were measured in Q140 mice and Q7/7 littermates freely walking across a walkway. (A) Representative stick diagram decompositions (5 ms between sticks) of the left iliac crest and toe motion during quadrupedal walking on a walkway. Average iliac crest height and the average stride duration are presented in (B) and (C), respectively. Statistics were performed using separate three-factor ANOVAs (GROUP [3] x SEX [2] x PUMP [2]) to determine whether there was an effect of the sex (SEX), the side at which the pump hang (PUMP) and the treatment group (GROUP) on the different kinematic measures. All graphs show mean values ± S.E. Significant differences ($p<0.05$) are denoted for main effects by asterisks (*). Figure produced by Juan Forero.

Figure 2.3: GM1 prevents body weight loss in R6/2 mice
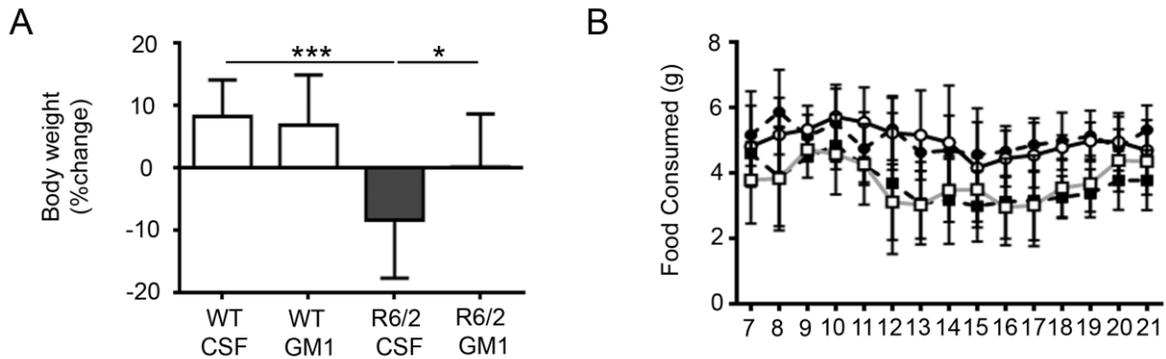
Figure 2.3 GM1 prevents body weight loss in R6/2 mice. (A) Percent change in body weight in 8-11 week WT and R6/2 mice after 21 days of treatment. Bars show mean values ±SD. N=23 WT CSF, 21 WT GM1, 14 R6/2 CSF, 11 R6/2 GM1. Two-way ANOVA with Bonferroni post-tests. *$p < 0.05$, ***$p < 0.001$. (B) Food consumption was monitored daily for each animal. Average food consumption from day 7 to 21 of treatment is shown. WT CSF N=16, WT GM1 14, R6/2 CSF 8, R6/2 GM1 7. Repeated measures Two-way ANOVA with Bonferonni post-test.

Figure 2.4: GM1 increases survival of R6/2 mice
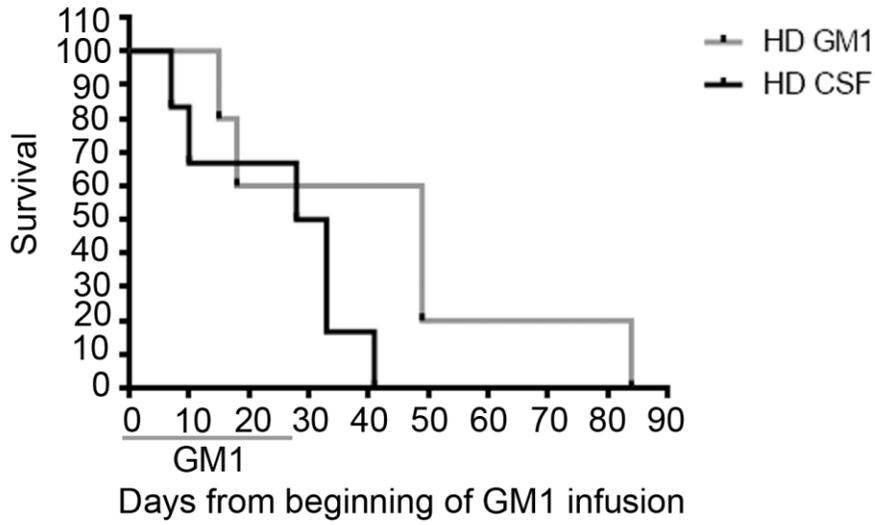
Figure 2.4 GM1 increases survival of R6/2 mice. Survival of R6/2 mice treated with CSF (N=6) and GM1 (N=5). Statistics performed using logrank analysis for the survival curve. Differences were not statistically significant.

Figure 2.5: GM1 slows neurodegeneration in R6/2 mice
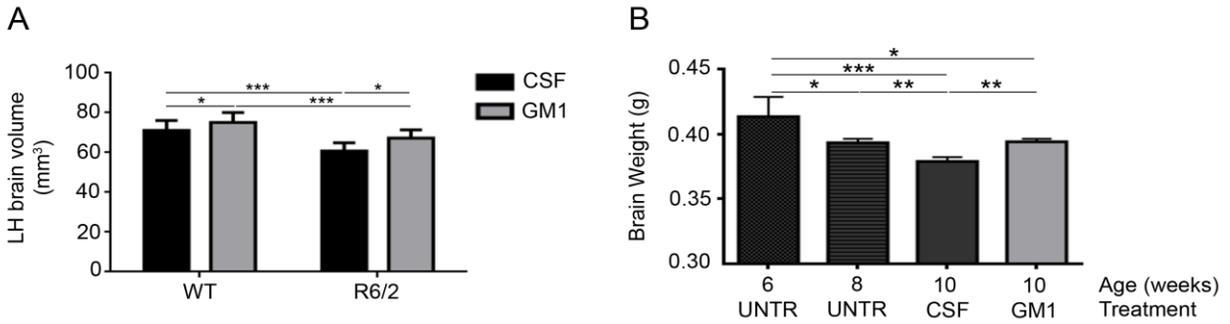
Figure 2.5 GM1 slows neurodegeneration in R6/2 mice. (A) LH brain volume from bregma 2.1 mm to -2.3 mm was measured after 28 days of treatment with CSF (vehicle) or GM1 in 10 week-old R6/2 mice. N=13 WT CSF, 11 WT GM1, 11 R6/2 CSF, 9 R6/2 GM1. (B) Time-course of brain weight loss in R6/2 mice. N=3 6-week R6/2, 10 8-week R6/2, 14 10-week R6/2 CSF and 10 10-week R6/2 GM1. Bars are means ± SD. One-way ANOVA with Holm-Sidak post-test. *$p<0.05$, **$p<0.01$, ***$p<0.001$

Figure 2.6: GM1 slows the neurodegenerative process in R6/2 mice
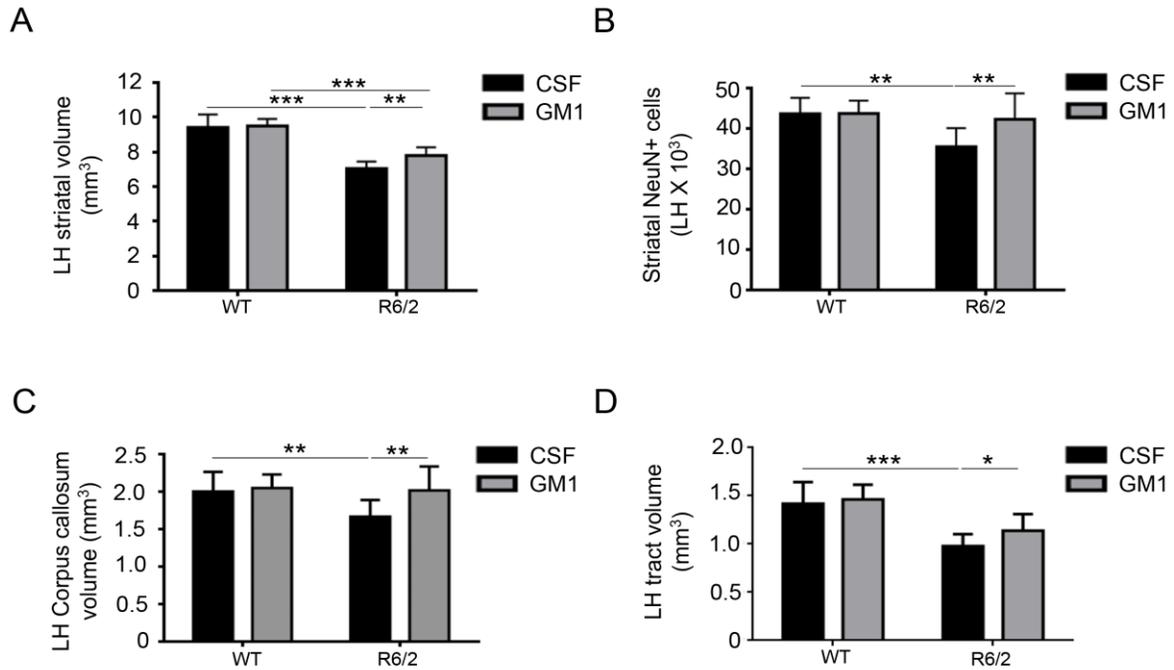
Figure 2.6 GM1 slows the neurodegenerative process in R6/2 mice. (A) LH striatal volume. For (A) and (B), the numbers of animals used in the analysis were: N=13 WT CSF, 11 WT GM1, 11 R6/2 CSF, 9 R6/2 GM1. (C) Neuron counts in the LH striatum between bregma 0.02 mm and -2.3 mm. N=8 WT CSF, 5 WT GM1, 7 R6/2 CSF, 6 R6/2 GM1. (D) LH corpus callosum volume between bregma 2.1 mm and 0.02 mm measured using eriochrome cyanine-stained sections. (E) LH striatal total white matter tract volume from 0.02 mm to bregma to -1.06 mm using eriochrome cyanine-stained brain sections. Bar represent means ± SD. Two-way ANOVA with Holm-Sidak post-test. $*p<0.05$, $**p<0.01$, $***p<0.001$.

Figure 2.7: GM1 increases levels of DARPP32 in Q140 mice
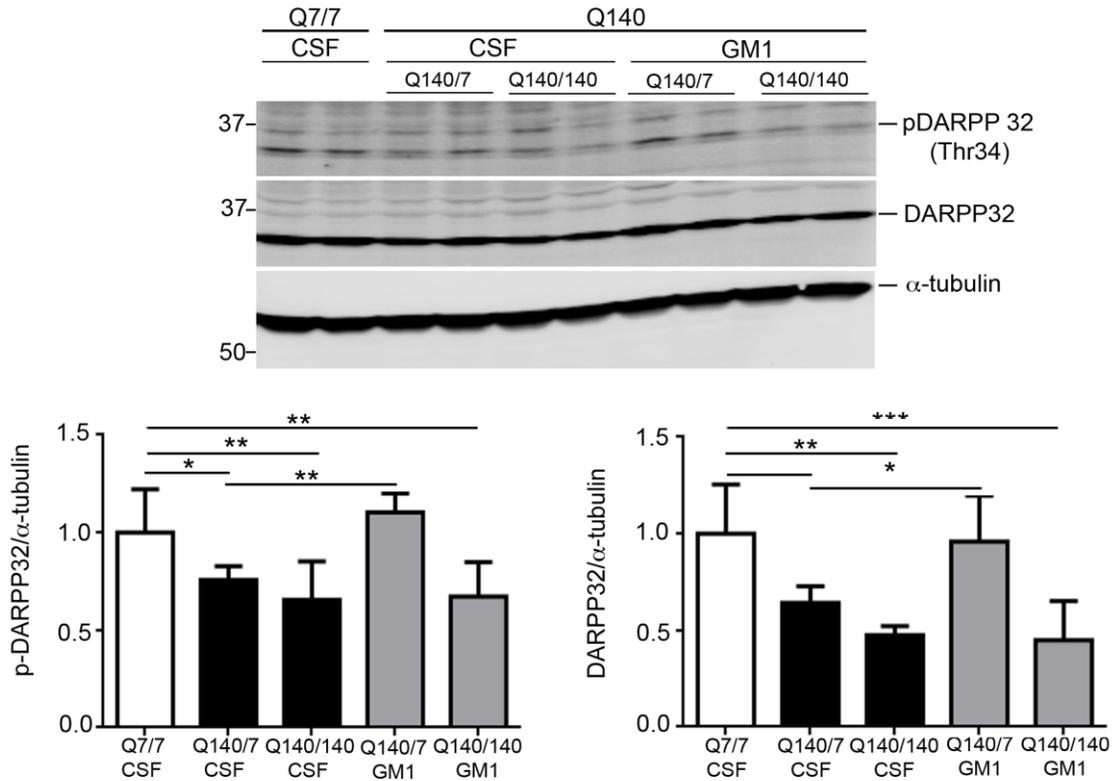
Figure 2.7 GM1 increases levels of DARPP32 in Q140 mice. Representative immunoblot analysis of DARPP32 expression and phospo-Thr34 DARPP32 (p-DARPP32) in 6-month-old male Q140 mice and Q7/7 littermates after 28 days of treatment with CSF or GM1. N=8 Q7/7, 5 Q140/7 CSF, 5 Q140/7 GM1, 4 Q140/140 CSF, 5 Q140/140 GM1. Graph bars show the densitometric analysis after normalization over α-tubulin. Bars represent mean values ± SD. One-way ANOVA with Holm-Sidak post-test. *$p<0.05$, **$p<0.01$.

Figure 2.8: GM1 increases expression of DARPP32 in R6/2 mice
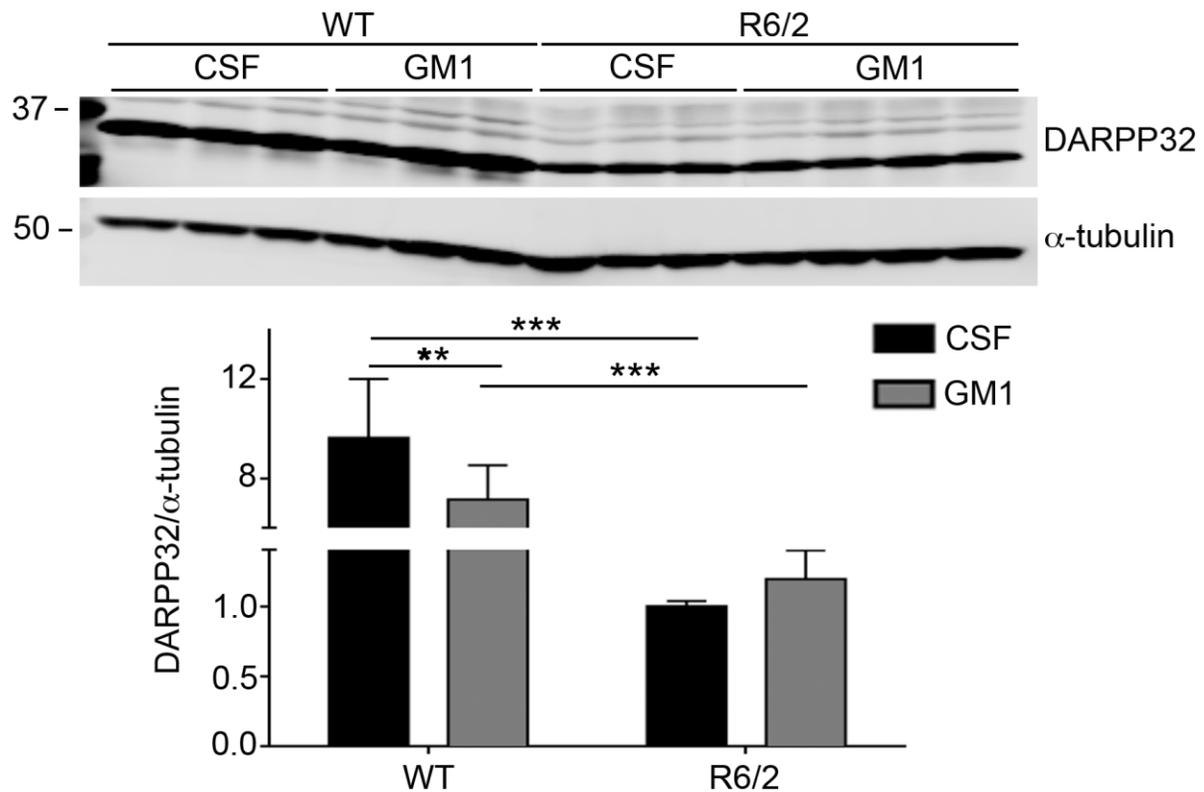
Figure 2.8 GM1 increases expression of DARPP32 in R6/2 mice. Representative western blot analysis of DARPP32 expression in 7-week-old male R6/2 after 28 days of treatment. DARPP32 expression is greatly reduced in R6/2 mice as compared to WT mice. GM1 decreased DARPP32 levels in WT. T-test analysis also shows an increased amount of DARPP32 in GM1-treated R6/2 mice. N=8 WT CSF, 7 WT GM1, 5 R6/2 CSF, or 5 R6/2 GM1. Statistical analysis performed by 2-way ANOVA with Bonferroni post-tests. ** $p<0.01$, ***$p<0.001$. Data produced by Danny Galleguillos.

Figure 2.9: GM1 decreases ferritin expression in R6/2 mice
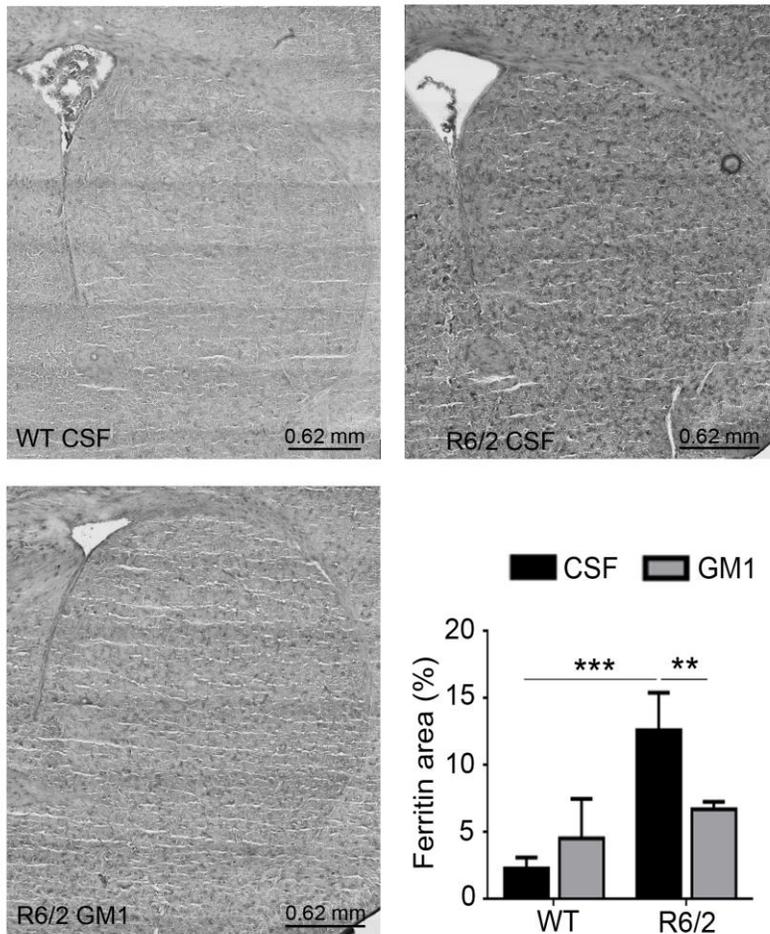
Figure 2.9 GM1 decreases ferritin expression in R6/2 mice. Representative microscopy images showing the striatum of 10 week-old WT and R6/2 mice treated with CSF or GM1, and immunostained with an anti-ferritin antibody. Quantification of the immunoreactive area is shown in the graph. N= 3 WT CSF, 4 WT GM1, 6 R6/2 CSF, 5 R6/2 GM1). Bars are means ± SD. Two-way ANOVA with Holm-Sidak post-test. $**p<0.01$, $***p<0.001$.

Figure 2.10: Effects of GM1 on astroglial and microglial markers
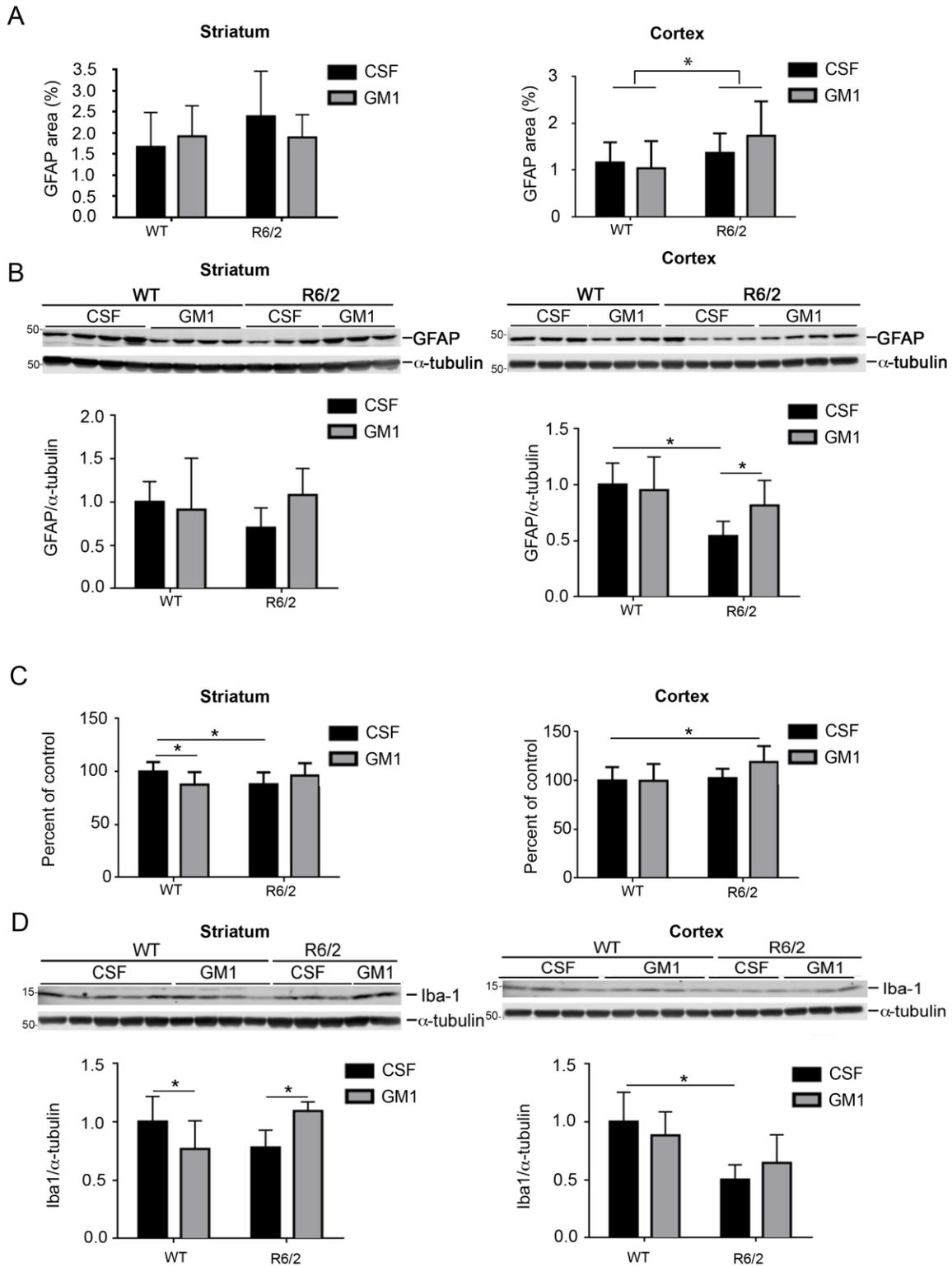
Figure 2.10 Effects of GM1 on astroglial and microglial markers. (A) Striatal and cortical sections (from left brain hemisphere, LH) from R6/2 mice and WT littermates were immunostained with anti-GFAP antibodies 28 days after treatment with CSF (vehicle) or GM1. Average immunoreactive area is shown (N=11 WT CSF, 9 WT GM1, 10 R6/2 CSF, 8 R6/2 GM1). (B) Representative immunoblots of GFAP in tissue lysates from R6/2 mice. The graphs show the densitometric analysis. GFAP signal was normalized over tubulin (N=7 WT CSF, 7 WT GM1, 7 R6/2 CSF, 7 R6/2 GM1). (C) Iba1$^+$ cell density in the cortex and striatum, normalized over Iba1$^+$ cell density in WT mice treated with CSF. All animals were 10 weeks old and analysis was performed from 8 serial sections in the left hemisphere of each mouse (N=11 WT CSF, 10 WT GM1, 10 R6/2 CSF, 9 R6/2 GM1). (D) Representative immunoblots for Iba1 expression in the striatum and cortex of WT and R6/2 mice after 28 days of treatment with CSF or GM1 (N=7 WT CSF, 7 WT GM1, 7 R6/2 CSF, 7 R6/2 GM1). Bars represent mean values ± SD. Two-way ANOVA with Holm-Sidak post-test. *$p<0.05$. Figure produced by myself and Danny Galleguillos.

Figure 2.11: HTT levels are reduced by administration of GM1
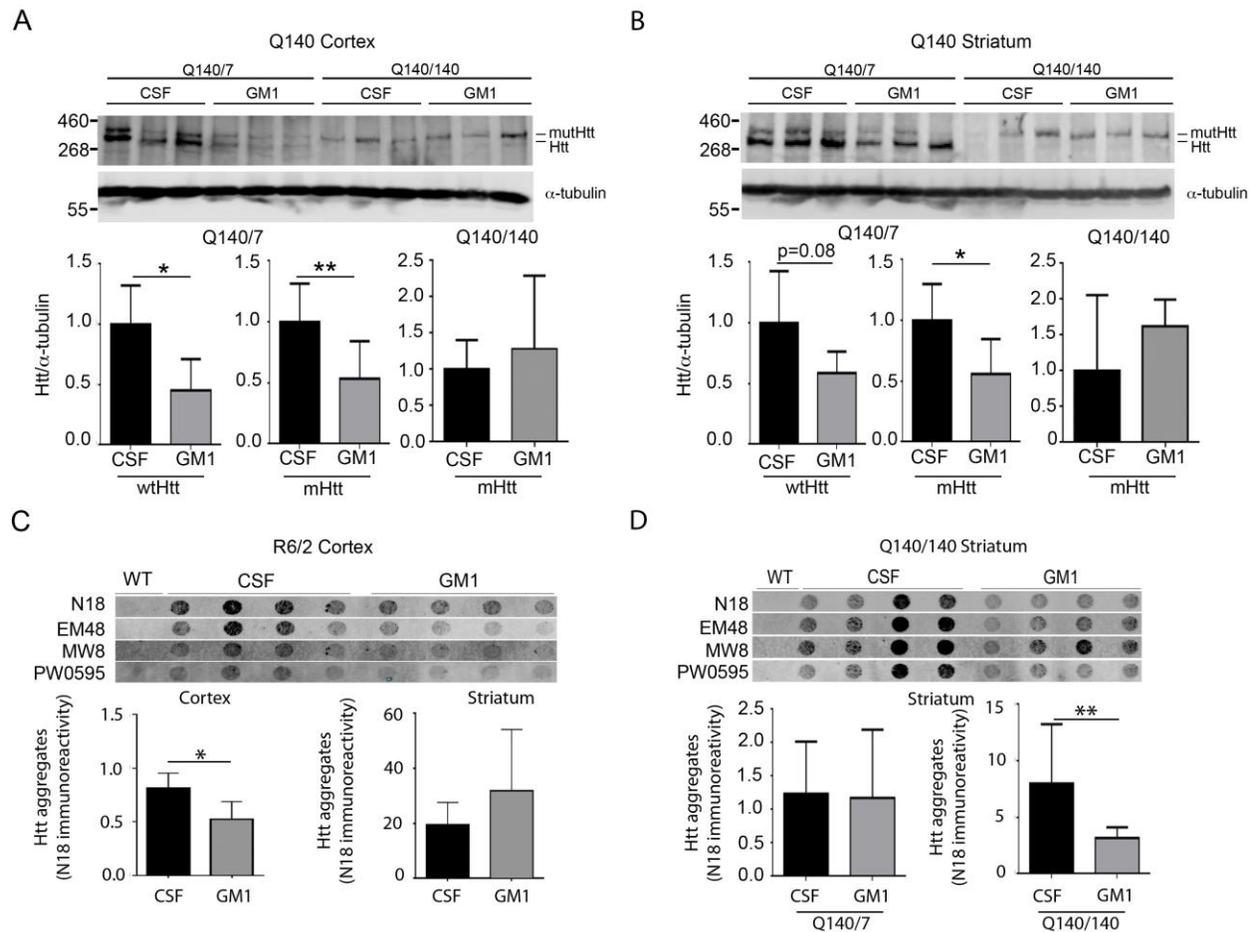
Figure 2.11 HTT levels are reduced by administration of GM1 (A) Representative immunoblots and densitometric analysis of wtHTT and mHTT in the cortex and striatum of Q140 mice after 28 days of treatment with CSF (vehicle) or GM1 (N= 5 Q140/7 CSF, 5-6 Q140/7, 3-7 Q140/140 CSF, 3-5 Q140/140 GM1). (B) and (C) Filter trap assay for mHTT insoluble aggregates in (N= 5 R6/2 CSF=5, 6 R6/2 GM1) and Q140 mice (N=5 Q140/7 CSF, 6 Q140/7 GM1, 4 Q140/140 CSF, 5 Q140/140 GM1) after 28 days of treatment with CSF or GM1. SDS-insoluble mHTT aggregates were detected with N-18 antibodies. Representative immunoblots and densitometric analysis are shown. Bars represent mean values ± SD. Student's *t*-test, *$p<0.05$, **$p<0.01$, ***$p<0.001$. Data collection for figure by Danny Galleguillos, Sebastian Lackey, and myself.

2.5 References

1.   A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. The Huntington's Disease Collaborative Research Group. Cell. 1993;72(6):971-83.

34.   Tabrizi SJ, Scahill RI, Durr A, Roos RA, Leavitt BR, Jones R, et al. Biological and clinical changes in premanifest and early stage Huntington's disease in the TRACK-HD study: the 12-month longitudinal analysis. The Lancet Neurology. 2011;10(1):31-42.

38.   Kegelmeyer DA, Kloos AD, Fritz NE, Fiumedora MM, White SE, Kostyk SK. Impact of tetrabenazine on gait and functional mobility in individuals with Huntington's disease. Journal of the neurological sciences. 2014;347(1-2):219-23.

41.   Vonsattel JP, Myers RH, Stevens TJ, Ferrante RJ, Bird ED, Richardson EP, Jr. Neuropathological classification of Huntington's disease. Journal of neuropathology and experimental neurology. 1985;44(6):559-77.

42.   Jech R, Klempir J, Vymazal J, Zidovska J, Klempirova O, Ruzicka E, et al. Variation of selective gray and white matter atrophy in Huntington's disease. Movement disorders : official journal of the Movement Disorder Society. 2007;22(12):1783-9.

52.   Singhrao SK, Neal JW, Morgan BP, Gasque P. Increased complement biosynthesis by microglia and complement activation on neurons in Huntington's disease. Experimental neurology. 1999;159(2):362-76.

61.   Mangiarini L, Sathasivam K, Seller M, Cozens B, Harper A, Hetherington C, et al. Exon 1 of the HD gene with an expanded CAG repeat is sufficient to cause a progressive neurological phenotype in transgenic mice. Cell. 1996;87(3):493-506.

62.   Davies SW, Turmaine M, Cozens BA, DiFiglia M, Sharp AH, Ross CA, et al. Formation of neuronal intranuclear inclusions underlies the neurological dysfunction in mice transgenic for the HD mutation. Cell. 1997;90(3):537-48.

69.   Menalled LB, Sison JD, Dragatsis I, Zeitlin S, Chesselet MF. Time course of early motor and neuropathological anomalies in a knock-in mouse model of Huntington's disease with 140 CAG repeats. The Journal of comparative neurology. 2003;465(1):11-26.

75.   Bates GP, Hockly E. Experimental therapeutics in Huntington's disease: are models useful for therapeutic trials? Current opinion in neurology. 2003;16(4):465-70.

76.   Menalled L, Brunner D. Animal models of Huntington's disease for translation to the clinic: best practices. Movement disorders : official journal of the Movement Disorder Society. 2014;29(11):1375-90.

137.   Maglione V, Marchi P, Di Pardo A, Lingrell S, Horkey M, Tidmarsh E, et al. Impaired ganglioside metabolism in Huntington's disease and neuroprotective role of GM1. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2010;30(11):4072-80.

138.   Denny CA, Desplats PA, Thomas EA, Seyfried TN. Cerebellar lipid differences between R6/1 transgenic mice and humans with Huntington's disease. Journal of neurochemistry. 2010;115(3):748-58.

139.   Desplats PA, Denny CA, Kass KE, Gilmartin T, Head SR, Sutcliffe JG, et al. Glycolipid and ganglioside metabolism imbalances in Huntington's disease. Neurobiology of disease. 2007;27(3):265-77.

148.   Posse de Chaves E, Sipione S. Sphingolipids and gangliosides of the nervous system in membrane function and dysfunction. FEBS letters. 2010;584(9):1748-59.

150.   Schengrund CL. Gangliosides: glycosphingolipids essential for normal neural development and function. Trends in biochemical sciences. 2015;40(7):397-406.

154.   Yang LJ, Zeller CB, Shaper NL, Kiso M, Hasegawa A, Shapiro RE, et al. Gangliosides are neuronal ligands for myelin-associated glycoprotein. Proceedings of the National Academy of Sciences of the United States of America. 1996;93(2):814-8.

172.   Arber C, Li A, Houlden H, Wray S. Insights into molecular mechanisms of disease in Neurodegeneration with Brain Iron Accumulation; unifying theories. Neuropathology and applied neurobiology. 2015.

198.   Lim ST, Esfahani K, Avdoshina V, Mocchetti I. Exogenous gangliosides increase the release of brain-derived neurotrophic factor. Neuropharmacology. 2011;60(7-8):1160-7.

210.   Gorria M, Huc L, Sergent O, Rebillard A, Gaboriau F, Dimanche-Boitrel MT, et al. Protective effect of monosialoganglioside GM1 against chemically induced apoptosis through targeting of mitochondrial function and iron transport. Biochemical pharmacology. 2006;72(10):1343-53.

212.   Di Pardo A, Maglione V, Alpaugh M, Horkey M, Atwal RS, Sassone J, et al. Ganglioside GM1 induces phosphorylation of mutant huntingtin and restores normal motor behavior in Huntington disease mice. Proceedings of the National Academy of Sciences of the United States of America. 2012;109(9):3528-33.

213.   Gu X, Greiner ER, Mishra R, Kodali R, Osmand A, Finkbeiner S, et al. Serines 13 and 16 are critical determinants of full-length human mutant huntingtin induced disease pathogenesis in HD mice. Neuron. 2009;64(6):828-40.

218.   Metz GA, Whishaw IQ. Cortical and subcortical lesions impair skilled walking in the ladder rung walking test: a new task to evaluate fore- and hindlimb stepping, placing, and co-ordination. Journal of neuroscience methods. 2002;115(2):169-79.

219.   De Souza RA, Leavitt BR. Neurobiology of Huntington's Disease. Current topics in behavioral neurosciences. 2015;22:81-100.

220.   Unschuld PG, Joel SE, Liu X, Shanahan M, Margolis RL, Biglan KM, et al. Impaired cortico-striatal functional connectivity in prodromal Huntington's Disease. Neuroscience letters. 2012;514(2):204-9.

221.   Vonsattel JP. Huntington disease models and human neuropathology: similarities and differences. Acta neuropathologica. 2008;115(1):55-69.

222.   Martindale D, Hackam A, Wieczorek A, Ellerby L, Wellington C, McCutcheon K, et al. Length of huntingtin and its polyglutamine tract influences localization and frequency of intracellular aggregates. Nature genetics. 1998;18(2):150-4.

223.   Caron NS, Desmond CR, Xia J, Truant R. Polyglutamine domain flexibility mediates the proximity between flanking sequences in huntingtin. Proceedings of the National Academy of Sciences of the United States of America. 2013;110(36):14610-5.

224.   Thompson LM, Aiken CT, Kaltenbach LS, Agrawal N, Illes K, Khoshnan A, et al. IKK phosphorylates Huntingtin and targets it for degradation by the proteasome and lysosome. The Journal of cell biology. 2009;187(7):1083-99.

225.   Atwal RS, Desmond CR, Caron N, Maiuri T, Xia J, Sipione S, et al. Kinase inhibitors modulate huntingtin cell localization and toxicity. Nat Chem Biol. 2011;7(7):453-60.

226.   Cummings JL. Defining and labeling disease-modifying treatments for Alzheimer's disease. Alzheimer's & dementia : the journal of the Alzheimer's Association. 2009;5(5):406-18.

227.   Carroll JB, Lerch JP, Franciosi S, Spreeuw A, Bissada N, Henkelman RM, et al. Natural history of disease in the YAC128 mouse reveals a discrete signature of pathology in Huntington disease. Neurobiology of disease. 2011;43(1):257-65.

228.   Hickey MA, Kosmalska A, Enayati J, Cohen R, Zeitlin S, Levine MS, et al. Extensive early motor and non-motor behavioral deficits are followed by striatal neuronal loss in knock-in Huntington's disease mice. Neuroscience. 2008;157(1):280-95.

229.   Leblond H, L'Esperance M, Orsal D, Rossignol S. Treadmill locomotion in the intact and spinal mouse. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2003;23(36):11411-9.

230.   Henery CC, Mayhew TM. The cerebrum and cerebellum of the fixed human brain: efficient and unbiased estimates of volumes and cortical surface areas. Journal of anatomy. 1989;167:167-80.

231.   Kiernan JA. Chromoxane cyanine R. II. Staining of animal tissues by the dye and its iron complexes. Journal of microscopy. 1984;134(Pt 1):25-39.

232.   Wanker EE, Scherzinger E, Heiser V, Sittler A, Eickhoff H, Lehrach H. Membrane filter assay for detection of amyloid-like polyglutamine-containing protein aggregates. Methods in enzymology. 1999;309:375-86.

233.   Li JY, Popovic N, Brundin P. The use of the R6 transgenic mouse models of Huntington's disease in attempts to develop novel therapeutic strategies. NeuroRx : the journal of the American Society for Experimental NeuroTherapeutics. 2005;2(3):447-64.

234.   Rattray I, Smith E, Gale R, Matsumoto K, Bates GP, Modo M. Correlations of behavioral deficits with brain pathology assessed through longitudinal MRI and histopathology in the R6/2 mouse model of HD. PloS one. 2013;8(4):e60012.

235.   Douaud G, Behrens TE, Poupon C, Cointepas Y, Jbabdi S, Gaura V, et al. In vivo evidence for the selective subcortical degeneration in Huntington's disease. NeuroImage. 2009;46(4):958-66.

236.   Greengard P, Allen PB, Nairn AC. Beyond the dopamine receptor: the DARPP-32/protein phosphatase-1 cascade. Neuron. 1999;23(3):435-47.

237.   Li Y, Yui D, Luikart BW, McKay RM, Li Y, Rubenstein JL, et al. Conditional ablation of brain-derived neurotrophic factor-TrkB signaling impairs striatal neuron development. Proceedings of the National Academy of Sciences of the United States of America. 2012;109(38):15491-6.

238.   Chen J, Marks E, Lai B, Zhang Z, Duce JA, Lam LQ, et al. Iron accumulates in Huntington's disease neurons: protection by deferoxamine. PloS one. 2013;8(10):e77023.

239.   Simmons DA, Casale M, Alcon B, Pham N, Narayan N, Lynch G. Ferritin accumulation in dystrophic microglia is an early event in the development of Huntington's disease. Glia. 2007;55(10):1074-84.

240.   Baune BT. Inflammation and neurodegenerative disorders: is there still hope for therapeutic intervention? Current opinion in psychiatry. 2015;28(2):148-54.

241.   Olejniczak M, Urbanek MO, Krzyzosiak WJ. The Role of the Immune System in Triplet Repeat Expansion Diseases. Mediators of inflammation. 2015;2015:873860.

242.   Abada YS, Nguyen HP, Schreiber R, Ellenbroek B. Assessment of motor function, sensory motor gating and recognition memory in a novel BACHD transgenic rat model for huntington disease. PloS one. 2013;8(7):e68584.

243.   Dai Y, Dudek NL, Li Q, Fowler SC, Muma NA. Striatal expression of a calmodulin fragment improved motor function, weight loss, and neuropathology in the R6/2 mouse model of Huntington's disease. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2009;29(37):11550-9.

244. Vandeputte C, Taymans JM, Casteels C, Coun F, Ni Y, Van Laere K, et al. Automated quantitative gait analysis in animal models of movement disorders. BMC neuroscience. 2010;11:92.

245. Delval A, Krystkowiak P, Delliaux M, Blatt JL, Derambure P, Destee A, et al. Effect of external cueing on gait in Huntington's disease. Movement disorders : official journal of the Movement Disorder Society. 2008;23(10):1446-52.

246. Delval A, Krystkowiak P, Delliaux M, Dujardin K, Blatt JL, Destee A, et al. Role of attentional resources on gait performance in Huntington's disease. Movement disorders : official journal of the Movement Disorder Society. 2008;23(5):684-9.

247. Casaca-Carreira J, Temel Y, van Zelst M, Jahanshahi A. Coexistence of Gait Disturbances and Chorea in Experimental Huntington's Disease. Behavioural neurology. 2015;2015:970204.

248. Cheng HM, Chern Y, Chen IH, Liu CR, Li SH, Chun SJ, et al. Effects on murine behavior and lifespan of selectively decreasing expression of mutant huntingtin allele by supt4h knockdown. PLoS genetics. 2015;11(3):e1005043.

249. Safren N, El Ayadi A, Chang L, Terrillion CE, Gould TD, Boehning DF, et al. Ubiquilin-1 overexpression increases the lifespan and delays accumulation of Huntingtin aggregates in the R6/2 mouse model of Huntington's disease. PloS one. 2014;9(1):e87513.

250. Aylward EH, Harrington DL, Mills JA, Nopoulos PC, Ross CA, Long JD, et al. Regional atrophy associated with cognitive and motor function in prodromal Huntington disease. Journal of Huntington's disease. 2013;2(4):477-89.

251. Tabrizi SJ, Reilmann R, Roos RA, Durr A, Leavitt B, Owen G, et al. Potential endpoints for clinical trials in premanifest and early Huntington's disease in the TRACK-HD study: analysis of 24 month observational data. The Lancet Neurology. 2012;11(1):42-53.

252. Dodds L, Chen J, Berggren K, Fox J. Characterization of Striatal Neuronal Loss and Atrophy in the R6/2 Mouse Model of Huntington's Disease. PLoS currents. 2014;6.

253. Paulsen JS, Magnotta VA, Mikos AE, Paulson HL, Penziner E, Andreasen NC, et al. Brain structure in preclinical Huntington's disease. Biological psychiatry. 2006;59(1):57-63.

254. Crawford HE, Hobbs NZ, Keogh R, Langbehn DR, Frost C, Johnson H, et al. Corpus callosal atrophy in premanifest and early Huntington's disease. Journal of Huntington's disease. 2013;2(4):517-26.

255. Matsui JT, Vaidya JG, Wassermann D, Kim RE, Magnotta VA, Johnson HJ, et al. Prefrontal cortex white matter tracts in prodromal Huntington disease. Human brain mapping. 2015;36(10):3717-32.

256. Novak MJ, Seunarine KK, Gibbard CR, Hobbs NZ, Scahill RI, Clark CA, et al. White matter integrity in premanifest and early Huntington's disease is related to caudate loss and disease progression. Cortex; a journal devoted to the study of the nervous system and behavior. 2014;52:98-112.

257. Sapp E, Penney J, Young A, Aronin N, Vonsattel JP, DiFiglia M. Axonal transport of N-terminal huntingtin suggests early pathology of corticostriatal projections in Huntington disease. Journal of neuropathology and experimental neurology. 1999;58(2):165-73.

258. Wolf RC, Sambataro F, Vasic N, Schonfeldt-Lecuona C, Ecker D, Landwehrmeyer B. Aberrant connectivity of lateral prefrontal networks in presymptomatic Huntington's disease. Experimental neurology. 2008;213(1):137-44.

259. Gatto RG, Chu Y, Ye AQ, Price SD, Tavassoli E, Buenaventura A, et al. Analysis of YFP(J16)-R6/2 reporter mice and postmortem brains reveals early pathology and increased

vulnerability of callosal axons in Huntington's disease. Human molecular genetics. 2015;24(18):5285-98.

260.   Deng YP, Wong T, Bricker-Anthony C, Deng B, Reiner A. Loss of corticostriatal and thalamostriatal synaptic terminals precedes striatal projection neuron pathology in heterozygous Q140 Huntington's disease mice. Neurobiology of disease. 2013;60:89-107.

261.   Vajn K, Viljetic B, Degmecic IV, Schnaar RL, Heffer M. Differential distribution of major brain gangliosides in the adult mouse central nervous system. PloS one. 2013;8(9):e75720.

262.   Zhang YP, Huang QL, Zhao CM, Tang JL, Wang YL. GM1 improves neurofascin155 association with lipid rafts and prevents rat brain myelin injury after hypoxia-ischemia. Brazilian journal of medical and biological research = Revista brasileira de pesquisas medicas e biologicas / Sociedade Brasileira de Biofisica [et al]. 2011;44(6):553-61.

263.   Rong X, Zhou W, Xiao-Wen C, Tao L, Tang J. Ganglioside GM1 reduces white matter damage in neonatal rats. Acta neurobiologiae experimentalis. 2013;73(3):379-86.

264.   Bibb JA, Yan Z, Svenningsson P, Snyder GL, Pieribone VA, Horiuchi A, et al. Severe deficiencies in dopamine signaling in presymptomatic Huntington's disease mice. Proceedings of the National Academy of Sciences of the United States of America. 2000;97(12):6809-14.

265.   Svenningsson P, Nishi A, Fisone G, Girault JA, Nairn AC, Greengard P. DARPP-32: an integrator of neurotransmission. Annual review of pharmacology and toxicology. 2004;44:269-96.

266.   Hajos F. Changes in glial fibrillary acidic protein (GFAP) immonureactivity reflect neuronal states. Neurochemical research. 2008;33(8):1643-50.

267.   Bode FJ, Stephan M, Suhling H, Pabst R, Straub RH, Raber KA, et al. Sex differences in a transgenic rat model of Huntington's disease: decreased 17beta-estradiol levels correlate with reduced numbers of DARPP32+ neurons in males. Human molecular genetics. 2008;17(17):2595-609.

268.   Giralt A, Carreton O, Lao-Peregrin C, Martin ED, Alberch J. Conditional BDNF release under pathological conditions improves Huntington's disease pathology by delaying neuronal dysfunction. Molecular neurodegeneration. 2011;6(1):71.

269.   Jin H, Xi G, Keep RF, Wu J, Hua Y. DARPP-32 to quantify intracerebral hemorrhage-induced neuronal death in basal ganglia. Translational stroke research. 2013;4(1):130-4.

270.   Bartzokis G, Lu PH, Tishler TA, Fong SM, Oluwadara B, Finn JP, et al. Myelin breakdown and iron changes in Huntington's disease: pathogenesis and treatment implications. Neurochemical research. 2007;32(10):1655-64.

271.   Sanchez-Castaneda C, Squitieri F, Di Paola M, Dayan M, Petrollini M, Sabatini U. The role of iron in gray matter degeneration in Huntington's disease: a magnetic resonance imaging study. Human brain mapping. 2015;36(1):50-66.

272.   Di Paola M, Phillips OR, Sanchez-Castaneda C, Di Pardo A, Maglione V, Caltagirone C, et al. MRI measures of corpus callosum iron and myelin in early Huntington's disease. Human brain mapping. 2014;35(7):3143-51.

273.   Zhang Z, Zhang Z, Lu H, Yang Q, Wu H, Wang J. Microglial Polarization and Inflammatory Mediators After Intracerebral Hemorrhage. Molecular neurobiology. 2016.

274.   Silvestroni A, Faull RL, Strand AD, Moller T. Distinct neuroinflammatory profile in post-mortem human Huntington's disease. Neuroreport. 2009;20(12):1098-103.

275.   Jeyakumar M, Williams I, Smith D, Cox TM, Platt FM. Critical role of iron in the pathogenesis of the murine gangliosidoses. Neurobiology of disease. 2009;34(3):406-16.

276.   Giampa C, Laurenti D, Anzilotti S, Bernardi G, Menniti FS, Fusco FR. Inhibition of the striatal specific phosphodiesterase PDE10A ameliorates striatal and cortical pathology in R6/2 mouse model of Huntington's disease. PloS one. 2010;5(10):e13417.

277.   Stack EC, Smith KM, Ryu H, Cormier K, Chen M, Hagerty SW, et al. Combination therapy using minocycline and coenzyme Q10 in R6/2 transgenic Huntington's disease mice. Biochimica et biophysica acta. 2006;1762(3):373-80.

278.   Ma L, Morton AJ, Nicholson LF. Microglia density decreases with age in a mouse model of Huntington's disease. Glia. 2003;43(3):274-80.

279.   Kanazawa H, Ohsawa K, Sasaki Y, Kohsaka S, Imai Y. Macrophage/microglia-specific protein Iba1 enhances membrane ruffling and Rac activation via phospholipase C-gamma -dependent pathway. The Journal of biological chemistry. 2002;277(22):20026-32.

280.   Hanisch UK, Kettenmann H. Microglia: active sensor and versatile effector cells in the normal and pathologic brain. Nature neuroscience. 2007;10(11):1387-94.

281.   Kwan W, Trager U, Davalos D, Chou A, Bouchard J, Andre R, et al. Mutant huntingtin impairs immune cell migration in Huntington disease. The Journal of clinical investigation. 2012;122(12):4737-47.

282.   Cong WN, Cai H, Wang R, Daimon CM, Maudsley S, Raber K, et al. Altered hypothalamic protein expression in a rat model of Huntington's disease. PloS one. 2012;7(10):e47240.

283.   Choi I, Choi DJ, Yang H, Woo JH, Chang MY, Kim JY, et al. PINK1 expression increases during brain development and stem cell differentiation, and affects the development of GFAP-positive astrocytes. Molecular brain. 2016;9(1):5.

284.   Middeldorp J, Hol EM. GFAP in health and disease. Progress in neurobiology. 2011;93(3):421-43.

285.   Bartzokis G, Lu PH, Nuechterlein KH, Gitlin M, Doi C, Edwards N, et al. Differential effects of typical and atypical antipsychotics on brain myelination in schizophrenia. Schizophrenia research. 2007;93(1-3):13-22.

286.   Weinstein DE, Shelanski ML, Liem RK. Suppression by antisense mRNA demonstrates a requirement for the glial fibrillary acidic protein in the formation of stable astrocytic processes in response to neurons. The Journal of cell biology. 1991;112(6):1205-13.

287.   Schneider JS, Sendek S, Daskalakis C, Cambi F. GM1 ganglioside in Parkinson's disease: Results of a five year open study. Journal of the neurological sciences. 2010;292(1-2):45-51.

288.   Schneider JS, Gollomp SM, Sendek S, Colcher A, Cambi F, Du W. A randomized, controlled, delayed start trial of GM1 ganglioside in treated Parkinson's disease patients. Journal of the neurological sciences. 2013;324(1-2):140-8.

289.   Schneider JS, Seyfried TN, Choi HS, Kidd SK. Intraventricular Sialidase Administration Enhances GM1 Ganglioside Expression and Is Partially Neuroprotective in a Mouse Model of Parkinson's Disease. PloS one. 2015;10(12):e0143351.

CHAPTER 3

GANGLIOSIDE GM1 RESTORES NORMAL NON-MOTOR BEHAVIOUR IN HD MOUSE MODELS

3.1 Introduction

HD is an inherited neurodegenerative disorder caused by the pathological expansion of a trinucleotide (CAG) repeat in the gene that codes for HTT (290). This mutation results in an abnormally long polyglutamine stretch (>36 Q) in the mHTT protein, which is consequently prone to misfolding and aggregation. MHTT triggers a plethora of molecular, neuronal and network dysfunctions culminating in neurodegeneration (291). Cerebral cortex and corpus striatum are the most affected brain regions (292, 293), while more subtle pathological changes occur in other brain areas (292-296).

Motor dysfunction such as chorea, dystonia and rigidity are the hallmark of HD and define disease onset (297). However, cognitive and psychiatric problems often precede the appearance of motor symptoms, and are frequently the most distressing for patients and their families (297, 298). Common early cognitive signs in HD include decreased verbal fluency and recognition memory as well as impaired ability to shift strategy and to plan (299-302). Deficits in procedural (303, 304), working (305) and long-term memory (306) also develop with disease progression.

Neuropsychiatric changes occur in nearly all HD patients (307), with depression being one of the most prevalent and debilitating symptoms of the disease (21, 308). Anxiety, irritability and agitation are also present in over 50% of patients (307). Cognitive dysfunction and increased anxiety and depression are also present in HD animal models where, similar to HD patients, they often predate motor impairment and overt neurodegeneration (309, 310).

To date there is no cure or disease-modifying therapy for HD. Clinical management of HD symptoms is possible to a certain extent with the use of tetrabenazine to reduce chorea, and with traditional anti-depressant and anti-psychotic drugs (311). However, the use of these

symptomatic treatments is often limited by their potential side effects (312-314), and the overall efficacy of antidepressants in HD patients is controversial (35). None of these treatments targets the underlying causes of dysfunction nor are able to slow down HD progression.

In previous studies we showed that the synthesis of gangliosides - sialic acid-containing glycosphingolipids - is affected in cellular and animal models of HD (137-139), resulting in lower levels of ganglioside GM1 and, to a lesser extent, other major brain gangliosides (137).

Gangliosides have a plethora of important modulatory functions in cell signaling, cell-cell interactions (315, 316) and calcium homeostasis (158, 205, 317), suggesting that reduced GM1 levels in HD may contribute to disease pathogenesis and/or progression. In support of this hypothesis, we showed that administration of exogenous GM1 decreases HD cell susceptibility to apoptosis *in vitro* (137), and corrects motor dysfunction in the YAC128 mouse model, after disease onset (212). The striking therapeutic effects of GM1 are accompanied by phosphorylation of HTT at amino acid residues Ser13 and Ser16 (212), a post-translational modification that decreases mHTT toxicity in cells and mice (213, 224, 225). The ability of GM1 to induce changes in the mHTT protein suggests that GM1 might have disease-modifying properties *in vivo*, and might be able to improve not only motor dysfunction, but also non-motor manifestations of the disease.

In this study we show that chronic administration of GM1 corrects behavioural abnormalities related to depression, anxiety and cognition across multiple HD mouse models. To the best of our knowledge, no other pharmacological treatment has been shown to have such profound effects across multiple HD models. We further show that these therapeutic effects are accompanied by neurochemical changes in the mouse corpus striatum and cerebral cortex, and

by restoration of normal concentrations of specific neuroactive amino acids and biogenic amines. Our data clearly demonstrate a therapeutic and disease-modifying role of GM1 in HD.

3.2 Materials and Methods

3.2.1 Animal models

YAC128 mice overexpressing the human HD gene with 128 CAG repeats (64) were originally purchased from the Jackson Laboratories (Jackson Laboratories, Bar Harbor, ME, USA) and subsequently maintained on FVB genetic background in our animal facility at the University of Alberta. Q140 knock-in mice expressing a chimeric mouse/human exon 1 gene with approximately 140 CAG repeats (69) were kindly donated by Cure HD Initiative (CHDI) and maintained on C57Bl/6J background in our animal facility. R6/2 mice overexpressing human *HTT* exon 1 (61) (B6CBA-Tg (HDExon1)62Gpb/3J) were obtained at 5 weeks of age from the Jackson Laboratory (stock number 006494) and used for experiments between 7 and 12 weeks of age. For practical reasons and to minimize the number of animals used we did not include WT Q7/Q7 mice treated with GM1 in our experiments. Previous experiments had shown that GM1 does not have any toxic effects and that it also does not affect motor performance in WT mice (C57Bl or FVB strain). All mice were maintained on a 14-10h light-dark cycle in a temperature and humidity-controlled room. All procedures involving animals were approved by the University of Alberta's Animal Care and Use Committee and were in accordance with the guidelines of the Canadian Council on Animal Care.

3.2.2 Intraventricular administration of GM1 and behavioural analysis

 Semi-synthetic GM1 was provided by Seneb BioSciences Inc. (Holliston, MA). GM1 treatment was performed by intraventricular infusion (0.25 µL/h) of a solution of 3.6 mM GM1

(approximately 33.8 mg GM1/day) in aCSF (Harvard Apparatus, Holliston, MA) for 28 or 42 days, using Alzet mini-osmotic pumps (model 2004, Cupertino, CA), as previously described (212). Control animals were infused with artificial CSF.

GM1 treatment was started at 6-6.5 months or 9-10 months of age for male YAC128 mice and WT littermates; at 6-8 months of age for Q140 and Q7/7 mice, and at 8 weeks of age for R6/2 mice and WT littermates. All mice were housed individually throughout the period of treatment to avoid accidental displacement or damage to the infusion kit and/or wound infection due to reciprocal grooming or fighting. Behavioural testing was conducted in the light phase of the cycle between 0800 h and 1800 h. Mice were allowed to acclimate to the testing room for 1 h prior to behaviour assessments, unless otherwise specified. All tests were performed by experimenters who were blind to genotype and treatment.

Behavioural testing started after day 14 of treatment for YAC128 mice, and after day 20 for Q140 mice. R6/2 mice were tested at day 7 of treatment. Homozygous and heterozygous Q140 mice performed similarly in most tests and, unless otherwise indicated, data from these groups were combined. Both male and female mice were used in most tests, as no significant sex-specific differences in behaviour were observed, except for the Y-maze and the nest building tests. In these tests female Q140 mice show no deficit and therefore they were excluded from the analysis.

3.2.3 Elevated plus maze

Mice were placed in the center of an elevated plus maze facing the open arm and left to freely explore for 5 min. Arm crosses into and out of open and closed arms, as well as time spent in each arm were recorded with a video camera and scored. A subset of mice was injected

intraperitoneally with adinazolam in saline solution (2.5 mg/kg, UpJohn Laboratories) 30 min prior to testing. Control animals received saline only.

3.2.4 Light-dark box test

The light-dark box test was performed as described in (318) with minor modifications. Mice were placed in the dark half of a 700 mm x 300 mm black (dark) and white (light) box containing a tunnel joining the two halves. Mice were allowed to freely explore the box for 5 min. Time spent in each box, number of entries into each box, and latency to first entry were recorded.

3.2.5 Novelty-suppressed feeding test

The test was performed as in (319), with modifications. Briefly, 24 h prior to testing mice were introduced to the target food (diluted sweetened condensed milk) in their home cage. Animals were then food-deprived overnight (10-12 h) to increase motivation to consume food. On the day of testing, mice were placed in a T-maze bated with diluted sweetened condensed milk at five places along the long arm. Mice were allowed to freely explore until they tasted the milk or for a maximum of 5 min. Time to approach and consume food was recorded.

3.2.6 Forced swim test

The forced swim test was performed as in (320). Mice were individually placed for 6 min in a 4 L beaker (25 cm tall, 16 cm wide) filled with 2.6 L of water pre-warmed to 23-25°C. The last 4 min of the test were scored using a time-sampling technique. Every 5 seconds mice were scored for swimming, climbing and immobility in the previous 5 seconds. Immobility was defined as no movement other than necessary to maintain the nose above water. A subset of mice received an

intraperitoneal injection of imipramine (10 mg/kg in saline) or saline only (controls) 30 min prior to testing.

3.2.7 Simple swim test

The simple swim test was used to control for motor deficits potentially interfering with the forced swim test (321). One day prior to testing, mice were trained to swim in a rectangular swimming chamber (90 cm long x 7 cm wide; water depth = 9 cm; with 6x7 cm platform at one end) and to reach a platform at one end of the swimming chamber in three consecutive trials separated by 5 min intervals. On the day of testing, swimming speed was calculated by averaging the time each mouse took to swim the length of the pool to the platform in 7 consecutive trials, after excluding from the count the best and worst trial for each animal (321).

3.2.8 Open pool test

The open pool test was performed 1-2 days after the forced swim test. Mice were placed in a pool (102 cm diameter, 21 cm high) filled with water at a temperature of 23-25ºC for 6 min. Swim activity in the last 4 min was measured as described above for the forced swim test.

3.2.9 Nest building

Nesting behaviour was assessed as described in (322) with modifications. Individually-housed mice were given a piece of paper towel and no other enrichment devices in their home cage. Percentage of paper towel shredded and height of the nest were scored according to a five point scale (322) after 10 days for YAC128 and 2 days for Q140 mice.

3.2.10 Open field habituation and spontaneous defecation

Mice were placed in an open field apparatus (90 cm x 90 cm) and filmed during 30 min sessions on two consecutive days as they explored the environment. Distance travelled in each 5 min interval was measured using EthoVision XT tracking software. Intrasession habituation was expressed as activity change ratio and calculated using the formula: *distance travelled in the last 5 min/sum of distance travelled in the first and in the last 5 min.* Intersession habituation was also expressed as activity change ratio between first session on day 1 and second session on day 2, and calculated as: *total distance travelled on day 2/sum of distance travelled on day 1 and day 2* (323, 324). At the end of each 30 min session, the number of fecal pellets dropped by each mouse was counted.

3.2.11 Social approach test

The social approach test was performed as described in (325). The test consisted of three 5 min sessions: 1) habituation, 2) social interaction and 3) social novelty. In session 1 each mouse was placed in the central compartment of an empty Plexiglas box divided into three compartments (left, center and right) and left to explore for 5 min. In session 2 (social interaction), a second unfamiliar mouse of the same age, sex and genotype (stranger 1) as the test mouse was placed inside a metal mesh container located in the left or right compartment of the box in an alternating pattern. In session 3 (social novelty) a third mouse of the same sex and genotype (stranger 2) was placed in the opposite compartment of the box, inside a metal mesh container. Time spent by the test mouse in each compartment in each session was recorded.

3.2.12 Y-maze

Mice were placed in a Y-maze with three identical arms (30.5 cm long x 5 cm wide, with 16 cm-high walls) and left to explore the maze for 5 min. Time of first entry and arm entries were recorded. An entry was defined as the front half of the mouse body being inside an arm. Percent correct alternations was calculated by giving one point for every correct alternation and applying the formula: *number of correct alternations/(total number of arm entries – 2)* (326). Animals that made fewer than five entries were excluded from the analysis (327). In our experiments only one mouse out of a total of forty-four met the criteria for exclusion (327).

3.2.13 Analysis of biogenic amines and amino acids

At the end of treatment and behavioural testing, mice were euthanized by cervical dislocation. Cortical and striatal tissue from the LH (contralateral to the site of cannulation) was immediately collected, flash-frozen in liquid nitrogen, and stored at -80ºC prior to neurochemical analysis. Analysis of amino acids was performed by HPLC according to (328) with minor modifications. Tissues were homogenized in 5 volumes of MeOH, let sit on ice for 10 min and then centrifuged at 10,000 x g for 4 min. Supernatants were diluted up to 30- or 60-fold in Milli-Q system-filtrated water. Aliquots of the diluted material were derivatized with o-pthaldialdehyde (OPA, Sigma-Aldrich) and N-isobutyryl-L-cysteine (IBC, Novachem) prior to HPLC analysis. Fluorescence detector was set at an excitation wavelength of 344 nm and emission at 433 nm. Calibration curves were prepared from standard amino acids for each individual run of samples. Biogenic amines and their metabolites were analyzed according to (329). Briefly, $1/10^{th}$ the volume of ice-cold 1N $HClO_4$ containing 500 µM ascorbic acid and ethylenediaminetetraacetic acid (EDTA) (100 mg%) was added to tissue aliquots in 5 volumes of water. Samples were then vortexed and centrifuged at 10,000g for 4 min. Supernatants were used for high performance

liquid chromatography (HPLC) analysis. Calibration curves were constructed for each HPLC run. Electrochemical detection was performed with an applied potential of 0.65V.

3.2.14 Statistical analysis

Two-way ANOVA followed by Bonferroni post-test was used to compare treatment groups in all behavioural tests that involved YAC128 or R6/2 mice. One-way ANOVA followed by Bonferroni post-test was used for tests involving Q140 mice. The chi square test was used to determine whether arm alternation by mice in the Y-maze was different than expected by chance.

3.3 Results

3.3.1 Treatment with GM1 normalizes anxiety-related behaviours in HD mice

We studied the effects of intraventricular infusion of GM1 in the YAC128, Q140 and R6/2 HD mouse models, which differ from one another in genetic background and disease severity (61, 64, 69). In the Q140 model no Q7/7 mice were treated with GM1 as in previous experiments GM1 did not have any effect on motor performance in WT mice and YAC128 WT littermates were included in all experiments to serve as a reference for the effects of GM1 on WT mice.

Anxiety-related behaviour was measured using a battery of well-established tests that included the elevated plus maze, the light/dark box, the novelty-suppressed feeding test and spontaneous defecation in an open-field arena (330, 331).

In the elevated plus maze, YAC128 mice spent significantly more time in the closed arm of the maze (genotype: $F_{1,57} = 5.701$, $p < 0.05$), and vehicle-treated YAC128 mice spent less in the open arm, compared to WT littermates (Fig. 3.1A), confirming that higher levels of anxiety than normal are present in these mice (331). Treatment with GM1 decreased time spent in closed arms

(treatment: $F_{1,57}=12.53$, $p<0.001$) and corrected the time vehicle-treated YAC128 mice spent in light arms (interaction: $F_{1,57}=4.31$, $p<0.05$) (Fig. 3.1A). The beneficial effects of GM1 were not due to changes in mouse motor activity and skills, since all YAC128 and WT mice showed similar motor activity in a control test where they were placed in an open field arena for the same amount of time as in the elevated plus maze test (Fig. 3.2); and because the deficit shown by YAC128 mice in the elevated plus maze was corrected by acute administration of an anxiolytic drug (adinazolam, 2.5 mg/kg) (332) (Fig. 3.3).

In the novelty-suppressed feeding test, which is based on an anxiogenic/depressive paradigm different from the elevated plus maze (333), vehicle-treated YAC128 mice took more time than WT littermates to approach and consume food placed in a novel environment, while GM1-treated YAC128 mice behaved as WT controls (interaction: $F_{1,28}=4.316$, $p<0.05$)(Fig. 3.1B).

In Q140 mice, anxiety-related behaviour was measured with the light/dark box test. Vehicle-treated Q140 mice (both heterozygous and homozygous) spent significantly more time in the dark chamber of a light/dark apparatus (treatment: $F_{2,80}=6.504$, $p<0.001$), less time in the light chamber (treatment: $F_{2,80}=7.712$, $p=0.009$) and moved between chambers less frequently than Q7/7 littermates (Fig. 3.1C) (treatment: $F_{2,80}=7.141$, $p=0.0014$). Upon treatment with GM1, the behaviour of Q140 mice became similar to Q7/7 control mice (Fig. 3.1C).

Treatment with GM1 also decreased open field defecation - a vegetative response to emotionality and fear - in both YAC128 (interaction: $F_{1,38}=6.980$, $p<0.05$) (Fig. 3.1D) and Q140 mice (treatment: $F_{2,77}=12.89$, $p<0.0001$) (Fig. 3.1E). Similar results were obtained in R6/2 mice (treatment: $F_{1,34}=4.302$, $p<0.05$; genotype: $F_{1,34}=4.302$, $p<0.05$) (Fig. 3.1F). Altogether our data

demonstrate that GM1 normalizes anxiety-related behaviours caused by the HD mutation across various mouse models.

3.3.2 GM1 decreases despair-related behaviour and improves nest building in HD mice

The forced swim test was used to assess depression-like behaviour. In line with previous reports (309), 6 and 9 month-old YAC128 mice spent more time immobile (9 month genotype: $F_{1,37}=6.696$, $p<0.05$) and less time swimming (9 month genotype: $F_{1,37}=4.924$, $p<0.05$) than WT littermates (Fig. 3.4A and 3.4B), a behaviour that indicates resignation and despair (334, 335). Behavioural differences between WT and YAC128 mice were abolished by treatment with GM1 (6 month immobile interaction: $F_{1,67}=5.740$, $p<0.05$; 6 month swimming interaction: $F_{1,67}=7.306$, $p<0.05$) (Fig. 3.4A and 3.4B). As in the case of anxiety tests, the therapeutic activity of GM1 was not due to its effects on motor activity, since all mice performed equally well in control tests that measured swimming speed (simple swim test, Fig. 3.5A) and swimming endurance (open pool test, Fig. 3.5B). Moreover, acute treatment of YAC128 mice with 10 mg/kg imipramine, a tricyclic antidepressant, dramatically decreased the time YAC128 mice spent immobile and increased time spent swimming, confirming the depression-like nature of YAC128 behaviour in the forced-swim test (Fig. 3.6). Differently from imipramine, however, GM1 required more than seven days of treatment to improve depression-like behaviour in YAC128 mice (data not shown).

GM1 treatment did not have any effect on the performance of 6 month-old WT mice in the forced swim test (Fig. 3.4A). However, it decreased depression-like behaviour in older WT mice (9 month-old) (9 month immobile treatment: $F_{1,37}=21.65$, $p<0.001$; 9 month swimming treatment: $F_{1,37}=15.26$, $p<0.001$), which on average spent more time immobile than younger mice (41% increase, $p=0.006$; compare Fig. 3.4B and Fig. 3.4A).

The performance of Q140 mice in the forced swim test was similar to Q7/7 littermates, as recently reported (336), but GM1 still decreased time immobile (treatment: $F_{2,57}$=5.955, p=0.0045) (Fig. 3.4C). As found in YAC128 mice, swimming endurance in the open pool test was similar across genotypes and treatments (Fig. 3.5C), thus excluding any potential confounding effect of motor dysfunction.

We next scored YAC128 and Q140 mice in the nest-building test. This test primarily assesses instinctual species-typical behaviour and general wellness in both male and female rodents (337), but it can also reveal nigrostriatal sensorimotor dysfunction (338, 339) and depression-like behaviour (340, 341). In this test, YAC128 (genotype: $F_{1,38}$=12.68, p<0.01) (Fig. 3.7A) and vehicle- treated Q140 mice (treatment: ($F_{2,36}$=4.002, p<0.05) (Fig. 3.7B) scored significantly lower than WT littermates, while GM1-treated YAC128 mice scored as well as WT animals (treatment: $F_{1,38}$=5.274, p<0.05) (Fig. 3.7A). In Q140 mice, the overall improvement observed in GM1-treated animals did not reach statistical significance (p=0.08; Fig. 3.7B).

3.3.3 GM1 improves social cognition/memory

GM1 effects on sociability and social cognition (or social memory) were measured with the Crawley's social approach test (342, 343). In the "sociability" session of the test - where the rodent instinctive preference to spend time with other mice rather than alone is assessed - all mice performed similarly, regardless of genotype and treatment, and spent more time in the compartment with the stranger mouse (stranger 1, social side) than in the empty compartment (non-social side, Fig. 3.8A). In the second part of the test, where social cognition/memory is measured as the propensity of mice to spend more time with unfamiliar mice rather than with familiar ones (342, 343), vehicle-treated YAC128 mice failed to show preference for a novel mouse (stranger 2, Fig 3.8B). Such a behaviour has been linked to impaired social cognition and

memory (342, 343). Treatment with GM1 did not affect WT mice, but restored normal behaviour in YAC128 mice (Fig. 3.8B) (interaction: $F_{1,53}=7.275$, $p<0.01$).

3.3.4 GM1 improves cognitive performance in HD mice

Habituation is a learning process whereby animals learn not to respond to repetitive, redundant and inconsequential stimuli (344). In a novel environment, habituation is manifested as a decrease in exploratory activity over time, once the animal has acquired enough information to form a "cognitive map" of the properties of the novel environment (345). In experimental settings, habituation can be measured as the change in exploratory activity that occurs over the time as mice explore a novel open field arena (346). Habituation that occurs within the same test session (intrasession) is considered a measure of non-associative learning, while habituation that occurs in subsequent test sessions (intersession) is dependent on memory of the previous session/s (346-348). None of the mice we tested showed intrasession habituation (which would have been indicated by an activity change ratio < 0.5; Fig. 3.9A), however, YAC128 mice had a higher activity change ratio than WT controls (genotype: $F_{1,64}=4.177$, $p<0.05$). Intersession habituation was evident on the second day of testing in both WT and GM1-treated YAC128 mice, but not in vehicle-treated YAC128 animals (Fig. 3.9B). These data suggest the presence of a memory deficit in YAC128 mice (genotype $F_{1,62}=4.471$, $p<0.05$) and a beneficial effect of GM1 on memory (treatment $F_{1,62}=6.518$, $p<0.05$).

We were not able to detect intrasession nor intersession habituation in Q140 and Q7/7 mice (Fig. 3.9C and D). However, vehicle-treated Q140 mice (heterozygous and homozygous together) displayed increased activity at the end of the first 30 min trial (as measured by increased activity change ratio) when compared to other groups (treatment: $F_{2,71}=5.578$, $p=0.0056$). This unusual behaviour, likely related to increased anxiety (346), was not present in GM1-treated Q140 mice

(Fig. 3.9C). Once again, these observations highlight the widespread restorative properties of GM1 in HD models.

Next, we assessed spatial working memory of Q7/7 and Q140 mice in the Y-maze. This test is based on the innate preference of mice to explore a novel environment, and their ability to remember the last explored arm of a Y-maze and to alternate arm entries. In this test, the fraction (or percent) of correct alternation is a measure of spatial working memory (349, 350). Interestingly, the Y-maze was the only test in our study where we were able to observe a difference in behaviour between heterozygous and homozygous Q140 mice. Consequently, this is the only test where data for the two groups are shown separately. WT mice usually score above chance level (50%). As shown in Fig. 3.10A, Q7/7 mice scored above chance level (correct alternation rate > 50%; chi-square test, $p<0.05$), as expected (351). Surprisingly, vehicle-treated homozygous Q140 mice performed as well as Q7/7 in this test, while the average alternation rate for vehicle-treated heterozygous Q140 mice was lower than 50% (although this average was not significantly different from chance; chi square test, $p=0.07$, it was significantly worse than Q7/7 mice; treatment $F_{4,50}=8.120$, $p<0.01$) and these mice made significantly more arm entries than Q7/7 mice prior to performing a correct alternation (treatment $F_{2,32}=6.373$ $p<0.01$) (Fig. 3.10B). This type of performance in the Y-maze has been linked to perseverative behaviour in rodents (352), and might reflect a common problem among HD patients (353, 354). GM1 restored a normal phenotype (Fig. 3.10B). Overall exploratory and locomotor activity in this test was similar across experimental groups (total arm entries, Fig. 3.10C). Data shown are for male mice only, as all females scored similarly in the Y-maze and always above chance level, regardless of genotype and treatment (data not shown).

3.3.5 GM1 treatment normalizes neurochemical changes in the brain of HD mice

To uncover neurochemical changes induced by treatment with GM1, we analyzed cortical and striatal levels of major neuroactive amino acids and biogenic amines. Only male animals were used, to avoid potential confounding effects of estrous cycle on the dopaminergic system (355, 356).

As shown in Table 3.1, levels of the neurotransmitter glutamate and were lower in cortical tissue from YAC128 mice compared to WT (genotype: $F_{1,27}=6.713$, $p<0.05$). Normal levels were restored with GM1 treatment (Table 3.1). In cortical tissue from YAC128 mice we also observed a significant effect of treatment for levels of GABA, glycine, D-serine (D-Ser) and L-serine (L-Ser) (treatment: $F_{1,27}=5.1$, $p<0.05$, $F_{1,27}=7.0$, $p<0.05$, $F_{1,27}=10.17$, $p<0.01$, and $F_{1,27}=6.79$, $p<0.05$ respectively). GM1 restored levels of GABA, L-Ser and D-Ser to normal, however, only GABA levels of HD CSF mice were significantly reduced. Glycine levels were also elevated by GM1, on average, but the difference with untreated animals did not reach statistical significance.

In the striatum of YAC128 mice, DA and metabolites resulting from DA turnover - 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) - were significantly decreased compared to WT (Table 3.2) (genotype: DA $F_{1,27}=6.16$, $p<0.05$, DOPAC $F_{1,27}=16.8$, $p<0.001$, HVA $F_{1,27}$)=18.9, $p<0.001$). GM1 treatment raised DA levels in both WT and YAC128 mice, modestly but significantly (treatment: $F_{1,27} = 5.09$, $p<0.05$), and at the same time decreased DOPAC levels (treatment: $F_{1,27} = 11.1$, $p<0.01$), DOPAC/DA ratio (treatment: $F_{1,27}=18.2$, $p<0.001$) and DOPAC + HVA/DA ratios (treatment: $F_{1,27} = 16.7$, p<0.001). These data suggest decreased activity of monoamine oxidase (MAO) enzymes in the striatum of animals treated with GM1.

In contrast, cortical levels of DA were similar across groups (Table 3.2), while DOPAC levels were significantly elevated in CSF treated YAC128 mice compared to WT. GM1 significantly decreased DOPAC levels in YAC128 mice (interaction: F $F_{1,27}$ = 14.2, $p<0.001$). In parallel, GM1 decreased HVA levels in YAC128 mice, but increased them in WT animals (interaction: $F_{1,27}$ = 6.4, $p<0.05$). Therefore, GM1 treatment appeared to have an overall modulatory action on the dopaminergic system and DA turnover, with the direction of the effects being dependent, likely, on overall tissue dopaminergic tone.

5HT and its metabolites were also affected by GM1 administration, in a manner likely depending on overall serotonergic tone. GM1 increased cortical but not striatal 5HT levels in YAC128 mice (treatment: $F_{1,26}$ = 4.6, $p<0.05$). Concomitantly, GM1 decreased 5-hydroxyindoleacetic acid (5HIAA), a product of 5HT catabolism by MAOs, both in the striatum (genotype: $F_{1,27}$=15.35, $p<0.01$ and treatment: $F_{1,27}$=5.96, $p<0.05$) and in the cortex of YAC128 mice (interaction: $F_{1,27}$=4.37, $p<0.05$) (Table 3.2). Overall, these changes suggest slower or decreased 5HT turnover (357), as a result of GM1 administration.

3.4 Discussion

Current treatments for HD are limited to the management of motor symptoms and psychiatric problems, and do not affect disease course. Disease-modifying therapies that ameliorate all symptoms of HD, not just chorea, and that can stop or slow down disease progression are urgently needed.

In this study, intraventricular infusion of GM1 for 28-42 days reversed behavioural and cognitive dysfunctions in YAC128 and Q140 HD mouse models, and corrected underlying neurochemical defects.

Administration of GM1 was able to restore WT behaviour in HD mice in a battery of tests that measure anxiety-like behaviours elicited by different anxiogenic paradigms. The tests we used are based on overlapping yet distinct psychological and neurobiological substrates (358) and, in fact, were shown to be differentially affected by anxiolytic drugs or mouse strain/genotype (359, 360). In addition, GM1 normalized open-field defecation, a sympathetic-driven response to anxiogenic stress that is shared by rodents (361-363) and humans (364, 365) and that is exacerbated in HD mouse models. The ability of GM1 to correct HD behaviour in each of these tests - and in mouse models with different genetic backgrounds - is a clear and powerful indication of the disease-modifying properties of GM1 in HD models and of its potential clinical relevance (360).

Depression is an important feature of the human HD pathology and one that, for obvious reasons, is difficult to recapitulate in all its complexity in animal models (366). Nevertheless, tests such as the forced swim test remain a gold standard in the pharmacological evaluation of molecules with antidepressant activity. Using this test, depression-like behaviour was reported in most HD models (367). Consistent with previous work (309), YAC128 mice displayed increased despair/resignation in the forced swim test compared to WT littermates. GM1 restored behaviour to WT. In 6 month-old mice the anti-depressant effects of GM1 were limited to HD mice. However, in 9 month-old mice, GM1 administration also reversed an age-dependent increase in depression-like behaviour (368, 369) which developed in older WT mice. Altogether, these data suggest that GM1 targets dysfunctions underlying depressive behaviour that can be triggered by expression of pathological mutant HTT in HD mice, or by normal aging in WT animals. GM1 improved the performance of HD mice also in the novelty-suppressed feeding test and in nest building. Although not specific for depression, these tests are affected by and can reveal

depression-like behaviours in rodents (340, 341, 370). The novelty-suppressed feeding test in particular has been widely used to assess the efficacy of anti-depressants (371-373). Therefore, the improvement observed in HD mice in these tests upon treatment with GM1 might be due to decrease of both anxiety and depression.

A large number of factors and mechanisms can contribute to anxiety and depression in the general population and in HD. These include alterations of the serotonergic system, dysfunction of the hypothalamic-pituitary-adrenal (HPA) axis, impaired neurogenesis and/or decreased neurotrophic support, among others (367, 374-376). In HD patients, these dysfunctions are secondary to expression of mutant HTT and the resulting cascade of cellular and molecular toxic effects, which include dysregulation of ganglioside synthesis (137). While we do not know whether the latter contributes to development of depression in HD, various studies have shown the involvement of gangliosides in depression and/or its underlying mechanisms. Mice lacking GD3 and other b-series gangliosides have impaired adult neurogenesis and display depression-like behaviour (377). It is also well established that gangliosides play a major role in neurotrophin signaling (148, 149, 160), which, in turn, could affect depression-like behaviour. Whether exogenously administered GM1 acts through one or more of these pathways in our models remains to be investigated.

In the forced swim test, GM1 treatment increased the time mice spent swimming, but not the time spent climbing. This is reminiscent of the effects of selective serotonin reuptake inhibitors (SSRI), and is in contrast to the effects of antidepressants that elevate noradrenaline levels, which increase time spent climbing (378-380). In line with these observations, GM1 significantly increased cortical levels of 5HT but not adrenaline in YAC128 mice (Table II). Concomitantly, GM1 also decreased 5HIAA levels in YAC128 mice, suggesting decreased 5HT turnover (357).

Of note, GM1 and other gangliosides can directly bind 5HT (157, 381, 382) and facilitate its interaction with 5HT receptors (156). Furthermore, GM1 was shown to increase functional coupling of 5HT receptors with adenylate cyclase (383), thereby increasing efficiency of serotonergic transmission. Altogether, our data and previous reports suggest that GM1 has a modulatory effect on the serotonergic system.

GM1 treatment improved cognitive behaviour in both YAC128 and Q140 mice, as shown by improved performance in Crawley's test, Y-maze and open-field habituation. In the Crawley's test (343), YAC128 mice spent more time with a familiar mouse rather than a new mouse. This behaviour is usually attributed to altered recognition memory and impaired social discrimination (343, 384) and can also be observed in AD mouse models (384, 385). Preference for the familiar animal could also reflect a perseverative behaviour and the inability to inhibit responding to an initial stimulus. Perseveration, also described as cognitive inflexibility, is a common executive dysfunction in HD patients (354). The presence of a perseverative phenotype in Q7/Q140 heterozygous mice was indeed suggested by an increase in repetitive choices made by these mice in the Y-maze test (386-388). A similar behaviour is also observed in models of obsessive-compulsive disorders (388, 389) or following impairment of serotonergic pathways (390). GM1 corrected the perseverative phenotype of Q7/Q140 heterozygous mice to WT. Note that the Y-maze was the only test where we observed a substantial difference between the behaviour of heterozygous (Q7/Q140) and homozygous (Q140/Q140) mice. The latter performed as well as Q7/Q7 mice in this test. The underlying reason for this is not known.

Administration of GM1 also improved HD mice intersession habituation in the open field. Intersession habituation is a complex behaviour that depends on non-associative learning and long-term memory (346, 348), and that is compromised in AD models (347) and by drugs that

affect memory (391, 392). Levels of 5HT, acetylcholine and glutamate play a major role in the habituation process (348). Although we did not measure acetylcholine levels, our study revealed changes in glutamate and 5HT levels that correlate with the beneficial effects of GM1 on habituation in HD mice. GM1 increased cortical levels of 5HT (discussed above) and glutamate in YAC128 mice along with D-Ser, an NMDA receptor co-agonist, and its precursor L-Ser (393). Of note, cortical glutamate levels were lower than normal in YAC128 mice – likely reflecting early cortical pathology in YAC128 mice and in HD patients (44, 394-397) - but were restored to normal by GM1.

It is not known whether decreased ganglioside levels (137) can contribute, per se, to cognitive dysfunction in HD. However, there is evidence that genetic or pharmacological perturbation of ganglioside synthesis in mice (162, 164, 398) leads to learning and memory deficits. Vice versa, a pharmacological activator of the ganglioside biosynthetic pathway (398-400) and exogenously administered GM1 (401) were shown to improve (long term potentiation) LTP and cognition in rodent models, and to improve learning and memory in models of AD (402) and aging (403). Other studies have suggested that GQ1b - a b-series ganglioside – but not GM1, has a crucial role in learning and memory (404, 405). Whether administration of GM1 in HD models also raises levels of GQ1b or other gangliosides is not yet known.

Psychiatric and cognitive problems, including cognitive inflexibility, have been linked to various changes in the monoaminergic systems, in both HD and non-HD populations (374, 406-408). However, the exact contribution of the DA system to HD pathology remains unclear (409, 410). DA levels are decreased in various HD animal models (411-413), while results in HD patients suggest dynamic and biphasic changes in the DA system (414-416). In this study, we found decreased DA levels in the striatum of YAC128 mice compared to WT. We also found region-

specific differences in the levels of DA metabolites between YAC128 and WT littermates, with DOPAC (and HVA) being lower than normal in the striatum, but higher than normal in the cortex. These findings might reflect changes in MAOs expression and/or activity, as reported in other studies (417-420), while the direction of the changes observed might depend on regional differences in dopaminergic tone. Of note, polymorphisms in the genes that code for monoamine oxidase A (MAOA) and catechol-O-methyltransferase (COMT) have been recently shown to act as modifiers of cognitive and psychiatric symptoms in a Danish HD population (421), thus strengthening the link between MAOA activity, dopaminergic tone and cognitive and psychiatric manifestations of HD.

GM1 treatment had somewhat variable effects on DA turnover, depending on brain region and mouse genotype. In fact, GM1 decreased DOPAC and DOPAC/DA ratio to normal levels in the cortex of YAC128 mice, but further decreased DOPAC and DOPAC/DA ratio below normal levels in the striatum. GM1 also affected DOPAC levels (and DOPAC/DA ratio) in WT animals. We speculate that changes induced by GM1 might reflect a modulatory, rather than inhibitory action of GM1 on DA turnover. A modulatory role of GM1 was also suggested in previous studies, where administration of GM1 was shown to improve dopaminergic transmission only in aged rats, but not in young animals (422-424).

Our study also revealed a small but significant decrease in GABA levels in the cortex of YAC128 mice compared to WT. These findings are in line with early studies showing decreased GABA levels in cortical tissue from HD patients (425), and with more recent reports of reduced GABAergic inhibitory input from cortical interneurons (426, 427) in HD models (426-428). GM1 restored GABA levels to normal.

It has been proposed that different combinations of molecular or cellular dysfunctions (367) and/or different patterns of cortical pathology (429, 430) underlie motor, psychiatric and cognitive manifestations in HD. The fact that GM1 is able to correct a large spectrum of emotionality-related and cognitive phenotypes in addition to motor symptoms in animal models (212) strongly supports a disease-modifying role for GM1 in HD. In addition, our rigorous studies validate the effects of GM1 in mice of different strains and with different genetic background. To the best of our knowledge, no other pharmacological treatment has ever been shown to have as widespread and remarkable therapeutic effects as GM1. Comparable results were only obtained with antisense oligonucleotide (ASO) approaches to decrease HTT expression (431).

The profound therapeutic effects exerted by GM1 can only be explained if the treatment targets mHTT itself or crucial downstream pathogenic steps. We previously demonstrated that administration of exogenous GM1 promotes HTT phosphorylation at amino acid residues Ser13 and Ser16 (212, 223), which in turn was shown to dramatically decrease mHTT toxicity (213, 224, 225). In addition to this crucial effect on HTT post-translational modification, exogenous GM1 corrects, at least in part, a ganglioside deficit which is present in HD models and that we proposed could play an important role in HD pathogenesis (137, 212). Finally, studies on other models of acute and chronic neurodegeneration suggest that GM1 administration might trigger several beneficial effects, from modulation of neurotrophin signaling to stimulation of neurogenesis and regulation of calcium homeostasis (148, 149, 160). It is not yet known whether these same mechanisms further contribute to the therapeutic effects of GM1 in HD.

The remarkable therapeutic effects of GM1 that we have shown in this study and in previous work in HD models warrant clinical investigations in HD patients. A neuroprotective role of

GM1 was shown in PD animal models (432-434). Later on, sustained benefits of the ganglioside were shown in a small randomized double blind placebo controlled trial, where PD patients receiving GM1 by daily subcutaneous injections improved on the UPDRS (288) and showed a decrease in the rate of loss of DA transporter in striatal regions (196). Importantly, in this trial and in a five-year open label extension study, administration of GM1 via subcutaneous injection was shown to be safe (196, 287). To which extent peripherally administered GM1 reaches therapeutic concentrations in the brain remains unclear (435, 436) and therefore it is possible that the clinical effects of GM1 could be better revealed by enhanced delivery of the drug to the brain.

Figure 3.1: GM1 normalizes anxiety-like behaviour in YAC128, Q140 and R6/2 mice
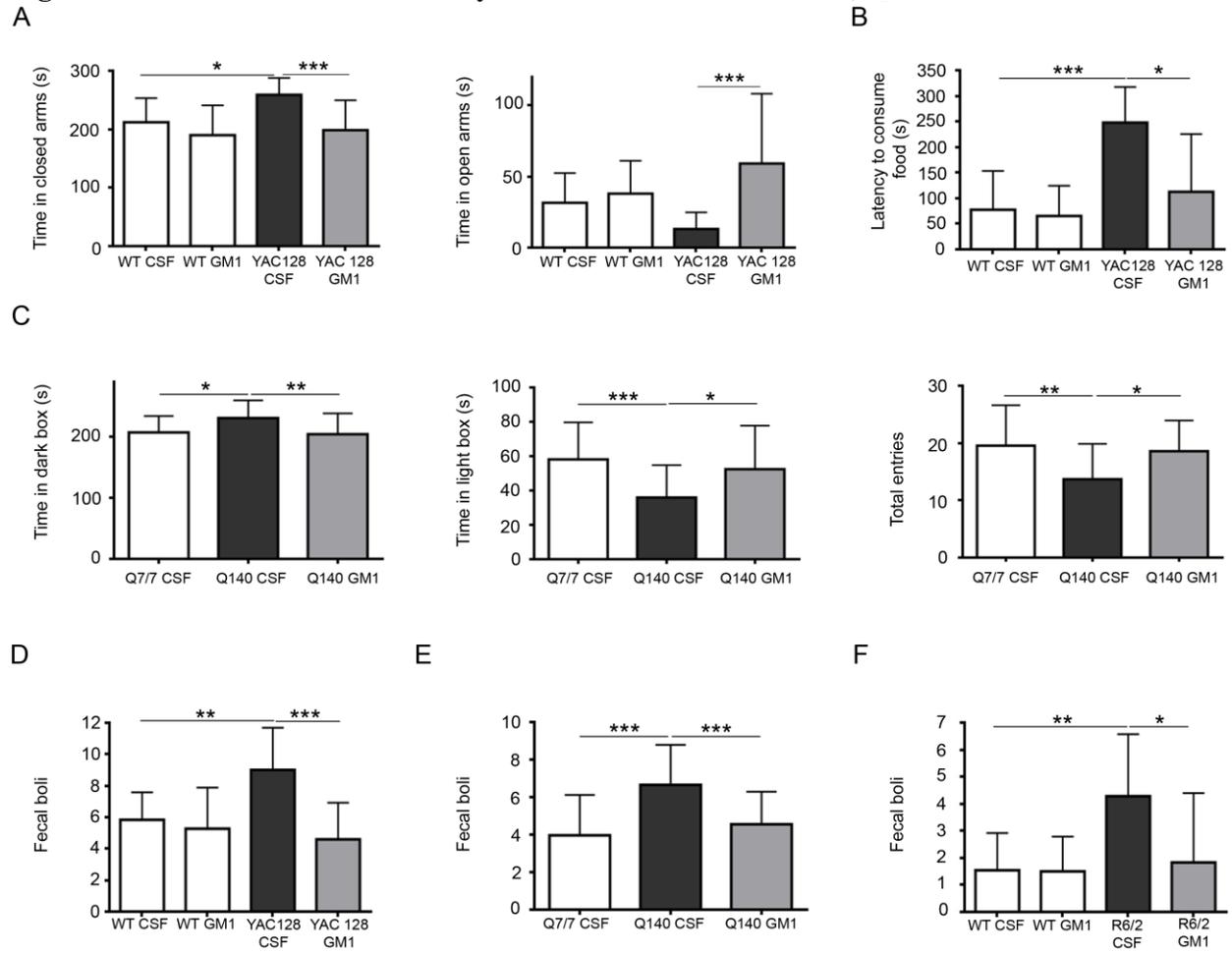
Figure 3.1 GM1 normalizes anxiety-like behaviour in YAC128, Q140 and R6/2 mice. (A)

Elevated plus maze. Six-month old YAC128 mice and WT littermates were placed in the center

of an elevated plus maze apparatus and left to explore for 5 min. Vehicle-treated YAC 128 mice

spent significantly more time in the closed arms and less in the open arms of the maze than WT

mice. GM1-treated YAC128 mice behaved as WT littermates. N= 15 WT CSF, 14 WT GM1, 13

YAC128 CSF, 19 YAC128 GM1. (B) Novelty-suppressed feeding. Nine month-old YAC128

mice showed increased latency to consume food in a novel environment, compared to WT

littermates. Treatment with GM1 reduced latency to WT levels. N = 10 WT CSF, 8 WT GM1, 6

YAC128 CSF, 8 YAC128 GM1. (C) Light/dark box test. Six to eight-month old Q140 mice

(heterozygous and homozygous combined) that were administered vehicle spent more time in the

dark box and less in the light box, and transitioned fewer times between boxes (total entries)

compared to Q7/7 littermates. GM1 restored WT performance. N = 27 Q7/7 CSF, 26 Q140 CSF,

29 Q140 GM1. (D) Fecal boli in open field arena. Nine month-old YAC128 mice were

individually placed in an open field arena for 30 min and the fecal boli dropped in the arena

during this time were counted. Vehicle-treated YAC128 mice dropped more fecal pellets than

WT mice. GM1 administration decreased the number of fecal boli to normal. N = 12 WT CSF,

11 WT GM1, 9 YAC128 CSF, 10 YAC128 GM1. (E) Same test as in (D) performed on 6-8

month-old Q140 mice (heterozygous and homozygous combined) and Q7/7 littermates. N= 24

Q7/7 CSF, 26 Q140CSF, 29 Q140 GM1. (F) Fecal boli excreted by 9-10 week-old R6/2 mice

after 5 min in an open field arena. N = 11 WT CSF, 14 WT GM1, 7 R6/2 CSF, 6 R6/2 GM1.

Statistical analysis was performed with Two-way (A, B, D and F) or one-way ANOVA (C and

E) followed by Bonferroni post-test. Bars represent mean values ± STDEV. *$p<0.05$, **$p<0.01$,

***$p<0.001$.

Figure 3.2: Distance travelled in an open field test is similar in WT and YAC128 mice.
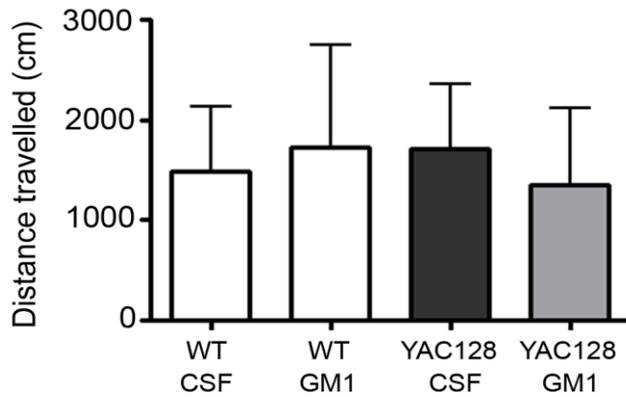
Figure 3.2 Distance travelled in an open field test is similar in WT and YAC128 mice.

Distance travelled by mice in 5 min in an open field arena was measured. Total distance travelled was similar for all groups. Bars represent mean values ± STDEV. N= 15 WT CSF, 14 WT GM1, 13 YAC128 CSF, 19 YAC128 GM1. Two-way ANOVA with Bonferroni post-test.

Figure 3.3: Acute adinazolam treatment decreases anxiety-like behaviour in YAC128 mice
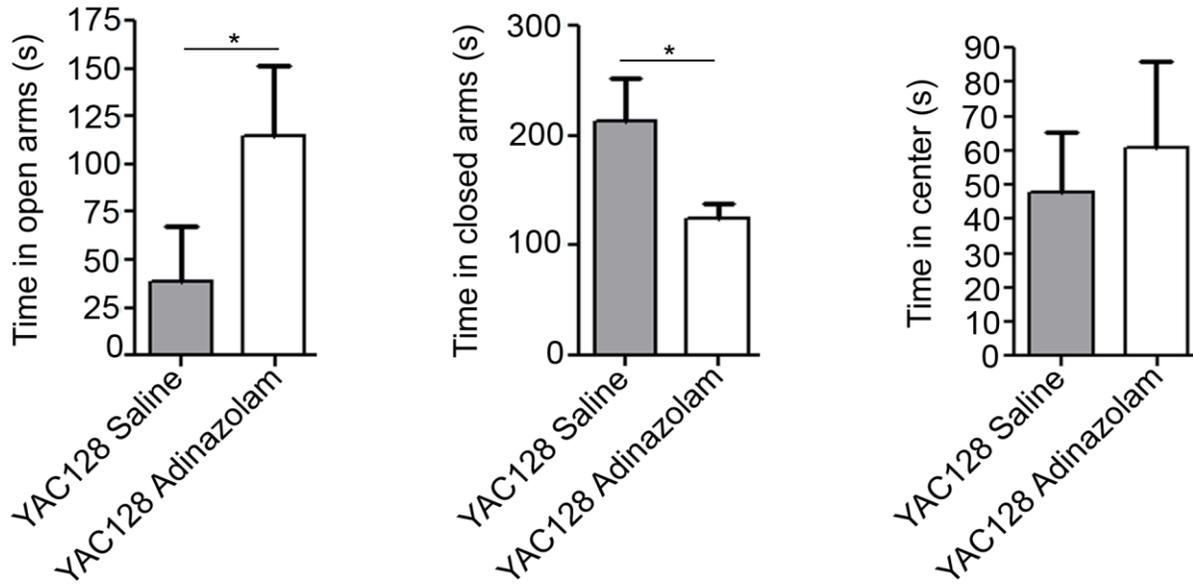
Figure 3.3 Acute adinazolam treatment decreases anxiety-like behaviour in YAC128 mice.

30 min prior to placement in the elevated plus maze, 6-10 month old YAC128 mice were injected intraperitoneally with saline or 2.5 mg/kg adinazolam. Time spent in each of the three compartments of the elevated plus maze was recorded. Bars are means ± STDEV. N= 4 YAC128 saline, 4 YAC128 adinazolam. Student's t-test. ***$p<0.001$

Figure 3.4: GM1 improves depression-like behaviour in YAC128 and Q140 mice.
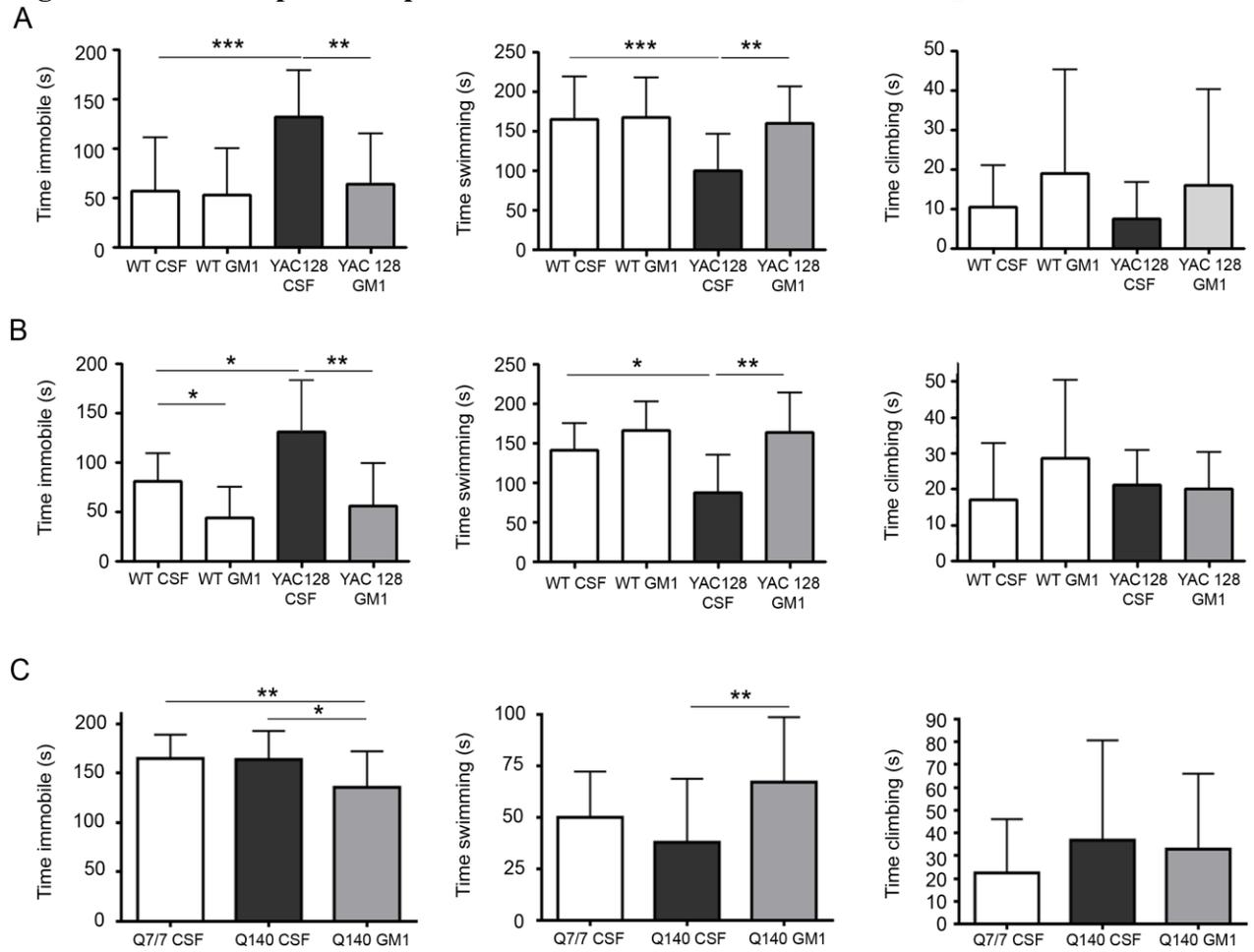
Figure 3.4 GM1 improves depression-like behaviour in YAC128 and Q140 mice. (A) 6 month-old YAC128 mice and WT littermates treated with vehicle (CSF) or GM1 were assessed in the forced swim test. Vehicle-treated YAC128 mice spent more time immobile and less time swimming than WT littermates. Treatment with GM1 reverted YAC128 behaviour to normal. N= 20 WT CSF, 19 WT GM1, 19 YAC128 CSF, 14 YAC128 GM1. (B) Nine month-old YAC128 mice were administered GM1 or CSF and tested as in (A). Treatment with GM1 decreased depression-like behaviour in both YAC128 and WT mice. N = 12 WT CSF, 12 WT GM1, 8 YAC128 CSF, 9 YAC128 GM1. (C) 6-8 month-old Q140 mice were assessed in the forced swim test as described in (A). GM1 decreased the time Q140 mice (heterozygous and homozygous combined) spent immobile and increased the time spent swimming. N = 22 Q7/7 CSF, 22 Q140 CSF, 18 Q140 GM1. Two-way (A and B) or one-way (C) ANOVA with Bonferroni correction were used to analyze data. Bars represent mean values ± STDEV. *$p<0.05$, **$p<0.01$, ***$p<0.001$

Figure 3.5: YAC128 and Q140 mice have normal performance in simple swim tests
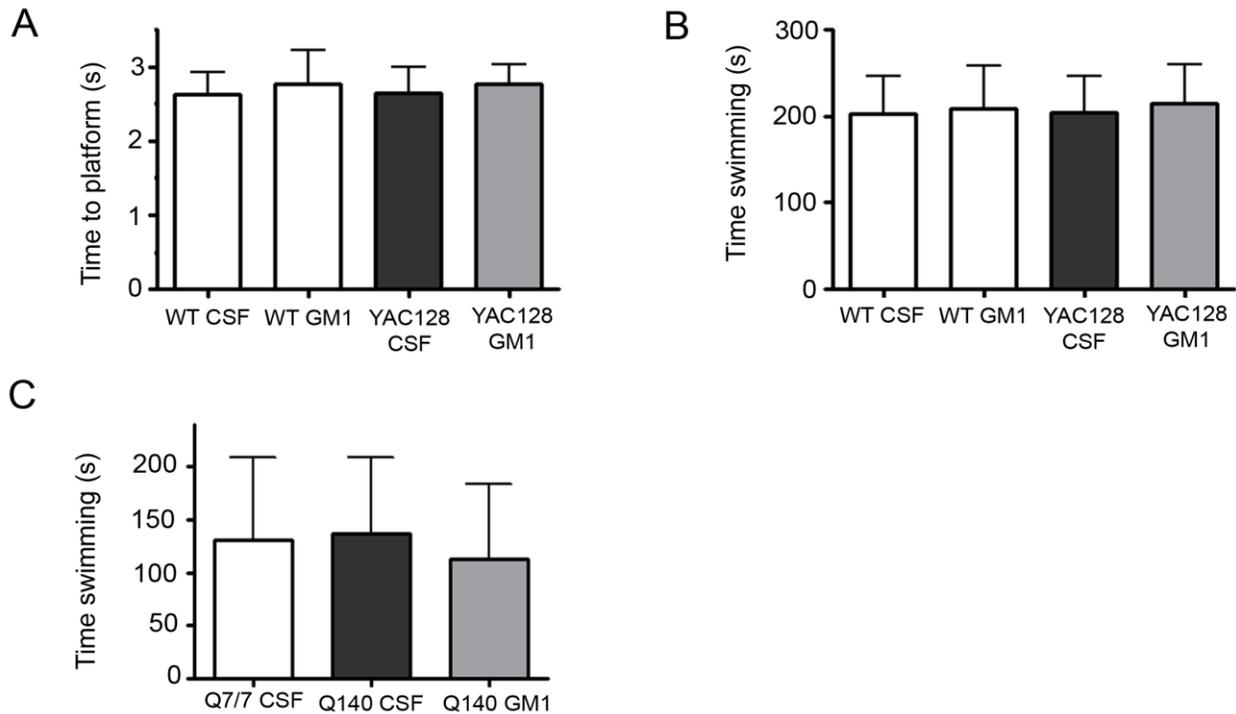
 Figure 3.5 YAC128 and Q140 mice have normal performance in simple swim tests. (A) YAC128 mice and WT littermates were trained to swim to a platform placed at one end of a narrow rectangular water pool. The time required to swim to the platform was recorded. All mice showed comparable performance, independently of genotype and treatment. (B) Swimming endurance of YAC128 and WT mice in a circular water pool was measure during a 6 min-session (same duration as the forced swim test). As in the forced swim test, time mice spent swimming or floating was measured in the last 4 min of the test. All groups of mice performed comparably in this test. Results for 6 and 9 month-old mice were similar and were combined. N = 21 WT CSF, 23 WT GM1, 17 YAC128 CSF, 16 YAC128 GM1. (C) Swimming endurance of Q7/7 and Q140 mice (heterozygous and homozygous combined). Test was performed as in (B). N= 22 Q7/7 CSF, 22 Q140 CSF, 19 Q140 GM1. Bars show mean values ± STDEV. Two-way (A and B) or one-way (C) ANOVA with Bonferroni post-test.

Figure 3.6: Acute imipramine treatment decreases depression-like behaviour of YAC128 mice in the forced swim test
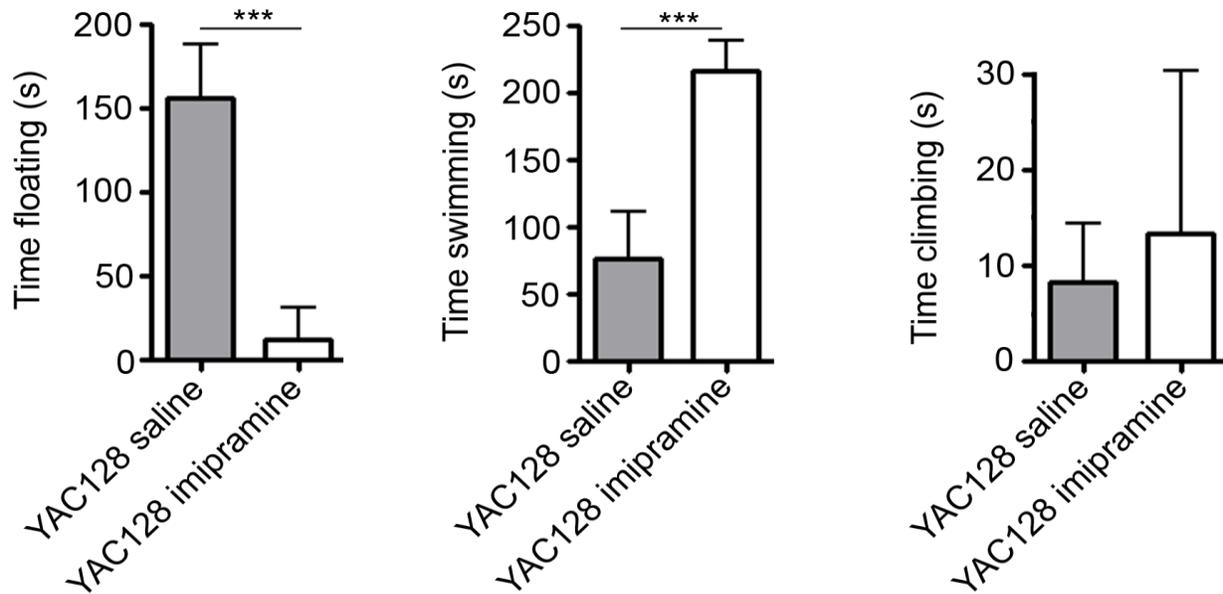
Figure 3.6 Acute imipramine treatment decreases depression-like behaviour of YAC128 mice in the forced swim test. 30 mins prior to placement in the forced swim test apparatus, 6-10 month-old YAC128 mice were administered saline or 10 mg/kg imipramine by intraperitoneal injection. The forced swim test was run for a total of 6 min and time spent swimming, floating or climbing was recorded in the last 4 min. Bars are mean values ± STDEV. N = 6 YAC128 saline, 6 YAC128 imipramine. Student's t test. ***$p<0.001$

Figure 3.7: GM1 improves nesting behaviour in YAC128 mice
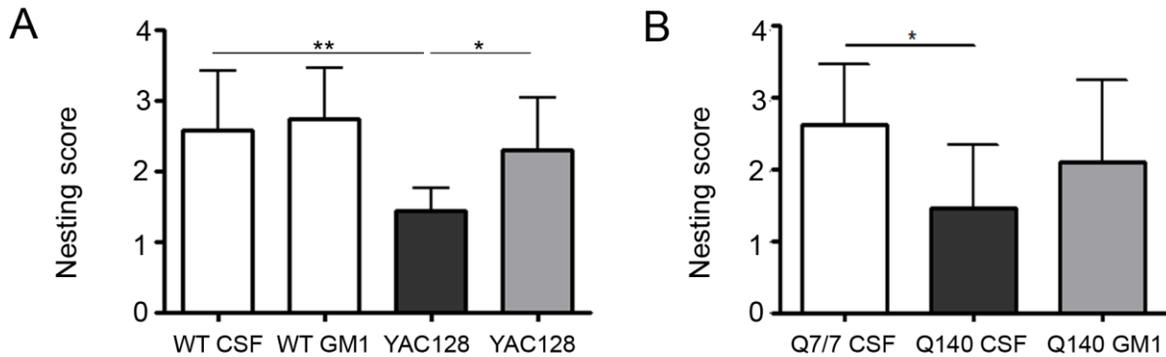
Figure 3.7 GM1 improves nesting behaviour in YAC128 mice. Mice ability to build a nest was scored on a 5 point scale (0-4) based on the amount of shredding of the material provided to build the nest and the height and shape of the nest. (A) YAC128 mice showed significant deficits on this task, but improved if treated with GM1. N = 12 WT CSF, 12 WT GM1, 8 YAC128 CSF, 10 YAC128 GM1. (B) Q140 mice scored less than Q7/7 littermates in the nest building test. The effects of GM1 were not statistically significant. N = 11 Q7/7 CSF, 12 Q140 CSF, 15 Q140 GM1. Bars are mean values ± STDEV. Two-way (A) or one-way (B) ANOVA with Bonferroni post-test. *p<0.05, **p<0.01, ***p<0.001.

Figure 3.8: GM1 improves social cognition and memory in YAC128 mice

A

Sociability

B

Preference for social novelty
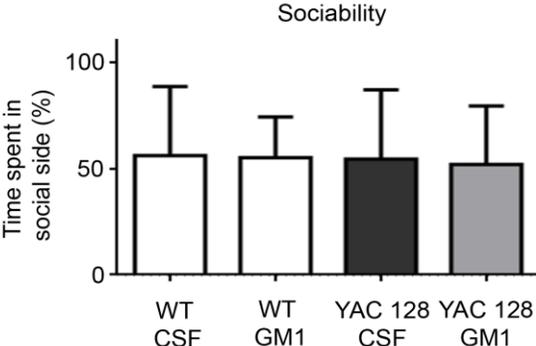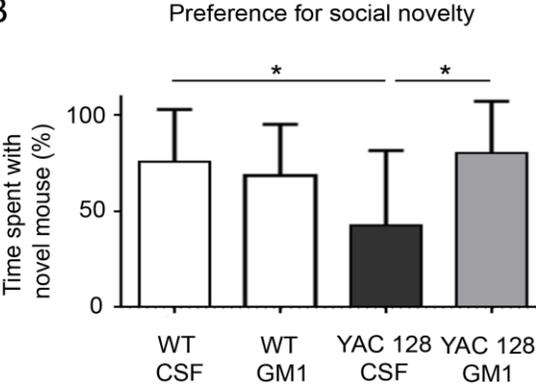
Figure 3.8 GM1 improves social cognition and memory in YAC128 mice. (A) Sociability test. YAC128 mice and WT littermates were placed in a three-chamber box containing two perforated metal containers in the left and right compartments and were left to explore the environment for 5 min. In the second 5 min period of the test, an age- and sex-matched mouse was placed in either the left or the right metal container and the time the experimental mouse spent in the social (side with the mouse) and in the non-social compartment (side without the mouse) was measured. All groups showed a natural preference for the social side, regardless of genotype and treatment. (B) Preference for social novelty. In the second part of the test, a novel mouse was introduced in the previously non-social compartment. WT mice, but not CSF-treated YAC128 mice, spent more time with the novel mice than with the old one, demonstrating a clear preference for social novelty. Normal behaviour was restored in YAC128 mice treated with GM1. Bars represent mean values ± STDEV. N = 18 WT CSF, 16 WT GM1, 16 YAC128 CSF, 17 YAC128 GM1. Bars are mean values ± STDEV. Two-way ANOVA with Bonferroni post-test. $*p<0.05$

Figure 3.9: GM1 improves open field habituation in YAC128 mice.
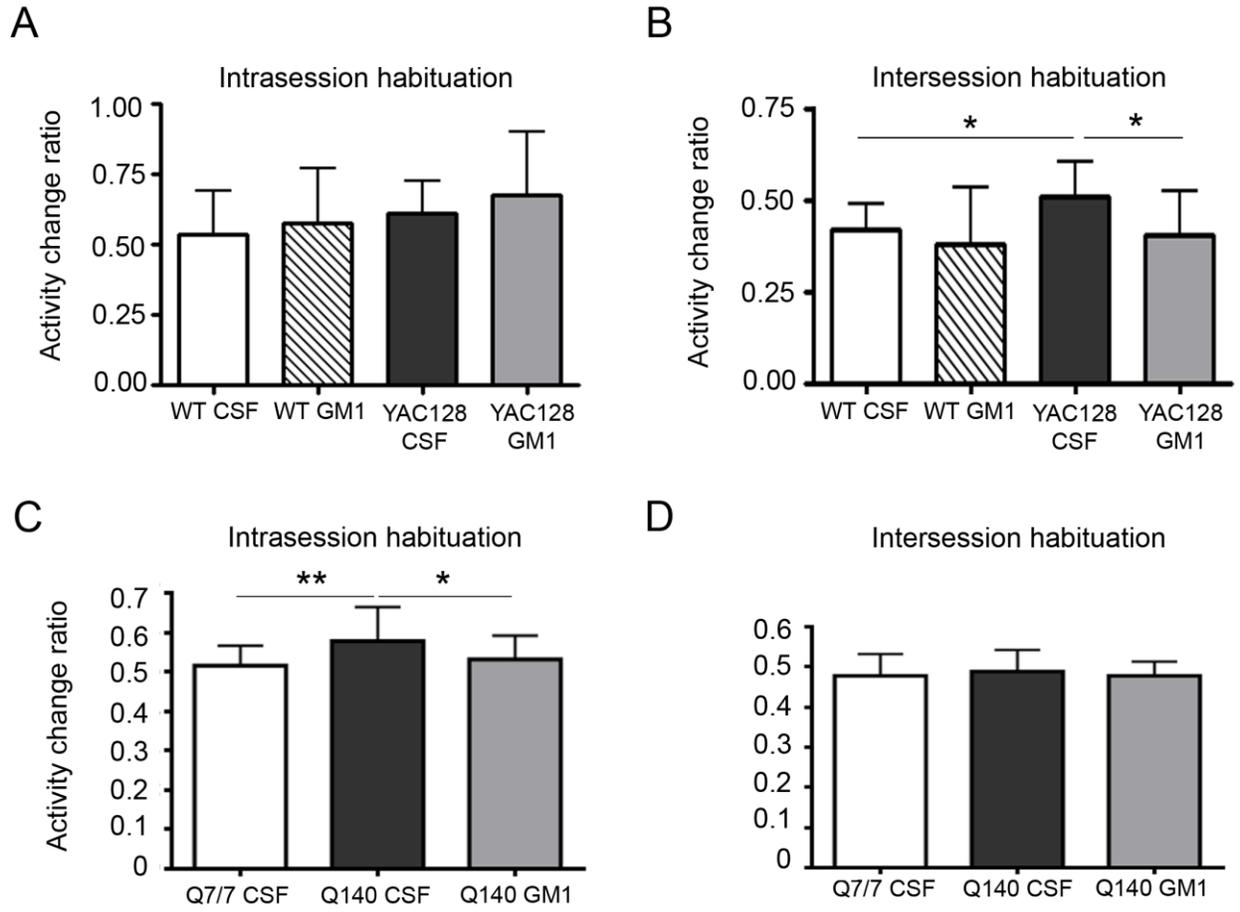
Figure 3.9 GM1 improves open field habituation in YAC128 mice. (A- B) 7-8 month-old YAC128 mice and WT littermates receiving GM1 or vehicle were placed in an open field apparatus on two consecutive days for 30 min each day. The change in activity between the first and last 5 min of testing on day 1 (*activity change ratio = distance travelled in the last 5 min/distance travelled in the first 5 min + last 5 min*) was used as a measure of intra-session habituation (A), while the change in total distance travelled between day 1 and day 2 (distance travelled on day 2/distance travelled on day 1 + day 2) reflected inter-session habituation (B). Mice did not show significant intra-session habituation, regardless of genotype and treatment. Vehicle-treated YAC128 mice showed a significant deficit in inter-session habituation, which was corrected by administration of GM1. N = 15 WT CSF, 14 WT GM1, 19 YAC128 CSF, 19 YAC128 GM1. (C-D) 6-8 month-old Q7/7 and Q140 (homozygous and heterozygous combined) receiving GM1 or vehicle were assessed in the open field habituation test as described in A and B. Mice did not show significant habituation in a 30 min session. However, vehicle-treated Q140 mice displayed a significant increase in the activity change ratio. This abnormal behaviour was not observed in YAC128 mice that received GM1. No differences in intersession habituation were observed among experimental groups. N = 25 WT CSF, 25 HD CSF, 24 HD GM. Bars are mean values ± STDEV. Two-way (A and B) or one-way (C and D) ANOVA with Bonferroni post-test. *$p<0.05$, **$p<0.01$.

Figure 3.10: GM1 improves spatial working memory in the Y-maze test in Q140 mice.
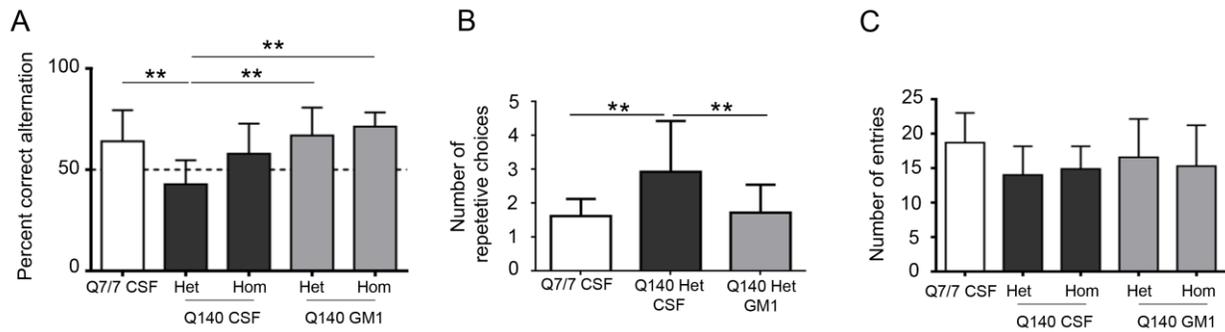
Figure 3.10 GM1 improves spatial working memory in the Y-maze test in Q140 mice. (A) 6-8 month-old male Q7/7 and Q140 mice were allowed to freely explore a Y-maze apparatus for 5 min, during which all arm entries were recorded. A correct alternation was defined as any sequence of three entries where no arm entry was repeated. Spatial working memory was assessed based on the percent correct alternation mice made. Q7/7 and homozygous Q140 mice alternated correctly above chance level (50%). Q140 heterozygous mice showed a perseverative behaviour and scored below 50% (or chance level). This aberrant behaviour was corrected by administration of GM1. (B) Q140 heterozygous mice made more repetitive choices in arm entries than Q7/7 littermates. GM1 normalized this perseverative behaviour. (C) Exploratory activity, as measured by the number of total arm entries, was similar among groups. Bars show mean values ± STDEV. N = 11 Q7/7 CSF, 12 Q140 CSF, 15 Q140 GM1. One-way ANOVA with Bonferroni post-test. **$p<0.01$.

Table 3.1: Amino acids

	WT		HD		Two-way ANOVA (Bonferroni post-test)
	CSF	GM1	CSF	GM1	
STRIATUM					
L-SER	100 ± 16.6	88.8 ± 11.4	96.6 ± 13.4	102.2 ± 8.8	N.S.
D-SER	100 ± 10.1	94.9 ± 9.9	99.3 ± 12.3	104.9 ± 7.6	N.S.
Glycine	100 ± 12.3	98.9 ± 10.6	104.4 ± 14.4	109.9 ± 7.8	N.S.
Glutamate	100 ± 12.3	86.6 ± 13.3	92.8 ± 13.4	97.4 ± 11.0	N.S.
Glutamine	100 ± 11.7	95.8 ± 16.8	98.4 ± 12.2	107.4 ± 10.6	N.S.
GABA	100 ± 3.1	93.4 ± 19.3	89.2 ± 4.3	104.1 ± 22.4	N.S.
CORTEX					
L-SER	100 ± 5.9	104.6 ± 12.2	88.4 ± 10.2	103.7 ± 12.9 ^	Effect of treatment: $F_{(1,27)}=6.79$, $p<0.05$ ^: HD GM1 ≠ HD CSF
D-SER	100 ± 5.8	112.0 ± 17.0	91.4 ± 9.9	108.6 ± 15.6 ^	Effect of treatment: $F_{(1,27)}=10.17$, $p<0.01$ ^: HD GM1 ≠ HD CSF
Glycine	100 ± 7.8	124.3 ± 54.9	88.1 ± 11.0	123.4 ± 31.5	Effect of treatment: $F_{(1,27)}=7.039$, $p<0.05$
Glutamate	100 ± 5.4	100.3 ± 4.9	88.0 ± 9.9 **	97.6 ± 8.8 ^	Effect of genotype: $F_{(1,26)}=6.713$; $p<0.05$ **: HD CSF ≠ WT CSF; ^: HD GM1 ≠ HD CSF
Glutamine	100 ± 5.3	106.5 ± 15.8	90.9 ± 9.4	101.8 ± 9.0	Effect of treatment: $F_{(1,27)}=5.516$; $p<0.05$
GABA	100 ± 9.3	101.2 ± 14.9	84.3 ± 7.5 *	103.4 ± 14.9 ^^	Effect of treatment: $F_{(1,27)}=5.12$, $p<0.05$ *: HD CSF ≠ WT CSF; ^^: HD GM1 ≠ HD CSF

Aminoacids levels were expressed as percentage of WT control (CSF). Values are means ± STDEV. Symbols used to indicate statistically significant differences between groups ($p<0.05$) were repeated twice or three times to indicate different p values ($p<0.01$ and $p<0.001$, respectively). N = 7-8 WT CSF, 6-7 WT GM1, 7-8 HD CSF, 7-8 HD GM1. N.S. = Not Significant

Table 3.2: Biogenic amines

	WT		YAC128		Two-way ANOVA (Bonferroni post-test)
	CSF	GM1	CSF	GM1	
STRIATUM					
DA	100 ± 8.7	105.2 ± 6.6	88.2 ± 12.0 ✓	99.2 ± 11.3	Effect of genotype: F (1,27)=6.16, $p<0.05$; Effect of treatment: F (1,27)=5.092, $p<0.05$ ✓: HD CSF ≠ WT GM1
DOPAC	100 ± 13.7	81.5 ± 9.8 §	78.0 ± 12.7 **	66.0 ± 13.9 ###	Effect of genotype F(1,27)=16.8, $p<0.001$; Effect of treatment F(1,27)=11.11, $p<0.01$ **: HD CSF ≠ WT CSF; §: WT GM1 ≠ WT CSF; ###: HD GM1≠
HVA	100 ± 11.7	97.9 ± 9.6	81.3 ± 6.7 **,✓	84.0 ± 12.6 #	Effect of genotype F(1,27)=18.92, $p<0.001$ **: HD CSF≠ WT CSF; ✓: HD CSF ≠ WT GM1; #: HD GM1 ≠ WT CSF
DOPAC/DA	100 ± 18.0	77.0 ± 12.4 §	88.0 ± 13.6	66.3 ± 13.0 ^,###	Effect of genotype F(1,27)=4.704, $p<0.05$; Effect of treatment F(1,27)=18.22, $p<0.001$ §: WT GM1 ≠ WT CSF; ^: HD GM1 ≠ HD
HVA/DA	100 ± 7.8	93.2 ± 9.8	92.8 ± 7.8	85.3 ± 15.0 #	#: HD GM1≠WT CSF
5HT	100 ± 14.8	97.5 ± 11.9	92.9 ± 22.1	93.3 ± 11.0	N.S.
5HIAA	100 ± 11.3	90.5 ± 11.3	85.1 ± 8.3 *	76.9 ± 9.3 ###	Effect of genotype F(1,27)=15.35, $p<0.001$; Effect of treatment: F(1,27)=5.961, $p<0.05$ *: HD CSF ≠ WT CSF; ###: HD GM1 ≠ WT CSF
5HIAA/5HT	100 ± 9.5	92.7 ± 10.2	95.8 ± 26.8	83.1 ± 17.2	N.S.
NA	100 ± 39.6	133.9 ± 64.4	137.3 ± 67.9	120.7 ± 42.1	N.S.
NME	N.D.	N.D.	N.D.	N.D.	
NME/NA	N.D.	N.D.	N.D.	N.D.	
CORTEX					
DA	100 ± 32.7	113.6 ± 19.0	106.4 ± 27.6	97.5 ± 14.1	N.S.
DOPAC	100 ± 26.7	123.1 ± 31.0	142.1 ± 31.7 **	92.1 ± 16.0 ^^	Interaction F(1,27)=14.26, $p<0.001$; **: HD CSF ≠ WT CSF; ^^: HD GM1 ≠ HD CSF
HVA	100 ± 9.9	118.3 ± 17.9 §	105.6 ± 17.6	97.2 ± 11.9 ∞	Interaction: F(1,27)=6.403, $p<0.05$; §: WT GM1 ≠ WT CSF; ∞: HD GM1 ≠ WT GM1
DOPAC/DA	100 ± 33.3	100.0 ± 19.4	133.3 ± 57.6	88.0 ± 5.9 ^	^: HD GM1≠ HD CSF
HVA/DA	100 ± 19.5	96.5 ± 16.6	95.7 ± 25.9	94.7 ± 15.1	N.S.
5HT	100 ± 14.0	101.6 ± 12.8	84.5 ± 19.2	105.4 ± 7.5 ^	Effect of treatment: F(1,26)=4.623, $p<0.01$; ^: HD GM1 ≠ HD CSF
5HIAA	100 ± 9.7	102.7 ± 19.1	104.5 ± 17.0	83.6 ± 15.8 ^	Interaction F(1,27)=4.369, $p<0.05$; ^: HD GM1 vs HD CSF
5HIAA/5HT	100 ± 23.0	98.4 ± 17.5	129.0 ± 52.2	80.4 ± 16.7 ^	Effect of treatment: F(1,27)=4.911, $p<0.05$; Interaction F(1,27)=4.324, $p<0.05$ ^: HD GM1 ≠ HD CSF
NA	100 ± 19.9	94.3 ± 12.9	95.3 ± 11.5	98.0 ± 14.8	N.S.
NME	100 ± 13.8	106.8 ± 12.9	112.4 ± 23.8	113.3 ± 9.7	N.S.
NME/NA	100 ± 25.3	110.6 ± 19.8	115.9 ± 33.6	107.8 ± 18.5	N.S.

Biogenic amine levels were expressed as percentage of WT control (CSF). Values are means ± STDEV. Symbols used to indicate statistically significant differences between groups ($p<0.05$) were repeated twice or three times to indicate different p values ($p<0.01$ and $p<0.001$, respectively). N = 7-8 WT CSF, 6-7 WT GM1, 7-8 HD CSF, 7-8 HD GM1. N.D. = Not Detected; N.S. = Not Significant.

3.5 References

21.	Ho AK, Gilbert AS, Mason SL, Goodman AO, Barker RA. Health-related quality of life in Huntington's disease: Which factors matter most? Movement disorders : official journal of the Movement Disorder Society. 2009;24(4):574-8.

35.	Moulton CD, Hopkins CW, Bevan-Jones WR. Systematic review of pharmacological treatments for depressive symptoms in Huntington's disease. Movement disorders : official journal of the Movement Disorder Society. 2014;29(12):1556-61.

44.	Rosas HD, Salat DH, Lee SY, Zaleta AK, Pappu V, Fischl B, et al. Cerebral cortex and the clinical expression of Huntington's disease: complexity and heterogeneity. Brain : a journal of neurology. 2008;131(Pt 4):1057-68.

61.	Mangiarini L, Sathasivam K, Seller M, Cozens B, Harper A, Hetherington C, et al. Exon 1 of the HD gene with an expanded CAG repeat is sufficient to cause a progressive neurological phenotype in transgenic mice. Cell. 1996;87(3):493-506.

64.	Slow EJ, van Raamsdonk J, Rogers D, Coleman SH, Graham RK, Deng Y, et al. Selective striatal neuronal loss in a YAC128 mouse model of Huntington disease. Human molecular genetics. 2003;12(13):1555-67.

69.	Menalled LB, Sison JD, Dragatsis I, Zeitlin S, Chesselet MF. Time course of early motor and neuropathological anomalies in a knock-in mouse model of Huntington's disease with 140 CAG repeats. The Journal of comparative neurology. 2003;465(1):11-26.

137.	Maglione V, Marchi P, Di Pardo A, Lingrell S, Horkey M, Tidmarsh E, et al. Impaired ganglioside metabolism in Huntington's disease and neuroprotective role of GM1. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2010;30(11):4072-80.

138.	Denny CA, Desplats PA, Thomas EA, Seyfried TN. Cerebellar lipid differences between R6/1 transgenic mice and humans with Huntington's disease. Journal of neurochemistry. 2010;115(3):748-58.

139.	Desplats PA, Denny CA, Kass KE, Gilmartin T, Head SR, Sutcliffe JG, et al. Glycolipid and ganglioside metabolism imbalances in Huntington's disease. Neurobiology of disease. 2007;27(3):265-77.

148.	Posse de Chaves E, Sipione S. Sphingolipids and gangliosides of the nervous system in membrane function and dysfunction. FEBS letters. 2010;584(9):1748-59.

149.	Mocchetti I. Exogenous gangliosides, neuronal plasticity and repair, and the neurotrophins. Cell Mol Life Sci. 2005;62(19-20):2283-94.

156.	Fantini J, Barrantes FJ. Sphingolipid/cholesterol regulation of neurotransmitter receptor conformation and function. Biochimica et biophysica acta. 2009;1788(11):2345-61.

157.	Krishnan KS, Balaram P. A nuclear magnetic resonance study of the interaction of serotonin with gangliosides. FEBS letters. 1976;63(2):313-5.

158.	Wu G, Xie X, Lu ZH, Ledeen RW. Sodium-calcium exchanger complexed with GM1 ganglioside in nuclear membrane transfers calcium from nucleoplasm to endoplasmic reticulum. Proceedings of the National Academy of Sciences of the United States of America. 2009;106(26):10829-34.

160.	Ledeen RW, Wu G. The multi-tasked life of GM1 ganglioside, a true factotum of nature. Trends in biochemical sciences. 2015;40(7):407-18.

162.	Takamiya K, Yamamoto A, Furukawa K, Yamashiro S, Shin M, Okada M, et al. Mice with disrupted GM2/GD2 synthase gene lack complex gangliosides but exhibit only subtle defects in their nervous system. Proceedings of the National Academy of Sciences of the United States of America. 1996;93(20):10662-7.

164. Sha S, Zhou L, Yin J, Takamiya K, Furukawa K, Furukawa K, et al. Deficits in cognitive function and hippocampal plasticity in GM2/GD2 synthase knockout mice. Hippocampus. 2014;24(4):369-82.

196. Schneider JS, Cambi F, Gollomp SM, Kuwabara H, Brasic JR, Leiby B, et al. GM1 ganglioside in Parkinson's disease: Pilot study of effects on dopamine transporter binding. Journal of the neurological sciences. 2015;356(1-2):118-23.

205. Wu G, Lu ZH, Obukhov AG, Nowycky MC, Ledeen RW. Induction of calcium influx through TRPC5 channels by cross-linking of GM1 ganglioside associated with alpha5beta1 integrin initiates neurite outgrowth. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2007;27(28):7447-58.

212. Di Pardo A, Maglione V, Alpaugh M, Horkey M, Atwal RS, Sassone J, et al. Ganglioside GM1 induces phosphorylation of mutant huntingtin and restores normal motor behavior in Huntington disease mice. Proceedings of the National Academy of Sciences of the United States of America. 2012;109(9):3528-33.

213. Gu X, Greiner ER, Mishra R, Kodali R, Osmand A, Finkbeiner S, et al. Serines 13 and 16 are critical determinants of full-length human mutant huntingtin induced disease pathogenesis in HD mice. Neuron. 2009;64(6):828-40.

223. Caron NS, Desmond CR, Xia J, Truant R. Polyglutamine domain flexibility mediates the proximity between flanking sequences in huntingtin. Proceedings of the National Academy of Sciences of the United States of America. 2013;110(36):14610-5.

224. Thompson LM, Aiken CT, Kaltenbach LS, Agrawal N, Illes K, Khoshnan A, et al. IKK phosphorylates Huntingtin and targets it for degradation by the proteasome and lysosome. The Journal of cell biology. 2009;187(7):1083-99.

225. Atwal RS, Desmond CR, Caron N, Maiuri T, Xia J, Sipione S, et al. Kinase inhibitors modulate huntingtin cell localization and toxicity. Nat Chem Biol. 2011;7(7):453-60.

287. Schneider JS, Sendek S, Daskalakis C, Cambi F. GM1 ganglioside in Parkinson's disease: Results of a five year open study. Journal of the neurological sciences. 2010;292(1-2):45-51.

288. Schneider JS, Gollomp SM, Sendek S, Colcher A, Cambi F, Du W. A randomized, controlled, delayed start trial of GM1 ganglioside in treated Parkinson's disease patients. Journal of the neurological sciences. 2013;324(1-2):140-8.

290. Group THsDCR. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. . Cell. 1993;72(6):971-83.

291. Imarisio S, Carmichael J, Korolchuk V, Chen CW, Saiki S, Rose C, et al. Huntington's disease: from pathology and genetics to potential therapies. Biochem J. 2008;412(2):191-209.

292. Vonsattel JP, DiFiglia M. Huntington disease. Journal of neuropathology and experimental neurology. 1998;57(5):369-84.

293. Vonsattel JP, Keller C, Cortes Ramirez EP. Huntington's disease - neuropathology. Handb Clin Neurol. 2011;100:83-100.

294. Aziz NA, Pijl H, Frolich M, van der Graaf AW, Roelfsema F, Roos RA. Increased hypothalamic-pituitary-adrenal axis activity in Huntington's disease. J Clin Endocrinol Metab. 2009;94(4):1223-8.

295. Petersen A, Bjorkqvist M. Hypothalamic-endocrine aspects in Huntington's disease. The European journal of neuroscience. 2006;24(4):961-7.

296. Spargo E, Everall IP, Lantos PL. Neuronal loss in the hippocampus in Huntington's disease: a comparison with HIV infection. Journal of neurology, neurosurgery, and psychiatry. 1993;56(5):487-91.

297.   Roos RA. Huntington's disease: a clinical review. Orphanet journal of rare diseases. 2010;5:40.

298.   Paulsen JS, Langbehn DR, Stout JC, Aylward E, Ross CA, Nance M, et al. Detection of Huntington's disease decades before diagnosis: the Predict-HD study. Journal of neurology, neurosurgery, and psychiatry. 2008;79(8):874-80.

299.   Berrios GE, Wagle AC, Markova IS, Wagle SA, Rosser A, Hodges JR. Psychiatric symptoms in neurologically asymptomatic Huntington's disease gene carriers: a comparison with gene negative at risk subjects. Acta Psychiatr Scand. 2002;105(3):224-30.

300.   Paulsen JS, Zhao H, Stout JC, Brinkman RR, Guttman M, Ross CA, et al. Clinical markers of early disease in persons near onset of Huntington's disease. Neurology. 2001;57(4):658-62.

301.   Snowden JS, Craufurd D, Thompson J, Neary D. Psychomotor, executive, and memory function in preclinical Huntington's disease. J Clin Exp Neuropsychol. 2002;24(2):133-45.

302.   Stout JC, Paulsen JS, Queller S, Solomon AC, Whitlock KB, Campbell JC, et al. Neurocognitive signs in prodromal Huntington disease. Neuropsychology. 2011;25(1):1-14.

303.   Knopman D, Nissen MJ. Procedural learning is impaired in Huntington's disease: evidence from the serial reaction time task. Neuropsychologia. 1991;29(3):245-54.

304.   Heindel WC, Butters N, Salmon DP. Impaired learning of a motor skill in patients with Huntington's disease. Behav Neurosci. 1988;102(1):141-7.

305.   Lawrence AD, Watkins LH, Sahakian BJ, Hodges JR, Robbins TW. Visual object and visuospatial cognition in Huntington's disease: implications for information processing in corticostriatal circuits. Brain : a journal of neurology. 2000;123 (Pt 7):1349-64.

306.   Wilson RS, Como PG, Garron DC, Klawans HL, Barr A, Klawans D. Memory failure in Huntington's disease. J Clin Exp Neuropsychol. 1987;9(2):147-54.

307.   Paulsen JS, Ready RE, Hamilton JM, Mega MS, Cummings JL. Neuropsychiatric aspects of Huntington's disease. Journal of neurology, neurosurgery, and psychiatry. 2001;71(3):310-4.

308.   Paulsen JS, Nehl C, Hoth KF, Kanz JE, Benjamin M, Conybeare R, et al. Depression and stages of Huntington's disease. J Neuropsychiatry Clin Neurosci. 2005;17(4):496-502.

309.   Pouladi MA, Graham RK, Karasinska JM, Xie Y, Santos RD, Petersen A, et al. Prevention of depressive behaviour in the YAC128 mouse model of Huntington disease by mutation at residue 586 of huntingtin. Brain : a journal of neurology. 2009;132(Pt 4):919-32.

310.   Lione LA, Carter RJ, Hunt MJ, Bates GP, Morton AJ, Dunnett SB. Selective discrimination learning impairments in mice expressing the human Huntington's disease mutation. The Journal of neuroscience : the official journal of the Society for Neuroscience. 1999;19(23):10428-37.

311.   Ross CA, Tabrizi SJ. Huntington's disease: from molecular pathogenesis to clinical treatment. The Lancet Neurology. 2011;10(1):83-98.

312.   Killoran A, Biglan KM. Current therapeutic options for Huntington's disease: Good clinical practice versus evidence-based approaches? Movement disorders : official journal of the Movement Disorder Society. 2014;29(11):1404-13.

313.   Reilmann R. Pharmacological treatment of chorea in Huntington's disease-good clinical practice versus evidence-based guideline. Movement disorders : official journal of the Movement Disorder Society. 2013;28(8):1030-3.

314.   Rosenblatt A. Neuropsychiatry of Huntington's disease. Dialogues in clinical neuroscience. 2007;9(2):191-7.

315.   Hakomori Si SI. The glycosynapse. Proceedings of the National Academy of Sciences of the United States of America. 2002;99(1):225-32.
316.   Sonnino S, Mauri L, Chigorno V, Prinetti A. Gangliosides as components of lipid membrane domains. Glycobiology. 2007;17(1):1R-13R.
317.   Ledeen R, Wu G. GM1 in the nuclear envelope regulates nuclear calcium through association with a nuclear sodium-calcium exchanger. Journal of neurochemistry. 2007;103 Suppl 1:126-34.
318.   Belzung C, Misslin R, Vogel E, Dodd RH, Chapouthier G. Anxiogenic effects of methyl-beta-carboline-3-carboxylate in a light/dark choice situation. Pharmacology, biochemistry, and behavior. 1987;28(1):29-33.
319.   Frye AAWaCA. The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. Nature Protocols. 2007:322-8.
320.   Porsolt RD, Le Pichon M, Jalfre M. Depression: a new animal model sensitive to antidepressant treatments. Nature. 1977;266(5604):730-2.
321.   Pouladi MA, Graham RK, Joshi P, Lu G, Deng Y, Wu N-P, et al. Diffeential susceptivility to excitotoxic stress in YAC128 mouse models of HD between initiation and progression of disease. Journal of Neuroscience. 2009:2193-204.
322.   Deacon RM. Assessing nest building in mice. Nat Protoc. 2006;1(3):1117-9.
323.   Bolivar VJ, Scott Ganus J, Messer A. The development of behavioral abnormalities in the motor neuron degeneration (mnd) mouse. Brain research. 2002;937(1-2):74-82.
324.   Cook MN, Bolivar VJ, McFadyen MP, Flaherty L. Behavioral differences among 129 substrains: implications for knockout and transgenic mice. Behav Neurosci. 2002;116(4):600-11.
325.   Ey E, Yang M, Katz AM, Woldeyohannes L, Silverman JL, Leblond CS, et al. Absence of deficits in social behaviors and ultrasonic vocalizations in later generations of mice lacking neuroligin4. Genes, brain, and behavior. 2012.
326.   Hughes R. The value of spontaeous alternation behavior (SAB) as a test of retention in pharmacological investigations of memory. Neuroscience and Biobehavioral Reviews 2004:497-505.
327.   Swonger AK, Rech RH. Serotonergic and cholinergic involvement in habituation of activity and spontaneous alternation of rats in a Y maze. Journal of comparative and physiological psychology. 1972;81(3):509-22.
328.   Grant SL, Shulman Y, Tibbo P, Hampson DR, Baker GB. Determination of d-serine and related neuroactive amino acids in human plasma by high-performance liquid chromatography with fluorimetric detection. Journal of chromatography B, Analytical technologies in the biomedical and life sciences. 2006;844(2):278-82.
329.   Parent M, Bush D, Rauw G, Master S, Vaccarino F, Baker G. Analysis of amino acids and catecholamines, 5-hydroxytryptamine and their metabolites in brain areas in the rat using in vivo microdialysis. Methods. 2001;23(1):11-20.
330.   Bailey KR, Crawley JN. Anxiety-Related Behaviors in Mice. In: Buccafusco JJ, editor. Methods of Behavior Analysis in Neuroscience. Frontiers in Neuroscience. 2nd ed. Boca Raton (FL)2009.
331.   Lister RG. The use of a plus-maze to measure anxiety in the mouse. Psychopharmacology. 1987;92(2):180-5.
332.   Owens MJ, Bissette G, Nemeroff CB. Acute effects of alprazolam and adinazolam on the concentrations of corticotropin-releasing factor in the rat brain. Synapse. 1989;4(3):196-202.

333. Gross C, Santarelli L, Brunner D, Zhuang X, Hen R. Altered fear circuits in 5-HT(1A) receptor KO mice. Biological psychiatry. 2000;48(12):1157-63.

334. Cryan JF, Markou A, Lucki I. Assessing antidepressant activity in rodents: recent developments and future needs. Trends in pharmacological sciences. 2002;23(5):238-45.

335. Petit-Demouliere B, Chenu F, Bourin M. Forced swimming test in mice: a review of antidepressant activity. Psychopharmacology. 2005;177(3):245-55.

336. Ciamei A, Detloff PJ, Morton AJ. Progression of behavioural despair in R6/2 and Hdh knock-in mouse models recapitulates depression in Huntington's disease. Behavioural brain research. 2015;291:140-6.

337. Deacon R. Assessing burrowing, nest construction, and hoarding in mice. J Vis Exp. 2012(59):e2607.

338. Sedelis M, Schwarting RK, Huston JP. Behavioral phenotyping of the MPTP mouse model of Parkinson's disease. Behavioural brain research. 2001;125(1-2):109-25.

339. Fleming SM, Salcedo J, Fernagut PO, Rockenstein E, Masliah E, Levine MS, et al. Early and progressive sensorimotor anomalies in mice overexpressing wild-type human alpha-synuclein. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2004;24(42):9434-40.

340. Belzung C. Innovative drugs to treat depression: did animal models fail to be predictive or did clinical trials fail to detect effects? Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology. 2014;39(5):1041-51.

341. Nollet M, Le Guisquet AM, Belzung C. Models of depression: unpredictable chronic mild stress in mice. Curr Protoc Pharmacol. 2013;Chapter 5:Unit 5 65.

342. Crawley JN. Mouse behavioral assays relevant to the symptoms of autism. Brain pathology. 2007;17(4):448-59.

343. Moy SS, Nadler JJ, Perez A, Barbaro RP, Johns JM, Magnuson TR, et al. Sociability and preference for social novelty in five inbred strains: an approach to assess autistic-like behavior in mice. Genes, brain, and behavior. 2004;3(5):287-302.

344. Groves PM, Thompson RF. Habituation: a dual-process theory. Psychol Rev. 1970;77(5):419-50.

345. O'Keefe. The Hippocampus as a Cognitive Map. Clarendon Press, Oxford. 1978.

346. Bolivar VJ. Intrasession and intersession habituation in mice: from inbred strain variability to linkage analysis. Neurobiol Learn Mem. 2009;92(2):206-14.

347. Muller U, Cristina N, Li ZW, Wolfer DP, Lipp HP, Rulicke T, et al. Behavioral and anatomical deficits in mice homozygous for a modified beta-amyloid precursor protein gene. Cell. 1994;79(5):755-65.

348. Leussis MP, Bolivar VJ. Habituation in rodents: a review of behavior, neurobiology, and genetics. Neurosci Biobehav Rev. 2006;30(7):1045-64.

349. Paul CM, Magda G, Abel S. Spatial memory: Theoretical basis and comparative review on experimental methods in rodents. Behavioural brain research. 2009;203(2):151-64.

350. Webster SJ, Bachstetter AD, Nelson PT, Schmitt FA, Van Eldik LJ. Using mice to model Alzheimer's dementia: an overview of the clinical disease and the preclinical behavioral changes in 10 mouse models. Frontiers in genetics. 2014;5:88.

351. Kokkinidis L, Anisman H. Interaction between cholinergic and catecholaminergic agents in a spontaneous alternation task. Psychopharmacology. 1976;48(3):261-70.

352.   Kokkinidis L, Anisman H. Perseveration and rotational behavior elicited by d-amphetamine in a Y-maze exploratory task: differential effects of intraperitoneal and unilateral intraventricular administration. Psychopharmacology. 1977;52(2):123-8.

353.   Lawrence AD, Sahakian BJ, Hodges JR, Rosser AE, Lange KW, Robbins TW. Executive and mnemonic functions in early Huntington's disease. Brain : a journal of neurology. 1996;119 (Pt 5):1633-45.

354.   Paulsen JS. Cognitive impairment in Huntington disease: diagnosis and treatment. Current neurology and neuroscience reports. 2011;11(5):474-83.

355.   Thompson TL, Moss RL. Modulation of mesolimbic dopaminergic activity over the rat estrous cycle. Neuroscience letters. 1997;229(3):145-8.

356.   Yu L, Liao PC. Sexual differences and estrous cycle in methamphetamine-induced dopamine and serotonin depletions in the striatum of mice. Journal of neural transmission. 2000;107(4):419-27.

357.   San-Martin-Clark O, Leza JC, Lizasoain I, Lorenzo P. Changes induced by sodium cromoglycate on brain serotonin turnover in morphine dependent and abstinent mice. Psychopharmacology. 1993;111(2):233-8.

358.   Cryan JF, Holmes A. The ascent of mouse: advances in modelling human depression and anxiety. Nature reviews Drug discovery. 2005;4(9):775-90.

359.   Vendruscolo LF, Takahashi RN, Bruske GR, Ramos A. Evaluation of the anxiolytic-like effect of NKP608, a NK1-receptor antagonist, in two rat strains that differ in anxiety-related behaviors. Psychopharmacology. 2003;170(3):287-93.

360.   Ramos A. Animal models of anxiety: do I need multiple tests? Trends in pharmacological sciences. 2008;29(10):493-8.

361.   Kinn AM, Gronli J, Fiske E, Kuipers S, Ursin R, Murison R, et al. A double exposure to social defeat induces sub-chronic effects on sleep and open field behaviour in rats. Physiology & behavior. 2008;95(4):553-61.

362.   Zhu X, Peng S, Zhang S, Zhang X. Stress-induced depressive behaviors are correlated with Par-4 and DRD2 expression in rat striatum. Behavioural brain research. 2011;223(2):329-35.

363.   Tache Y, Brunnhuber S. From Hans Selye's discovery of biological stress to the identification of corticotropin-releasing factor signaling pathways: implication in stress-related functional bowel diseases. Annals of the New York Academy of Sciences. 2008;1148:29-41.

364.   Narducci F, Snape WJ, Jr., Battle WM, London RL, Cohen S. Increased colonic motility during exposure to a stressful situation. Digestive diseases and sciences. 1985;30(1):40-4.

365.   Rao SS, Hatfield RA, Suls JM, Chamberlain MJ. Psychological and physical stress induce differential effects on human colonic motility. The American journal of gastroenterology. 1998;93(6):985-90.

366.   Nestler EJ, Hyman SE. Animal models of neuropsychiatric disorders. Nature neuroscience. 2010;13(10):1161-9.

367.   Pla P, Orvoen S, Saudou F, David DJ, Humbert S. Mood disorders in Huntington's disease: from behavior to cellular and molecular mechanisms. Frontiers in behavioral neuroscience. 2014;8:135.

368.   Turner RC, Seminerio MJ, Naser ZJ, Ford JN, Martin SJ, Matsumoto RR, et al. Effects of aging on behavioral assessment performance: implications for clinically relevant models of neurological disease. Journal of neurosurgery. 2012;117(3):629-37.

369.   Bogdanova OV, Kanekar S, D'Anci KE, Renshaw PF. Factors influencing behavior in the forced swim test. Physiology & behavior. 2013;118:227-39.

370.   Samuels BAaH, R. Novelty-suppressed feeding in the mouse. In: Gould TD, editor. Mood and Anxiety Related Phenotypes in Mice: Characterization Using Behavioral Tests. Neuromethods. II: Springer Science+Business Media; 2011. p. 107-22.

371.   David DJ, Samuels BA, Rainer Q, Wang JW, Marsteller D, Mendez I, et al. Neurogenesis-dependent and -independent effects of fluoxetine in an animal model of anxiety/depression. Neuron. 2009;62(4):479-93.

372.   Santarelli L, Saxe M, Gross C, Surget A, Battaglia F, Dulawa S, et al. Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. Science. 2003;301(5634):805-9.

373.   Bodnoff SR, Suranyi-Cadotte B, Aitken DH, Quirion R, Meaney MJ. The effects of chronic antidepressant treatment in an animal model of anxiety. Psychopharmacology. 1988;95(3):298-302.

374.   Du X, Pang TY, Hannan AJ. A Tale of Two Maladies? Pathogenesis of Depression with and without the Huntington's Disease Gene Mutation. Front Neurol. 2013;4:81.

375.   Ben M'Barek K, Pla P, Orvoen S, Benstaali C, Godin JD, Gardier AM, et al. Huntingtin mediates anxiety/depression-related behaviors and hippocampal neurogenesis. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2013;33(20):8608-20.

376.   Pla P, Orvoen S, Benstaali C, Dodier S, Gardier AM, David DJ, et al. Huntingtin acts non cell-autonomously on hippocampal neurogenesis and controls anxiety-related behaviors in adult mouse. PloS one. 2013;8(9):e73902.

377.   Wang J, Cheng A, Wakade C, Yu RK. Ganglioside GD3 is required for neurogenesis and long-term maintenance of neural stem cells in the postnatal mouse brain. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2014;34(41):13790-800.

378.   Cryan JF, Valentino RJ, Lucki I. Assessing substrates underlying the behavioral effects of antidepressants using the modified rat forced swimming test. Neurosci Biobehav Rev. 2005;29(4-5):547-69.

379.   Detke MJ, Rickels M, Lucki I. Active behaviors in the rat forced swimming test differentially produced by serotonergic and noradrenergic antidepressants. Psychopharmacology. 1995;121(1):66-72.

380.   Page ME, Detke MJ, Dalvi A, Kirby LG, Lucki I. Serotonergic mediation of the effects of fluoxetine, but not desipramine, in the rat forced swimming test. Psychopharmacology. 1999;147(2):162-7.

381.   Yandrasitz JR, Cohn RM, Masley B, DelRowe D. Evaluation of the binding of serotonin by isolated CNS acidic lipids. Neurochemical research. 1980;5(5):465-77.

382.   Matinyan NS, Melikyan GB, Arakelyan VB, Kocharov SL, Prokazova NV, Avakian TM. Interaction of ganglioside-containing planar bilayers with serotonin and inorganic cations. Biochimica et biophysica acta. 1989;984(3):313-8.

383.   Berry-Kravis E, Dawson G. Possible role of gangliosides in regulating an adenylate cyclase-linked 5-hydroxytryptamine (5-HT1) receptor. Journal of neurochemistry. 1985;45(6):1739-47.

384.   Faizi M, Bader PL, Saw N, Nguyen TV, Beraki S, Wyss-Coray T, et al. Thy1-hAPP(Lond/Swe+) mouse model of Alzheimer's disease displays broad behavioral deficits in sensorimotor, cognitive and social function. Brain and behavior. 2012;2(2):142-54.

385. Cheng D, Low JK, Logge W, Garner B, Karl T. Novel behavioural characteristics of female APPSwe/PS1DeltaE9 double transgenic mice. Behavioural brain research. 2014;260:111-8.

386. Kokkinidis L. Amphetamine-elicited perseverative and rotational behavior: Evaluation of directional preference. Mar 1987. Pharmacology, Biochemistry and Behavior. 1987;.26(3):pp.

387. Katz RJ, Schmaltz K. Dopaminergic involvement in attention. A novel animal model. Progress in neuro-psychopharmacology. 1980;4(6):585-90.

388. Ulloa RE, Nicolini H, Fernandez-Guasti A. Sex differences on spontaneous alternation in prepubertal rats: implications for an animal model of obsessive-compulsive disorder. Progress in neuro-psychopharmacology & biological psychiatry. 2004;28(4):687-92.

389. Yadin E, Friedman E, Bridger WH. Spontaneous alternation behavior: an animal model for obsessive-compulsive disorder? Pharmacology, biochemistry, and behavior. 1991;40(2):311-5.

390. Geyer MA, Puerto A, Menkes DB, Segal DS, Mandell AJ. Behavioral studies following lesions of the mesolimbic and mesostriatal serotonergic pathways. Brain research. 1976;106(2):257-69.

391. Platel A, Porsolt RD. Habituation of exploratory activity in mice: a screening test for memory enhancing drugs. Psychopharmacology. 1982;78(4):346-52.

392. Hess EJ, Albers LJ, Le H, Creese I. Effects of chronic SCH23390 treatment on the biochemical and behavioral properties of D1 and D2 dopamine receptors: potentiated behavioral responses to a D2 dopamine agonist after selective D1 dopamine receptor upregulation. The Journal of pharmacology and experimental therapeutics. 1986;238(3):846-54.

393. Van Horn MR, Sild M, Ruthazer ES. D-serine as a gliotransmitter and its roles in brain development and disease. Frontiers in cellular neuroscience. 2013;7:39.

394. Rosas HD, Liu AK, Hersch S, Glessner M, Ferrante RJ, Salat DH, et al. Regional and progressive thinning of the cortical ribbon in Huntington's disease. Neurology. 2002;58(5):695-701.

395. Rosas HD, Reuter M, Doros G, Lee SY, Triggs T, Malarick K, et al. A tale of two factors: what determines the rate of progression in Huntington's disease? A longitudinal MRI study. Movement disorders : official journal of the Movement Disorder Society. 2011;26(9):1691-7.

396. Estrada-Sanchez AM, Rebec GV. Role of cerebral cortex in the neuropathology of Huntington's disease. Frontiers in neural circuits. 2013;7:19.

397. Padowski JM, Weaver KE, Richards TL, Laurino MY, Samii A, Aylward EH, et al. Neurochemical correlates of caudate atrophy in Huntington's disease. Movement disorders : official journal of the Movement Disorder Society. 2014;29(3):327-35.

398. Fujiwara H, Ikarashi K, Yamazaki Y, Goto J, Kaneko K, Sugita M, et al. Impairment of hippocampal long-term potentiation and failure of learning in mice treated with d-threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol. Biomedical research. 2012;33(5):265-71.

399. Usuki S, Hamanoue M, Kohsaka S, Inokuchi J. Induction of ganglioside biosynthesis and neurite outgrowth of primary cultured neurons by L-threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol. Journal of neurochemistry. 1996;67(5):1821-30.

400. Inokuchi J, Mizutani A, Jimbo M, Usuki S, Yamagishi K, Mochizuki H, et al. Up-regulation of ganglioside biosynthesis, functional synapse formation, and memory retention by a synthetic ceramide analog (L-PDMP). Biochemical and biophysical research communications. 1997;237(3):595-600.

401.   Furuse H, Waki H, Kaneko K, Fujii S, Miura M, Sasaki H, et al. Effect of the mono- and tetra-sialogangliosides, GM1 and GQ1b, on long-term potentiation in the CA1 hippocampal neurons of the guinea pig. Experimental brain research. 1998;123(3):307-14.

402.   Yang R, Wang Q, Min L, Sui R, Li J, Liu X. Monosialoanglioside improves memory deficits and relieves oxidative stress in the hippocampus of rat model of Alzheimer's disease. Neurol Sci. 2013;34(8):1447-51.

403.   Fong TG, Neff NH, Hadjiconstantinou M. GM1 ganglioside improves spatial learning and memory of aged rats. Behavioural brain research. 1997;85(2):203-11.

404.   Fujii S, Igarashi K, Sasaki H, Furuse H, Ito K, Kaneko K, et al. Effects of the mono- and tetrasialogangliosides GM1 and GQ1b on ATP-induced long-term potentiation in hippocampal CA1 neurons. Glycobiology. 2002;12(5):339-44.

405.   Jung WR, Kim HG, Kim KL. Ganglioside GQ1b improves spatial learning and memory of rats as measured by the Y-maze and the Morris water maze tests. Neuroscience letters. 2008;439(2):220-5.

406.   Yohrling IG, Jiang GC, DeJohn MM, Robertson DJ, Vrana KE, Cha JH. Inhibition of tryptophan hydroxylase activity and decreased 5-HT1A receptor binding in a mouse model of Huntington's disease. Journal of neurochemistry. 2002;82(6):1416-23.

407.   Castro ME, Pascual J, Romon T, Berciano J, Figols J, Pazos A. 5-HT1B receptor binding in degenerative movement disorders. Brain research. 1998;790(1-2):323-8.

408.   Dang LC, Donde A, Madison C, O'Neil JP, Jagust WJ. Striatal dopamine influences the default mode network to affect shifting between object features. Journal of cognitive neuroscience. 2012;24(9):1960-70.

409.   Schwab LC, Garas SN, Drouin-Ouellet J, Mason SL, Stott SR, Barker RA. Dopamine and Huntington's disease. Expert review of neurotherapeutics. 2015;15(4):445-58.

410.   Chen JY, Wang EA, Cepeda C, Levine MS. Dopamine imbalance in Huntington's disease: a mechanism for the lack of behavioral flexibility. Frontiers in neuroscience. 2013;7:114.

411.   Callahan JW, Abercrombie ED. In vivo Dopamine Efflux is Decreased in Striatum of both Fragment (R6/2) and Full-Length (YAC128) Transgenic Mouse Models of Huntington's Disease. Frontiers in systems neuroscience. 2011;5:61.

412.   Johnson MA, Rajan V, Miller CE, Wightman RM. Dopamine release is severely compromised in the R6/2 mouse model of Huntington's disease. Journal of neurochemistry. 2006;97(3):737-46.

413.   Hickey MA, Reynolds GP, Morton AJ. The role of dopamine in motor symptoms in the R6/2 transgenic mouse model of Huntington's disease. Journal of neurochemistry. 2002;81(1):46-59.

414.   Garrett MC, Soares-da-Silva P. Increased cerebrospinal fluid dopamine and 3,4-dihydroxyphenylacetic acid levels in Huntington's disease: evidence for an overactive dopaminergic brain transmission. Journal of neurochemistry. 1992;58(1):101-6.

415.   Spokes EG. Neurochemical alterations in Huntington's chorea: a study of post-mortem brain tissue. Brain : a journal of neurology. 1980;103(1):179-210.

416.   Kish SJ, Shannak K, Hornykiewicz O. Elevated serotonin and reduced dopamine in subregionally divided Huntington's disease striatum. Annals of neurology. 1987;22(3):386-9.

417.   Mann JJ, Kaplan RD, Bird ED. Elevated postmortem monoamine oxidase B activity in the caudate nucleus in Huntington's disease compared to schizophrenics and controls. Journal of neural transmission. 1986;65(3-4):277-83.

418.   Richards G, Messer J, Waldvogel HJ, Gibbons HM, Dragunow M, Faull RL, et al. Up-regulation of the isoenzymes MAO-A and MAO-B in the human basal ganglia and pons in Huntington's disease revealed by quantitative enzyme radioautography. Brain research. 2011;1370:204-14.

419.   Laprairie RB, Bagher AM, Precious SV, Denovan-Wright EM. Components of the endocannabinoid and dopamine systems are dysregulated in Huntington's disease: analysis of publicly available microarray datasets. Pharmacology research & perspectives. 2015;3(1):e00104.

420.   Ooi J, Hayden MR, Pouladi MA. Inhibition of Excessive Monoamine Oxidase A/B Activity Protects Against Stress-induced Neuronal Death in Huntington Disease. Molecular neurobiology. 2014.

421.   Vinther-Jensen T, Nielsen TT, Budtz-Jorgensen E, Larsen IU, Hansen MM, Hasholt L, et al. Psychiatric and cognitive symptoms in Huntington's disease are modified by polymorphisms in catecholamine regulating enzyme genes. Clinical genetics. 2015.

422.   Goettl VM, Wemlinger TA, Colvin AE, Neff NH, Hadjiconstantinou M. Motoric behavior in aged rats treated with GM1. Brain research. 2001;906(1-2):92-100.

423.   Goettl VM, Wemlinger TA, Duchemin AM, Neff NH, Hadjiconstantinou M. GM1 ganglioside restores dopaminergic neurochemical and morphological markers in aged rats. Neuroscience. 1999;92(3):991-1000.

424.   Goettl VM, Zhang H, Burrows AC, Wemlinger TA, Neff NH, Hadjiconstantinou M. GM1 enhances dopaminergic markers in the brain of aged rats. Experimental neurology. 2003;183(2):665-72.

425.   Pearson SJ, Reynolds GP. Neocortical neurotransmitter markers in Huntington's disease. Journal of neural transmission General section. 1994;98(3):197-207.

426.   Cummings DM, Andre VM, Uzgil BO, Gee SM, Fisher YE, Cepeda C, et al. Alterations in cortical excitation and inhibition in genetic mouse models of Huntington's disease. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2009;29(33):10371-86.

427.   Gu X, Li C, Wei W, Lo V, Gong S, Li SH, et al. Pathological cell-cell interactions elicited by a neuropathogenic form of mutant Huntingtin contribute to cortical pathogenesis in HD mice. Neuron. 2005;46(3):433-44.

428.   Spampanato J, Gu X, Yang XW, Mody I. Progressive synaptic pathology of motor cortical neurons in a BAC transgenic mouse model of Huntington's disease. Neuroscience. 2008;157(3):606-20.

429.   Waldvogel HJ, Kim EH, Thu DC, Tippett LJ, Faull RL. New Perspectives on the Neuropathology in Huntington's Disease in the Human Brain and its Relation to Symptom Variation. Journal of Huntington's disease. 2012;1(2):143-53.

430.   Thu DC, Oorschot DE, Tippett LJ, Nana AL, Hogg VM, Synek BJ, et al. Cell loss in the motor and cingulate cortex correlates with symptomatology in Huntington's disease. Brain : a journal of neurology. 2010;133(Pt 4):1094-110.

431.   Kordasiewicz HB, Stanek LM, Wancewicz EV, Mazur C, McAlonis MM, Pytel KA, et al. Sustained therapeutic reversal of Huntington's disease by transient repression of huntingtin synthesis. Neuron. 2012;74(6):1031-44.

432.   Schneider JS, Pope A, Simpson K, Taggart J, Smith MG, DiStefano L. Recovery from experimental parkinsonism in primates with GM1 ganglioside treatment. Science. 1992;256(5058):843-6.

433.   Schneider JS, Kean A, DiStefano L. GM1 ganglioside rescues substantia nigra pars compacta neurons and increases dopamine synthesis in residual nigrostriatal dopaminergic neurons in MPTP-treated mice. J Neurosci Res. 1995;42(1):117-23.

434.   Hadjiconstantinou M, Neff NH. Treatment with GM1 ganglioside restores striatal dopamine in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-treated mouse. Journal of neurochemistry. 1988;51(4):1190-6.

435.   Saulino MF, Schengrund CL. Differential accumulation of gangliosides by the brains of MPTP-lesioned mice. J Neurosci Res. 1994;37(3):384-91.

436.   Ghidoni R, Trinchera M, Venerando B, Fiorilli A, Sonnino S, Tettamanti G. Incorporation and metabolism of exogenous GM1 ganglioside in rat liver. Biochem J. 1986;237(1):147-55.

CHAPTER 4

GANGLIOMIMMETIC COMPOUNDS AS A POTENTIAL THERAPY FOR HD

4.1 Introduction

Gangliosides are a complex family of sialic acid-containing glycosphingolipids that are integral components of the plasma membrane and highly enriched in lipid rafts. Consequently, gangliosides influence many cell signaling pathways (150). Structurally, gangliosides are comprised of two distinct groups, a sugar glycan head group and the ceramide moiety which can be subdivided into a sphingoid base arm and a fatty acid arm (155, 437). In general the sphingoid base is sphingosine with 18 carbons and one double bond (18:1) and the fatty acid is usually stearic acid (18:0) (143, 438), although other fatty acids can be present as well (439). Alterations to any of these components can dramatically affect function (440). Synthesis of gangliosides begins with ceramide formation in the endoplasmic reticulum (ER) and continues with the sequential addition of various glycan groups in the Golgi network prior to ganglioside transport to the plasma membrane (150, 441). GM3 is the precursor of all complex gangliosides and is produced by the sequential addition of glucose, galactose and a sialic acid to ceramide (441). GM3 and GD3 synthesis is the rate limiting steps in the production of a-series and b-series gangliosides respectively (442, 443). A-series gangliosides include GM3, GM2, GM1 and GD1a, while b-series gangliosides include GD3, GD2, GD1b, and GT1b. GM1, GD1a, GD1b, and GT1b are present in the highest concentration in the adult nervous system (444), while GM3 and GD3 are the most abundant gangliosides in the periphery and at early developmental stages (155, 437). Although gangliosides have very similar structures it has been demonstrated that the variability in the sugar head groups is sufficient to confer functional diversity (150). To further complicate the understanding of the roles of gangliosides, many studies have demonstrated that changes in the ceramide moieties can also contribute to functional differences (445-447).

The study of the function of individual gangliosides is complicated by the fact that enzymatic conversion from one ganglioside to another can be rapid. Furthermore, knock-out of key enzymes in the ganglioside biosynthetic pathway leads to the simultaneous disappearance of multiple ganglioside species, not just one (159, 162, 448, 449). Additionally, pharmacological use of individual species of exogenous gangliosides might reveal pharmacological effects of gangliosides which are not necessarily equivalent to their physiological roles (149).

Despite these difficulties, analysis of genetic mouse models have generated insightful results that support an important role for gangliosides in healthy brain physiology, particularly in myelin stabilization, and in immune functions (155, 159, 450, 451). The most dramatic phenotype is shown by mice that lack both GM2/GD2 synthase and GM3 synthase. These mice lack the ability to synthesize any ganglio-series gangliosides (167) and have reduced life-span, less than 50% of mice surviving to 1 month of age (167), and severe neurodegeneration by 3 months of age, which is accompanied by increased activation of astrocytes (167). Interestingly, mice generated by the double knock-out of GM2/GD2 synthase and GD3 synthase (DKO mice), which express only GM3 demonstrate a similar, although less severe phenotype. DKO mice die suddenly beginning after 4 weeks of age and have severe neurodegeneration after 30 weeks of age (166). A third common, but less severe, model lacks all complex gangliosides due to knock-out of the *Galgt1* gene which encodes for GM2/GD2 synthase (162). These mice can only synthesize GM3 and GD3 (164). These animals develop an adult onset neurodegenerative phenotype, with generalized brain atrophy and marked reduction of the volume of the substantia nigra. For this reason it has been proposed that these mice could represent a model of PD (165).

Genetic models demonstrate that gangliosides are clearly important for proper functioning of the nervous system, however, the exact mechanisms behind these neurotrophic factors have not yet been fully elucidated.

Dysregulation of ganglioside metabolism has been implicated in HD (137-139, 452), a dominantly inherited neurodegenerative disease caused by the expansion of a CAG trinucleotide repeat in the gene that codes for *HTT*. HD patients present with motor dysfunction, cognitive deficits and psychiatric disturbances due to progressive neurodegeneration in specific areas of the brain, in particular the corpus striatum and the cortex (34).

Decreased levels of gangliosides, GM1 in particular, and ganglioside biosynthetic enzymes were found in HD patients and in multiple cell and mouse models (137-139) of the disease. Intraventricular administration of exogenous GM1 has dramatic therapeutic effects in HD models, including reversal of motor symptoms (212)(Chapter 2), improvement of psychiatric-like and cognitive deficits (Alpaugh et al., submitted- Chapter 3) and attenuated neurodegeneration (Chapter 2). These effects are accompanied by activation of the PI3K/Akt pathway, a major pathway for neuronal survival (453) and Akt phosphorylation *in vitro* (137), as well as phosphorylation of HTT at Ser13 and Ser16 (212), a post-translational modification that was shown to dramatically decrease mHTT toxicity (213).

GM1 was shown to have beneficial effects in PD as well (195, 196). However, one major obstacle with ganglioside therapies is the poor bioavailability and pharmacokinetics of these large amphiphilic molecules, which do not efficiently cross the BBB (454). One potential solution to this problem would be to design membrane- and BBB- permeant structural analogues. This has been tried in the past, with some success. The Liga series of molecules contain the same

glycan group of GM1, but the stearic acid component of the ceramide moiety was replaced with a more hydrophilic group (455). The best characterized of these compounds, Liga20, contains N-dichloroacetylsphingosine in place of ceramide (456). Liga20 has been shown to have improved efficacy at reducing the duration and severity of kainite induced seizures (449) and the amount of cell death after glutamate induced excitotoxicity (454, 455), additionally, Liga20 has improved pharmacokinetics compared to GM1 (449, 454-457). Whether these improvements would also be present in HD models is not known. Liga20, however, would not be suitable to replace GM1 in clinical settings, as it has been suggested that long term use produces toxicity (160). Nevertheless, Liga20 does serve as proof of principle that it is possible to produce membrane permeant analogues of GM1 with preserved therapeutic effects.

The aim of this study was to identify what structural features of the GM1 molecule are necessary for its therapeutic effects in HD, with the ultimate goal of informing the design of second generation compounds with the same therapeutic potential of HD but with improved pharmacokinetic properties. To this end, we tested a number of different gangliosides and gangliomimetic compounds and determined their neuroprotective effects in knock-in immortalized striatal-derived cells expressing either full-length wtHTT or mHTT. We showed that although a few other gangliosides maintain the neuroprotective activity of GM1 *in vitro*, suggesting that small modifications of the glycan structures are tolerated, modifications of the ceramide moiety can have significant effects on the ability of the ganglioside to provide neuroprotection. Furthermore, we show that, although several molecules were able to provide some neuroprotection *in vitro*, only a few structures maintained the ability to induce phosphorylation of HTT at crucial amino acid residues, Ser13 and Ser16, which decrease mHTT

toxicity. Finally, we show that at least one gangliomimetic compound has beneficial effects *in vivo*, after intraperitoneal administration in an animal model of HD.

4.2 Materials and Methods

4.2.1 Animal Models

YAC128 mice overexpressing the human *HTT* gene with 128 CAG repeats (64) were originally purchased from the Jackson Laboratories (Jackson Laboratories, Bar Harbor, ME, USA) and subsequently maintained on FVB genetic background in our animal facility at the University of Alberta. All mice were maintained on a 14-10 h light-dark cycle in a temperature and humidity-controlled room. All procedures involving animals were approved by the University of Alberta's Animal Care and Use Committee and were in accordance with the guidelines of the Canadian Council on Animal Care. Only male mice were analyzed as previous testing with GM1 in HD mice showed no differences between male and female mice in terms of behavioural response to therapy (*Alpaugh et al.*; in preparation). Treatment started between 5 and 9 months of age and 10 days after the start of treatment motor testing began. All mice were group housed for the duration of treatment.

4.2.2 Intraperitoneal drug administration

Mice were injected with isotonic saline, semi-synthetic GM1 (30 mg/kg) dissolved in isotonic saline or compound SNB-6100 (3 mg/kg) dissolved in isotonic saline. All treatments were administered by intraperitoneal injection using a 26-gauge needle in a volume of 10 ml/kg. Injections were given at 1600 h daily to alternating sides of the abdomen. Compounds SNB-6100 and semi-synthetic GM1 were provided by Seneb BioSciences INC. (Holliston, MA).

4.2.3 Behavioural Testing

Behavioural testing was conducted in the light phase of the light cycle between 0800 h and 1800 h. For all behavioural training and testing mice were allowed to acclimate to the testing room for 1 h before testing, unless otherwise specified. In each experiment, each behavioural test was performed on the same day after beginning of treatment. All mice received training for 2 consecutive days on each instrument and task before performing motor behavior measurements, and these days of training were preceded by one day of acclimation to the testing room. All experiments were performed by experimenters who were blind to the genotype and treatment of the animals.

Rotarod:

Mice were tested at fixed speed (12 RPM) for a maximum of 3 min. Each mouse was tested in three consecutive trials with 1 min rest in between trials. The time spent on the rotarod for each of the three trials was averaged to give the overall latency to fall for each mouse. A similar testing protocol was used to analyze mouse performance on an accelerating rotarod (4-40 RPM in 2 min), although in this test mice were allowed to remain on the rotarod until they spontaneously fell.

Narrow beam:

Mice were placed at the extremity of a 100-cm-long wooden narrow beam (0.75 cm wide, suspended 30 cm above the floor) and allowed to traverse the beam from one end to the other three times. Each animals' performance was recorded with a video camera and footfalls, body balance, and motor coordination were analyze using a footfall scoring system (212).

Horizontal ladder:

Mice were scored live as they spontaneously walked along a horizontal ladder with variable and irregular spacing between rungs. In each test session, the performance of the mouse was evaluated using an established footfall scoring system (212), which allows for qualitative and quantitative evaluation of forelimb and hind limb placement on the ladder rungs.

Elevated plus maze:

Mice were placed in the center of an elevated plus maze facing the open arm and left to freely explore for 5 min. Arm crosses into and out of open and closed arms, as well as time spent in each arm were recorded with a video camera and scored. An entry was defined as ¾ of the body of the mouse excluding the tail entering into a compartment.

Forced swim test:

The forced swim test was performed as in (320). Mice were individually placed for 6 min in a 4 L beaker (25 cm tall, 16 cm wide) filled with 2.6 L of water pre-warmed to 23-25°C. The last 4 min of the test were scored using a time-sampling technique. Every 5 seconds mice were scored for swimming, climbing and immobility in the previous 5 seconds. Immobility was defined as no movement other than what is necessary to maintain the nose above water.

Simple swim test:

The simple swim test was used to control for motor deficits potentially interfering with the forced swim test (321). One day prior to testing, mice were trained to swim in a rectangular swimming chamber (90 cm long x 7 cm wide; water depth = 9 cm; with 6x7 cm platform at one end) and to reach a platform at one end of the swimming chamber in three consecutive trials separated by 5 min intervals. On the day of testing, swimming speed was calculated by averaging

the time each mouse took to swim the length of the pool to the platform in 7 consecutive trials, after excluding from the count the best and worst trial for each animal (321).

Open pool test:

The open pool test was performed 1-2 days after the forced swim test. Mice were placed in a pool (102 cm diameter, 21 cm high) filled with water for 6 min. Swim activity in the last 4 min was measured as described above for the forced swim test.

4.2.4 Cell Models

Conditionally immortalized mouse striatal knock-in cells expressing endogenous levels of WT (STHdh7/7) or mHTT (STHdh111/111) were a gift from M. E. MacDonald (Massachusetts General Hospital, Boston) and were maintained in culture as previously described (458).

4.2.5 Cell viability

To test the effects of various compounds on cell viability, STHdh7/7 cells (7/7) or STHdh111/111 (111/111) cells were plated in a 12-well cell culture dish (Falcon 353043) at a density of 0.13 and 0.14 x 10^6 cells per well, respectively, and left to attach overnight. 14 h later, cells were washed once with Dulbecco-PBS (D-PBS, Hyclone SH30028.02) and then incubated in serum-free medium (High glucose DMEM with 400 µg/ml geneticin, 2 mM L-glutamine, and 1 mM sodium pyruvate) containing one of the following compounds at the indicated concentration: semi-synthetic GM1 (50 µM, Seneb Biosciences Inc.), Liga20 (1-5 µM, provided by Dr. Ledeen), GA1 (0.5-50 µM, Enzo Alx-302-013-M001), stearyl-truncated GM1 (0.01-10 µM, provided by Dr. Bundle, University of Alberta), truncated GM1 (10-100 µM, provided by Dr. Bundle, University of Alberta), lysoGM1 (1-10 µM, Sigma G5660). SNB-6100 (1-5 µM, Seneb Biosciences Inc.), SNB-2140 (0.01- 0.5 µM, Seneb Biosciences Inc.), and SNB-711 (0.1-1

μM, Seneb Biosciences Inc.). All other gangliosides were tested at concentrations from 10 μM to 100 μM and were: GM3 (Enzo Alx-302-005-M001), GM2 (Enzo Alx-302-003-M001), GD1a (Enzo Alx-302-007-M001), GT1b (Enzo Alx-302-011-M001), GD3 (Enzo Alx-302-010-M001), and GD1b (Enzo Alx-302-009-M001). Cells were treated with the aforementioned compounds for 9 h (gangliosides and modified gangliosides) or 12 h (gangliomimetic compounds) at 39°C. These conditions cause a greater degree of cell death in the mutant 111/111 cells as compared to WT 7/7 cells (212). All treatments were performed in quadruplicate. Cell viability was assessed by flow cytometry analysis of annexin V-positive cells.

Briefly, cells were washed and then collected by trypsinization. Cells were centrifuged at 4°C at 1,100 RPM for 5 min. The cell pellet was resuspended in PBS and transferred into a 96-well v-bottomed plate. Cell were centrifuged again and washed once in PBS prior to incubation with 5 μl annexin V PE (BD Pharmingen 556421) in 45 μL of 1X annexin binding buffer (ABB) (BD Pharmingen 51-66121E) for 15 min at RT, protected from the light. After incubation, cells were centrifuged and washed once with 1X ABB prior to fixation with 2% PFA in 1X ABB. For experiments where only annexin-V staining was performed, cells were left in fixative overnight.

In double-labelling experiments using annexin-V and anti-active caspase 3, cells were fixed in 2% PFA for 30 min at 4°C. Cells were then washed twice using PBS, re-suspended in 1% BSA in PBS, centrifuged at 1,300 RPM at 4°C for 5 min, and permeabilized with 50 μl of 0.3% saponin in PBS for 10 min at RT. Next, 50 μl of antibody solution containing anti-active caspase 3-FITC (Clone C92-605 (BD Pharmingen™) 1/100 in 0.3% saponin (Sigma S7900-25G) in PBS was added and incubated at 4°C O/N. The following morning 100 μl of 0.1% saponin in PBS were added to each well and cells were centrifuged for 4 min at 1,400 RPM at 4°C. Cells were washed once in 0.1% saponin and once in PBS and finally resuspended in PBS for flow

cytometry analysis. Cells were analyzed using a CANTO II cell analyzer (10,000 events acquired for each sample) and either CellQuestPro or FlowJo software.

4.2.6 Western Blotting

For western blot analysis, 3.6 and 4 x 10^5 STHdh7/7 and STHdh111/111 cells, respectively were plated in 35 mm cell culture dishes (Falcon 353001) and left to attach overnight. 14 h later, cells were washed once with Dulbeco phosphate-buffered saline (D-PBS) (Hyclone SH30028.02) prior to pre-incubation in serum-free medium (High glucose Dulbeco modified Eagle's medium (DMEM)) with 400 µg/ml geneticin, 2 mM L-glutamine, and 1 mM sodium pyruvate) at 33°C for 4-5 h. Cells were then treated with the indicated compounds for 1 h or 10 min, at 33°C, prior to cell lysis. Cells were scraped in ice-cold lysis buffer (20 mM Tris, pH 7.4, 1% NP40, 1 mM EDTA, 1 mM EGTA, 50 µM MG132, 1X cOmplete protease inhibitor cocktail and 1X PhosStop phosphatase inhibitor cocktail, Roche). Samples were sonicated two times for 10 sec each at power 1 using a Sonic Dismembrator Model 100. Cells were then left on ice for 30 min prior to centrifugation at 20,000 x g and 4°C for 10 min. Protein concentration in the supernatants was measured using the BCA assay. For extracellular signal-regulate kinases (Erk)/pErk and Akt/pAkt analysis, 30 µg of proteins were electrophoresed on a 4-12% SDS-polyacrylamide gel and transferred onto an Immobilon-FL PVDF membrane (Millipore). Membranes were then blocked with 5% BSA in TBS-T for 1 h and then incubated overnight at 4°C with antibodies against pErk and pAkt. Primary antibodies used were rabbit anti-Akt (phospho-) ser473 (clone193H12, 1:1,000, Cell Signaling 4058), rabbit anti-Akt (1:2,000, Cell Signaling #9272), rabbit anti-Erk 1 (1:2,000, Santa Cruz #C-16), mouse anti-Erk (phospho-) thr202/tyr204 (1:1,000, Cell Signaling #9106), mouse anti-alpha tubulin (1:10,000, Sigma #T5168). After 3 washes in TBS-T for 10 min each, membranes were incubated with the appropriate IRDye

secondary antibodies (1:10,000, LI-COR Biotechnology) for 45 min at RT. Membranes were washed 3 X 10 min in TBS-T and 1 X 10 min in TBS and then infrared signal was acquired and quantified using the Odyssey Imaging System. For antibody stripping, membranes were incubated in 62.5 mM TRIS-HCL, pH 6.7 containing 2% SDS and 100 mM β-mercaptoethanol for 45 min in a 60°C water-bath with agitation. All membranes were rinsed quickly with TBS and then blocked in TBS-based odyssey blocking buffer for 30 min. stripping was confirmed by a 30 min incubation in secondary antibody and visualization at a LiCor Odyssey System with scanning intensity 10 for both 680 nm and 800 nm channel.

4.2.7 Immunocytochemistry

For immunocytochemistry analysis of HTT phosphorylation, cells were seeded onto poly-L-lysine-coated coverslips in 24-well cell culture dishes. 7.5×10^4 cells were plated into each well and left to attach overnight. In the morning, cells were washed once with D-PBS (Hyclone SH30028.02), pre-incubated with serum-free medium (High glucose DMEM with 400 µg/ml geneticin, 2mM L-glutamine, and 1mM sodium pyruvate) at 33°C for 4 h and 40 min and then treated with the indicated compounds for 20 min. After 3 washes in PBS, cells were fixed with freshly made 4% PFA for 10 min. Cells were then washed 3 X 30 sec in PBS and permeabilized with 0.1% Triton X-100 (BDH) in PBS for 5 min, followed by 4 washes in PBS. Cells were then blocked in 4% normal donkey serum (Sigma cat. #Dp663) for 1 h at RT, followed by incubation in primary antibodies, (pHTT, 2nd batch, 1:1000, provided by Dr. Truant, McMaster University; and mAB2166, 1:200), for 1 h at RT. Cells were washed 3 X 4 min with PBS prior to incubation with secondary antibodies (goat anti-mouse A488, Invitrogen #A11001; and donkey anti-rabbit A594, Invitrogen #A21207) for 1 h at RT. After 3 X 4 min washes with PBS, cell nuclei were counterstained with 2 µM 4',6-Diamidino-2-phenylindole (DAPI) (Invitrogen D1306). After one

wash for 30 sec in PBS, coverslips were mounted using ProLong Gold mounting media (Life Technologies, #P36934). Quantitative analysis of immunofluorescence was performed using photomicrographs taken at 20X magnification with 1.6 optical variance using a Zeiss Observer.Z1 microscope coupled to a Zeiss AxioCam MRm camera. Five images containing approximately 80 cells per field were taken per coverslip. Regions were selected randomly while observing DAPI staining in the blue channel. Images were opened in ImageJ as JPEG files with all channels shown separately. Files were converted to greyscale and positive staining was selected using a threshold set to detect almost no staining in the untreated condition. The DAPI channel was used to set the nuclear regions of interest for each image to obtain the signal intensity specifically for the nucleus. The sum of the integrated density for each image was calculated and divided by the total number of cells as detected by the nuclei count. This allowed for the calculating of the mean fluorescence intensity (MFI) per cell.

4.2.8 Statistical analysis

All statistical analyses for biochemistry experiments were performed using one-way ANOVA with Bonferroni post-tests. All statistical analyses for animal experiments were performed using two-way ANOVA with Bonferroni post-tests.

4.3 Results

4.3.1 A and B-series gangliosides improve survival of HD cells

We analyzed various a-series and b-series gangliosides to determine whether gangliosides other than GM1 have neuroprotective effects in HD cells and test whether the latter depend on a specific glycan head group.

Apoptosis was induced by incubating cells in serum-free medium and measured by flow cytometry analysis of phosphatidylserine externalization after cell labelling with annexin V-PE. Annexin V binds to phosphatidylserine, which, in healthy cells is exclusively present on the inner leaflet of the plasma membrane and therefore inaccessible to annexin V (459). During apoptosis, phosphatidylserine is translocated to the outer leaflet of the plasma membrane where it can be detected by annexin V.

For each ganglioside, three different concentrations were tested: lower (10 µM), equal (50 µM) and greater (100 µM) than the most effective concentration of GM1 (50 µM). Each ganglioside was tested in both STdh7/7 (WT, 7/7) and STdh111/111 (HD, 111/111) cells. Ganglioside GM3, the predominant ganglioside species in the periphery and a precursor to all more complex gangliosides (460) (Fig. 1.1), reduced cell death in both HD and WT cells (Fig. 4.1A). In WT cells 50 µM GM3 equaled the reduction in cell death observed after treatment with GM1. In HD cells all three concentrations displayed some ability to decrease cell death, however, no concentration achieved the same degree of protection as GM1. Ganglioside GM2, the direct precursor of GM1 (461) (Fig. 1.1), did not reduce cell death at any concentration tested in either cell line (Fig. 4.1B). In fact, the highest concentration (100 µM) resulted in significantly increased cell death in both HD and WT cells. Ganglioside GD1a, a common complex a-series ganglioside which can be converted to GM1 by the action of Neu3 sialidase (436), was as protective as GM1 in HD and WT cells at 50 µM (Fig. 4.1C). No beneficial effect was observed at the lower concentration.

Next the common b-series gangliosides were tested. Ganglioside GD3, the precursor for all other b-series gangliosides (Fig. 1.1), was found to be protective at concentrations of 50 µM and 100 µM in both WT and HD cell lines (Fig. 4.1D). In WT cells 50 µM GD3 was more effective than

100 µM GD3. In 111/111 cells, 100 µM GD3 reduced cell death significantly more than the 50

µM concentration and showed a reduction in cell death that was not significantly different from

that produced by GM1. Ganglioside GD1b, one of the most highly expressed gangliosides in the

CNS (261), reduced cell death to the same extent as GM1 in 111/111 cells at a dose of 100 µM

(Fig. 4.1E). No reduction in cell death was observed in 7/7 cells, however, no reduction in cell

death was observed with GM1 in 7/7 cells in these experiments so the lack of effect of GD1b

may reflect the low levels of cell death in 7/7 cells and does not necessarily indicate that the

effect of GD1b is restricted to 111/111 cells. GT1b, another common brain ganglioside, was not

able to reduce cell death in either WT or HD cells at any concentration tested (Fig. 4.1F).

Because several gangliosides were able to reduce cell death to the extent of GM1, our data

suggest that the exact sugar composition of GM1 head group is not necessary for the protective

effects of GM1. Of the four most common brain gangliosides only GT1b was unable to reduce

cell death. No significant difference was found between the efficacies of GD1b, GD1a and GM1

suggesting that their therapeutic actions might be mediated through a common mechanism.

4.3.2 Lyso-derivatives of GM1, but not asialo-GM1 or other truncated forms of the ganglioside decrease cell death

We next analyzed various truncated derivatives of GM1 where either the sialic acid residue or

portions of the ceramide moiety (e.g. the fatty acid or the sphingosine chain, or both) were

eliminated. First, we tested the anti-apoptotic activity of asialo-GM1 (GA1). No beneficial effect

was observed at any concentration tested (Fig. 4.2A). The sialic acid residue of GM1, therefore,

is essential for GM1 neuroprotective activity. This is especially interesting since many other

changes to the glycan head groups were insufficient to prevent the action of GM1. We next

tested whether the glycan head group by itself (truncated GM1, tGM1) was sufficient to reduce

cell death, but it was not (Fig. 4.2B). This might be due to fact that the glycan head group alone is unlikely to distribute within membranes and inside the cells the same way as GM1, since it misses the lipophilic group (represented by ceramide in the natural GM1 molecule) that allows insertion of GM1 within biological membranes. Therefore, a second truncated version of GM1 (stearyl-truncated GM1, stGM1) containing the sugar head group attached to stearic acid (but missing the sphingosine arm of the ceramide moiety) was analyzed (Fig. 4.2C). This compound was also unable to reduce cell death in HD cells. To test whether changes in the stearic acid branch of the ceramide moiety of GM1 was better tolerated than loss of the sphingosine arm, two different lyso-derivatives of GM1 were tested, lysoGM1 (Fig. 4.2D) and Liga20, a semi-synthetic ganglioside which contains N-dichloroacetylsphingosine in place of ceramide (Fig. 4.2E). Both lysoGM1 and Liga20 were as, or more, protective than GM1 at one tenth of the concentration (5 µM) in HD cells. LysoGM1, but not Liga20, was also able to reduce cell death to the same extent as GM1 in WT cells. Interestingly, when both lyso-derivatives were tested at a concentration of 10 µM cells were observed to round up and detach from the cell culture plate. This population of cells was difficult to pellet, but annexin V staining indicates that this phenotype did not correspond to an increase in cell death (data not shown). This change in cell morphology and attachment was also observed when high concentration of GM1 were tested (≥100 µM) (data not shown).

The increased potency of lyso-derivatives of GM1 is promising for the development of second generation molecules with neuroprotective activity in HD, since the lyso-derivatives show improved membrane permeability and increased ability to cross the BBB as compared to GM1 (449).

To further confirm the anti-apoptotic effects of lysoGM1, and since annexin V could also potentially label necrotic cells with compromised membrane integrity (459), in addition to apoptotic cells, we performed double-staining of cells with annexin V and with antibodies that detect active caspase 3, a specific marker of apoptosis (Fig. 4.3). The number of dead cells detected with both markers was found to be very similar, indicating that in the serum-deprivation condition that we used, cells were primarily undergoing apoptosis. This is in line with our previous studies using GM1 (137). For the remainder of the experiments only staining for annexin V was performed.

4.3.3 Gangliomimetic compounds with neuroprotective activity in vitro

We then tested three rationally designed GM1 mimetics provided by Seneb Biosciences Inc. (US) that were designed to potentially increase bioavailability and BBB permeability as compared to GM1. These compounds all have the same glycan structure as GM1, but chemical substitutions in the ceramide moiety cannot be disclosed. SNB-2140 (Fig. 4.4A) and SNB-711 (Fig. 4.4B) were both partially effective at reducing cell death in HD cells at a concentration of 500 nM. Neither compound demonstrated an effect as pronounced as the reduction observed after administration of GM1. Both compounds were found to be toxic (as determined by annexin V staining) and/or ineffective at concentrations above 1 µM (data not shown). Interestingly, SNB-2140 was more effective than GM1 at reducing cell death in 7/7 cells. A third compound, SNB-6100, was as effective in preventing HD cell death as GM1, at one tenth of the concentration (5 µM). Similarly to what was observed with both lyso-derivatives of GM1, SNB-6100 caused cells to change shape and detach from the cell culture plate at concentrations ≥ 10 µM (cells remained viable as measured by annexin V staining).

Efficacy of all gangliosides and gangliomimetic compounds at reducing the number annexin V$^+$ cells in 111/111 cells is summarized in Fig. 4.5.

4.3.4 Non-GM1 gangliosides and gangliomimetic compounds do not increase phosphorylation of Akt or Erk after 10 minutes

Gangliosides have been described to activate tyrosine receptor kinases by direct binding or through induction of release of neurotrophins (198, 199). The activation of trk receptors by gangliosides, and ganglioside GM1 in particular, has been shown to converge on neuroprotective pathways such as the PI3K/Akt and the extracellular signal-regulated kinases (Erk) pathways (462). We have previously demonstrated that GM1 is able to increase phosphorylation of both Akt and Erk within 10 min of administration in serum free medium (137). To determine if non-GM1 gangliosides and protective gangliomimetic compounds act through a similar mechanism, the ability of each compound to increase phosphorylation of Akt and Erk at each compounds optimal concentration was tested. Surprisingly, GM1 was the only compound to increase phosphorylation of Akt in both WT and HD cell lines (Fig. 4.6 A and B). GD1b showed a trend towards increasing levels of pAkt in HD cells, but most other compounds with similar anti-apoptotic activity as GM1 showed a trend towards a decrease in phosphorylation of Akt, rather than an increase, with Liga20 and GD1a significantly decreasing the levels of pAkt as compared to untreated cells. The results for Erk phosphorylation matched closely those obtained for Akt. Only GD1b was able to increase phosphorylation of Erk, and this effect was specific to HD cells (Fig. 4.6D). Other compounds did not have any effect on Erk, except for GT1b, which significantly reduced pErk levels. When tested for a longer period of time (1 hour incubation) (Fig. 4.7), most compounds generally behaved in a similar manner as in the short treatment. At 1 hour GT1b significantly decreased the amount of pAkt in both 7/7 and 111/111 cells and GD1a

had the same effect in 7/7 cells. However, lysoGM1 was now able to increase phosphorylation of Erk to the same levels as done by GM1, although this effect was limited to WT cells.

4.3.5 Protective non-GM1 gangliosides and gangliomimetic compounds increase phosphorylation of HTT

Work from our lab has previously indicated that GM1 administration leads to increased phosphorylation of HTT at Ser13 and Ser16 (212). Since phosphorylation at these amino acid residues dramatically decreases mHTT toxicity (213), the ability of GM1 to trigger HTT phosphorylation is a crucial property that should be mirrored in any gangliomimetic compound considered as a potential treatment for HD. Because localization of phosphorylated forms of HTT has shown to influence its function and toxicity (463), we analyzed the levels of HTT phosphorylation both in the nucleus and in entire cells, by immunocytochemistry, in cells treated with two of the most promising compounds identified in our studies, i.e. SNB-6100 and Liga20. In WT cells, SNB-6100 triggered a significant increase in HTT phosphorylation at Ser13 and Ser16, both in the nucleus and in the cytoplasm of cells. This increase, however, did not quite equal the increase present after treatment with GM1. In HD cells, Liga20 increased HTT phosphorylation to the same extent as GM1 (Fig. 4.8), while SNB-6100 resulted in almost twice as much signal for pHTT when considering whole cells and about one and half times as much signal in the nucleus.

4.3.6 Peripheral administration of SNB-6100 and GM1 improves motor and non-motor performance in YAC128 mice

The ability of non-GM1 gangliosides and gangliomimetic compounds to reduce cell death does not necessarily indicate that the compounds will be protective *in vivo*. Thus, validation of the

therapeutic effects of any compound *in vivo* is essential. In this study we analyzed the effects of SNB-6100 administration in YAC128 mice and compared them to the effects of GM1. SNB-6100 was tested as it was one of the most protective compounds *in vitro* and it retained the ability to trigger phosphorylation of HTT. Liga20 was not analyzed as it has previously been shown to be toxic after long periods of administration (160).

We initially tested SNB-6100 by chronic intraventricular infusion for 28 days, as previously done for GM1, however, in these conditions the drug did not equal the beneficial effects of GM1 in YAC128 mice. We attributed this to poor stability of the compound, compared to GM1, which might have lost its efficacy during the prolonged storage inside the micro osmotic pumps used for chronic intraventricular administration. We then analyzed the effects of SNB-6100 after daily administration by intraperitoneal injection for 28 days. GM1 was included in these studies, to determine whether peripheral administration would provide benefits in HD models, in spite of poor BBB permeability to the ganglioside. Motor performance was assessed using a battery of tests after 28 days of treatment. The accelerated rotarod was used to assess motor coordination and motor endurance. HD mice injected with saline demonstrated worse performance than littermate controls, as expected (Fig. 4.9A). Injection with SNB-6100, but not GM1, restored performance to that observed in WT mice. Motor endurance was further analyzed using the fixed rotarod. Once again, motor performance of YAC128 mice was restored to normal levels by administration of SNB-6100 (Fig. 4.9B). GM1 had no significant effect on the performance of either WT or HD mice. Fine motor control was assessed using the horizontal ladder task. In this test, although HD mice showed a similar performance as WT littermates, SNB6100 still led to a significant decrease in the number of errors made by HD mice (Fig. 4.9C), indicating an improvement in motor coordination. An improvement by SNB-6100 was also observed in the

narrow beam test for balance and motor coordination. (Fig. 4.9D). The effects of SNB-6100 on non-motor symptoms of HD were also assessed. Patients frequently display non-motor symptoms prior to the onset of motor symptoms, and non-motor symptoms contribute more heavily to disease burden (21). Anxiety-like behaviour was assessed in YAC128 mice using the elevated plus maze, a well validated test for anxiety in rodents. No group differences were observed in this test (Fig. 4.10). Depression-like behaviour was analyzed using the forced swim test. YAC128 mice displayed decreased time swimming (Fig. 4.9E) and increased time immobile (Fig. 4.9E) as compared to WT controls. YAC128 mice treated with SNB-6100 spent significantly more time swimming, and less time immobile than saline-treated YAC128 mice, suggesting that SNB-6100 has an anti-depressant-like action in HD mice. No change was detected in WT mice suggesting that the anti-depressant effect was specific to HD animals. To confirm that performance on the forced swim test was not confounded by swimming deficits, mice were assessed on two motor controls of swimming performance, the open pool test and the simple swim test. No significant differences between any groups were observed on either test (Fig. 4.12).

4.4 Discussion

Gangliosides are of great functional importance in the central nervous system. The type and arrangement of sugar residues in the glycan head group is responsible for most of the functional diversity and for the heterogeneity of interactions displayed by the various gangliosides (148, 150). In certain brain structures, however, a significant variability in the fatty acid component of GM1 has been detected, suggesting that the fatty acid moiety of the ganglioside may have more functional relevance than previously thought (464). In a search for key structural components of the GM1 molecule that are necessary and sufficient to provide neuroprotection in HD models,

we began our analysis by testing different gangliosides to assess the functional consequences of varying the composition of the sugar head group of GM1 (440). We found that a few common gangliosides could replicate, at least partially, the effects of GM1 on cell survival, indicating that the precise glycan structure of GM1 is not essential for its protective effects. However, one important finding was that the presence of sialic acid in the head group is crucial, as asialo-GM1 (GA1) had no protective effects on HD cells. This finding is in line with previous studies that demonstrate that elimination of the sialic acid residue abrogates the effect of GM1 on neurotrophin signaling and calcium homeostasis (203, 465, 466).

In our model, terminal sialylation (attachment of sialic acid to the outermost galactose residue of complex gangliosides) did not seem to be a modifier of ganglioside efficacy when considering protection from cell death, as GD1a increased cell survival while GT1b did not. The total number of sialic acid residues, however, may contribute to the efficacy of different gangliosides. Asialo and trisialogangliosides had no protective effects in a cell death assay, while all mono and disialogangliosides were at least partially effective. Interestingly, the two disialogangliosides tested that had α2,8-conjugated sialic acid residues, GD3 and GD1b, demonstrated reduced potency as compared to GM1. GD3 and GD1b both matched the efficacy of GM1 at 100 μM concentration but were only half as effective as GM1 when administered at a concentration of 50 μM. This suggests that when the two sialic acid residues are linked to one another the interaction between the ganglioside and potential effector proteins is less efficient. Furthermore, a terminal galactose residue seems to be required (but not sufficient) to mediate anti-apoptotic effects, as ganglioside GM2, the glycan chain of which terminates with an N-acetylgalactosamine residue, was not effective.

GM3, GM2, GM1 and GD1a are the most common a-series gangliosides, of these only GM1 and GD1a reduce cell death back to the level observed in WT cells. GM1 and GD1a are the two a-series gangliosides that are present at the highest concentrations in the brain (261). The greater functional relevance of these two compounds in the nervous system may explain their beneficial effects. However, we cannot exclude the possibility that the beneficial effects of GD1a are at least partially mediated through conversion to GM1. Neu3 sialidase, the enzyme responsible for conversion of GD1a to GM1, is present in multiple cellular compartments including the plasma membrane, where it is functionally active and capable of mediating conversion from GD1a to GM1 without going through the lysosomal pathway (467, 468).

The number of sialic acid residues and their position within the glycan head group of gangliosides is of clear functional importance in our assay. In contrast to this, there does not appear to be any relationship between efficacy and ganglioside series. It is not uncommon for ganglioside functions to be specific to one series (469, 470), as it has previously been shown that b-series gangliosides are specifically required for repairing nerve damage. However, the anti-apoptotic effects of gangliosides in our experiments did not seem to be bound to a particular series as gangliosides from both pathways were able to reduce cell death. In summary, our data suggests that the number and position of sialic acid residues and the presence of a terminal galactose residue are the most critical structural components of the sugar head group in regards to reducing cell death.

Next, we dissected the contribution of the ceramide moiety to the beneficial actions of GM1. The lack of beneficial effects of truncated GM1 (which misses the hydrophobic hydrocarbon chains that anchor the molecule to membranes) suggest that membrane localization of GM1 is critical for its beneficial effects. On the other hand, the fact that stearyl truncated GM1 (which could still

integrate within membranes thanks to its fatty acid chain) is ineffective, suggests that the sphingosine arm is a crucial determinant of GM1 activity in our model system, likely because of its unsaturated carbon chain.

Lyso-derivatives of GM1, Liga20 and lysoGM1, where the fatty acid of ceramide is substituted with a smaller and more polar group or removed (respectively) were as effective as GM1 at preventing cell death in HD cells, but at one tenth of the concentration. Increased potency of these compounds is likely due to their reduced hydrophobicity, which results in a higher membrane permeability and more efficient incorporation of the compound into membranes.

Based on our findings, the most effective type of synthetic compound would be a lyso-derivative containing the sugar head group of either GM1 or GD1a. Unfortunately, Liga20 was shown to be toxic after prolonged administration in animal models (160). The potential toxicity profile of lysoGM1 is not known yet. However lyso-derivatives of gangliosides and phospholipids have detergent-like properties that might affect, above certain concentrations, membrane integrity and cell viability (471). Whether lysoGM1 would display toxicity at therapeutic concentrations in HD models remains to be determined.

In addition to the aforementioned compounds, we also tested a small group of gangliomimetics bearing the same glycan head group as GM1, but with specific substitutions in the ceramide moiety that were designed to increase membrane permeability while retaining low toxicity. The exact nature of these substitutions in the gangliomimetic compounds tested in this study cannot be disclosed due to a confidentiality agreement with the providing company (Seneb Biosciences Inc., US). These compounds retained the ability to decrease HD cell death in our assay, and showed increased potency compared to GM1.

While protection of HD cells from apoptosis *in vitro* is an important readout of the therapeutic effects of GM1 in HD, it is not the only one and perhaps not even the most relevant to neuroprotection *in vivo*. Therefore, although testing other gangliosides and gangliomimetic compounds in an *in vitro* cell death assay is informative and suggests that many gangliosides share similar action with GM1, it is not enough, by itself, to determine whether the therapeutic role of GM1 in HD is fully mimicked. Therefore, to further characterize the action of non-GM1 gangliosides and gangliomimetics we determined whether they were able to activate protective cell signaling pathways, the Akt and the Erk pathways, that are known to be activated by GM1 in our cell models. The P13K/Akt and MEK/Erk pathways are both pro-survival pathways that are activated downstream of growth factors and neurotrophin receptors (472). In previous studies, GM1 was shown to protect cells from serum deprivation by activation of neurotrophins/trk receptors signaling (151, 462, 473). Surprisingly, although in our cell lines various gangliosides were able to provide protection from cell death induced by growth factor withdrawal, only GM1 was able to increase activation (phosphorylation) of both AKT and ERK. Not even Liga20 and lysoGM1, which only differ from GM1 by the absence of stearic acid, were able to activate Akt and/or Erk. This suggests that i) either the protective compounds identified in this study act through a mechanism that is different from that activated by GM1, or that ii) activation of Akt and Erk is an epiphenomenon of GM1 activity, but not required for the anti-apoptotic effects of this or other gangliosides. In line with these observations, previous studies showed that pharmacological inhibition of the PI3K/Akt pathway only partially decreases the neuroprotective activity of GM1 in an HD cell model (137).

Based on the pattern of deficits observed in KO mice with different genes targeted in the ganglioside synthetic pathway it has been hypothesized that gangliosides have both conserved

and specific functions (474). Our results support a specific role of GM1 in increasing phosphorylation of Akt and Erk. They also suggest that, in our model, increased signaling via Akt and Erk by GM1 is not mediated through activation of the trk B, trk C or GDNF receptors. These receptors can all be activated by GM1 or Liga20 to a similar extent in other models (190, 199, 475), while Akt and Erk are only activated by GM1 in our study. This is also in line with previous studies that showed that a pan-trk receptor inhibitor does not block the protective action of GM1 in HD cells (137).

An important aspect of the therapeutic effects of GM1 in HD models is its ability to induce phosphorylation of HTT at Ser13 and Ser16 (212), a post-translational modification that decreases the toxicity of mHTT (213) through mechanisms that are not yet clear, but that might involve the transport of pHTT to the nucleus and a beneficial role for pHTT in the response to cellular stress (213, 463, 476). Thus, the ability of GM1-mimetics to trigger phosphorylation of HTT is crucial to their potential clinical use in HD. Similar to GM1, both Liga20 and SNB-6100 increased level of pHTT in the whole cell and in the nucleus, in both WT and HD cells. The mechanism of GM1-mediated increase in HTT phosphorylation is currently unclear. In one report it was suggested that activation of casein kinase 2 (CK2) could enhance HTT phosphorylation at Ser13 and Ser16 (463), while in another study suggested that I kappa B kinase (IKK) could be involved (224). Whether GM1 or other gangliosides could activate any of these kinases is not known.

Our *in vitro* data suggests that structural analogues of GM1, while they may not share the exact mechanism, are able to replicate the anti-apoptotic effects of GM1. While these results are promising, the most relevant question is whether these analogues can improve HD symptoms and neuropathology *in vivo*. The dissociable nature of cell death and Akt/Erk signaling in the

protective action of non-GM1 gangliosides, suggests that other actions of GM1 may not be conserved despite the rescue of cell death.

Therefore, we tested the most promising compound identified in this study, SNB-6100, *in vivo*, in YAC128 mice. Our results indicate the SNB-6100 can revert to normal motor symptoms and improve non-motor symptoms such as depression-like behavior when administered to HD mice for 28 days by intraperitoneal injection. In parallel, we tested the effects of intraperitoneal administration of GM1, and confirmed that the ganglioside cannot exert therapeutic activity when administered peripherally, likely because of its inability to cross the BBB in therapeutically relevant amounts (454).

The rescue of motor performance by SNB-6100 in YAC128 mice suggests that the ability of a drug to provide neuroprotection *in vitro* and to trigger phosphorylation of HTT at Ser13 and Ser16 is a better predictor of therapeutic activity in animal models than is its ability to activate Akt and Erk.

Although much still need to be done to prove that SNB-6100 or similar compounds could have the same dramatic therapeutic effects of GM1 upon peripheral administration with no toxicity, our studies provide proof-of-principle that GM1-mimetic compounds can be designed that have conserved efficacy but improved potency and pharmacokinetics. Furthermore, our studies provide a framework for the design of additional GM1-mimetics with clinical relevance in HD.

Figure 4.1: A and B-series gangliosides improve survival of HD cells
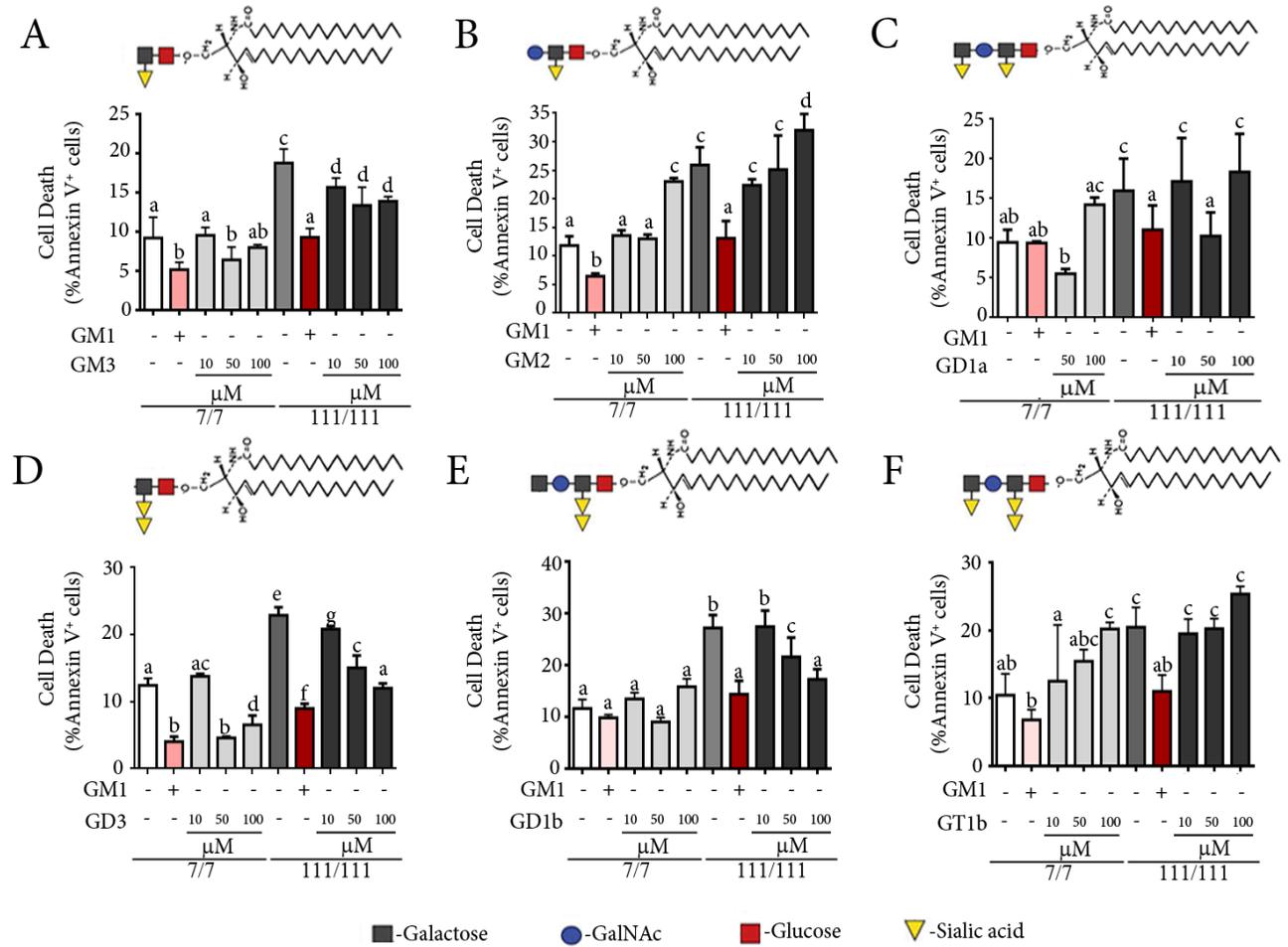
Figure 4.1 A and B-series gangliosides improve survival of HD cells. WT (7/7) and HD (111/111) cells were incubated for 9 h at 39°C in serum-free medium in the presence of the indicated gangliosides at the indicated concentrations. GM1 was used at a concentration of 50 µM in all experiments. Cell death was measured after labeling with Annexin V and FACS analysis. (A) Ganglioside GM3 decreased cell death in HD cells but to a lesser extent than GM1. (B) Ganglioside GM2 does not prevent cell death. (C) GD1a was as effective as GM1 at a concentration of 50 µM in both cell lines. N=8 replicates from two independent experiments for 7/7 UNTR, 111/111 UNTR, GM1 and GM2 50 µM. N=4 replicates from one experiment for 7/7 GM1, 7/7 and 111/111 10 µM, 7/7 and 111/111 100 µM. GD3 (D) and GD1b (E) were as effective as GM1 at reducing cell death in both cell lines at a concentration of 100 µM. F) GT1b was not able to significantly reduce cell death in either cell line. Data shown are means of 1-4 independent experiments each performed in triplicates or quadruplicates. One-way ANOVA with Bonferroni post-tests. Different letters indicate significant differences between groups, $p<0.05$.

Figure 4.2: Asialo-GM1 and truncated GM1 analogues that lack the sphingosine arm do not protect HD cells from apoptosis, while lyso-ganglioside derivatives are more potent than GM1
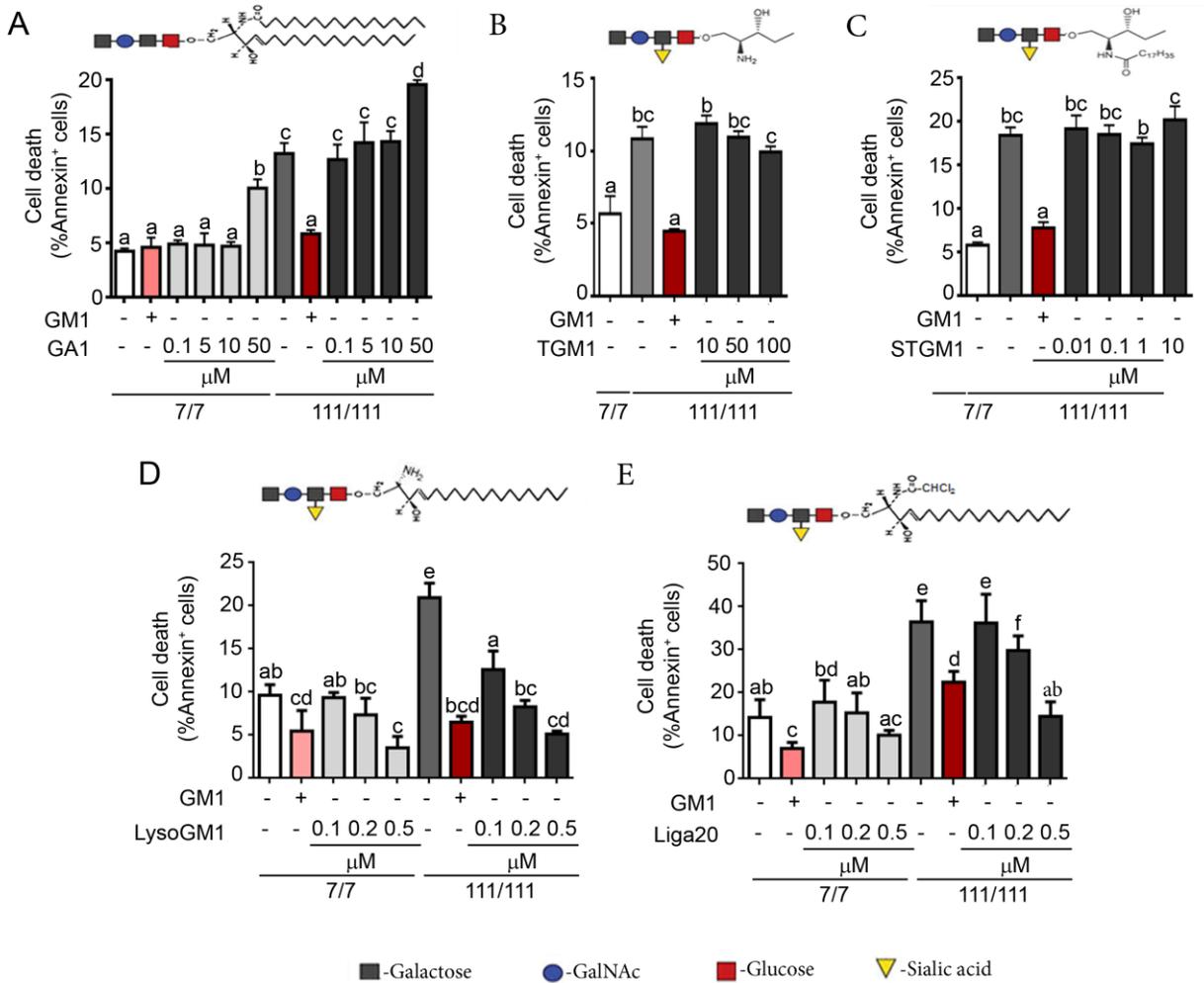
Figure 4.2 Asialo-GM1 and truncated GM1 analogues that lack the sphingosine arm do not protect HD cells from apoptosis, while lyso-ganglioside derivatives are more potent than GM1. Cells were treated as in Fig. 4.2. (A) Ganglioside GM1 lacking the sialic aside residue (GA1) was unable to reduce cell death in either WT (7/7) or HD (111/111) cells. (B) Truncated GM1 (TGM1, lacking both fatty acid and sphingosine hydrophobic chains) and (C) Stearyl truncated GM1 (StGM1 lacking the sphingosine arm) were not able to reduce HD cell death. (D) lysoGM1 was as effective as GM1 at reducing cell death in both cell lines at a concentration of 5 µM. Data in A-D are means of quadruplicates from one experiment. (F) Liga20 was more effective than GM1 at reducing cell death in HD cells at a concentration of 5 µM but did not reduce cell death in WT cells. Data are means of 8 replicates from two independent experiments. GM1 was used at the concentration of 50 µM in all experiments. One-way ANOVA with Bonferroni post-tests. Different letters indicate significant differences between groups, $p<0.05$.

Figure 4.3: Caspase 3 and annexin V expression show similar results in a cell death assay
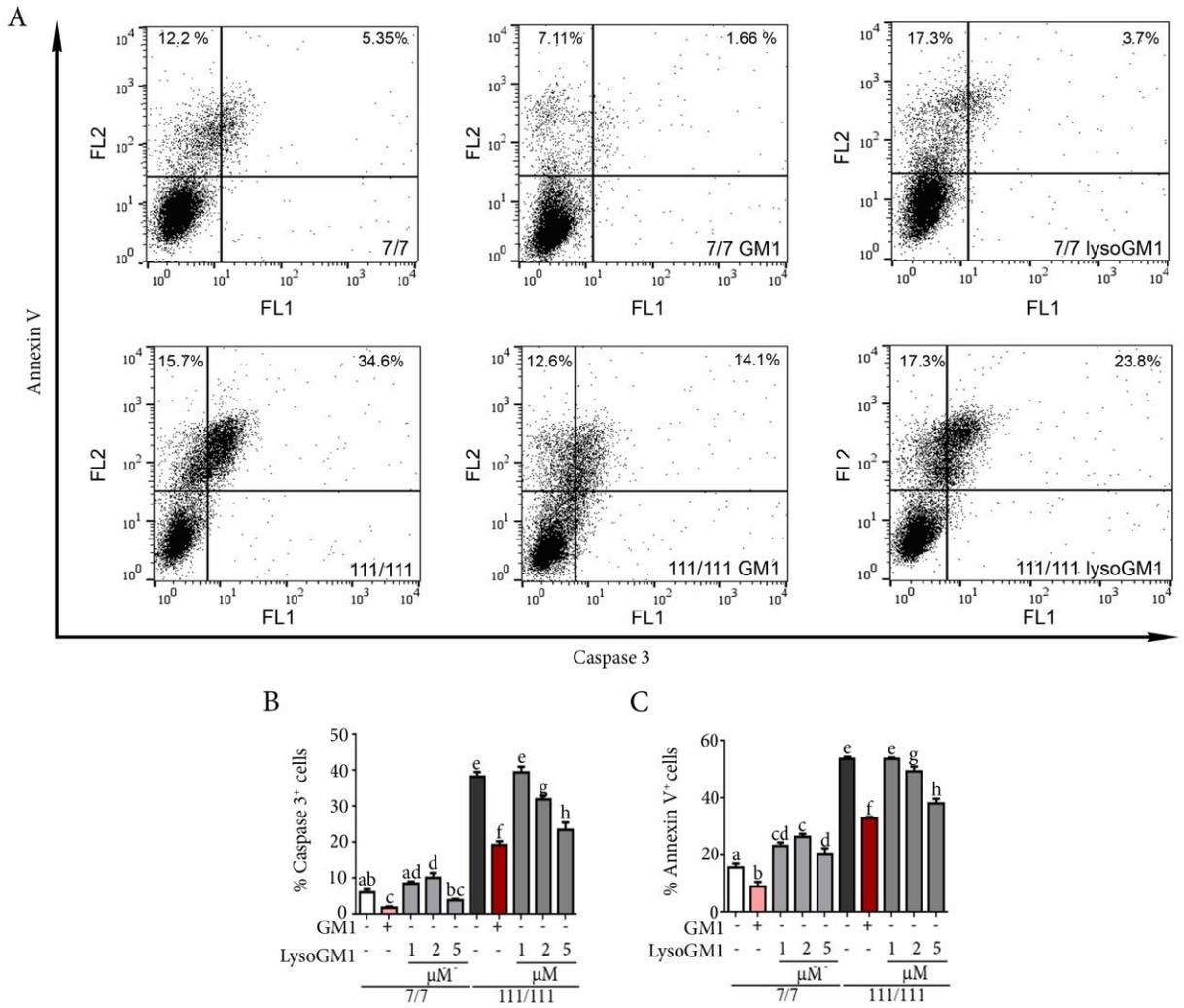
Figure 4.3 Caspase 3 and annexin V expression show similar results in a cell death assay.

(A) Representative flow cytometry profiles of 7/7 (WT) and 111/111 (HD) cells after 12 h incubation at 39°C in serum free media with lysoGM1 (5 µM) and GM1 (50 µM). FL1 and Fl2 indicate caspase 3^+ cells and Annexin V^+ cells respectively. Cells in the upper right quadrant (Double$^+$ cells) are apoptotic cells. Cells in the upper left quadrant (annexin V^+, but active caspase 3^- cells) are early apoptotic cells. The numbers reported in the top quadrants indicate the percentage of the total population of cells present in each quadrant. (B) Percent caspase 3^+ Stdh7/7 and Stdh111/111 cells after 12 h incubation at 39°C in serum free media with lysoGM1 as measured by flow cytometry. (C) Percent annexin V^+ Stdh7/7 and Stdh111/111 cells after 12 h incubation at 39°C in serum free media with lysoGM1 as measured by flow cytometry. Error bars show standard deviation. Each graph is the average of four technical replicates from one experiment, with 10,000 cells counted per replicate. Statistics performed using one-way ANOVA with Bonferroni post-tests. Different letters indicate significant differences between groups, $p<0.05$.

Figure 4.4: Gangliomimetic compounds with protective activity on HD cells
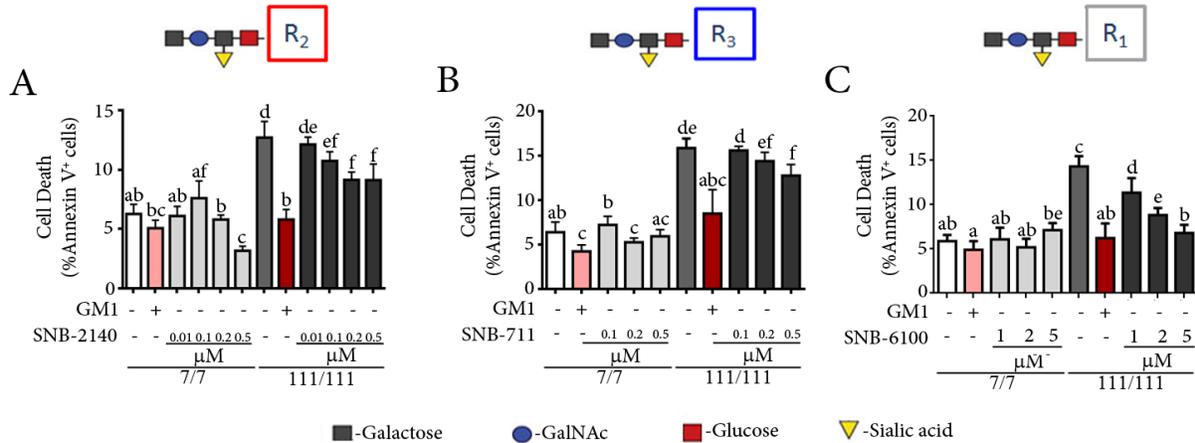
Figure 4.4 Gangliomimetic compounds with protective activity on HD cells. Cells were treated as in Fig. 4.2. (A) SNB-2140 reduced cell death in both WT (7/7) or HD (111/111) cells at concentration of 200 nM and 500 nM. Data are means of 4 replicates from one experiment. (B) SNB-711 was less effective than SNB-2140, but was able to reduce HD cell death at a concentration of 500 nM. (C) SNB-6100 was the most protective gangliomimetic compound and demonstrated equal rescue to GM1 at a concentration of 5 µM in HD cells. Data in B and C are means of 8 replicates from two independent experiments. GM1 was used at a concentration of 50 µM in all experiments. One-way ANOVA with Bonferroni post-tests. Different letters indicate significant differences between groups, $p<0.05$.

Figure 4.5: summary of ganglioside and gangliomimetic compound efficacy at reducing cell death
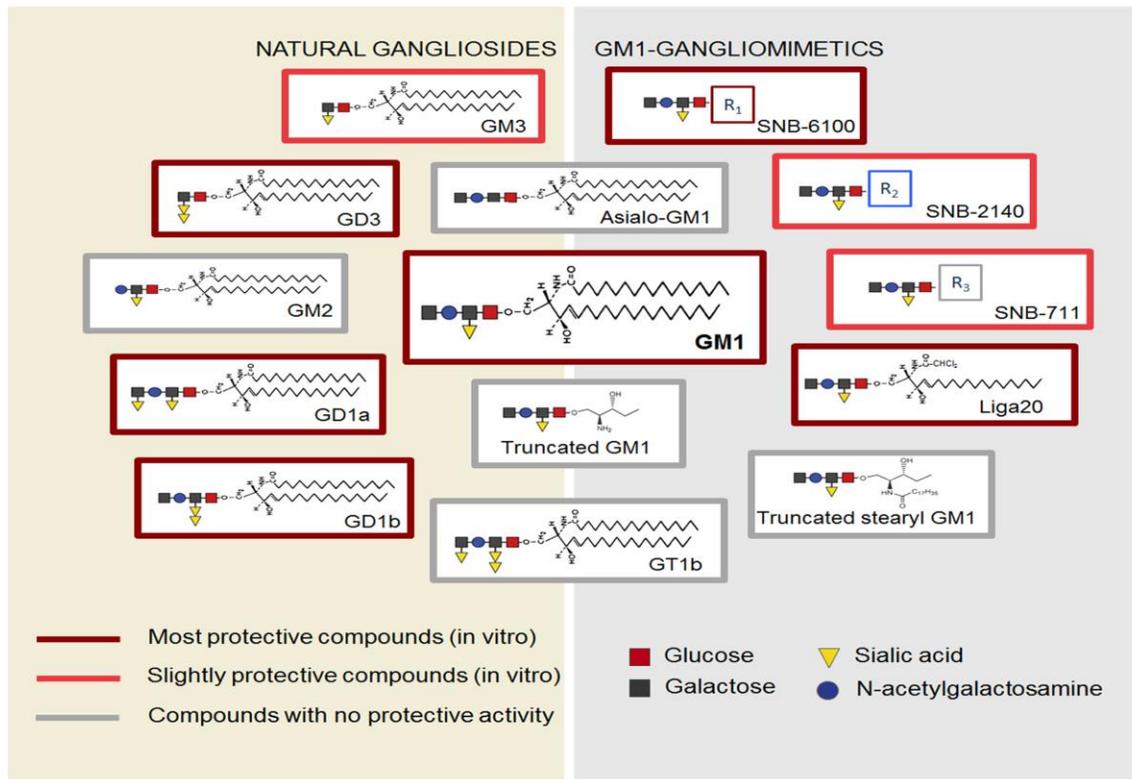
Figure 4.5 Ganglioside biosynthetic pathway and summary of ganglioside and gangliomimetic compound efficacy at reducing cell death. Summary of the protective actions of gangliosides and gangliomimetic compounds. All comparisons are based on the number of annexin V^+ cells after 6-9 h of serum deprivation.

Figure 4.6: Differential effects of GM1 and other gangliosides and gangliomimetic compounds on AKT and ERK activation
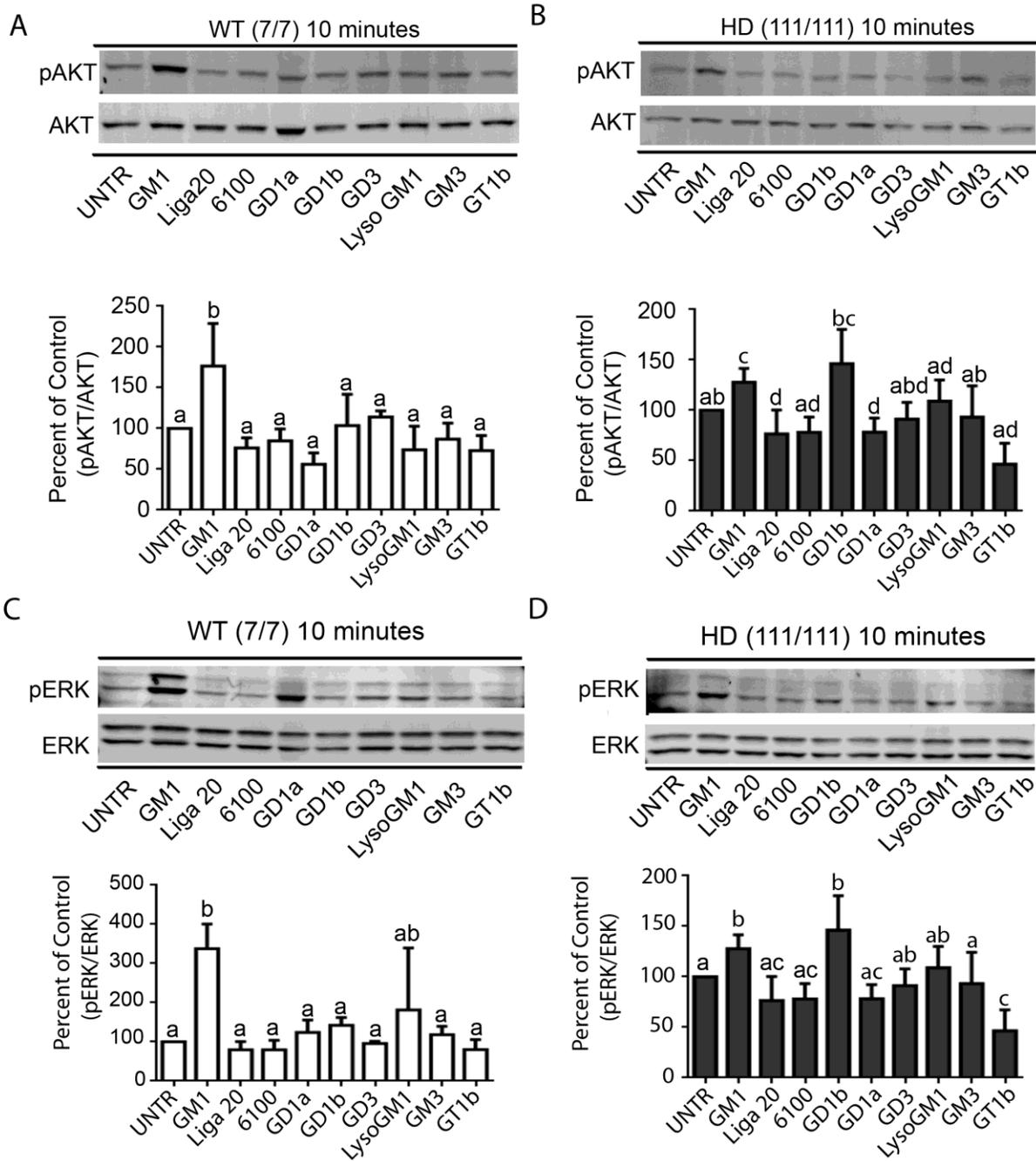
Figure 4.6 Differential effects of GM1 and other gangliosides and gangliomimetic compounds on AKT and ERK activation. WT (7/7) and HD (111/111) cells were pre-incubated for 4-5 h in serum-free medium and then treated for 10 min with the indicated compounds at the following concentrations: 50 µM GM1, 5 µM Liga20, 5 µM SNB-6100, 50 µM GD1b, 50 µM GD1a, 50 µM GD3, 5 µM lysoGM1, 50 µM GM3, and 50 µM GT1b. Representative immunoblots and densitometric analysis are shown. (A) GM1 was the only compound to increase the amount of pAkt detected in WT cells. (B) GM1 and GD1b increased pAkt levels in HD cells. (C) Large increases in the levels of pErk were detected in WT cells after treatment with GM1 and LysoGM1. (D) In HD cells, only GM1 was able to increase the amount of pErk, while GT1b, decreased pErk levels. Bars show mean value of 3-4 independent experiments ± SD. One-way ANOVA with Bonferroni post-tests. Different letters indicate significant differences between groups, $p<0.05$

Figure 4.7: Non-GM1 gangliosides and gangliomimetic compounds do not increase phosphorylation of AKT or ERK after one hour of administration
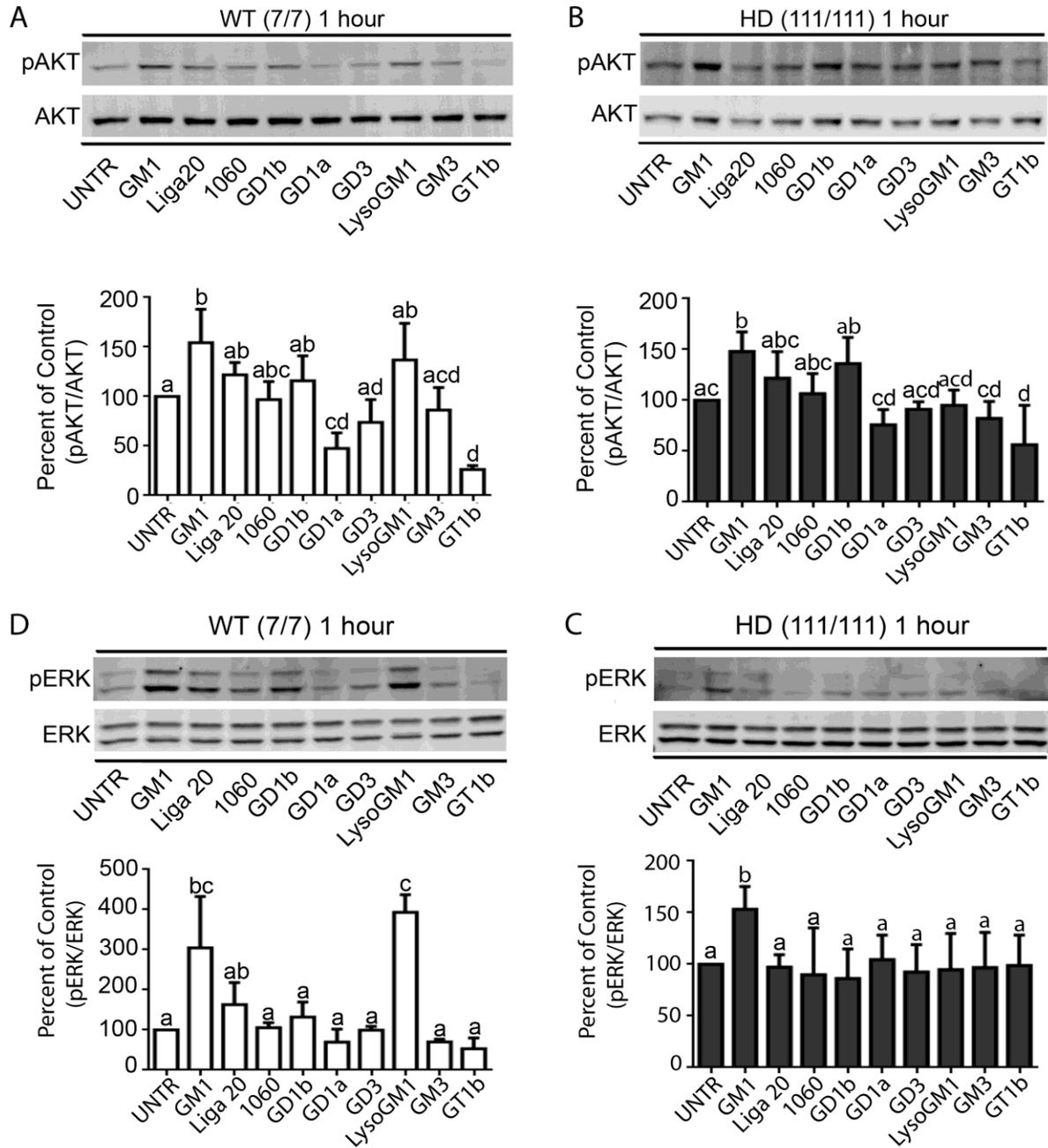
Figure 4.7 Non-GM1 gangliosides and gangliomimetic compounds do not increase phosphorylation of AKT or ERK after one hour of administration. 7/7 (WT) and 111/111 (HD) cells were treated for 10 min at 33°C after 4 h and 50 min of pre-treatment with serum free media. Cells were treated with GM3 and GT1b in addition to all compounds that demonstrated efficacy at reducing the percentage of annexin V$^+$ in the cell death assay. Cells were lysed and lysates were analyzed by western blot to determine the levels of pAkt and pErk. Compounds were tested at the following concentrations GM1 50 µM, Liga20 5 µM, SNB-6100 5 µM, GD1b 50 µM, GD1a 50 µM, GD3 50 µM, LysoGM1 5 µM, GM3 50 µM, and GT1b 50 µM. (A) GM1 was the only compound to increase the amount of pAkt detected in WT cells. (B) GM1 and GD1b increased the amount of pAkt detected in HD cells. (C) Large increases in the amount of pErk was detected in WT cells after treatment with GM1 and LysoGM1. (D) Only GM1 was able to increase the amount of pErk in HD cells. Error bars indicate standard deviation and each graph is the average of 3-4 independent experiments. Statistics performed using one-way ANOVA with Bonferroni post-tests. Different letters indicate significant differences between groups, $p<0.05$.

Figure 4.8: Liga20 and SNB-6100 increase phosphorylation of HTT at Ser13 and Ser16
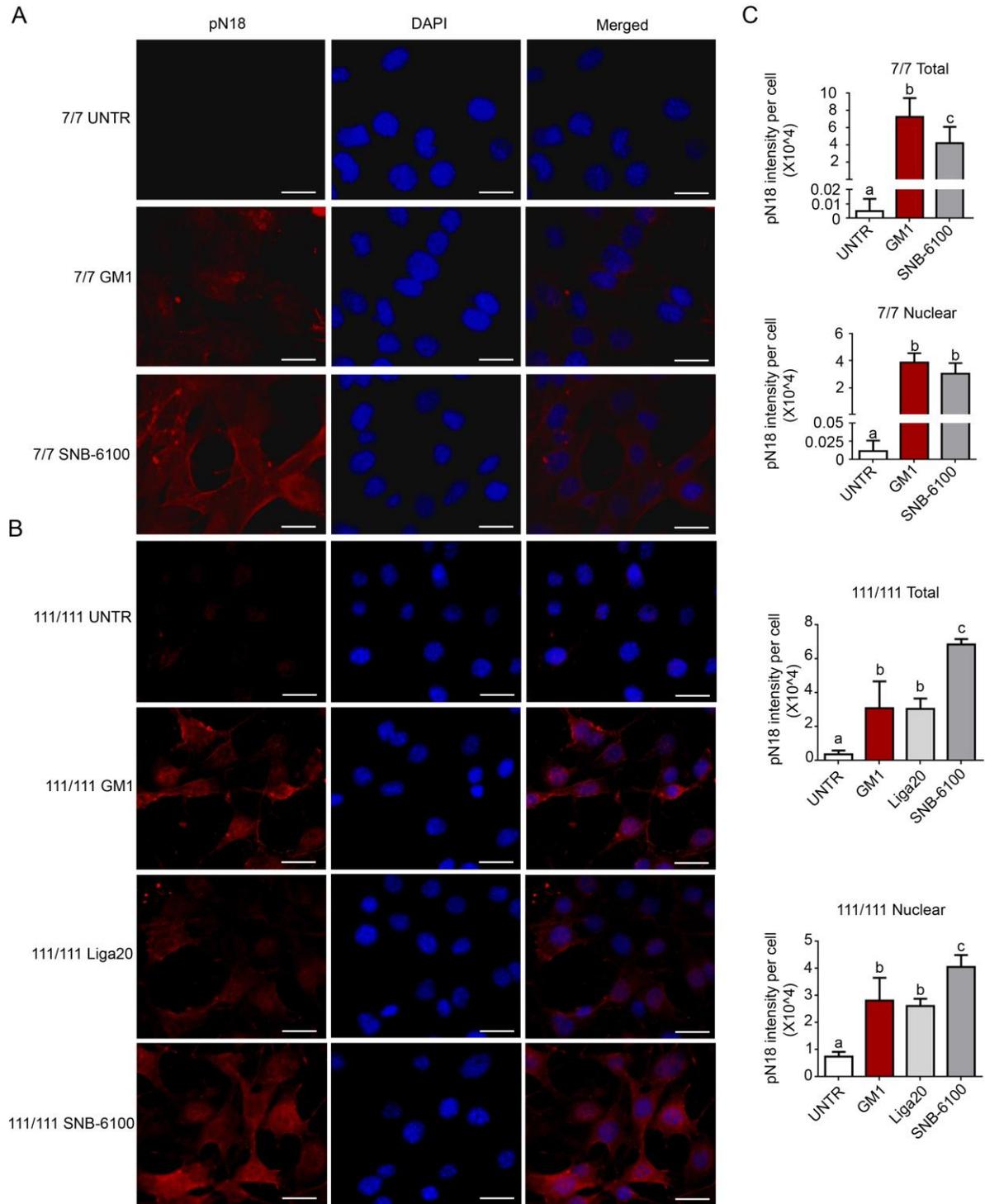
Figure 4.8 Liga20 and SNB-6100 increase phosphorylation of HTT at Ser13 and Ser16. 7/7 (WT) (A) and 111/111 (HD) cells (B) were pre-incubated in serum-free medium for 4 h and then treated with 50 µM GM1, 5 µM Liga20 or 2 µM SNB-6100 for 20 min at 33°C. Cells were treated with GM1, Liga20 or SNB-6100. HTT phosphorylation at Ser13 and Ser16 was detected with pHTT N17 2nd batch. Nuclei were visualized with DAPI staining. Representative epifluorescence microscopy images are shown. Scale bars indicate 16 µm. (C) Graphs show the mean fluorescence intensity (MFI) per cell. Approximately 400 cells per conditions were analyzed. Both GM1 and SNB-6100 increased HTT phosphorylation in whole cells as well as in the nucleus of WT cells, however, GM1 increased the levels of pHTT significantly more than did SNB-6100. (B) In HD cells all compounds increased HTT phosphorylation, with SNB-6100 showing the largest effect. Different letters indicate significant differences between groups, *p*<0.05

Figure 4.9: SNB-6100 improves motor and non-motor behaviour in YAC128 mice
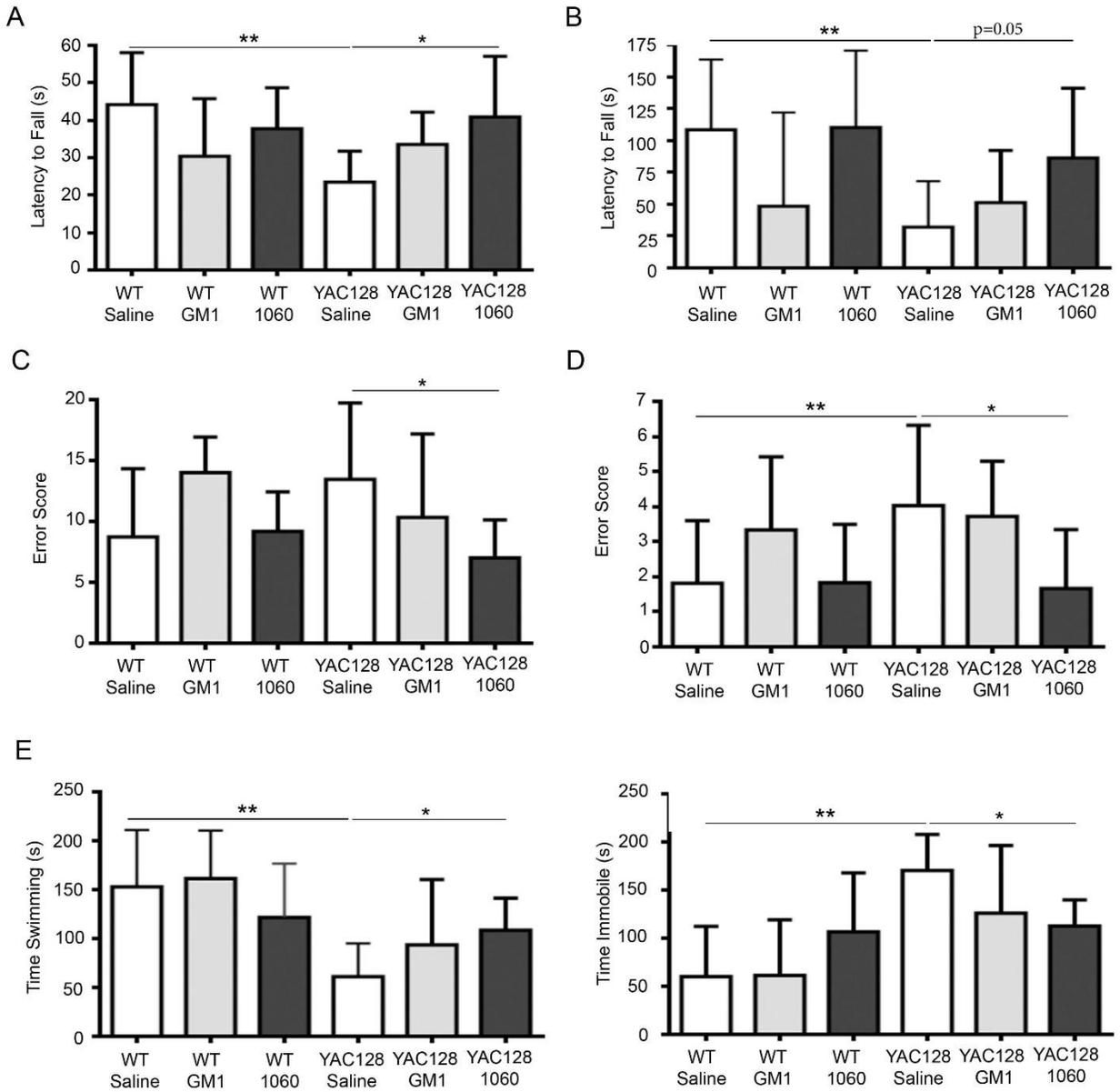
Figure 4.9 Peripheral administration of SNB-6100 and GM1 improves motor and non-motor performance in YAC128 mice. YAC128 mice and WT littermates where administered 30 mg/kg/day GM1, 3 mg/kg/day SNB-1060 or saline for 28 days, by intraperitoneal injection. (A) Mice were tested on the accelerating rotarod (4-40 RPM in 2 min), for 3 consecutive trials which were averaged to determine the latency to fall. SNB-6100 significantly improved the performance of YAC128 mice, while GM1 did not have any effect after intraperitoneal administration. (B) After 24 days of treatment mice were tested on the fixed rotarod at 12 RPM for three consecutive trial which were averaged to determine the latency to fall. SNB-6100 significantly improved the performance of YAC128 mice. N=11 WT saline, 5 WT GM1, 11 WT SNB-6100, 11 YAC128 saline, 6 YAC128 GM1, 9 YAC128 SNB-6100. (C) Horizontal ladder. Each mouse walked across the ladder 5 times and were scored for the number of mistakes made for each pass, which were added together to determine the error score for each animal. WT saline mice and YAC128 saline treated mice were not significantly different, however, treatment with SNB-6100 significantly decreased the number of errors made by YAC128 mice. N=11 WT saline, 4 WT GM1, 11 WT SNB-6100, 11 YAC128 saline, 6 YAC128 GM1, 9 YAC128 SNB-6100 (D) After 24 days of treatment mice were tested on the narrow beam. Each mouse walked across the narrow beam 3 times and were scored for the number of mistakes made for each pass, which were averaged together to determine the error score for each animal. Treatment with SNB-6100 reduced the number of errors. YAC128 mice. N=11 WT saline, 5 WT GM1, 10 WT SNB-6100, 11 YAC128 saline, 6 YAC128 GM1, 8 YAC128 SNB-6100 (E) After 28 days of treatment mice were tested for depression-like behaviour in the forced swim test. Testing lasted for 6 min and the last 4 min were scored for the presence or absence of swimming. Saline treated YAC128 mice spent significantly less time swimming and more time immobile (F) than WT

mice. Time swimming was increased, and time immobile decreased by the administration of SNB-6100 to YAC128 mice. Error bars are standard deviation. N=11 WT saline, 4 WT GM1, 11 WT SNB-6100, 11 YAC128 saline, 6 YAC128 GM1, 9 YAC128 SNB-6100. Statistics performed using a two-way ANOVA and Bonferroni post-tests. * $p<0.05$, **$p<0.01$.

Figure 4.10: SNB-6100 and GM1 cause a trend towards decreased anxiety-like behaviour
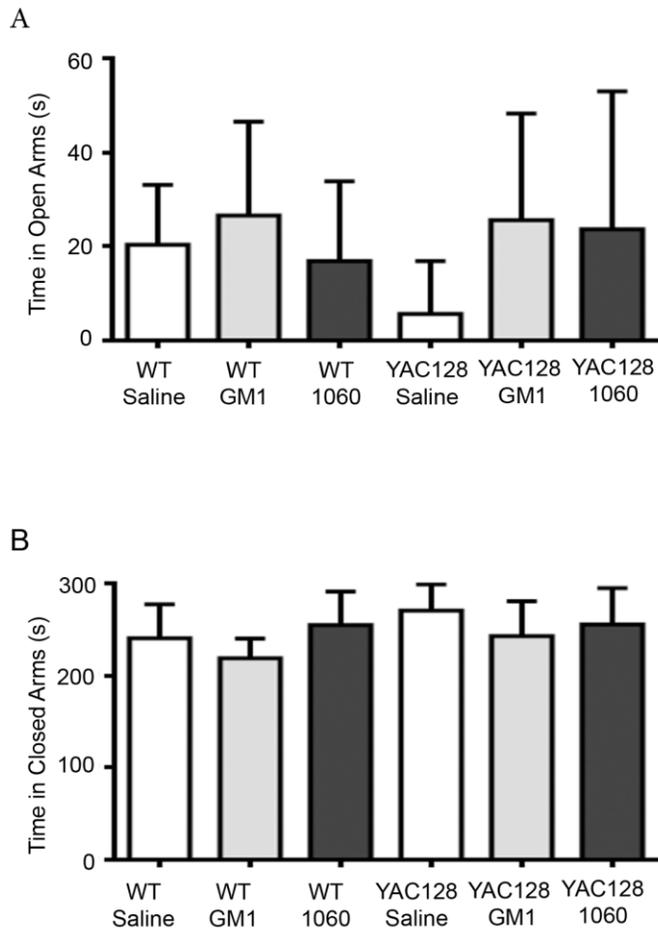
Figure 4.10 SNB-6100 and GM1 cause a trend towards decreased anxiety-like behaviour.

After 24 days of treatment mice were assessed for anxiety like-behaviour using the elevated plus maze. No significant differences in time in the open arms (A) or time in the closed arms (B) was observed between any groups. Error bars show standard deviation. N=11 WT saline, 5 WT GM1, 11 WT SNB-6100, 10 YAC128 saline, 6 YAC128 GM1, 9 YAC128 SNB-6100. Statistics performed using a two-way ANOVA and Bonferroni post-tests.

Figure 4.11: No deficits in swimming ability were detected in YAC128 mice
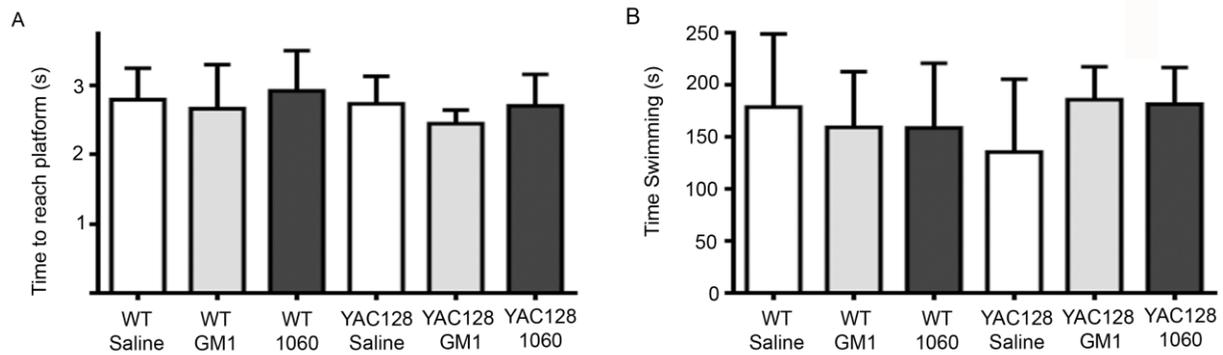
Figure 4.11 no deficits in swimming ability were detected in YAC128 mice. (A) Mice were tested in the simple swim test to confirm intact swimming ability. No differences between groups were observed. (B) Mice were additionally placed in an open pool for 6 min and the last 4 min were assessed for time swimming as a second motor control. No group differences were observed. Error bars are standard deviation. N=11 WT saline, 5 WT GM1, 11 WT SNB-6100, 11 YAC128 saline, 6 YAC128 GM1, 9 YAC128 SNB-6100. Statistics performed using a two-way ANOVA and Bonferroni post-tests.

4.5 References

21.   Ho AK, Gilbert AS, Mason SL, Goodman AO, Barker RA. Health-related quality of life in Huntington's disease: Which factors matter most? Movement disorders : official journal of the Movement Disorder Society. 2009;24(4):574-8.

34.   Tabrizi SJ, Scahill RI, Durr A, Roos RA, Leavitt BR, Jones R, et al. Biological and clinical changes in premanifest and early stage Huntington's disease in the TRACK-HD study: the 12-month longitudinal analysis. The Lancet Neurology. 2011;10(1):31-42.

64.   Slow EJ, van Raamsdonk J, Rogers D, Coleman SH, Graham RK, Deng Y, et al. Selective striatal neuronal loss in a YAC128 mouse model of Huntington disease. Human molecular genetics. 2003;12(13):1555-67.

137.   Maglione V, Marchi P, Di Pardo A, Lingrell S, Horkey M, Tidmarsh E, et al. Impaired ganglioside metabolism in Huntington's disease and neuroprotective role of GM1. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2010;30(11):4072-80.

138.   Denny CA, Desplats PA, Thomas EA, Seyfried TN. Cerebellar lipid differences between R6/1 transgenic mice and humans with Huntington's disease. Journal of neurochemistry. 2010;115(3):748-58.

139.   Desplats PA, Denny CA, Kass KE, Gilmartin T, Head SR, Sutcliffe JG, et al. Glycolipid and ganglioside metabolism imbalances in Huntington's disease. Neurobiology of disease. 2007;27(3):265-77.

143.   Sonnino S, Chigorno V. Ganglioside molecular species containing C18- and C20-sphingosine in mammalian nervous tissues and neuronal cell cultures. Biochimica et biophysica acta. 2000;1469(2):63-77.

148.   Posse de Chaves E, Sipione S. Sphingolipids and gangliosides of the nervous system in membrane function and dysfunction. FEBS letters. 2010;584(9):1748-59.

149.   Mocchetti I. Exogenous gangliosides, neuronal plasticity and repair, and the neurotrophins. Cell Mol Life Sci. 2005;62(19-20):2283-94.

150.   Schengrund CL. Gangliosides: glycosphingolipids essential for normal neural development and function. Trends in biochemical sciences. 2015;40(7):397-406.

151.   Ferrari G, Anderson BL, Stephens RM, Kaplan DR, Greene LA. Prevention of apoptotic neuronal death by GM1 ganglioside. Involvement of Trk neurotrophin receptors. The Journal of biological chemistry. 1995;270(7):3074-80.

155.   Sheikh KA, Sun J, Liu Y, Kawai H, Crawford TO, Proia RL, et al. Mice lacking complex gangliosides develop Wallerian degeneration and myelination defects. Proceedings of the National Academy of Sciences of the United States of America. 1999;96(13):7532-7.

159.   Ohmi Y, Tajima O, Ohkawa Y, Mori A, Sugiura Y, Furukawa K, et al. Gangliosides play pivotal roles in the regulation of complement systems and in the maintenance of integrity in nerve tissues. Proceedings of the National Academy of Sciences of the United States of America. 2009;106(52):22405-10.

160.   Ledeen RW, Wu G. The multi-tasked life of GM1 ganglioside, a true factotum of nature. Trends in biochemical sciences. 2015;40(7):407-18.

162.   Takamiya K, Yamamoto A, Furukawa K, Yamashiro S, Shin M, Okada M, et al. Mice with disrupted GM2/GD2 synthase gene lack complex gangliosides but exhibit only subtle defects in their nervous system. Proceedings of the National Academy of Sciences of the United States of America. 1996;93(20):10662-7.

164.   Sha S, Zhou L, Yin J, Takamiya K, Furukawa K, Furukawa K, et al. Deficits in cognitive function and hippocampal plasticity in GM2/GD2 synthase knockout mice. Hippocampus. 2014;24(4):369-82.

165.   Wu G, Lu ZH, Kulkarni N, Amin R, Ledeen RW. Mice lacking major brain gangliosides develop parkinsonism. Neurochemical research. 2011;36(9):1706-14.

166.   Tajima O, Egashira N, Ohmi Y, Fukue Y, Mishima K, Iwasaki K, et al. Reduced motor and sensory functions and emotional response in GM3-only mice: emergence from early stage of life and exacerbation with aging. Behavioural brain research. 2009;198(1):74-82.

167.   Yamashita T, Wu YP, Sandhoff R, Werth N, Mizukami H, Ellis JM, et al. Interruption of ganglioside synthesis produces central nervous system degeneration and altered axon-glial interactions. Proceedings of the National Academy of Sciences of the United States of America. 2005;102(8):2725-30.

190.   Bachis A, Rabin SJ, Del Fiacco M, Mocchetti I. Gangliosides prevent excitotoxicity through activation of TrkB receptor. Neurotoxicity research. 2002;4(3):225-34.

195.   Schneider JS, Roeltgen DP, Mancall EL, Chapas-Crilly J, Rothblat DS, Tatarian GT. Parkinson's disease: improved function with GM1 ganglioside treatment in a randomized placebo-controlled study. Neurology. 1998;50(6):1630-6.

196.   Schneider JS, Cambi F, Gollomp SM, Kuwabara H, Brasic JR, Leiby B, et al. GM1 ganglioside in Parkinson's disease: Pilot study of effects on dopamine transporter binding. Journal of the neurological sciences. 2015;356(1-2):118-23.

198.   Lim ST, Esfahani K, Avdoshina V, Mocchetti I. Exogenous gangliosides increase the release of brain-derived neurotrophic factor. Neuropharmacology. 2011;60(7-8):1160-7.

199.   Rabin SJ, Bachis A, Mocchetti I. Gangliosides activate Trk receptors by inducing the release of neurotrophins. The Journal of biological chemistry. 2002;277(51):49466-72.

203.   Duchemin AM, Ren Q, Mo L, Neff NH, Hadjiconstantinou M. GM1 ganglioside induces phosphorylation and activation of Trk and Erk in brain. Journal of neurochemistry. 2002;81(4):696-707.

212.   Di Pardo A, Maglione V, Alpaugh M, Horkey M, Atwal RS, Sassone J, et al. Ganglioside GM1 induces phosphorylation of mutant huntingtin and restores normal motor behavior in Huntington disease mice. Proceedings of the National Academy of Sciences of the United States of America. 2012;109(9):3528-33.

213.   Gu X, Greiner ER, Mishra R, Kodali R, Osmand A, Finkbeiner S, et al. Serines 13 and 16 are critical determinants of full-length human mutant huntingtin induced disease pathogenesis in HD mice. Neuron. 2009;64(6):828-40.

224.   Thompson LM, Aiken CT, Kaltenbach LS, Agrawal N, Illes K, Khoshnan A, et al. IKK phosphorylates Huntingtin and targets it for degradation by the proteasome and lysosome. The Journal of cell biology. 2009;187(7):1083-99.

261.   Vajn K, Viljetic B, Degmecic IV, Schnaar RL, Heffer M. Differential distribution of major brain gangliosides in the adult mouse central nervous system. PloS one. 2013;8(9):e75720.

320.   Porsolt RD, Le Pichon M, Jalfre M. Depression: a new animal model sensitive to antidepressant treatments. Nature. 1977;266(5604):730-2.

321.   Pouladi MA, Graham RK, Joshi P, Lu G, Deng Y, Wu N-P, et al. Diffeential susceptivility to excitotoxic stress in YAC128 mouse models of HD between initiation and progression of disease. Journal of Neuroscience. 2009:2193-204.

436.   Ghidoni R, Trinchera M, Venerando B, Fiorilli A, Sonnino S, Tettamanti G. Incorporation and metabolism of exogenous GM1 ganglioside in rat liver. Biochem J. 1986;237(1):147-55.

437.   Mitchell MD, Henare K, Balakrishnan B, Lowe E, Fong BY, McJarrow P. Transfer of gangliosides across the human placenta. Placenta. 2012;33(4):312-6.

438.   Palestini P, Masserini M, Fiorilli A, Calappi E, Tettamanti G. Age-related changes in the ceramide composition of the major gangliosides present in rat brain subcellular fractions enriched in plasma membranes of neuronal and myelin origin. Journal of neurochemistry. 1993;61(3):955-60.

439.   Mansson JE, Vanier MT, Svennerholm L. Changes in the fatty acid and sphingosine composition of the major gangliosides of human brain with age. Journal of neurochemistry. 1978;30(1):273-5.

440.   Wiegandt H. The chemical constitution of gangliosides of the vertebrate nervous system. Behavioural brain research. 1995;66(1-2):85-97.

441.   Hussain MM, Jin W, Jiang XC. Mechanisms involved in cellular ceramide homeostasis. Nutrition & metabolism. 2012;9(1):71.

442.   Yu RK, Bieberich E, Xia T, Zeng G. Regulation of ganglioside biosynthesis in the nervous system. Journal of lipid research. 2004;45(5):783-93.

443.   Pohlentz G, Klein D, Schwarzmann G, Schmitz D, Sandhoff K. Both GA2, GM2, and GD2 synthases and GM1b, GD1a, and GT1b synthases are single enzymes in Golgi vesicles from rat liver. Proceedings of the National Academy of Sciences of the United States of America. 1988;85(19):7044-8.

444.   Svennerholm L, Bostrom K, Fredman P, Mansson JE, Rosengren B, Rynmark BM. Human brain gangliosides: developmental changes from early fetal stage to advanced age. Biochimica et biophysica acta. 1989;1005(2):109-17.

445.   Kiarash A, Boyd B, Lingwood CA. Glycosphingolipid receptor function is modified by fatty acid content. Verotoxin 1 and verotoxin 2c preferentially recognize different globotriaosyl ceramide fatty acid homologues. The Journal of biological chemistry. 1994;269(15):11138-46.

446.   Iwabuchi K, Prinetti A, Sonnino S, Mauri L, Kobayashi T, Ishii K, et al. Involvement of very long fatty acid-containing lactosylceramide in lactosylceramide-mediated superoxide generation and migration in neutrophils. Glycoconjugate journal. 2008;25(4):357-74.

447.   Saslowsky DE, te Welscher YM, Chinnapen DJ, Wagner JS, Wan J, Kern E, et al. Ganglioside GM1-mediated transcytosis of cholera toxin bypasses the retrograde pathway and depends on the structure of the ceramide domain. The Journal of biological chemistry. 2013;288(36):25804-9.

448.   Yamashita T, Hashiramoto A, Haluzik M, Mizukami H, Beck S, Norton A, et al. Enhanced insulin sensitivity in mice lacking ganglioside GM3. Proceedings of the National Academy of Sciences of the United States of America. 2003;100(6):3445-9.

449.   Wu G, Lu ZH, Wang J, Wang Y, Xie X, Meyenhofer MF, et al. Enhanced susceptibility to kainate-induced seizures, neuronal apoptosis, and death in mice lacking gangliotetraose gangliosides: protection with LIGA 20, a membrane-permeant analog of GM1. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2005;25(47):11014-22.

450.   Ohmi Y, Tajima O, Ohkawa Y, Yamauchi Y, Sugiura Y, Furukawa K, et al. Gangliosides are essential in the protection of inflammation and neurodegeneration via maintenance of lipid rafts: elucidation by a series of ganglioside-deficient mutant mice. Journal of neurochemistry. 2011;116(5):926-35.

451.   Wang J, Lu ZH, Gabius HJ, Rohowsky-Kochan C, Ledeen RW, Wu G. Cross-linking of GM1 ganglioside by galectin-1 mediates regulatory T cell activity involving TRPC5 channel activation: possible role in suppressing experimental autoimmune encephalomyelitis. Journal of immunology. 2009;182(7):4036-45.

452.   Martinez Z, Zhu M, Han S, Fink AL. GM1 specifically interacts with alpha-synuclein and inhibits fibrillation. Biochemistry. 2007;46(7):1868-77.

453.   Ahn JY. Neuroprotection signaling of nuclear Akt in neuronal cells. Experimental neurobiology. 2014;23(3):200-6.

454.   Polo A, Kirschner G, Guidotti A, Costa E. Brain content of glycosphingolipids after oral administration of monosialogangliosides GM1 and LIGA20 to rats. Molecular and chemical neuropathology / sponsored by the International Society for Neurochemistry and the World Federation of Neurology and research groups on neurochemistry and cerebrospinal fluid. 1994;21(1):41-53.

455.   Manev H, Favaron M, Vicini S, Guidotti A, Costa E. Glutamate-induced neuronal death in primary cultures of cerebellar granule cells: protection by synthetic derivatives of endogenous sphingolipids. The Journal of pharmacology and experimental therapeutics. 1990;252(1):419-27.

456.   de Erausquin GA, Manev H, Guidotti A, Costa E, Brooker G. Gangliosides normalize distorted single-cell intracellular free Ca2+ dynamics after toxic doses of glutamate in cerebellar granule cells. Proceedings of the National Academy of Sciences of the United States of America. 1990;87(20):8017-21.

457.   Lipartiti M, Lazzaro A, Manev H. Ganglioside derivative LIGA20 reduces NMDA neurotoxicity in neonatal rat brain. Neuroreport. 1992;3(10):919-21.

458.   Trettel F, Rigamonti D, Hilditch-Maguire P, Wheeler VC, Sharp AH, Persichetti F, et al. Dominant phenotypes produced by the HD mutation in STHdh(Q111) striatal cells. Human molecular genetics. 2000;9(19):2799-809.

459.   Vermes I, Haanen C, Steffens-Nakken H, Reutelingsperger C. A novel assay for apoptosis. Flow cytometric detection of phosphatidylserine expression on early apoptotic cells using fluorescein labelled Annexin V. Journal of immunological methods. 1995;184(1):39-51.

460.   Prokazova NV, Samovilova NN, Gracheva EV, Golovanova NK. Ganglioside GM3 and its biological functions. Biochemistry Biokhimiia. 2009;74(3):235-49.

461.   Tettamanti G. Ganglioside/glycosphingolipid turnover: new concepts. Glycoconjugate journal. 2004;20(5):301-17.

462.   Zakharova IO, Sokolova TV, Vlasova YA, Furaev VV, Rychkova MP, Avrova NF. GM1 ganglioside activates ERK1/2 and Akt downstream of Trk tyrosine kinase and protects PC12 cells against hydrogen peroxide toxicity. Neurochemical research. 2014;39(11):2262-75.

463.   Atwal RS, Desmond CR, Caron N, Maiuri T, Xia J, Sipione S, et al. Kinase inhibitors modulate huntingtin cell localization and toxicity. Nature chemical biology. 2011;7(7):453-60.

464.   Serb AF, Sisu E, Vukelic Z, Zamfir AD. Profiling and sequencing of gangliosides from human caudate nucleus by chip-nanoelectrospray mass spectrometry. Journal of mass spectrometry : JMS. 2012;47(12):1561-70.

465.   Newburn EN, Duchemin AM, Neff NH, Hadjiconstantinou M. GM1 ganglioside enhances Ret signaling in striatum. Journal of neurochemistry. 2014;130(4):541-54.

466.   Ginzburg L, Li SC, Li YT, Futerman AH. An exposed carboxyl group on sialic acid is essential for gangliosides to inhibit calcium uptake via the sarco/endoplasmic reticulum Ca2+-ATPase: relevance to gangliosidoses. Journal of neurochemistry. 2008;104(1):140-6.

467. Papini N, Anastasia L, Tringali C, Croci G, Bresciani R, Yamaguchi K, et al. The plasma membrane-associated sialidase MmNEU3 modifies the ganglioside pattern of adjacent cells supporting its involvement in cell-to-cell interactions. The Journal of biological chemistry. 2004;279(17):16989-95.

468. Zanchetti G, Colombi P, Manzoni M, Anastasia L, Caimi L, Borsani G, et al. Sialidase NEU3 is a peripheral membrane protein localized on the cell surface and in endosomal structures. Biochem J. 2007;408(2):211-9.

469. Itoh M, Fukumoto S, Iwamoto T, Mizuno A, Rokutanda A, Ishida HK, et al. Specificity of carbohydrate structures of gangliosides in the activity to regenerate the rat axotomized hypoglossal nerve. Glycobiology. 2001;11(2):125-30.

470. Okada M, Itoh Mi M, Haraguchi M, Okajima T, Inoue M, Oishi H, et al. b-series Ganglioside deficiency exhibits no definite changes in the neurogenesis and the sensitivity to Fas-mediated apoptosis but impairs regeneration of the lesioned hypoglossal nerve. The Journal of biological chemistry. 2002;277(3):1633-6.

471. Kobayashi T, Goto I. A sensitive assay of lysogangliosides using high-performance liquid chromatography. Biochimica et biophysica acta. 1991;1081(2):159-66.

472. Numakawa T, Adachi N, Richards M, Chiba S, Kunugi H. Brain-derived neurotrophic factor and glucocorticoids: reciprocal influence on the central nervous system. Neuroscience. 2013;239:157-72.

473. Ferrari G, Greene LA. Promotion of neuronal survival by GM1 ganglioside. Phenomenology and mechanism of action. Annals of the New York Academy of Sciences. 1998;845:263-73.

474. Furukawa K, Ohmi Y, Ohkawa Y, Tajima O, Furukawa K. Glycosphingolipids in the regulation of the nervous system. Advances in neurobiology. 2014;9:307-20.

475. Hadaczek P, Wu G, Sharma N, Ciesielska A, Bankiewicz K, Davidow AL, et al. GDNF signaling implemented by GM1 ganglioside; failure in Parkinson's disease and GM1-deficient murine model. Experimental neurology. 2015;263:177-89.

476. Mishra R, Hoop CL, Kodali R, Sahoo B, van der Wel PC, Wetzel R. Serine phosphorylation suppresses huntingtin amyloid accumulation by altering protein aggregation properties. Journal of molecular biology. 2012;424(1-2):1-14.

CHAPTER 5

DISCUSSION AND CONCLUSIONS

5.1 Summary of findings

5.1.1 Ganglioside GM1 improves behavioural, neuropathological, and biochemical hallmarks of HD

HD is characterized by motor dysfunctions, cognitive deficits and psychiatric disturbances. In the second chapter of this thesis I demonstrated that ganglioside GM1 is able to revert all three behavioural aspects of disease pathology in well validated mouse models of HD with different genetic backgrounds. According to guidelines established by the National Institute of Health (NIH) together with the HD scientific community, the use of diverse models is an essential element of pre-clinical trials in HD (75, 76). By using a combination of knock-in (Q140) and transgenic (YAC128 and R6/2) mouse models we were able to safely conclude that the beneficial effects of GM1 extend to all models, regardless of genetic background, expression levels of mHTT and disease severity. The use of different genetic backgrounds further reduces the probability of the observed therapeutic benefits being an epiphenomenon or artifact resulting from the particular background strain of the mouse interacting with the treatment.

NIH recently recommended the use of both male and female mice in pre-clinical testing of therapeutic compounds (477). To meet this new requirement and to ensure the effects of GM1 were not specific to male animals we included female Q140 mice in our behavioural and biochemical studies whenever possible. While there were some biochemical differences in the effect of GM1 between the two sexes, the behavioural benefits were clearly conserved between males and females, suggesting that while the underlying biochemical correlates may not be identical the outcome is the same and that GM1 is a viable treatment option in both sexes.

In addition to assessing the effects of GM1 on mouse behavior, we also tested the effects of GM1 on neurodegeneration by measuring the volume of brain structures that are known to be severely affected in HD, namely the striatum, white matter tracts, and total brain volume (34). In all measures of neurodegeneration we observed a reduction in volume loss after GM1 treatment, which indicated the ability of GM1 to slow down disease progression by about two weeks in the R6/2 mice. This is a rather significant slowing of disease, as R6/2 mice generally do not survive past 13-16 weeks of age (233). Two weeks is also the estimated time period required for GM1 to mediate behaviour rescue in YAC128 mice, which suggests that disease progression might have been stalled once GM1 reached full therapeutic effect. Further studies with longer duration of treatment would be necessary to determine the degree to which administration of GM1 can slow down or even stop neurodegeneration.

Regardless of the exact timeline of GM1 action, the decreased volume loss observed in the brains of R6/2 mice is of great functional importance. Brain volume loss correlates well with behavioural aspects of the disease. Striatal atrophy correlates particularly well with motor deficits, while white matter integrity loss correlates well with apathy and with measures of fine motor control (42, 45). Behavioural changes can always occur as a result of symptomatic benefit without necessarily indicating any change in disease course. Therefore, our findings that GM1 is able to slow brain atrophy provides compelling evidence that GM1 can modify disease course.

The mechanism of GM1 action is still not firmly established. Based on the strong connection in the literature between exogenous GM1 and BDNF signaling and the well-described loss of BDNF trophic support to striatal neurons in HD brains (85, 149), it was initially hypothesized that GM1 would affect BDNF signaling. However, we found no evidence to support this hypothesis in Q140 mice or R6/2 mice after administration with GM1 (data not shown in this

thesis). We did find evidence that GM1 can affect other prototypically dysfunctional HD pathways. DARPP32 and pDARPP32 levels are well described indicators of striatal health and functionality (265). We observed an increase in DARPP32 in the striatum of R6/2 and heterozygous Q140 mice. The fact that GM1 was able to increase DARPP32 levels in heterozygous Q140 mice, which express one copy of the normal *Htt* allele but not in homozygous which express two copies of the mutant gene, suggests that the presence of the WT protein is necessary to mediate this specific effect of GM1. Since similar results were obtained concerning the effects of GM1 on HTT protein levels, it is possible that the changes in pDARPP32 and DARPP32 levels in heterozygous mice are secondary to the decrease in mHTT expression as it has previously been described that reduction of mHTT expression through the use of short hair RNAs is able to increase levels of DARPP32 (478).

The effects of GM1 on HTT are of particular interest and relevance. While many biochemical pathways have been implicated early on in disease progression, the mHTT protein is the only factor that is both necessary and sufficient to cause the disease. Direct effects on the mutant protein are the only certain way to slow or prevent disease progression and are therefore crucial to define disease-modifying treatments in HD.

5.1.2 Rational design of second generation gangliomimetics with improved pharmacokinetics is a feasible approach for the treatment of HD

One major difficulty in the translation of gangliosides to the clinic is their poor pharmacokinetics (454). Structural changes to the ceramide component of GM1 can increase membrane permeability and preserve many of the functions of endogenous GM1 (455). Unfortunately, the previously created Liga series of compounds has been demonstrated to display toxicity over extended treatments (160). The existence of these compounds, however, does provide proof-of-

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principle for development of ganglioside analogues with conserved neuroprotective properties and improved pharmacokinetics. To facilitate the development of second generation gangliomimetic molecules with relevance for HD, we assessed the effects of gangliosides and gangliomimetics with various chemical structures in assays where GM1 has known effects. These experiments provided evidence that specific features of both the glycan head group and the hydrophobic tail of gangliosides are required for protective actions. There were three non-GM1 gangliosides that shared with GM1 the ability to protect HD cells from apoptosis in an *in vitro* assay, namely GD3, GD1a, and GD1b. These gangliosides, however, were not able to activate the same signaling pathways activated by GM1. Further studies are required to determine whether they can recapitulate other effects of GM1 in HD models, including the profound therapeutic effects *in vivo*. Gangliomimetic compounds with changes to the sphingosine or stearic acid arm, but containing the same sugar head group as GM1, were able to mimic the actions of GM1 on cell death with increased potency. These findings suggest that gangliomimetic compounds with structural changes that increase membrane permeability are viable therapeutic options for HD. This point was further supported by the beneficial effects of SNB-6100 on the behaviour of YAC128 mice after 28 days of peripheral administration. While SNB-6100 was unable to completely replicate all protective actions of GM1, as it did not increase activation of Akt and Erk pathways, it was able to trigger a beneficial phosphorylation of HTT and to provide functional benefits *in vivo.* One limitation of this study is that only one concentration of SNB-6100 was tested *in vivo,* and although no obvious signs of toxicity were observed during the treatment with SNB-6100, a thorough toxicological examination was not conducted. Additional studies are needed to determine whether higher doses of the compound are

required to fully recapitulate the therapeutic effects of GM1 *in vivo*, and to exclude potential toxicity.

5.2 Overall significance of findings

HD is a complex disorder causing deficits, disturbances or dysfunctions in many different behavioural domains, including cognition, mood, and movement. Currently management of symptoms is the best intervention that can be provided (311, 479). Even this degree of intervention is mitigated by the lack of efficacy of standard treatments of symptoms such as cognitive deficits and depression (28-30, 35). Additionally, some of the pharmacological interventions which have been approved for use in HD patients are contraindicated in individuals with psychiatric symptoms, which occur frequently even prior to disease onset (40).Even greater are the challenges in finding therapeutic interventions with the ability to reverse or slow neurodegeneration. To date no pharmacological treatment has shown the ability to modify parameters associated with disease progression (311, 480). The data presented in this thesis suggest that ganglioside GM1 could be a disease modifying therapy, with the ability to slow or prevent neurodegeneration in addition to improving all symptoms.

Currently, the most promising strategy for altering HD disease course involves targeting the expression of mHTT with small interfering RNA (siRNA), ASOs, and ribozymes (480, 481). Reduction of the expression of mHTT using both ASO and siRNA methods has been shown to reduce mHTT mRNA *in vivo* and to result in improved in animal behaviour in mouse models (431, 482). Both ASO's and siRNA strategies pose some difficulties in terms of method of administration, however, a phase I/IIa clinical trial was started in 2015 by Ionis Pharmaceuticals in collaboration with Roche to test ASOs in HD patients by intrathecal administration. Despite the many advantages of this approach there are still many challenges. For one, currently none of

the siRNAs or ASO compounds are able to cross the BBB and consequently, require surgical approaches to deliver the drugs into the central nervous system (481). Additionally, potential detrimental side effects of these treatments could arise from the inability of current ASOs and siRNAs to target the mutant *HTT* allele specifically, without causing a corresponding decrease in the expression of wtHTT. WtHTT has important functions in the nervous system, and its conditional knock-out in post-natal mice is sufficient to result in a neurodegenerative phenotype (85, 87). Although the possibility exists to design ASOs that target specific single nucleotide polymorphisms (SNP)s that are linked to the mutant *HTT* allele only, this approach is currently limited to a subset of the clinical HD population (483). Thus additional disease-modifying treatments for HD are still highly desirable.

Ganglioside GM1 or gangliomimetic compounds could have some potentially important advantages over the ASO approach. In chapter 2 of this thesis I present data showing that GM1 is capable of reducing the expression of mHTT, albeit it with a corresponding decrease in wtHTT, in both the striatum and cortex of heterozygous Q140 mice. Additionally, aggregate formation was reduced in the homozygous Q140 striatum and in the cortex of R6/2 mice. In terms of direct targeting of the mHTT protein GM1 showed encouraging effects, by decreasing overall HTT levels by approximately 50%, compared to 25-66% reduction mediated by RNAi and ASOs (480). Additionally, GM1 can reduce the number of mutant aggregates, an effect that has not been replicated in all studies with siRNAs and ASOs under all conditions (480). Additionally, GM1 facilitates phosphorylation of HTT at Ser13 and Ser16 (212), which in turn could affect mHTT aggregation and clearance (213, 224) but also promote beneficial functions of the remaining protein in cellular stress responses (463, 484). Furthermore, in addition to the

aforementioned HD-specific effects, GM1 has pleiotropic and more general neuroprotective activities that could benefit a wide range of neurodegenerative diseases (149).

The neuroprotective effects of GM1 are well illustrated by its disease modifying activity in clinical trials in PD. In PD, GM1 was able to reduce the motor deficits of treated patients as measured by the UPDRS (287, 288). At the end of a five year open label study, patient's demonstrated lower UPDRS scores than at the start of treatment. Additionally, no detrimental effects of GM1 were observed (287).

Further studies with ganglioside GM1 in PD suggested that in addition to the symptomatic improvement GM1 was also able to slow disease progression (288). A follow up study assessing the effects of GM1 on the expression of a DA transporter, as measured by positron emission tomography (PET) labelling, in the striatum showed that GM1 treatment reduced the loss of methylphenidate binding to the DA transporter. Since reduced expression of DA transporter is a marker of disease progression in PD, these results further supported the claim that GM1 can modify disease course in PD (196).

To the best of my knowledge GM1 is the only pharmacotherapy that has demonstrated disease modifying effects in two separate neurodegenerative disorders. In HD GM1 is likely to be even more effective than in PD, since GM1 modulates the amount and toxicity of the mutant protein that causes the disease, in addition to its pleiotropic neuroprotective effects.

Despite the many beneficial properties of GM1, clinical translation is hindered by its chemical properties that make this ganglioside a poor drug candidate according to classic pharmacology criteria. GM1 is a large molecule with poor pharmacokinetics and bioavailability (454). Central administration is necessary for GM1 to rescue behavioural deficits, as demonstrated in chapter 4

of this thesis. Due to the devastating and chronic nature of HD, central administration of the drug might still be a viable option. However, drugs that can be administered peripherally would be much more desirable. Research in the PD field has attempted to overcome these difficulties by administering sialidase, an enzyme that is known to convert endogenous GD1a, GD1b and GT1b to ganglioside GM1 (224, 289).

In the fourth chapter of this thesis I describe an alternative strategy: the synthesis of second generation molecules with improved pharmacokinetics. By elucidating the structural motifs of GM1 that are essential for execution of its beneficial effects I provide information for rational design of second generation compounds. I additionally provide proof of principle that gangliomimetic compounds can be designed that couple the therapeutic benefits of GM1 with improved potency, bioavailability, and pharmacokinetics. This claim is further supported by previous work with Liga20, a membrane permeant analogue of GM1, which was shown to retain the therapeutic effects of GM1 in various models of neurodegeneration, at lower concentrations and after peripheral administration (192, 449). Together this body of evidence suggests that membrane permeant ganglioside analogues are a viable option for clinical development for the treatment of HD and other neurodegenerative diseases.

5.3 Future directions

The work in this thesis strongly suggests that GM1 could be a disease modifying therapy for HD. However, the mechanism of GM1 action is still relatively poorly understood, and this hampers progress towards clinical translation. Future work will need to fill this important gap in knowledge. A better understanding of the mechanisms of action of GM1 could also help the development of second generation compounds, or more targeted therapies that specifically address GM1-mediated mechanisms.

Development of safe gangliomimetic compounds will require considerable efforts for thorough preclinical testing (as done for GM1 in this and previous studies) and toxicological profiling.

5.4 Concluding statements

HD is a devastating neurodegenerative disorder, for which there is currently no disease-modifying therapy. Ganglioside GM1 is a neuroprotective compound with critically important roles in the physiology of the healthy nervous system. GM1 has previously been demonstrated to confer significant benefit in multiple disorders of the nervous system, including PD. Previously, our lab has demonstrated that ganglioside metabolism is affected in HD and that exogenous GM1 administration is beneficial *in vitro* and *in vivo.* In my thesis I show that GM1 improves all aspects of disease pathology in multiple HD mouse models, including beneficial effects on motor symptoms, non-motor symptoms and brain atrophy. Furthermore, I demonstrate that GM1 is capable of directly reducing the toxicity of mHTT, both through alterations in post-translational modifications and reduction of soluble and insoluble protein levels. These HD-specific effects, in combination with other potential pleiotropic neuroprotective actions of the ganglioside, suggest that GM1 represents an exciting and novel disease-modifying therapy for HD. Additionally, I provide evidence that membrane-permeant analogues of GM1, particularly SNB-6100, conserve many of the therapeutic actions of GM1, demonstrating the potential of developing second generation compounds with improved bioavailability.

5.5 References

28.   Cubo E, Shannon KM, Tracy D, Jaglin JA, Bernard BA, Wuu J, et al. Effect of donepezil on motor and cognitive function in Huntington disease. Neurology. 2006;67(7):1268-71.

29.   Ondo WG, Mejia NI, Hunter CB. A pilot study of the clinical efficacy and safety of memantine for Huntington's disease. Parkinsonism & related disorders. 2007;13(7):453-4.

30.   Vattakatuchery JJ, Kurien R. Acetylcholinesterase inhibitors in cognitive impairment in Huntington's disease: A brief review. World journal of psychiatry. 2013;3(3):62-4.

34.   Tabrizi SJ, Scahill RI, Durr A, Roos RA, Leavitt BR, Jones R, et al. Biological and clinical changes in premanifest and early stage Huntington's disease in the TRACK-HD study: the 12-month longitudinal analysis. The Lancet Neurology. 2011;10(1):31-42.

35.   Moulton CD, Hopkins CW, Bevan-Jones WR. Systematic review of pharmacological treatments for depressive symptoms in Huntington's disease. Movement disorders : official journal of the Movement Disorder Society. 2014;29(12):1556-61.

40.   Burgunder JM, Guttman M, Perlman S, Goodman N, van Kammen DP, Goodman L. An International Survey-based Algorithm for the Pharmacologic Treatment of Chorea in Huntington's Disease. PLoS currents. 2011;3:RRN1260.

42.   Jech R, Klempir J, Vymazal J, Zidovska J, Klempirova O, Ruzicka E, et al. Variation of selective gray and white matter atrophy in Huntington's disease. Movement disorders : official journal of the Movement Disorder Society. 2007;22(12):1783-9.

45.   Delmaire C, Dumas EM, Sharman MA, van den Bogaard SJ, Valabregue R, Jauffret C, et al. The structural correlates of functional deficits in early huntington's disease. Human brain mapping. 2013;34(9):2141-53.

75.   Bates GP, Hockly E. Experimental therapeutics in Huntington's disease: are models useful for therapeutic trials? Current opinion in neurology. 2003;16(4):465-70.

76.   Menalled L, Brunner D. Animal models of Huntington's disease for translation to the clinic: best practices. Movement disorders : official journal of the Movement Disorder Society. 2014;29(11):1375-90.

85.   Zuccato C, Valenza M, Cattaneo E. Molecular mechanisms and potential therapeutical targets in Huntington's disease. Physiological reviews. 2010;90(3):905-81.

87.   Dragatsis I, Levine MS, Zeitlin S. Inactivation of Hdh in the brain and testis results in progressive neurodegeneration and sterility in mice. Nature genetics. 2000;26(3):300-6.

149.   Mocchetti I. Exogenous gangliosides, neuronal plasticity and repair, and the neurotrophins. Cell Mol Life Sci. 2005;62(19-20):2283-94.

160.   Ledeen RW, Wu G. The multi-tasked life of GM1 ganglioside, a true factotum of nature. Trends in biochemical sciences. 2015;40(7):407-18.

192.   Saito M, Mao RF, Wang R, Vadasz C, Saito M. Effects of gangliosides on ethanol-induced neurodegeneration in the developing mouse brain. Alcoholism, clinical and experimental research. 2007;31(4):665-74.

196.   Schneider JS, Cambi F, Gollomp SM, Kuwabara H, Brasic JR, Leiby B, et al. GM1 ganglioside in Parkinson's disease: Pilot study of effects on dopamine transporter binding. Journal of the neurological sciences. 2015;356(1-2):118-23.

212.   Di Pardo A, Maglione V, Alpaugh M, Horkey M, Atwal RS, Sassone J, et al. Ganglioside GM1 induces phosphorylation of mutant huntingtin and restores normal motor behavior in Huntington disease mice. Proceedings of the National Academy of Sciences of the United States of America. 2012;109(9):3528-33.

213.   Gu X, Greiner ER, Mishra R, Kodali R, Osmand A, Finkbeiner S, et al. Serines 13 and 16 are critical determinants of full-length human mutant huntingtin induced disease pathogenesis in HD mice. Neuron. 2009;64(6):828-40.

224.   Thompson LM, Aiken CT, Kaltenbach LS, Agrawal N, Illes K, Khoshnan A, et al. IKK phosphorylates Huntingtin and targets it for degradation by the proteasome and lysosome. The Journal of cell biology. 2009;187(7):1083-99.

233.   Li JY, Popovic N, Brundin P. The use of the R6 transgenic mouse models of Huntington's disease in attempts to develop novel therapeutic strategies. NeuroRx : the journal of the American Society for Experimental NeuroTherapeutics. 2005;2(3):447-64.

265.   Svenningsson P, Nishi A, Fisone G, Girault JA, Nairn AC, Greengard P. DARPP-32: an integrator of neurotransmission. Annual review of pharmacology and toxicology. 2004;44:269-96.

287.   Schneider JS, Sendek S, Daskalakis C, Cambi F. GM1 ganglioside in Parkinson's disease: Results of a five year open study. Journal of the neurological sciences. 2010;292(1-2):45-51.

288.   Schneider JS, Gollomp SM, Sendek S, Colcher A, Cambi F, Du W. A randomized, controlled, delayed start trial of GM1 ganglioside in treated Parkinson's disease patients. Journal of the neurological sciences. 2013;324(1-2):140-8.

289.   Schneider JS, Seyfried TN, Choi HS, Kidd SK. Intraventricular Sialidase Administration Enhances GM1 Ganglioside Expression and Is Partially Neuroprotective in a Mouse Model of Parkinson's Disease. PloS one. 2015;10(12):e0143351.

311.   Ross CA, Tabrizi SJ. Huntington's disease: from molecular pathogenesis to clinical treatment. The Lancet Neurology. 2011;10(1):83-98.

431.   Kordasiewicz HB, Stanek LM, Wancewicz EV, Mazur C, McAlonis MM, Pytel KA, et al. Sustained therapeutic reversal of Huntington's disease by transient repression of huntingtin synthesis. Neuron. 2012;74(6):1031-44.

449.   Wu G, Lu ZH, Wang J, Wang Y, Xie X, Meyenhofer MF, et al. Enhanced susceptibility to kainate-induced seizures, neuronal apoptosis, and death in mice lacking gangliotetraose gangliosides: protection with LIGA 20, a membrane-permeant analog of GM1. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2005;25(47):11014-22.

454.   Polo A, Kirschner G, Guidotti A, Costa E. Brain content of glycosphingolipids after oral administration of monosialogangliosides GM1 and LIGA20 to rats. Molecular and chemical neuropathology / sponsored by the International Society for Neurochemistry and the World Federation of Neurology and research groups on neurochemistry and cerebrospinal fluid. 1994;21(1):41-53.

455.   Manev H, Favaron M, Vicini S, Guidotti A, Costa E. Glutamate-induced neuronal death in primary cultures of cerebellar granule cells: protection by synthetic derivatives of endogenous sphingolipids. The Journal of pharmacology and experimental therapeutics. 1990;252(1):419-27.

463.   Atwal RS, Desmond CR, Caron N, Maiuri T, Xia J, Sipione S, et al. Kinase inhibitors modulate huntingtin cell localization and toxicity. Nature chemical biology. 2011;7(7):453-60.

477.   Clayton JA, Collins FS. Policy: NIH to balance sex in cell and animal studies. Nature. 2014;509(7500):282-3.

478.   Rodriguez-Lebron E, Denovan-Wright EM, Nash K, Lewin AS, Mandel RJ. Intrastriatal rAAV-mediated delivery of anti-huntingtin shRNAs induces partial reversal of disease progression in R6/1 Huntington's disease transgenic mice. Molecular therapy : the journal of the American Society of Gene Therapy. 2005;12(4):618-33.

479.   Frank S. Treatment of Huntington's disease. Neurotherapeutics : the journal of the American Society for Experimental NeuroTherapeutics. 2014;11(1):153-60.

480.   Godinho BM, Malhotra M, O'Driscoll CM, Cryan JF. Delivering a disease-modifying treatment for Huntington's disease. Drug discovery today. 2015;20(1):50-64.

481.   Aronin N, DiFiglia M. Huntingtin-lowering strategies in Huntington's disease: antisense oligonucleotides, small RNAs, and gene editing. Movement disorders : official journal of the Movement Disorder Society. 2014;29(11):1455-61.

482.   Wang YL, Liu W, Wada E, Murata M, Wada K, Kanazawa I. Clinico-pathological rescue of a model mouse of Huntington's disease by siRNA. Neuroscience research. 2005;53(3):241-9.

483.   Skotte NH, Southwell AL, Ostergaard ME, Carroll JB, Warby SC, Doty CN, et al. Allele-specific suppression of mutant huntingtin using antisense oligonucleotides: providing a therapeutic option for all Huntington disease patients. PloS one. 2014;9(9):e107434.

484.   Maiuri T, Woloshansky T, Xia J, Truant R. The huntingtin N17 domain is a multifunctional CRM1 and Ran-dependent nuclear and cilial export signal. Human molecular genetics. 2013;22(7):1383-94.

BIBLIOGRAPHY

1.   A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. The Huntington's Disease Collaborative Research Group. Cell. 1993;72(6):971-83.

2.   Pringsheim T, Wiltshire K, Day L, Dykeman J, Steeves T, Jette N. The incidence and prevalence of Huntington's disease: a systematic review and meta-analysis. Movement disorders : official journal of the Movement Disorder Society. 2012;27(9):1083-91.

3.   Langbehn DR, Brinkman RR, Falush D, Paulsen JS, Hayden MR, International Huntington's Disease Collaborative G. A new model for prediction of the age of onset and penetrance for Huntington's disease based on CAG length. Clinical genetics. 2004;65(4):267-77.

4.   Brinkman RR, Mezei MM, Theilmann J, Almqvist E, Hayden MR. The likelihood of being affected with Huntington disease by a particular age, for a specific CAG size. American journal of human genetics. 1997;60(5):1202-10.

5.   Garcia-Ruiz PJ, Garcia-Caldentey J, Feliz C, Del Val J, Herranz A, Martinez-Castrillo JC. Late onset Huntington's disease with 29 CAG repeat expansion. Journal of the neurological sciences. 2016;363:114-5.

6.   Groen JL, de Bie RM, Foncke EM, Roos RA, Leenders KL, Tijssen MA. Late-onset Huntington disease with intermediate CAG repeats: true or false? Journal of neurology, neurosurgery, and psychiatry. 2010;81(2):228-30.

7.   Falush D, Almqvist EW, Brinkmann RR, Iwasa Y, Hayden MR. Measurement of mutational flow implies both a high new-mutation rate for Huntington disease and substantial underascertainment of late-onset cases. American journal of human genetics. 2001;68(2):373-85.

8.   Castilhos RM, Augustin MC, Santos JA, Perandones C, Saraiva-Pereira ML, Jardim LB, et al. Genetic aspects of Huntington's disease in Latin America. A systematic review. Clinical genetics. 2016;89(3):295-303.

9.   Squitieri F, Andrew SE, Goldberg YP, Kremer B, Spence N, Zeisler J, et al. DNA haplotype analysis of Huntington disease reveals clues to the origins and mechanisms of CAG expansion and reasons for geographic variations of prevalence. Human molecular genetics. 1994;3(12):2103-14.

10.   Warby SC, Visscher H, Collins JA, Doty CN, Carter C, Butland SL, et al. HTT haplotypes contribute to differences in Huntington disease prevalence between Europe and East Asia. European journal of human genetics : EJHG. 2011;19(5):561-6.

11.   Sipila JO, Hietala M, Siitonen A, Paivarinta M, Majamaa K. Epidemiology of Huntington's disease in Finland. Parkinsonism & related disorders. 2015;21(1):46-9.

12.   Baine FK, Krause A, Greenberg LJ. The Frequency of Huntington Disease and Huntington Disease-Like 2 in the South African Population. Neuroepidemiology. 2016;46(3):198-202.

13.   Weydt P, Soyal SM, Gellera C, Didonato S, Weidinger C, Oberkofler H, et al. The gene coding for PGC-1alpha modifies age at onset in Huntington's Disease. Molecular neurodegeneration. 2009;4:3.

14.   Alberch J, Lopez M, Badenas C, Carrasco JL, Mila M, Munoz E, et al. Association between BDNF Val66Met polymorphism and age at onset in Huntington disease. Neurology. 2005;65(6):964-5.

15.   Arning L, Kraus PH, Valentin S, Saft C, Andrich J, Epplen JT. NR2A and NR2B receptor gene variations modify age at onset in Huntington disease. Neurogenetics. 2005;6(1):25-8.

16.   Arning L, Epplen JT. Genetic modifiers in Huntington's disease: fiction or fact? Neurogenetics. 2013;14(3-4):171-2.

17.   Genetic Modifiers of Huntington's Disease C. Identification of Genetic Factors that Modify Clinical Onset of Huntington's Disease. Cell. 2015;162(3):516-26.

18.   Unified Huntington's Disease Rating Scale: reliability and consistency. Huntington Study Group. Movement disorders : official journal of the Movement Disorder Society. 1996;11(2):136-42.

19.   Reilmann R, Leavitt BR, Ross CA. Diagnostic criteria for Huntington's disease based on natural history. Movement disorders : official journal of the Movement Disorder Society. 2014;29(11):1335-41.

20.   Novak MJ, Tabrizi SJ. Huntington's disease: clinical presentation and treatment. International review of neurobiology. 2011;98:297-323.

21.   Ho AK, Gilbert AS, Mason SL, Goodman AO, Barker RA. Health-related quality of life in Huntington's disease: Which factors matter most? Movement disorders : official journal of the Movement Disorder Society. 2009;24(4):574-8.

22.   Sitek EJ, Thompson JC, Craufurd D, Snowden JS. Unawareness of deficits in Huntington's disease. Journal of Huntington's disease. 2014;3(2):125-35.

23.   Guo Z, Rudow G, Pletnikova O, Codispoti KE, Orr BA, Crain BJ, et al. Striatal neuronal loss correlates with clinical motor impairment in Huntington's disease. Movement disorders : official journal of the Movement Disorder Society. 2012;27(11):1379-86.

24.   Rothlind JC, Bylsma FW, Peyser C, Folstein SE, Brandt J. Cognitive and motor correlates of everyday functioning in early Huntington's disease. The Journal of nervous and mental disease. 1993;181(3):194-9.

25.   Williams JK, Kim JI, Downing N, Farias S, Harrington DL, Long JD, et al. Everyday cognition in prodromal Huntington disease. Neuropsychology. 2015;29(2):255-67.

26.   Dumas EM, van den Bogaard SJ, Middelkoop HA, Roos RA. A review of cognition in Huntington's disease. Frontiers in bioscience. 2013;5:1-18.

27.   Duff K, Paulsen JS, Beglinger LJ, Langbehn DR, Wang C, Stout JC, et al. "Frontal" behaviors before the diagnosis of Huntington's disease and their relationship to markers of disease progression: evidence of early lack of awareness. The Journal of neuropsychiatry and clinical neurosciences. 2010;22(2):196-207.

28.   Cubo E, Shannon KM, Tracy D, Jaglin JA, Bernard BA, Wuu J, et al. Effect of donepezil on motor and cognitive function in Huntington disease. Neurology. 2006;67(7):1268-71.

29.   Ondo WG, Mejia NI, Hunter CB. A pilot study of the clinical efficacy and safety of memantine for Huntington's disease. Parkinsonism & related disorders. 2007;13(7):453-4.

30.   Vattakatuchery JJ, Kurien R. Acetylcholinesterase inhibitors in cognitive impairment in Huntington's disease: A brief review. World journal of psychiatry. 2013;3(3):62-4.

31.   Cankurtaran ES, Ozalp E, Soygur H, Cakir A. Clinical experience with risperidone and memantine in the treatment of Huntington's disease. Journal of the National Medical Association. 2006;98(8):1353-5.

32.   Beister A, Kraus P, Kuhn W, Dose M, Weindl A, Gerlach M. The N-methyl-D-aspartate antagonist memantine retards progression of Huntington's disease. Journal of neural transmission Supplementum. 2004(68):117-22.

33.   Stout JC, Queller S, Baker KN, Cowlishaw S, Sampaio C, Fitzer-Attas C, et al. HD-CAB: a cognitive assessment battery for clinical trials in Huntington's disease 1,2,3. Movement disorders : official journal of the Movement Disorder Society. 2014;29(10):1281-8.

34.   Tabrizi SJ, Scahill RI, Durr A, Roos RA, Leavitt BR, Jones R, et al. Biological and clinical changes in premanifest and early stage Huntington's disease in the TRACK-HD study: the 12-month longitudinal analysis. The Lancet Neurology. 2011;10(1):31-42.

35.   Moulton CD, Hopkins CW, Bevan-Jones WR. Systematic review of pharmacological treatments for depressive symptoms in Huntington's disease. Movement disorders : official journal of the Movement Disorder Society. 2014;29(12):1556-61.

36.   Pettibone DJ, Totaro JA, Pflueger AB. Tetrabenazine-induced depletion of brain monoamines: characterization and interaction with selected antidepressants. European journal of pharmacology. 1984;102(3-4):425-30.

37.   Podurgiel SJ, Yohn SE, Dortche K, Correa M, Salamone JD. The MAO-B inhibitor deprenyl reduces the oral tremor and the dopamine depletion induced by the VMAT-2 inhibitor tetrabenazine. Behavioural brain research. 2016;298(Pt B):188-91.

38.   Kegelmeyer DA, Kloos AD, Fritz NE, Fiumedora MM, White SE, Kostyk SK. Impact of tetrabenazine on gait and functional mobility in individuals with Huntington's disease. Journal of the neurological sciences. 2014;347(1-2):219-23.

39.   Fekete R, Davidson A, Jankovic J. Clinical assessment of the effect of tetrabenazine on functional scales in huntington disease: a pilot open label study. Tremor and other hyperkinetic movements. 2012;2.

40.   Burgunder JM, Guttman M, Perlman S, Goodman N, van Kammen DP, Goodman L. An International Survey-based Algorithm for the Pharmacologic Treatment of Chorea in Huntington's Disease. PLoS currents. 2011;3:RRN1260.

41.   Vonsattel JP, Myers RH, Stevens TJ, Ferrante RJ, Bird ED, Richardson EP, Jr. Neuropathological classification of Huntington's disease. Journal of neuropathology and experimental neurology. 1985;44(6):559-77.

42.   Jech R, Klempir J, Vymazal J, Zidovska J, Klempirova O, Ruzicka E, et al. Variation of selective gray and white matter atrophy in Huntington's disease. Movement disorders : official journal of the Movement Disorder Society. 2007;22(12):1783-9.

43.   Aylward EH, Liu D, Nopoulos PC, Ross CA, Pierson RK, Mills JA, et al. Striatal volume contributes to the prediction of onset of Huntington disease in incident cases. Biological psychiatry. 2012;71(9):822-8.

44.   Rosas HD, Salat DH, Lee SY, Zaleta AK, Pappu V, Fischl B, et al. Cerebral cortex and the clinical expression of Huntington's disease: complexity and heterogeneity. Brain : a journal of neurology. 2008;131(Pt 4):1057-68.

45.   Delmaire C, Dumas EM, Sharman MA, van den Bogaard SJ, Valabregue R, Jauffret C, et al. The structural correlates of functional deficits in early huntington's disease. Human brain mapping. 2013;34(9):2141-53.

46.   Rosas HD, Hevelone ND, Zaleta AK, Greve DN, Salat DH, Fischl B. Regional cortical thinning in preclinical Huntington disease and its relationship to cognition. Neurology. 2005;65(5):745-7.

47.   van den Bogaard SJ, Dumas EM, Ferrarini L, Milles J, van Buchem MA, van der Grond J, et al. Shape analysis of subcortical nuclei in Huntington's disease, global versus local atrophy--results from the TRACK-HD study. Journal of the neurological sciences. 2011;307(1-2):60-8.

48.   van den Bogaard SJ, Dumas EM, Acharya TP, Johnson H, Langbehn DR, Scahill RI, et al. Early atrophy of pallidum and accumbens nucleus in Huntington's disease. Journal of neurology. 2011;258(3):412-20.

49.   Thieben MJ, Duggins AJ, Good CD, Gomes L, Mahant N, Richards F, et al. The distribution of structural neuropathology in pre-clinical Huntington's disease. Brain : a journal of neurology. 2002;125(Pt 8):1815-28.

50.   Drouin-Ouellet J, Sawiak SJ, Cisbani G, Lagace M, Kuan WL, Saint-Pierre M, et al. Cerebrovascular and blood-brain barrier impairments in Huntington's disease: Potential implications for its pathophysiology. Annals of neurology. 2015;78(2):160-77.

51.   Guncova I, Latr I, Mazurova Y. The neurodegenerative process in a neurotoxic rat model and in patients with Huntington's disease: histopathological parallels and differences. Acta histochemica. 2011;113(8):783-92.

52.   Singhrao SK, Neal JW, Morgan BP, Gasque P. Increased complement biosynthesis by microglia and complement activation on neurons in Huntington's disease. Experimental neurology. 1999;159(2):362-76.

53.   Tai YF, Pavese N, Gerhard A, Tabrizi SJ, Barker RA, Brooks DJ, et al. Microglial activation in presymptomatic Huntington's disease gene carriers. Brain : a journal of neurology. 2007;130(Pt 7):1759-66.

54.   Sapp E, Kegel KB, Aronin N, Hashikawa T, Uchiyama Y, Tohyama K, et al. Early and progressive accumulation of reactive microglia in the Huntington disease brain. Journal of neuropathology and experimental neurology. 2001;60(2):161-72.

55.   Dalrymple A, Wild EJ, Joubert R, Sathasivam K, Bjorkqvist M, Petersen A, et al. Proteomic profiling of plasma in Huntington's disease reveals neuroinflammatory activation and biomarker candidates. Journal of proteome research. 2007;6(7):2833-40.

56.   Bjorkqvist M, Wild EJ, Thiele J, Silvestroni A, Andre R, Lahiri N, et al. A novel pathogenic pathway of immune activation detectable before clinical onset in Huntington's disease. The Journal of experimental medicine. 2008;205(8):1869-77.

57.   Beal MF, Kowall NW, Ellison DW, Mazurek MF, Swartz KJ, Martin JB. Replication of the neurochemical characteristics of Huntington's disease by quinolinic acid. Nature. 1986;321(6066):168-71.

58.   Borlongan CV, Koutouzis TK, Randall TS, Freeman TB, Cahill DW, Sanberg PR. Systemic 3-nitropropionic acid: behavioral deficits and striatal damage in adult rats. Brain research bulletin. 1995;36(6):549-56.

59.   Wang LH, Qin ZH. Animal models of Huntington's disease: implications in uncovering pathogenic mechanisms and developing therapies. Acta pharmacologica Sinica. 2006;27(10):1287-302.

60.   Morton AJ, Howland DS. Large genetic animal models of Huntington's Disease. Journal of Huntington's disease. 2013;2(1):3-19.

61.   Mangiarini L, Sathasivam K, Seller M, Cozens B, Harper A, Hetherington C, et al. Exon 1 of the HD gene with an expanded CAG repeat is sufficient to cause a progressive neurological phenotype in transgenic mice. Cell. 1996;87(3):493-506.

62.   Davies SW, Turmaine M, Cozens BA, DiFiglia M, Sharp AH, Ross CA, et al. Formation of neuronal intranuclear inclusions underlies the neurological dysfunction in mice transgenic for the HD mutation. Cell. 1997;90(3):537-48.

63.   Schilling G, Becher MW, Sharp AH, Jinnah HA, Duan K, Kotzuk JA, et al. Intranuclear inclusions and neuritic aggregates in transgenic mice expressing a mutant N-terminal fragment of huntingtin. Human molecular genetics. 1999;8(3):397-407.

64.   Slow EJ, van Raamsdonk J, Rogers D, Coleman SH, Graham RK, Deng Y, et al. Selective striatal neuronal loss in a YAC128 mouse model of Huntington disease. Human molecular genetics. 2003;12(13):1555-67.

65.   Gray M, Shirasaki DI, Cepeda C, Andre VM, Wilburn B, Lu XH, et al. Full-length human mutant huntingtin with a stable polyglutamine repeat can elicit progressive and selective neuropathogenesis in BACHD mice. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2008;28(24):6182-95.

66.   Pouladi MA, Stanek LM, Xie Y, Franciosi S, Southwell AL, Deng Y, et al. Marked differences in neurochemistry and aggregates despite similar behavioural and neuropathological features of Huntington disease in the full-length BACHD and YAC128 mice. Human molecular genetics. 2012;21(10):2219-32.

67.   Pouladi MA, Xie Y, Skotte NH, Ehrnhoefer DE, Graham RK, Kim JE, et al. Full-length huntingtin levels modulate body weight by influencing insulin-like growth factor 1 expression. Human molecular genetics. 2010;19(8):1528-38.

68.   Sanberg PR, Fibiger HC, Mark RF. Body weight and dietary factors in Huntington's disease patients compared with matched controls. The Medical journal of Australia. 1981;1(8):407-9.

69.   Menalled LB, Sison JD, Dragatsis I, Zeitlin S, Chesselet MF. Time course of early motor and neuropathological anomalies in a knock-in mouse model of Huntington's disease with 140 CAG repeats. The Journal of comparative neurology. 2003;465(1):11-26.

70.   Menalled LB, Kudwa AE, Miller S, Fitzpatrick J, Watson-Johnson J, Keating N, et al. Comprehensive behavioral and molecular characterization of a new knock-in mouse model of Huntington's disease: zQ175. PloS one. 2012;7(12):e49838.

71.   Levine MS, Klapstein GJ, Koppel A, Gruen E, Cepeda C, Vargas ME, et al. Enhanced sensitivity to N-methyl-D-aspartate receptor activation in transgenic and knockin mouse models of Huntington's disease. Journal of neuroscience research. 1999;58(4):515-32.

72.   Woodman B, Butler R, Landles C, Lupton MK, Tse J, Hockly E, et al. The Hdh(Q150/Q150) knock-in mouse model of HD and the R6/2 exon 1 model develop comparable and widespread molecular phenotypes. Brain research bulletin. 2007;72(2-3):83-97.

73.   Wheeler VC, Auerbach W, White JK, Srinidhi J, Auerbach A, Ryan A, et al. Length-dependent gametic CAG repeat instability in the Huntington's disease knock-in mouse. Human molecular genetics. 1999;8(1):115-22.

74.   Wheeler VC, Gutekunst CA, Vrbanac V, Lebel LA, Schilling G, Hersch S, et al. Early phenotypes that presage late-onset neurodegenerative disease allow testing of modifiers in Hdh CAG knock-in mice. Human molecular genetics. 2002;11(6):633-40.

75.   Bates GP, Hockly E. Experimental therapeutics in Huntington's disease: are models useful for therapeutic trials? Current opinion in neurology. 2003;16(4):465-70.

76.   Menalled L, Brunner D. Animal models of Huntington's disease for translation to the clinic: best practices. Movement disorders : official journal of the Movement Disorder Society. 2014;29(11):1375-90.

77.   Zeef DH, Jahanshahi A, Vlamings R, Casaca-Carreira J, Santegoeds RG, Janssen ML, et al. An experimental model for Huntington's chorea? Behavioural brain research. 2014;262:31-4.

78.   von Horsten S, Schmitt I, Nguyen HP, Holzmann C, Schmidt T, Walther T, et al. Transgenic rat model of Huntington's disease. Human molecular genetics. 2003;12(6):617-24.

79.   Jacobsen JC, Bawden CS, Rudiger SR, McLaughlan CJ, Reid SJ, Waldvogel HJ, et al. An ovine transgenic Huntington's disease model. Human molecular genetics. 2010;19(10):1873-82.

80.   Baxa M, Hruska-Plochan M, Juhas S, Vodicka P, Pavlok A, Juhasova J, et al. A transgenic minipig model of Huntington's Disease. Journal of Huntington's disease. 2013;2(1):47-68.

81.   Yang D, Wang CE, Zhao B, Li W, Ouyang Z, Liu Z, et al. Expression of Huntington's disease protein results in apoptotic neurons in the brains of cloned transgenic pigs. Human molecular genetics. 2010;19(20):3983-94.

82.   Yang SH, Cheng PH, Banta H, Piotrowska-Nitsche K, Yang JJ, Cheng EC, et al. Towards a transgenic model of Huntington's disease in a non-human primate. Nature. 2008;453(7197):921-4.

83.   Chan AW, Jiang J, Chen Y, Li C, Prucha MS, Hu Y, et al. Progressive cognitive deficit, motor impairment and striatal pathology in a transgenic Huntington disease monkey model from infancy to adulthood. PloS one. 2015;10(5):e0122335.

84.   Li XJ, Li S. Large Animal Models of Huntington's Disease. Current topics in behavioral neurosciences. 2015;22:149-60.

85.   Zuccato C, Valenza M, Cattaneo E. Molecular mechanisms and potential therapeutical targets in Huntington's disease. Physiological reviews. 2010;90(3):905-81.

86.   Saudou F, Humbert S. The Biology of Huntingtin. Neuron. 2016;89(5):910-26.

87.   Dragatsis I, Levine MS, Zeitlin S. Inactivation of Hdh in the brain and testis results in progressive neurodegeneration and sterility in mice. Nature genetics. 2000;26(3):300-6.

88.   Gauthier LR, Charrin BC, Borrell-Pages M, Dompierre JP, Rangone H, Cordelieres FP, et al. Huntingtin controls neurotrophic support and survival of neurons by enhancing BDNF vesicular transport along microtubules. Cell. 2004;118(1):127-38.

89.   Zala D, Colin E, Rangone H, Liot G, Humbert S, Saudou F. Phosphorylation of mutant huntingtin at S421 restores anterograde and retrograde transport in neurons. Human molecular genetics. 2008;17(24):3837-46.

90.   Zuccato C, Tartari M, Crotti A, Goffredo D, Valenza M, Conti L, et al. Huntingtin interacts with REST/NRSF to modulate the transcription of NRSE-controlled neuronal genes. Nature genetics. 2003;35(1):76-83.

91.   Marcora E, Kennedy MB. The Huntington's disease mutation impairs Huntingtin's role in the transport of NF-kappaB from the synapse to the nucleus. Human molecular genetics. 2010;19(22):4373-84.

92.   Hoffner G, Djian P. Monomeric, oligomeric and polymeric proteins in huntington disease and other diseases of polyglutamine expansion. Brain sciences. 2014;4(1):91-122.

93.   Seidel K, Siswanto S, Fredrich M, Bouzrou M, Brunt ER, van Leeuwen FW, et al. Polyglutamine aggregation in Huntington's disease and spinocerebellar ataxia type 3: similar mechanisms in aggregate formation. Neuropathology and applied neurobiology. 2016;42(2):153-66.

94.   Arrasate M, Mitra S, Schweitzer ES, Segal MR, Finkbeiner S. Inclusion body formation reduces levels of mutant huntingtin and the risk of neuronal death. Nature. 2004;431(7010):805-10.

95.   Slow EJ, Graham RK, Osmand AP, Devon RS, Lu G, Deng Y, et al. Absence of behavioral abnormalities and neurodegeneration in vivo despite widespread neuronal huntingtin

inclusions. Proceedings of the National Academy of Sciences of the United States of America. 2005;102(32):11402-7.

96.   Hackam AS, Singaraja R, Wellington CL, Metzler M, McCutcheon K, Zhang T, et al. The influence of huntingtin protein size on nuclear localization and cellular toxicity. The Journal of cell biology. 1998;141(5):1097-105.

97.   Sun CS, Lee CC, Li YN, Yao-Chen Yang S, Lin CH, Chang YC, et al. Conformational switch of polyglutamine-expanded huntingtin into benign aggregates leads to neuroprotective effect. Scientific reports. 2015;5:14992.

98.   Lee WC, Yoshihara M, Littleton JT. Cytoplasmic aggregates trap polyglutamine-containing proteins and block axonal transport in a Drosophila model of Huntington's disease. Proceedings of the National Academy of Sciences of the United States of America. 2004;101(9):3224-9.

99.   Duim WC, Jiang Y, Shen K, Frydman J, Moerner WE. Super-resolution fluorescence of huntingtin reveals growth of globular species into short fibers and coexistence of distinct aggregates. ACS chemical biology. 2014;9(12):2767-78.

100.   Chen S, Berthelier V, Yang W, Wetzel R. Polyglutamine aggregation behavior in vitro supports a recruitment mechanism of cytotoxicity. Journal of molecular biology. 2001;311(1):173-82.

101.   Altar CA, Cai N, Bliven T, Juhasz M, Conner JM, Acheson AL, et al. Anterograde transport of brain-derived neurotrophic factor and its role in the brain. Nature. 1997;389(6653):856-60.

102.   Kells AP, Fong DM, Dragunow M, During MJ, Young D, Connor B. AAV-mediated gene delivery of BDNF or GDNF is neuroprotective in a model of Huntington disease. Molecular therapy : the journal of the American Society of Gene Therapy. 2004;9(5):682-8.

103.   Baquet ZC, Gorski JA, Jones KR. Early striatal dendrite deficits followed by neuron loss with advanced age in the absence of anterograde cortical brain-derived neurotrophic factor. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2004;24(17):4250-8.

104.   Martin EJ, Kim M, Velier J, Sapp E, Lee HS, Laforet G, et al. Analysis of Huntingtin-associated protein 1 in mouse brain and immortalized striatal neurons. The Journal of comparative neurology. 1999;403(4):421-30.

105.   Trushina E, Dyer RB, Badger JD, 2nd, Ure D, Eide L, Tran DD, et al. Mutant huntingtin impairs axonal trafficking in mammalian neurons in vivo and in vitro. Molecular and cellular biology. 2004;24(18):8195-209.

106.   Barnhart EL. Mechanics of mitochondrial motility in neurons. Current opinion in cell biology. 2016;38:90-9.

107.   Cui L, Jeong H, Borovecki F, Parkhurst CN, Tanese N, Krainc D. Transcriptional repression of PGC-1alpha by mutant huntingtin leads to mitochondrial dysfunction and neurodegeneration. Cell. 2006;127(1):59-69.

108.   Shirendeb U, Reddy AP, Manczak M, Calkins MJ, Mao P, Tagle DA, et al. Abnormal mitochondrial dynamics, mitochondrial loss and mutant huntingtin oligomers in Huntington's disease: implications for selective neuronal damage. Human molecular genetics. 2011;20(7):1438-55.

109.   Wang H, Lim PJ, Karbowski M, Monteiro MJ. Effects of overexpression of huntingtin proteins on mitochondrial integrity. Human molecular genetics. 2009;18(4):737-52.

110.	Guo X, Disatnik MH, Monbureau M, Shamloo M, Mochly-Rosen D, Qi X. Inhibition of mitochondrial fragmentation diminishes Huntington's disease-associated neurodegeneration. The Journal of clinical investigation. 2013;123(12):5371-88.

111.	Gu M, Gash MT, Mann VM, Javoy-Agid F, Cooper JM, Schapira AH. Mitochondrial defect in Huntington's disease caudate nucleus. Annals of neurology. 1996;39(3):385-9.

112.	El-Hattab AW, Scaglia F. Mitochondrial cytopathies. Cell calcium. 2016.

113.	Baumgartner HK, Gerasimenko JV, Thorne C, Ferdek P, Pozzan T, Tepikin AV, et al. Calcium elevation in mitochondria is the main Ca2+ requirement for mitochondrial permeability transition pore (mPTP) opening. The Journal of biological chemistry. 2009;284(31):20796-803.

114.	Graham RK, Pouladi MA, Joshi P, Lu G, Deng Y, Wu NP, et al. Differential susceptibility to excitotoxic stress in YAC128 mouse models of Huntington disease between initiation and progression of disease. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2009;29(7):2193-204.

115.	Sun Y, Savanenin A, Reddy PH, Liu YF. Polyglutamine-expanded huntingtin promotes sensitization of N-methyl-D-aspartate receptors via post-synaptic density 95. The Journal of biological chemistry. 2001;276(27):24713-8.

116.	Milnerwood AJ, Gladding CM, Pouladi MA, Kaufman AM, Hines RM, Boyd JD, et al. Early increase in extrasynaptic NMDA receptor signaling and expression contributes to phenotype onset in Huntington's disease mice. Neuron. 2010;65(2):178-90.

117.	Muller M, Leavitt BR. Iron dysregulation in Huntington's disease. Journal of neurochemistry. 2014;130(3):328-50.

118.	Jurgens CK, Jasinschi R, Ekin A, Witjes-Ane MN, Middelkoop H, van der Grond J, et al. MRI T2 Hypointensities in basal ganglia of premanifest Huntington's disease. PLoS currents. 2010;2.

119.	Bartzokis G, Tishler TA. MRI evaluation of basal ganglia ferritin iron and neurotoxicity in Alzheimer's and Huntingon's disease. Cellular and molecular biology. 2000;46(4):821-33.

120.	Shoham S, Youdim MB. Iron involvement in neural damage and microgliosis in models of neurodegenerative diseases. Cellular and molecular biology. 2000;46(4):743-60.

121.	Rathore KI, Redensek A, David S. Iron homeostasis in astrocytes and microglia is differentially regulated by TNF-alpha and TGF-beta1. Glia. 2012;60(5):738-50.

122.	Neher JJ, Neniskyte U, Brown GC. Primary phagocytosis of neurons by inflamed microglia: potential roles in neurodegeneration. Frontiers in pharmacology. 2012;3:27.

123.	Sipione S, Rigamonti D, Valenza M, Zuccato C, Conti L, Pritchard J, et al. Early transcriptional profiles in huntingtin-inducible striatal cells by microarray analyses. Human molecular genetics. 2002;11(17):1953-65.

124.	Allen JA, Halverson-Tamboli RA, Rasenick MM. Lipid raft microdomains and neurotransmitter signalling. Nature reviews Neuroscience. 2007;8(2):128-40.

125.	Valenza M, Cattaneo E. Emerging roles for cholesterol in Huntington's disease. Trends in neurosciences. 2011;34(9):474-86.

126.	Goedeke L, Fernandez-Hernando C. MicroRNAs: a connection between cholesterol metabolism and neurodegeneration. Neurobiology of disease. 2014;72 Pt A:48-53.

127.	Xue-Shan Z, Juan P, Qi W, Zhong R, Li-Hong P, Zhi-Han T, et al. Imbalanced cholesterol metabolism in Alzheimer's disease. Clinica chimica acta; international journal of clinical chemistry. 2016;456:107-14.

128.	Leoni V, Caccia C. The impairment of cholesterol metabolism in Huntington disease. Biochimica et biophysica acta. 2015;1851(8):1095-105.

129.   Leoni V, Mariotti C, Tabrizi SJ, Valenza M, Wild EJ, Henley SM, et al. Plasma 24S-hydroxycholesterol and caudate MRI in pre-manifest and early Huntington's disease. Brain : a journal of neurology. 2008;131(Pt 11):2851-9.

130.   Valenza M, Leoni V, Tarditi A, Mariotti C, Bjorkhem I, Di Donato S, et al. Progressive dysfunction of the cholesterol biosynthesis pathway in the R6/2 mouse model of Huntington's disease. Neurobiology of disease. 2007;28(1):133-42.

131.   Valenza M, Rigamonti D, Goffredo D, Zuccato C, Fenu S, Jamot L, et al. Dysfunction of the cholesterol biosynthetic pathway in Huntington's disease. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2005;25(43):9932-9.

132.   Kreilaus F, Spiro AS, McLean CA, Garner B, Jenner AM. Evidence for altered cholesterol metabolism in Huntington's disease post mortem brain tissue. Neuropathology and applied neurobiology. 2015.

133.   Leoni V, Long JD, Mills JA, Di Donato S, Paulsen JS, group P-Hs. Plasma 24S-hydroxycholesterol correlation with markers of Huntington disease progression. Neurobiology of disease. 2013;55:37-43.

134.   Yu RK, Tsai YT, Ariga T, Yanagisawa M. Structures, biosynthesis, and functions of gangliosides--an overview. Journal of oleo science. 2011;60(10):537-44.

135.   Higatsberger MR, Sperk G, Bernheimer H, Shannak KS, Hornykiewicz O. Striatal ganglioside levels in the rat following kainic acid lesions: comparison with Huntington's disease. Experimental brain research. 1981;44(1):93-6.

136.   Scorrano L, Petronilli V, Di Lisa F, Bernardi P. Commitment to apoptosis by GD3 ganglioside depends on opening of the mitochondrial permeability transition pore. The Journal of biological chemistry. 1999;274(32):22581-5.

137.   Maglione V, Marchi P, Di Pardo A, Lingrell S, Horkey M, Tidmarsh E, et al. Impaired ganglioside metabolism in Huntington's disease and neuroprotective role of GM1. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2010;30(11):4072-80.

138.   Denny CA, Desplats PA, Thomas EA, Seyfried TN. Cerebellar lipid differences between R6/1 transgenic mice and humans with Huntington's disease. Journal of neurochemistry. 2010;115(3):748-58.

139.   Desplats PA, Denny CA, Kass KE, Gilmartin T, Head SR, Sutcliffe JG, et al. Glycolipid and ganglioside metabolism imbalances in Huntington's disease. Neurobiology of disease. 2007;27(3):265-77.

140.   Svennerholm L. Designation and schematic structure of gangliosides and allied glycosphingolipids. Progress in brain research. 1994;101:XI-XIV.

141.   Kolter T. Ganglioside biochemistry. ISRN biochemistry. 2012;2012:506160.

142.   Wang H, Wang A, Wang D, Bright A, Sency V, Zhou A, et al. Early growth and development impairment in patients with ganglioside GM3 synthase deficiency. Clinical genetics. 2015.

143.   Sonnino S, Chigorno V. Ganglioside molecular species containing C18- and C20-sphingosine in mammalian nervous tissues and neuronal cell cultures. Biochimica et biophysica acta. 2000;1469(2):63-77.

144.   Kracun I, Rosner H, Drnovsek V, Vukelic Z, Cosovic C, Trbojevic-Cepe M, et al. Gangliosides in the human brain development and aging. Neurochemistry international. 1992;20(3):421-31.

145.   Rosner H. Developmental expression and possible roles of gangliosides in brain development. Progress in molecular and subcellular biology. 2003;32:49-73.

146.   Weishaupt N, Caughlin S, Yeung KK, Whitehead SN. Differential Anatomical Expression of Ganglioside GM1 Species Containing d18:1 or d20:1 Sphingosine Detected by MALDI Imaging Mass Spectrometry in Mature Rat Brain. Frontiers in neuroanatomy. 2015;9:155.
147.   Schnaar RL. Gangliosides of the Vertebrate Nervous System. Journal of molecular biology. 2016.
148.   Posse de Chaves E, Sipione S. Sphingolipids and gangliosides of the nervous system in membrane function and dysfunction. FEBS letters. 2010;584(9):1748-59.
149.   Mocchetti I. Exogenous gangliosides, neuronal plasticity and repair, and the neurotrophins. Cell Mol Life Sci. 2005;62(19-20):2283-94.
150.   Schengrund CL. Gangliosides: glycosphingolipids essential for normal neural development and function. Trends in biochemical sciences. 2015;40(7):397-406.
151.   Ferrari G, Anderson BL, Stephens RM, Kaplan DR, Greene LA. Prevention of apoptotic neuronal death by GM1 ganglioside. Involvement of Trk neurotrophin receptors. The Journal of biological chemistry. 1995;270(7):3074-80.
152.   Kabayama K, Sato T, Saito K, Loberto N, Prinetti A, Sonnino S, et al. Dissociation of the insulin receptor and caveolin-1 complex by ganglioside GM3 in the state of insulin resistance. Proceedings of the National Academy of Sciences of the United States of America. 2007;104(34):13678-83.
153.   Sekino-Suzuki N, Yuyama K, Miki T, Kaneda M, Suzuki H, Yamamoto N, et al. Involvement of gangliosides in the process of Cbp/PAG phosphorylation by Lyn in developing cerebellar growth cones. Journal of neurochemistry. 2013;124(4):514-22.
154.   Yang LJ, Zeller CB, Shaper NL, Kiso M, Hasegawa A, Shapiro RE, et al. Gangliosides are neuronal ligands for myelin-associated glycoprotein. Proceedings of the National Academy of Sciences of the United States of America. 1996;93(2):814-8.
155.   Sheikh KA, Sun J, Liu Y, Kawai H, Crawford TO, Proia RL, et al. Mice lacking complex gangliosides develop Wallerian degeneration and myelination defects. Proceedings of the National Academy of Sciences of the United States of America. 1999;96(13):7532-7.
156.   Fantini J, Barrantes FJ. Sphingolipid/cholesterol regulation of neurotransmitter receptor conformation and function. Biochimica et biophysica acta. 2009;1788(11):2345-61.
157.   Krishnan KS, Balaram P. A nuclear magnetic resonance study of the interaction of serotonin with gangliosides. FEBS letters. 1976;63(2):313-5.
158.   Wu G, Xie X, Lu ZH, Ledeen RW. Sodium-calcium exchanger complexed with GM1 ganglioside in nuclear membrane transfers calcium from nucleoplasm to endoplasmic reticulum. Proceedings of the National Academy of Sciences of the United States of America. 2009;106(26):10829-34.
159.   Ohmi Y, Tajima O, Ohkawa Y, Mori A, Sugiura Y, Furukawa K, et al. Gangliosides play pivotal roles in the regulation of complement systems and in the maintenance of integrity in nerve tissues. Proceedings of the National Academy of Sciences of the United States of America. 2009;106(52):22405-10.
160.   Ledeen RW, Wu G. The multi-tasked life of GM1 ganglioside, a true factotum of nature. Trends in biochemical sciences. 2015;40(7):407-18.
161.   Tajima O, Egashira N, Ohmi Y, Fukue Y, Mishima K, Iwasaki K, et al. Dysfunction of muscarinic acetylcholine receptors as a substantial basis for progressive neurological deterioration in GM3-only mice. Behavioural brain research. 2010;206(1):101-8.

162.	Takamiya K, Yamamoto A, Furukawa K, Yamashiro S, Shin M, Okada M, et al. Mice with disrupted GM2/GD2 synthase gene lack complex gangliosides but exhibit only subtle defects in their nervous system. Proceedings of the National Academy of Sciences of the United States of America. 1996;93(20):10662-7.

163.	Chiavegatto S, Sun J, Nelson RJ, Schnaar RL. A functional role for complex gangliosides: motor deficits in GM2/GD2 synthase knockout mice. Experimental neurology. 2000;166(2):227-34.

164.	Sha S, Zhou L, Yin J, Takamiya K, Furukawa K, Furukawa K, et al. Deficits in cognitive function and hippocampal plasticity in GM2/GD2 synthase knockout mice. Hippocampus. 2014;24(4):369-82.

165.	Wu G, Lu ZH, Kulkarni N, Amin R, Ledeen RW. Mice lacking major brain gangliosides develop parkinsonism. Neurochemical research. 2011;36(9):1706-14.

166.	Tajima O, Egashira N, Ohmi Y, Fukue Y, Mishima K, Iwasaki K, et al. Reduced motor and sensory functions and emotional response in GM3-only mice: emergence from early stage of life and exacerbation with aging. Behavioural brain research. 2009;198(1):74-82.

167.	Yamashita T, Wu YP, Sandhoff R, Werth N, Mizukami H, Ellis JM, et al. Interruption of ganglioside synthesis produces central nervous system degeneration and altered axon-glial interactions. Proceedings of the National Academy of Sciences of the United States of America. 2005;102(8):2725-30.

168.	Goldman JE, Katz D, Rapin I, Purpura DP, Suzuki K. Chronic GM1 gangliosidosis presenting as dystonia: I. Clinical and pathological features. Annals of neurology. 1981;9(5):465-75.

169.	Fernandes Filho JA, Shapiro BE. Tay-Sachs disease. Archives of neurology. 2004;61(9):1466-8.

170.	Bley AE, Giannikopoulos OA, Hayden D, Kubilus K, Tifft CJ, Eichler FS. Natural history of infantile G(M2) gangliosidosis. Pediatrics. 2011;128(5):e1233-41.

171.	Simpson MA, Cross H, Proukakis C, Priestman DA, Neville DC, Reinkensmeier G, et al. Infantile-onset symptomatic epilepsy syndrome caused by a homozygous loss-of-function mutation of GM3 synthase. Nature genetics. 2004;36(11):1225-9.

172.	Arber C, Li A, Houlden H, Wray S. Insights into molecular mechanisms of disease in Neurodegeneration with Brain Iron Accumulation; unifying theories. Neuropathology and applied neurobiology. 2015.

173.	Harlalka GV, Lehman A, Chioza B, Baple EL, Maroofian R, Cross H, et al. Mutations in B4GALNT1 (GM2 synthase) underlie a new disorder of ganglioside biosynthesis. Brain : a journal of neurology. 2013;136(Pt 12):3618-24.

174.	Boukhris A, Schule R, Loureiro JL, Lourenco CM, Mundwiller E, Gonzalez MA, et al. Alteration of ganglioside biosynthesis responsible for complex hereditary spastic paraplegia. American journal of human genetics. 2013;93(1):118-23.

175.	Taki T. An approach to glycobiology from glycolipidomics: ganglioside molecular scanning in the brains of patients with Alzheimer's disease by TLC-blot/matrix assisted laser desorption/ionization-time of flight MS. Biological & pharmaceutical bulletin. 2012;35(10):1642-7.

176.	Gizaw ST, Ohashi T, Tanaka M, Hinou H, Nishimura S. Glycoblotting method allows for rapid and efficient glycome profiling of human Alzheimer's disease brain, serum and cerebrospinal fluid towards potential biomarker discovery. Biochimica et biophysica acta. 2016;1860(8):1716-27.

177.   Wu G, Lu ZH, Kulkarni N, Ledeen RW. Deficiency of ganglioside GM1 correlates with Parkinson's disease in mice and humans. Journal of neuroscience research. 2012;90(10):1997-2008.

178.   Miyatani N, Saito M, Ariga T, Yoshino H, Yu RK. Glycosphingolipids in the cerebrospinal fluid of patients with multiple sclerosis. Molecular and chemical neuropathology / sponsored by the International Society for Neurochemistry and the World Federation of Neurology and research groups on neurochemistry and cerebrospinal fluid. 1990;13(3):205-16.

179.   Dodge JC, Treleaven CM, Pacheco J, Cooper S, Bao C, Abraham M, et al. Glycosphingolipids are modulators of disease pathogenesis in amyotrophic lateral sclerosis. Proceedings of the National Academy of Sciences of the United States of America. 2015;112(26):8100-5.

180.   Kristjansdottir R, Uvebrant P, Lekman A, Mansson JE. Cerebrospinal fluid markers in children with cerebral white matter abnormalities. Neuropediatrics. 2001;32(4):176-82.

181.   Yanagisawa K. GM1 ganglioside and Alzheimer's disease. Glycoconjugate journal. 2015;32(3-4):87-91.

182.   Calamai M, Pavone FS. Partitioning and confinement of GM1 ganglioside induced by amyloid aggregates. FEBS letters. 2013;587(9):1385-91.

183.   Kreutz F, Scherer EB, Ferreira AG, Petry Fdos S, Pereira CL, Santana F, et al. Alterations on Na(+),K(+)-ATPase and acetylcholinesterase activities induced by amyloid-beta peptide in rat brain and GM1 ganglioside neuroprotective action. Neurochemical research. 2013;38(11):2342-50.

184.   Svennerholm L, Brane G, Karlsson I, Lekman A, Ramstrom I, Wikkelso C. Alzheimer disease - effect of continuous intracerebroventricular treatment with GM1 ganglioside and a systematic activation programme. Dementia and geriatric cognitive disorders. 2002;14(3):128-36.

185.   Ariga T. Pathogenic role of ganglioside metabolism in neurodegenerative diseases. J Neurosci Res. 2014;92(10):1227-42.

186.   Sadatipour BT, Greer JM, Pender MP. Increased circulating antiganglioside antibodies in primary and secondary progressive multiple sclerosis. Annals of neurology. 1998;44(6):980-3.

187.   Head VA, Wakerley BR. Guillain-Barre syndrome in general practice: clinical features suggestive of early diagnosis. The British journal of general practice : the journal of the Royal College of General Practitioners. 2016;66(645):218-9.

188.   Pender MP, Greer JM. Immunology of multiple sclerosis. Current allergy and asthma reports. 2007;7(4):285-92.

189.   Chapman J, Sela BA, Wertman E, Michaelson DM. Antibodies to ganglioside GM1 in patients with Alzheimer's disease. Neuroscience letters. 1988;86(2):235-40.

190.   Bachis A, Rabin SJ, Del Fiacco M, Mocchetti I. Gangliosides prevent excitotoxicity through activation of TrkB receptor. Neurotoxicity research. 2002;4(3):225-34.

191.   Lazzaro A, Seren MS, Koga T, Zanoni R, Schiavo N, Manev H. GM1 reduces infarct volume after focal cerebral ischemia. Experimental neurology. 1994;125(2):278-85.

192.   Saito M, Mao RF, Wang R, Vadasz C, Saito M. Effects of gangliosides on ethanol-induced neurodegeneration in the developing mouse brain. Alcoholism, clinical and experimental research. 2007;31(4):665-74.

193.   Rothblat DS, Schneider JS. Effects of GM1 ganglioside treatment on dopamine innervation of the striatum of MPTP-treated mice. Annals of the New York Academy of Sciences. 1998;845:274-7.

194.   Wu G, Lu ZH, Xie X, Ledeen RW. Susceptibility of cerebellar granule neurons from GM2/GD2 synthase-null mice to apoptosis induced by glutamate excitotoxicity and elevated KCl: rescue by GM1 and LIGA20. Glycoconjugate journal. 2004;21(6):305-13.

195.   Schneider JS, Roeltgen DP, Mancall EL, Chapas-Crilly J, Rothblat DS, Tatarian GT. Parkinson's disease: improved function with GM1 ganglioside treatment in a randomized placebo-controlled study. Neurology. 1998;50(6):1630-6.

196.   Schneider JS, Cambi F, Gollomp SM, Kuwabara H, Brasic JR, Leiby B, et al. GM1 ganglioside in Parkinson's disease: Pilot study of effects on dopamine transporter binding. Journal of the neurological sciences. 2015;356(1-2):118-23.

197.   Ceni C, Unsain N, Zeinieh MP, Barker PA. Neurotrophins in the regulation of cellular survival and death. Handbook of experimental pharmacology. 2014;220:193-221.

198.   Lim ST, Esfahani K, Avdoshina V, Mocchetti I. Exogenous gangliosides increase the release of brain-derived neurotrophic factor. Neuropharmacology. 2011;60(7-8):1160-7.

199.   Rabin SJ, Bachis A, Mocchetti I. Gangliosides activate Trk receptors by inducing the release of neurotrophins. The Journal of biological chemistry. 2002;277(51):49466-72.

200.   Valdomero A, Perondi MC, Orsingher OA, Cuadra GR. Exogenous GM1 ganglioside increases accumbal BDNF levels in rats. Behavioural brain research. 2015;278:303-6.

201.   Rabin SJ, Mocchetti I. GM1 ganglioside activates the high-affinity nerve growth factor receptor trkA. Journal of neurochemistry. 1995;65(1):347-54.

202.   Farooqui T, Yates AJ. Effect of GM1 on TrkA dimerization. Annals of the New York Academy of Sciences. 1998;845:407.

203.   Duchemin AM, Ren Q, Mo L, Neff NH, Hadjiconstantinou M. GM1 ganglioside induces phosphorylation and activation of Trk and Erk in brain. Journal of neurochemistry. 2002;81(4):696-707.

204.   Ledeen RW, Wu G. Ganglioside function in calcium homeostasis and signaling. Neurochemical research. 2002;27(7-8):637-47.

205.   Wu G, Lu ZH, Obukhov AG, Nowycky MC, Ledeen RW. Induction of calcium influx through TRPC5 channels by cross-linking of GM1 ganglioside associated with alpha5beta1 integrin initiates neurite outgrowth. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2007;27(28):7447-58.

206.   Favaron M, Manev H, Alho H, Bertolino M, Ferret B, Guidotti A, et al. Gangliosides prevent glutamate and kainate neurotoxicity in primary neuronal cultures of neonatal rat cerebellum and cortex. Proceedings of the National Academy of Sciences of the United States of America. 1988;85(19):7351-5.

207.   Nakamura K, Wu G, Ledeen RW. Protection of neuro-2a cells against calcium ionophore cytotoxicity by gangliosides. J Neurosci Res. 1992;31(2):245-53.

208.   Gavella M, Garaj-Vrhovac V, Lipovac V, Antica M, Gajski G, Car N. Ganglioside GT1b protects human spermatozoa from hydrogen peroxide-induced DNA and membrane damage. International journal of andrology. 2010;33(3):536-44.

209.   Kruszewski M. Labile iron pool: the main determinant of cellular response to oxidative stress. Mutation research. 2003;531(1-2):81-92.

210.   Gorria M, Huc L, Sergent O, Rebillard A, Gaboriau F, Dimanche-Boitrel MT, et al. Protective effect of monosialoganglioside GM1 against chemically induced apoptosis through targeting of mitochondrial function and iron transport. Biochemical pharmacology. 2006;72(10):1343-53.

211.   Brito V, Puigdellivol M, Giralt A, del Toro D, Alberch J, Gines S. Imbalance of p75(NTR)/TrkB protein expression in Huntington's disease: implication for neuroprotective therapies. Cell death & disease. 2013;4:e595.

212.   Di Pardo A, Maglione V, Alpaugh M, Horkey M, Atwal RS, Sassone J, et al. Ganglioside GM1 induces phosphorylation of mutant huntingtin and restores normal motor behavior in Huntington disease mice. Proceedings of the National Academy of Sciences of the United States of America. 2012;109(9):3528-33.

213.   Gu X, Greiner ER, Mishra R, Kodali R, Osmand A, Finkbeiner S, et al. Serines 13 and 16 are critical determinants of full-length human mutant huntingtin induced disease pathogenesis in HD mice. Neuron. 2009;64(6):828-40.

214.   Fienberg AA, Hiroi N, Mermelstein PG, Song W, Snyder GL, Nishi A, et al. DARPP-32: regulator of the efficacy of dopaminergic neurotransmission. Science. 1998;281(5378):838-42.

215.   Young D, Mayer F, Vidotto N, Schweizer T, Berth R, Abramowski D, et al. Mutant huntingtin gene-dose impacts on aggregate deposition, DARPP32 expression and neuroinflammation in HdhQ150 mice. PloS one. 2013;8(9):e75108.

216.   Valencia A, Sapp E, Kimm JS, McClory H, Ansong KA, Yohrling G, et al. Striatal synaptosomes from Hdh140Q/140Q knock-in mice have altered protein levels, novel sites of methionine oxidation, and excess glutamate release after stimulation. Journal of Huntington's disease. 2013;2(4):459-75.

217.   Van Raamsdonk JM, Pearson J, Rogers DA, Lu G, Barakauskas VE, Barr AM, et al. Ethyl-EPA treatment improves motor dysfunction, but not neurodegeneration in the YAC128 mouse model of Huntington disease. Experimental neurology. 2005;196(2):266-72.

218.   Metz GA, Whishaw IQ. Cortical and subcortical lesions impair skilled walking in the ladder rung walking test: a new task to evaluate fore- and hindlimb stepping, placing, and co-ordination. Journal of neuroscience methods. 2002;115(2):169-79.

219.   De Souza RA, Leavitt BR. Neurobiology of Huntington's Disease. Current topics in behavioral neurosciences. 2015;22:81-100.

220.   Unschuld PG, Joel SE, Liu X, Shanahan M, Margolis RL, Biglan KM, et al. Impaired cortico-striatal functional connectivity in prodromal Huntington's Disease. Neuroscience letters. 2012;514(2):204-9.

221.   Vonsattel JP. Huntington disease models and human neuropathology: similarities and differences. Acta neuropathologica. 2008;115(1):55-69.

222.   Martindale D, Hackam A, Wieczorek A, Ellerby L, Wellington C, McCutcheon K, et al. Length of huntingtin and its polyglutamine tract influences localization and frequency of intracellular aggregates. Nature genetics. 1998;18(2):150-4.

223.   Caron NS, Desmond CR, Xia J, Truant R. Polyglutamine domain flexibility mediates the proximity between flanking sequences in huntingtin. Proceedings of the National Academy of Sciences of the United States of America. 2013;110(36):14610-5.

224.   Thompson LM, Aiken CT, Kaltenbach LS, Agrawal N, Illes K, Khoshnan A, et al. IKK phosphorylates Huntingtin and targets it for degradation by the proteasome and lysosome. The Journal of cell biology. 2009;187(7):1083-99.

225.   Atwal RS, Desmond CR, Caron N, Maiuri T, Xia J, Sipione S, et al. Kinase inhibitors modulate huntingtin cell localization and toxicity. Nat Chem Biol. 2011;7(7):453-60.

226.   Cummings JL. Defining and labeling disease-modifying treatments for Alzheimer's disease. Alzheimer's & dementia : the journal of the Alzheimer's Association. 2009;5(5):406-18.

227.   Carroll JB, Lerch JP, Franciosi S, Spreeuw A, Bissada N, Henkelman RM, et al. Natural history of disease in the YAC128 mouse reveals a discrete signature of pathology in Huntington disease. Neurobiology of disease. 2011;43(1):257-65.

228.   Hickey MA, Kosmalska A, Enayati J, Cohen R, Zeitlin S, Levine MS, et al. Extensive early motor and non-motor behavioral deficits are followed by striatal neuronal loss in knock-in Huntington's disease mice. Neuroscience. 2008;157(1):280-95.

229.   Leblond H, L'Esperance M, Orsal D, Rossignol S. Treadmill locomotion in the intact and spinal mouse. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2003;23(36):11411-9.

230.   Henery CC, Mayhew TM. The cerebrum and cerebellum of the fixed human brain: efficient and unbiased estimates of volumes and cortical surface areas. Journal of anatomy. 1989;167:167-80.

231.   Kiernan JA. Chromoxane cyanine R. II. Staining of animal tissues by the dye and its iron complexes. Journal of microscopy. 1984;134(Pt 1):25-39.

232.   Wanker EE, Scherzinger E, Heiser V, Sittler A, Eickhoff H, Lehrach H. Membrane filter assay for detection of amyloid-like polyglutamine-containing protein aggregates. Methods in enzymology. 1999;309:375-86.

233.   Li JY, Popovic N, Brundin P. The use of the R6 transgenic mouse models of Huntington's disease in attempts to develop novel therapeutic strategies. NeuroRx : the journal of the American Society for Experimental NeuroTherapeutics. 2005;2(3):447-64.

234.   Rattray I, Smith E, Gale R, Matsumoto K, Bates GP, Modo M. Correlations of behavioral deficits with brain pathology assessed through longitudinal MRI and histopathology in the R6/2 mouse model of HD. PloS one. 2013;8(4):e60012.

235.   Douaud G, Behrens TE, Poupon C, Cointepas Y, Jbabdi S, Gaura V, et al. In vivo evidence for the selective subcortical degeneration in Huntington's disease. NeuroImage. 2009;46(4):958-66.

236.   Greengard P, Allen PB, Nairn AC. Beyond the dopamine receptor: the DARPP-32/protein phosphatase-1 cascade. Neuron. 1999;23(3):435-47.

237.   Li Y, Yui D, Luikart BW, McKay RM, Li Y, Rubenstein JL, et al. Conditional ablation of brain-derived neurotrophic factor-TrkB signaling impairs striatal neuron development. Proceedings of the National Academy of Sciences of the United States of America. 2012;109(38):15491-6.

238.   Chen J, Marks E, Lai B, Zhang Z, Duce JA, Lam LQ, et al. Iron accumulates in Huntington's disease neurons: protection by deferoxamine. PloS one. 2013;8(10):e77023.

239.   Simmons DA, Casale M, Alcon B, Pham N, Narayan N, Lynch G. Ferritin accumulation in dystrophic microglia is an early event in the development of Huntington's disease. Glia. 2007;55(10):1074-84.

240.   Baune BT. Inflammation and neurodegenerative disorders: is there still hope for therapeutic intervention? Current opinion in psychiatry. 2015;28(2):148-54.

241.   Olejniczak M, Urbanek MO, Krzyzosiak WJ. The Role of the Immune System in Triplet Repeat Expansion Diseases. Mediators of inflammation. 2015;2015:873860.

242.   Abada YS, Nguyen HP, Schreiber R, Ellenbroek B. Assessment of motor function, sensory motor gating and recognition memory in a novel BACHD transgenic rat model for huntington disease. PloS one. 2013;8(7):e68584.

243.   Dai Y, Dudek NL, Li Q, Fowler SC, Muma NA. Striatal expression of a calmodulin fragment improved motor function, weight loss, and neuropathology in the R6/2 mouse model of

Huntington's disease. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2009;29(37):11550-9.

244.   Vandeputte C, Taymans JM, Casteels C, Coun F, Ni Y, Van Laere K, et al. Automated quantitative gait analysis in animal models of movement disorders. BMC neuroscience. 2010;11:92.

245.   Delval A, Krystkowiak P, Delliaux M, Blatt JL, Derambure P, Destee A, et al. Effect of external cueing on gait in Huntington's disease. Movement disorders : official journal of the Movement Disorder Society. 2008;23(10):1446-52.

246.   Delval A, Krystkowiak P, Delliaux M, Dujardin K, Blatt JL, Destee A, et al. Role of attentional resources on gait performance in Huntington's disease. Movement disorders : official journal of the Movement Disorder Society. 2008;23(5):684-9.

247.   Casaca-Carreira J, Temel Y, van Zelst M, Jahanshahi A. Coexistence of Gait Disturbances and Chorea in Experimental Huntington's Disease. Behavioural neurology. 2015;2015:970204.

248.   Cheng HM, Chern Y, Chen IH, Liu CR, Li SH, Chun SJ, et al. Effects on murine behavior and lifespan of selectively decreasing expression of mutant huntingtin allele by supt4h knockdown. PLoS genetics. 2015;11(3):e1005043.

249.   Safren N, El Ayadi A, Chang L, Terrillion CE, Gould TD, Boehning DF, et al. Ubiquilin-1 overexpression increases the lifespan and delays accumulation of Huntingtin aggregates in the R6/2 mouse model of Huntington's disease. PloS one. 2014;9(1):e87513.

250.   Aylward EH, Harrington DL, Mills JA, Nopoulos PC, Ross CA, Long JD, et al. Regional atrophy associated with cognitive and motor function in prodromal Huntington disease. Journal of Huntington's disease. 2013;2(4):477-89.

251.   Tabrizi SJ, Reilmann R, Roos RA, Durr A, Leavitt B, Owen G, et al. Potential endpoints for clinical trials in premanifest and early Huntington's disease in the TRACK-HD study: analysis of 24 month observational data. The Lancet Neurology. 2012;11(1):42-53.

252.   Dodds L, Chen J, Berggren K, Fox J. Characterization of Striatal Neuronal Loss and Atrophy in the R6/2 Mouse Model of Huntington's Disease. PLoS currents. 2014;6.

253.   Paulsen JS, Magnotta VA, Mikos AE, Paulson HL, Penziner E, Andreasen NC, et al. Brain structure in preclinical Huntington's disease. Biological psychiatry. 2006;59(1):57-63.

254.   Crawford HE, Hobbs NZ, Keogh R, Langbehn DR, Frost C, Johnson H, et al. Corpus callosal atrophy in premanifest and early Huntington's disease. Journal of Huntington's disease. 2013;2(4):517-26.

255.   Matsui JT, Vaidya JG, Wassermann D, Kim RE, Magnotta VA, Johnson HJ, et al. Prefrontal cortex white matter tracts in prodromal Huntington disease. Human brain mapping. 2015;36(10):3717-32.

256.   Novak MJ, Seunarine KK, Gibbard CR, Hobbs NZ, Scahill RI, Clark CA, et al. White matter integrity in premanifest and early Huntington's disease is related to caudate loss and disease progression. Cortex; a journal devoted to the study of the nervous system and behavior. 2014;52:98-112.

257.   Sapp E, Penney J, Young A, Aronin N, Vonsattel JP, DiFiglia M. Axonal transport of N-terminal huntingtin suggests early pathology of corticostriatal projections in Huntington disease. Journal of neuropathology and experimental neurology. 1999;58(2):165-73.

258.   Wolf RC, Sambataro F, Vasic N, Schonfeldt-Lecuona C, Ecker D, Landwehrmeyer B. Aberrant connectivity of lateral prefrontal networks in presymptomatic Huntington's disease. Experimental neurology. 2008;213(1):137-44.

259. Gatto RG, Chu Y, Ye AQ, Price SD, Tavassoli E, Buenaventura A, et al. Analysis of YFP(J16)-R6/2 reporter mice and postmortem brains reveals early pathology and increased vulnerability of callosal axons in Huntington's disease. Human molecular genetics. 2015;24(18):5285-98.

260. Deng YP, Wong T, Bricker-Anthony C, Deng B, Reiner A. Loss of corticostriatal and thalamostriatal synaptic terminals precedes striatal projection neuron pathology in heterozygous Q140 Huntington's disease mice. Neurobiology of disease. 2013;60:89-107.

261. Vajn K, Viljetic B, Degmecic IV, Schnaar RL, Heffer M. Differential distribution of major brain gangliosides in the adult mouse central nervous system. PloS one. 2013;8(9):e75720.

262. Zhang YP, Huang QL, Zhao CM, Tang JL, Wang YL. GM1 improves neurofascin155 association with lipid rafts and prevents rat brain myelin injury after hypoxia-ischemia. Brazilian journal of medical and biological research = Revista brasileira de pesquisas medicas e biologicas / Sociedade Brasileira de Biofisica [et al]. 2011;44(6):553-61.

263. Rong X, Zhou W, Xiao-Wen C, Tao L, Tang J. Ganglioside GM1 reduces white matter damage in neonatal rats. Acta neurobiologiae experimentalis. 2013;73(3):379-86.

264. Bibb JA, Yan Z, Svenningsson P, Snyder GL, Pieribone VA, Horiuchi A, et al. Severe deficiencies in dopamine signaling in presymptomatic Huntington's disease mice. Proceedings of the National Academy of Sciences of the United States of America. 2000;97(12):6809-14.

265. Svenningsson P, Nishi A, Fisone G, Girault JA, Nairn AC, Greengard P. DARPP-32: an integrator of neurotransmission. Annual review of pharmacology and toxicology. 2004;44:269-96.

266. Hajos F. Changes in glial fibrillary acidic protein (GFAP) immonureactivity reflect neuronal states. Neurochemical research. 2008;33(8):1643-50.

267. Bode FJ, Stephan M, Suhling H, Pabst R, Straub RH, Raber KA, et al. Sex differences in a transgenic rat model of Huntington's disease: decreased 17beta-estradiol levels correlate with reduced numbers of DARPP32+ neurons in males. Human molecular genetics. 2008;17(17):2595-609.

268. Giralt A, Carreton O, Lao-Peregrin C, Martin ED, Alberch J. Conditional BDNF release under pathological conditions improves Huntington's disease pathology by delaying neuronal dysfunction. Molecular neurodegeneration. 2011;6(1):71.

269. Jin H, Xi G, Keep RF, Wu J, Hua Y. DARPP-32 to quantify intracerebral hemorrhage-induced neuronal death in basal ganglia. Translational stroke research. 2013;4(1):130-4.

270. Bartzokis G, Lu PH, Tishler TA, Fong SM, Oluwadara B, Finn JP, et al. Myelin breakdown and iron changes in Huntington's disease: pathogenesis and treatment implications. Neurochemical research. 2007;32(10):1655-64.

271. Sanchez-Castaneda C, Squitieri F, Di Paola M, Dayan M, Petrollini M, Sabatini U. The role of iron in gray matter degeneration in Huntington's disease: a magnetic resonance imaging study. Human brain mapping. 2015;36(1):50-66.

272. Di Paola M, Phillips OR, Sanchez-Castaneda C, Di Pardo A, Maglione V, Caltagirone C, et al. MRI measures of corpus callosum iron and myelin in early Huntington's disease. Human brain mapping. 2014;35(7):3143-51.

273. Zhang Z, Zhang Z, Lu H, Yang Q, Wu H, Wang J. Microglial Polarization and Inflammatory Mediators After Intracerebral Hemorrhage. Molecular neurobiology. 2016.

274. Silvestroni A, Faull RL, Strand AD, Moller T. Distinct neuroinflammatory profile in post-mortem human Huntington's disease. Neuroreport. 2009;20(12):1098-103.

275.   Jeyakumar M, Williams I, Smith D, Cox TM, Platt FM. Critical role of iron in the pathogenesis of the murine gangliosidoses. Neurobiology of disease. 2009;34(3):406-16.

276.   Giampa C, Laurenti D, Anzilotti S, Bernardi G, Menniti FS, Fusco FR. Inhibition of the striatal specific phosphodiesterase PDE10A ameliorates striatal and cortical pathology in R6/2 mouse model of Huntington's disease. PloS one. 2010;5(10):e13417.

277.   Stack EC, Smith KM, Ryu H, Cormier K, Chen M, Hagerty SW, et al. Combination therapy using minocycline and coenzyme Q10 in R6/2 transgenic Huntington's disease mice. Biochimica et biophysica acta. 2006;1762(3):373-80.

278.   Ma L, Morton AJ, Nicholson LF. Microglia density decreases with age in a mouse model of Huntington's disease. Glia. 2003;43(3):274-80.

279.   Kanazawa H, Ohsawa K, Sasaki Y, Kohsaka S, Imai Y. Macrophage/microglia-specific protein Iba1 enhances membrane ruffling and Rac activation via phospholipase C-gamma - dependent pathway. The Journal of biological chemistry. 2002;277(22):20026-32.

280.   Hanisch UK, Kettenmann H. Microglia: active sensor and versatile effector cells in the normal and pathologic brain. Nature neuroscience. 2007;10(11):1387-94.

281.   Kwan W, Trager U, Davalos D, Chou A, Bouchard J, Andre R, et al. Mutant huntingtin impairs immune cell migration in Huntington disease. The Journal of clinical investigation. 2012;122(12):4737-47.

282.   Cong WN, Cai H, Wang R, Daimon CM, Maudsley S, Raber K, et al. Altered hypothalamic protein expression in a rat model of Huntington's disease. PloS one. 2012;7(10):e47240.

283.   Choi I, Choi DJ, Yang H, Woo JH, Chang MY, Kim JY, et al. PINK1 expression increases during brain development and stem cell differentiation, and affects the development of GFAP-positive astrocytes. Molecular brain. 2016;9(1):5.

284.   Middeldorp J, Hol EM. GFAP in health and disease. Progress in neurobiology. 2011;93(3):421-43.

285.   Bartzokis G, Lu PH, Nuechterlein KH, Gitlin M, Doi C, Edwards N, et al. Differential effects of typical and atypical antipsychotics on brain myelination in schizophrenia. Schizophrenia research. 2007;93(1-3):13-22.

286.   Weinstein DE, Shelanski ML, Liem RK. Suppression by antisense mRNA demonstrates a requirement for the glial fibrillary acidic protein in the formation of stable astrocytic processes in response to neurons. The Journal of cell biology. 1991;112(6):1205-13.

287.   Schneider JS, Sendek S, Daskalakis C, Cambi F. GM1 ganglioside in Parkinson's disease: Results of a five year open study. Journal of the neurological sciences. 2010;292(1-2):45-51.

288.   Schneider JS, Gollomp SM, Sendek S, Colcher A, Cambi F, Du W. A randomized, controlled, delayed start trial of GM1 ganglioside in treated Parkinson's disease patients. Journal of the neurological sciences. 2013;324(1-2):140-8.

289.   Schneider JS, Seyfried TN, Choi HS, Kidd SK. Intraventricular Sialidase Administration Enhances GM1 Ganglioside Expression and Is Partially Neuroprotective in a Mouse Model of Parkinson's Disease. PloS one. 2015;10(12):e0143351.

290.   Group THsDCR. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. . Cell. 1993;72(6):971-83.

291.   Imarisio S, Carmichael J, Korolchuk V, Chen CW, Saiki S, Rose C, et al. Huntington's disease: from pathology and genetics to potential therapies. Biochem J. 2008;412(2):191-209.

292.   Vonsattel JP, DiFiglia M. Huntington disease. Journal of neuropathology and experimental neurology. 1998;57(5):369-84.

293. Vonsattel JP, Keller C, Cortes Ramirez EP. Huntington's disease - neuropathology. Handb Clin Neurol. 2011;100:83-100.

294. Aziz NA, Pijl H, Frolich M, van der Graaf AW, Roelfsema F, Roos RA. Increased hypothalamic-pituitary-adrenal axis activity in Huntington's disease. J Clin Endocrinol Metab. 2009;94(4):1223-8.

295. Petersen A, Bjorkqvist M. Hypothalamic-endocrine aspects in Huntington's disease. The European journal of neuroscience. 2006;24(4):961-7.

296. Spargo E, Everall IP, Lantos PL. Neuronal loss in the hippocampus in Huntington's disease: a comparison with HIV infection. Journal of neurology, neurosurgery, and psychiatry. 1993;56(5):487-91.

297. Roos RA. Huntington's disease: a clinical review. Orphanet journal of rare diseases. 2010;5:40.

298. Paulsen JS, Langbehn DR, Stout JC, Aylward E, Ross CA, Nance M, et al. Detection of Huntington's disease decades before diagnosis: the Predict-HD study. Journal of neurology, neurosurgery, and psychiatry. 2008;79(8):874-80.

299. Berrios GE, Wagle AC, Markova IS, Wagle SA, Rosser A, Hodges JR. Psychiatric symptoms in neurologically asymptomatic Huntington's disease gene carriers: a comparison with gene negative at risk subjects. Acta Psychiatr Scand. 2002;105(3):224-30.

300. Paulsen JS, Zhao H, Stout JC, Brinkman RR, Guttman M, Ross CA, et al. Clinical markers of early disease in persons near onset of Huntington's disease. Neurology. 2001;57(4):658-62.

301. Snowden JS, Craufurd D, Thompson J, Neary D. Psychomotor, executive, and memory function in preclinical Huntington's disease. J Clin Exp Neuropsychol. 2002;24(2):133-45.

302. Stout JC, Paulsen JS, Queller S, Solomon AC, Whitlock KB, Campbell JC, et al. Neurocognitive signs in prodromal Huntington disease. Neuropsychology. 2011;25(1):1-14.

303. Knopman D, Nissen MJ. Procedural learning is impaired in Huntington's disease: evidence from the serial reaction time task. Neuropsychologia. 1991;29(3):245-54.

304. Heindel WC, Butters N, Salmon DP. Impaired learning of a motor skill in patients with Huntington's disease. Behav Neurosci. 1988;102(1):141-7.

305. Lawrence AD, Watkins LH, Sahakian BJ, Hodges JR, Robbins TW. Visual object and visuospatial cognition in Huntington's disease: implications for information processing in corticostriatal circuits. Brain : a journal of neurology. 2000;123 (Pt 7):1349-64.

306. Wilson RS, Como PG, Garron DC, Klawans HL, Barr A, Klawans D. Memory failure in Huntington's disease. J Clin Exp Neuropsychol. 1987;9(2):147-54.

307. Paulsen JS, Ready RE, Hamilton JM, Mega MS, Cummings JL. Neuropsychiatric aspects of Huntington's disease. Journal of neurology, neurosurgery, and psychiatry. 2001;71(3):310-4.

308. Paulsen JS, Nehl C, Hoth KF, Kanz JE, Benjamin M, Conybeare R, et al. Depression and stages of Huntington's disease. J Neuropsychiatry Clin Neurosci. 2005;17(4):496-502.

309. Pouladi MA, Graham RK, Karasinska JM, Xie Y, Santos RD, Petersen A, et al. Prevention of depressive behaviour in the YAC128 mouse model of Huntington disease by mutation at residue 586 of huntingtin. Brain : a journal of neurology. 2009;132(Pt 4):919-32.

310. Lione LA, Carter RJ, Hunt MJ, Bates GP, Morton AJ, Dunnett SB. Selective discrimination learning impairments in mice expressing the human Huntington's disease mutation. The Journal of neuroscience : the official journal of the Society for Neuroscience. 1999;19(23):10428-37.

311.   Ross CA, Tabrizi SJ. Huntington's disease: from molecular pathogenesis to clinical treatment. The Lancet Neurology. 2011;10(1):83-98.

312.   Killoran A, Biglan KM. Current therapeutic options for Huntington's disease: Good clinical practice versus evidence-based approaches? Movement disorders : official journal of the Movement Disorder Society. 2014;29(11):1404-13.

313.   Reilmann R. Pharmacological treatment of chorea in Huntington's disease-good clinical practice versus evidence-based guideline. Movement disorders : official journal of the Movement Disorder Society. 2013;28(8):1030-3.

314.   Rosenblatt A. Neuropsychiatry of Huntington's disease. Dialogues in clinical neuroscience. 2007;9(2):191-7.

315.   Hakomori Si SI. The glycosynapse. Proceedings of the National Academy of Sciences of the United States of America. 2002;99(1):225-32.

316.   Sonnino S, Mauri L, Chigorno V, Prinetti A. Gangliosides as components of lipid membrane domains. Glycobiology. 2007;17(1):1R-13R.

317.   Ledeen R, Wu G. GM1 in the nuclear envelope regulates nuclear calcium through association with a nuclear sodium-calcium exchanger. Journal of neurochemistry. 2007;103 Suppl 1:126-34.

318.   Belzung C, Misslin R, Vogel E, Dodd RH, Chapouthier G. Anxiogenic effects of methyl-beta-carboline-3-carboxylate in a light/dark choice situation. Pharmacology, biochemistry, and behavior. 1987;28(1):29-33.

319.   Frye AAWaCA. The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. Nature Protocols. 2007:322-8.

320.   Porsolt RD, Le Pichon M, Jalfre M. Depression: a new animal model sensitive to antidepressant treatments. Nature. 1977;266(5604):730-2.

321.   Pouladi MA, Graham RK, Joshi P, Lu G, Deng Y, Wu N-P, et al. Diffential susceptivility to excitotoxic stress in YAC128 mouse models of HD between initiation and progression of disease. Journal of Neuroscience. 2009:2193-204.

322.   Deacon RM. Assessing nest building in mice. Nat Protoc. 2006;1(3):1117-9.

323.   Bolivar VJ, Scott Ganus J, Messer A. The development of behavioral abnormalities in the motor neuron degeneration (mnd) mouse. Brain research. 2002;937(1-2):74-82.

324.   Cook MN, Bolivar VJ, McFadyen MP, Flaherty L. Behavioral differences among 129 substrains: implications for knockout and transgenic mice. Behav Neurosci. 2002;116(4):600-11.

325.   Ey E, Yang M, Katz AM, Woldeyohannes L, Silverman JL, Leblond CS, et al. Absence of deficits in social behaviors and ultrasonic vocalizations in later generations of mice lacking neuroligin4. Genes, brain, and behavior. 2012.

326.   Hughes R. The value of spontaeous alternation behavior (SAB) as a test of retention in pharmacological investigations of memory. Neuroscience and Biobehavioral Reviews 2004:497-505.

327.   Swonger AK, Rech RH. Serotonergic and cholinergic involvement in habituation of activity and spontaneous alternation of rats in a Y maze. Journal of comparative and physiological psychology. 1972;81(3):509-22.

328.   Grant SL, Shulman Y, Tibbo P, Hampson DR, Baker GB. Determination of d-serine and related neuroactive amino acids in human plasma by high-performance liquid chromatography with fluorimetric detection. Journal of chromatography B, Analytical technologies in the biomedical and life sciences. 2006;844(2):278-82.

329.   Parent M, Bush D, Rauw G, Master S, Vaccarino F, Baker G. Analysis of amino acids and catecholamines, 5-hydroxytryptamine and their metabolites in brain areas in the rat using in vivo microdialysis. Methods. 2001;23(1):11-20.

330.   Bailey KR, Crawley JN. Anxiety-Related Behaviors in Mice. In: Buccafusco JJ, editor. Methods of Behavior Analysis in Neuroscience. Frontiers in Neuroscience. 2nd ed. Boca Raton (FL)2009.

331.   Lister RG. The use of a plus-maze to measure anxiety in the mouse. Psychopharmacology. 1987;92(2):180-5.

332.   Owens MJ, Bissette G, Nemeroff CB. Acute effects of alprazolam and adinazolam on the concentrations of corticotropin-releasing factor in the rat brain. Synapse. 1989;4(3):196-202.

333.   Gross C, Santarelli L, Brunner D, Zhuang X, Hen R. Altered fear circuits in 5-HT(1A) receptor KO mice. Biological psychiatry. 2000;48(12):1157-63.

334.   Cryan JF, Markou A, Lucki I. Assessing antidepressant activity in rodents: recent developments and future needs. Trends in pharmacological sciences. 2002;23(5):238-45.

335.   Petit-Demouliere B, Chenu F, Bourin M. Forced swimming test in mice: a review of antidepressant activity. Psychopharmacology. 2005;177(3):245-55.

336.   Ciamei A, Detloff PJ, Morton AJ. Progression of behavioural despair in R6/2 and Hdh knock-in mouse models recapitulates depression in Huntington's disease. Behavioural brain research. 2015;291:140-6.

337.   Deacon R. Assessing burrowing, nest construction, and hoarding in mice. J Vis Exp. 2012(59):e2607.

338.   Sedelis M, Schwarting RK, Huston JP. Behavioral phenotyping of the MPTP mouse model of Parkinson's disease. Behavioural brain research. 2001;125(1-2):109-25.

339.   Fleming SM, Salcedo J, Fernagut PO, Rockenstein E, Masliah E, Levine MS, et al. Early and progressive sensorimotor anomalies in mice overexpressing wild-type human alpha-synuclein. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2004;24(42):9434-40.

340.   Belzung C. Innovative drugs to treat depression: did animal models fail to be predictive or did clinical trials fail to detect effects? Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology. 2014;39(5):1041-51.

341.   Nollet M, Le Guisquet AM, Belzung C. Models of depression: unpredictable chronic mild stress in mice. Curr Protoc Pharmacol. 2013;Chapter 5:Unit 5 65.

342.   Crawley JN. Mouse behavioral assays relevant to the symptoms of autism. Brain pathology. 2007;17(4):448-59.

343.   Moy SS, Nadler JJ, Perez A, Barbaro RP, Johns JM, Magnuson TR, et al. Sociability and preference for social novelty in five inbred strains: an approach to assess autistic-like behavior in mice. Genes, brain, and behavior. 2004;3(5):287-302.

344.   Groves PM, Thompson RF. Habituation: a dual-process theory. Psychol Rev. 1970;77(5):419-50.

345.   O'Keefe. The Hippocampus as a Cognitive Map. Clarendon Press, Oxford. 1978.

346.   Bolivar VJ. Intrasession and intersession habituation in mice: from inbred strain variability to linkage analysis. Neurobiol Learn Mem. 2009;92(2):206-14.

347.   Muller U, Cristina N, Li ZW, Wolfer DP, Lipp HP, Rulicke T, et al. Behavioral and anatomical deficits in mice homozygous for a modified beta-amyloid precursor protein gene. Cell. 1994;79(5):755-65.

348.   Leussis MP, Bolivar VJ. Habituation in rodents: a review of behavior, neurobiology, and genetics. Neurosci Biobehav Rev. 2006;30(7):1045-64.
349.   Paul CM, Magda G, Abel S. Spatial memory: Theoretical basis and comparative review on experimental methods in rodents. Behavioural brain research. 2009;203(2):151-64.
350.   Webster SJ, Bachstetter AD, Nelson PT, Schmitt FA, Van Eldik LJ. Using mice to model Alzheimer's dementia: an overview of the clinical disease and the preclinical behavioral changes in 10 mouse models. Frontiers in genetics. 2014;5:88.
351.   Kokkinidis L, Anisman H. Interaction between cholinergic and catecholaminergic agents in a spontaneous alternation task. Psychopharmacology. 1976;48(3):261-70.
352.   Kokkinidis L, Anisman H. Perseveration and rotational behavior elicited by d-amphetamine in a Y-maze exploratory task: differential effects of intraperitoneal and unilateral intraventricular administration. Psychopharmacology. 1977;52(2):123-8.
353.   Lawrence AD, Sahakian BJ, Hodges JR, Rosser AE, Lange KW, Robbins TW. Executive and mnemonic functions in early Huntington's disease. Brain : a journal of neurology. 1996;119 (Pt 5):1633-45.
354.   Paulsen JS. Cognitive impairment in Huntington disease: diagnosis and treatment. Current neurology and neuroscience reports. 2011;11(5):474-83.
355.   Thompson TL, Moss RL. Modulation of mesolimbic dopaminergic activity over the rat estrous cycle. Neuroscience letters. 1997;229(3):145-8.
356.   Yu L, Liao PC. Sexual differences and estrous cycle in methamphetamine-induced dopamine and serotonin depletions in the striatum of mice. Journal of neural transmission. 2000;107(4):419-27.
357.   San-Martin-Clark O, Leza JC, Lizasoain I, Lorenzo P. Changes induced by sodium cromoglycate on brain serotonin turnover in morphine dependent and abstinent mice. Psychopharmacology. 1993;111(2):233-8.
358.   Cryan JF, Holmes A. The ascent of mouse: advances in modelling human depression and anxiety. Nature reviews Drug discovery. 2005;4(9):775-90.
359.   Vendruscolo LF, Takahashi RN, Bruske GR, Ramos A. Evaluation of the anxiolytic-like effect of NKP608, a NK1-receptor antagonist, in two rat strains that differ in anxiety-related behaviors. Psychopharmacology. 2003;170(3):287-93.
360.   Ramos A. Animal models of anxiety: do I need multiple tests? Trends in pharmacological sciences. 2008;29(10):493-8.
361.   Kinn AM, Gronli J, Fiske E, Kuipers S, Ursin R, Murison R, et al. A double exposure to social defeat induces sub-chronic effects on sleep and open field behaviour in rats. Physiology & behavior. 2008;95(4):553-61.
362.   Zhu X, Peng S, Zhang S, Zhang X. Stress-induced depressive behaviors are correlated with Par-4 and DRD2 expression in rat striatum. Behavioural brain research. 2011;223(2):329-35.
363.   Tache Y, Brunnhuber S. From Hans Selye's discovery of biological stress to the identification of corticotropin-releasing factor signaling pathways: implication in stress-related functional bowel diseases. Annals of the New York Academy of Sciences. 2008;1148:29-41.
364.   Narducci F, Snape WJ, Jr., Battle WM, London RL, Cohen S. Increased colonic motility during exposure to a stressful situation. Digestive diseases and sciences. 1985;30(1):40-4.
365.   Rao SS, Hatfield RA, Suls JM, Chamberlain MJ. Psychological and physical stress induce differential effects on human colonic motility. The American journal of gastroenterology. 1998;93(6):985-90.

366.   Nestler EJ, Hyman SE. Animal models of neuropsychiatric disorders. Nature neuroscience. 2010;13(10):1161-9.

367.   Pla P, Orvoen S, Saudou F, David DJ, Humbert S. Mood disorders in Huntington's disease: from behavior to cellular and molecular mechanisms. Frontiers in behavioral neuroscience. 2014;8:135.

368.   Turner RC, Seminerio MJ, Naser ZJ, Ford JN, Martin SJ, Matsumoto RR, et al. Effects of aging on behavioral assessment performance: implications for clinically relevant models of neurological disease. Journal of neurosurgery. 2012;117(3):629-37.

369.   Bogdanova OV, Kanekar S, D'Anci KE, Renshaw PF. Factors influencing behavior in the forced swim test. Physiology & behavior. 2013;118:227-39.

370.   Samuels BAaH, R. Novelty-suppressed feeding in the mouse. In: Gould TD, editor. Mood and Anxiety Related Phenotypes in Mice: Characterization Using Behavioral Tests. Neuromethods. II: Springer Science+Business Media; 2011. p. 107-22.

371.   David DJ, Samuels BA, Rainer Q, Wang JW, Marsteller D, Mendez I, et al. Neurogenesis-dependent and -independent effects of fluoxetine in an animal model of anxiety/depression. Neuron. 2009;62(4):479-93.

372.   Santarelli L, Saxe M, Gross C, Surget A, Battaglia F, Dulawa S, et al. Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. Science. 2003;301(5634):805-9.

373.   Bodnoff SR, Suranyi-Cadotte B, Aitken DH, Quirion R, Meaney MJ. The effects of chronic antidepressant treatment in an animal model of anxiety. Psychopharmacology. 1988;95(3):298-302.

374.   Du X, Pang TY, Hannan AJ. A Tale of Two Maladies? Pathogenesis of Depression with and without the Huntington's Disease Gene Mutation. Front Neurol. 2013;4:81.

375.   Ben M'Barek K, Pla P, Orvoen S, Benstaali C, Godin JD, Gardier AM, et al. Huntingtin mediates anxiety/depression-related behaviors and hippocampal neurogenesis. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2013;33(20):8608-20.

376.   Pla P, Orvoen S, Benstaali C, Dodier S, Gardier AM, David DJ, et al. Huntingtin acts non cell-autonomously on hippocampal neurogenesis and controls anxiety-related behaviors in adult mouse. PloS one. 2013;8(9):e73902.

377.   Wang J, Cheng A, Wakade C, Yu RK. Ganglioside GD3 is required for neurogenesis and long-term maintenance of neural stem cells in the postnatal mouse brain. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2014;34(41):13790-800.

378.   Cryan JF, Valentino RJ, Lucki I. Assessing substrates underlying the behavioral effects of antidepressants using the modified rat forced swimming test. Neurosci Biobehav Rev. 2005;29(4-5):547-69.

379.   Detke MJ, Rickels M, Lucki I. Active behaviors in the rat forced swimming test differentially produced by serotonergic and noradrenergic antidepressants. Psychopharmacology. 1995;121(1):66-72.

380.   Page ME, Detke MJ, Dalvi A, Kirby LG, Lucki I. Serotonergic mediation of the effects of fluoxetine, but not desipramine, in the rat forced swimming test. Psychopharmacology. 1999;147(2):162-7.

381.   Yandrasitz JR, Cohn RM, Masley B, DelRowe D. Evaluation of the binding of serotonin by isolated CNS acidic lipids. Neurochemical research. 1980;5(5):465-77.

382.   Matinyan NS, Melikyan GB, Arakelyan VB, Kocharov SL, Prokazova NV, Avakian TM. Interaction of ganglioside-containing planar bilayers with serotonin and inorganic cations. Biochimica et biophysica acta. 1989;984(3):313-8.

383.   Berry-Kravis E, Dawson G. Possible role of gangliosides in regulating an adenylate cyclase-linked 5-hydroxytryptamine (5-HT1) receptor. Journal of neurochemistry. 1985;45(6):1739-47.

384.   Faizi M, Bader PL, Saw N, Nguyen TV, Beraki S, Wyss-Coray T, et al. Thy1-hAPP(Lond/Swe+) mouse model of Alzheimer's disease displays broad behavioral deficits in sensorimotor, cognitive and social function. Brain and behavior. 2012;2(2):142-54.

385.   Cheng D, Low JK, Logge W, Garner B, Karl T. Novel behavioural characteristics of female APPSwe/PS1DeltaE9 double transgenic mice. Behavioural brain research. 2014;260:111-8.

386.   Kokkinidis L. Amphetamine-elicited perseverative and rotational behavior: Evaluation of directional preference. Mar 1987. Pharmacology, Biochemistry and Behavior. 1987;.26(3):pp.

387.   Katz RJ, Schmaltz K. Dopaminergic involvement in attention. A novel animal model. Progress in neuro-psychopharmacology. 1980;4(6):585-90.

388.   Ulloa RE, Nicolini H, Fernandez-Guasti A. Sex differences on spontaneous alternation in prepubertal rats: implications for an animal model of obsessive-compulsive disorder. Progress in neuro-psychopharmacology & biological psychiatry. 2004;28(4):687-92.

389.   Yadin E, Friedman E, Bridger WH. Spontaneous alternation behavior: an animal model for obsessive-compulsive disorder? Pharmacology, biochemistry, and behavior. 1991;40(2):311-5.

390.   Geyer MA, Puerto A, Menkes DB, Segal DS, Mandell AJ. Behavioral studies following lesions of the mesolimbic and mesostriatal serotonergic pathways. Brain research. 1976;106(2):257-69.

391.   Platel A, Porsolt RD. Habituation of exploratory activity in mice: a screening test for memory enhancing drugs. Psychopharmacology. 1982;78(4):346-52.

392.   Hess EJ, Albers LJ, Le H, Creese I. Effects of chronic SCH23390 treatment on the biochemical and behavioral properties of D1 and D2 dopamine receptors: potentiated behavioral responses to a D2 dopamine agonist after selective D1 dopamine receptor upregulation. The Journal of pharmacology and experimental therapeutics. 1986;238(3):846-54.

393.   Van Horn MR, Sild M, Ruthazer ES. D-serine as a gliotransmitter and its roles in brain development and disease. Frontiers in cellular neuroscience. 2013;7:39.

394.   Rosas HD, Liu AK, Hersch S, Glessner M, Ferrante RJ, Salat DH, et al. Regional and progressive thinning of the cortical ribbon in Huntington's disease. Neurology. 2002;58(5):695-701.

395.   Rosas HD, Reuter M, Doros G, Lee SY, Triggs T, Malarick K, et al. A tale of two factors: what determines the rate of progression in Huntington's disease? A longitudinal MRI study. Movement disorders : official journal of the Movement Disorder Society. 2011;26(9):1691-7.

396.   Estrada-Sanchez AM, Rebec GV. Role of cerebral cortex in the neuropathology of Huntington's disease. Frontiers in neural circuits. 2013;7:19.

397.   Padowski JM, Weaver KE, Richards TL, Laurino MY, Samii A, Aylward EH, et al. Neurochemical correlates of caudate atrophy in Huntington's disease. Movement disorders : official journal of the Movement Disorder Society. 2014;29(3):327-35.

398.   Fujiwara H, Ikarashi K, Yamazaki Y, Goto J, Kaneko K, Sugita M, et al. Impairment of hippocampal long-term potentiation and failure of learning in mice treated with d-threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol. Biomedical research. 2012;33(5):265-71.

399.   Usuki S, Hamanoue M, Kohsaka S, Inokuchi J. Induction of ganglioside biosynthesis and neurite outgrowth of primary cultured neurons by L-threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol. Journal of neurochemistry. 1996;67(5):1821-30.

400.   Inokuchi J, Mizutani A, Jimbo M, Usuki S, Yamagishi K, Mochizuki H, et al. Up-regulation of ganglioside biosynthesis, functional synapse formation, and memory retention by a synthetic ceramide analog (L-PDMP). Biochemical and biophysical research communications. 1997;237(3):595-600.

401.   Furuse H, Waki H, Kaneko K, Fujii S, Miura M, Sasaki H, et al. Effect of the mono- and tetra-sialogangliosides, GM1 and GQ1b, on long-term potentiation in the CA1 hippocampal neurons of the guinea pig. Experimental brain research. 1998;123(3):307-14.

402.   Yang R, Wang Q, Min L, Sui R, Li J, Liu X. Monosialoanglioside improves memory deficits and relieves oxidative stress in the hippocampus of rat model of Alzheimer's disease. Neurol Sci. 2013;34(8):1447-51.

403.   Fong TG, Neff NH, Hadjiconstantinou M. GM1 ganglioside improves spatial learning and memory of aged rats. Behavioural brain research. 1997;85(2):203-11.

404.   Fujii S, Igarashi K, Sasaki H, Furuse H, Ito K, Kaneko K, et al. Effects of the mono- and tetrasialogangliosides GM1 and GQ1b on ATP-induced long-term potentiation in hippocampal CA1 neurons. Glycobiology. 2002;12(5):339-44.

405.   Jung WR, Kim HG, Kim KL. Ganglioside GQ1b improves spatial learning and memory of rats as measured by the Y-maze and the Morris water maze tests. Neuroscience letters. 2008;439(2):220-5.

406.   Yohrling IG, Jiang GC, DeJohn MM, Robertson DJ, Vrana KE, Cha JH. Inhibition of tryptophan hydroxylase activity and decreased 5-HT1A receptor binding in a mouse model of Huntington's disease. Journal of neurochemistry. 2002;82(6):1416-23.

407.   Castro ME, Pascual J, Romon T, Berciano J, Figols J, Pazos A. 5-HT1B receptor binding in degenerative movement disorders. Brain research. 1998;790(1-2):323-8.

408.   Dang LC, Donde A, Madison C, O'Neil JP, Jagust WJ. Striatal dopamine influences the default mode network to affect shifting between object features. Journal of cognitive neuroscience. 2012;24(9):1960-70.

409.   Schwab LC, Garas SN, Drouin-Ouellet J, Mason SL, Stott SR, Barker RA. Dopamine and Huntington's disease. Expert review of neurotherapeutics. 2015;15(4):445-58.

410.   Chen JY, Wang EA, Cepeda C, Levine MS. Dopamine imbalance in Huntington's disease: a mechanism for the lack of behavioral flexibility. Frontiers in neuroscience. 2013;7:114.

411.   Callahan JW, Abercrombie ED. In vivo Dopamine Efflux is Decreased in Striatum of both Fragment (R6/2) and Full-Length (YAC128) Transgenic Mouse Models of Huntington's Disease. Frontiers in systems neuroscience. 2011;5:61.

412.   Johnson MA, Rajan V, Miller CE, Wightman RM. Dopamine release is severely compromised in the R6/2 mouse model of Huntington's disease. Journal of neurochemistry. 2006;97(3):737-46.

413.   Hickey MA, Reynolds GP, Morton AJ. The role of dopamine in motor symptoms in the R6/2 transgenic mouse model of Huntington's disease. Journal of neurochemistry. 2002;81(1):46-59.

414.   Garrett MC, Soares-da-Silva P. Increased cerebrospinal fluid dopamine and 3,4-dihydroxyphenylacetic acid levels in Huntington's disease: evidence for an overactive dopaminergic brain transmission. Journal of neurochemistry. 1992;58(1):101-6.

415.   Spokes EG. Neurochemical alterations in Huntington's chorea: a study of post-mortem brain tissue. Brain : a journal of neurology. 1980;103(1):179-210.

416.   Kish SJ, Shannak K, Hornykiewicz O. Elevated serotonin and reduced dopamine in subregionally divided Huntington's disease striatum. Annals of neurology. 1987;22(3):386-9.

417.   Mann JJ, Kaplan RD, Bird ED. Elevated postmortem monoamine oxidase B activity in the caudate nucleus in Huntington's disease compared to schizophrenics and controls. Journal of neural transmission. 1986;65(3-4):277-83.

418.   Richards G, Messer J, Waldvogel HJ, Gibbons HM, Dragunow M, Faull RL, et al. Up-regulation of the isoenzymes MAO-A and MAO-B in the human basal ganglia and pons in Huntington's disease revealed by quantitative enzyme radioautography. Brain research. 2011;1370:204-14.

419.   Laprairie RB, Bagher AM, Precious SV, Denovan-Wright EM. Components of the endocannabinoid and dopamine systems are dysregulated in Huntington's disease: analysis of publicly available microarray datasets. Pharmacology research & perspectives. 2015;3(1):e00104.

420.   Ooi J, Hayden MR, Pouladi MA. Inhibition of Excessive Monoamine Oxidase A/B Activity Protects Against Stress-induced Neuronal Death in Huntington Disease. Molecular neurobiology. 2014.

421.   Vinther-Jensen T, Nielsen TT, Budtz-Jorgensen E, Larsen IU, Hansen MM, Hasholt L, et al. Psychiatric and cognitive symptoms in Huntington's disease are modified by polymorphisms in catecholamine regulating enzyme genes. Clinical genetics. 2015.

422.   Goettl VM, Wemlinger TA, Colvin AE, Neff NH, Hadjiconstantinou M. Motoric behavior in aged rats treated with GM1. Brain research. 2001;906(1-2):92-100.

423.   Goettl VM, Wemlinger TA, Duchemin AM, Neff NH, Hadjiconstantinou M. GM1 ganglioside restores dopaminergic neurochemical and morphological markers in aged rats. Neuroscience. 1999;92(3):991-1000.

424.   Goettl VM, Zhang H, Burrows AC, Wemlinger TA, Neff NH, Hadjiconstantinou M. GM1 enhances dopaminergic markers in the brain of aged rats. Experimental neurology. 2003;183(2):665-72.

425.   Pearson SJ, Reynolds GP. Neocortical neurotransmitter markers in Huntington's disease. Journal of neural transmission General section. 1994;98(3):197-207.

426.   Cummings DM, Andre VM, Uzgil BO, Gee SM, Fisher YE, Cepeda C, et al. Alterations in cortical excitation and inhibition in genetic mouse models of Huntington's disease. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2009;29(33):10371-86.

427.   Gu X, Li C, Wei W, Lo V, Gong S, Li SH, et al. Pathological cell-cell interactions elicited by a neuropathogenic form of mutant Huntingtin contribute to cortical pathogenesis in HD mice. Neuron. 2005;46(3):433-44.

428.   Spampanato J, Gu X, Yang XW, Mody I. Progressive synaptic pathology of motor cortical neurons in a BAC transgenic mouse model of Huntington's disease. Neuroscience. 2008;157(3):606-20.

429.   Waldvogel HJ, Kim EH, Thu DC, Tippett LJ, Faull RL. New Perspectives on the Neuropathology in Huntington's Disease in the Human Brain and its Relation to Symptom Variation. Journal of Huntington's disease. 2012;1(2):143-53.

430.   Thu DC, Oorschot DE, Tippett LJ, Nana AL, Hogg VM, Synek BJ, et al. Cell loss in the motor and cingulate cortex correlates with symptomatology in Huntington's disease. Brain : a journal of neurology. 2010;133(Pt 4):1094-110.

431.   Kordasiewicz HB, Stanek LM, Wancewicz EV, Mazur C, McAlonis MM, Pytel KA, et al. Sustained therapeutic reversal of Huntington's disease by transient repression of huntingtin synthesis. Neuron. 2012;74(6):1031-44.

432.   Schneider JS, Pope A, Simpson K, Taggart J, Smith MG, DiStefano L. Recovery from experimental parkinsonism in primates with GM1 ganglioside treatment. Science. 1992;256(5058):843-6.

433.   Schneider JS, Kean A, DiStefano L. GM1 ganglioside rescues substantia nigra pars compacta neurons and increases dopamine synthesis in residual nigrostriatal dopaminergic neurons in MPTP-treated mice. J Neurosci Res. 1995;42(1):117-23.

434.   Hadjiconstantinou M, Neff NH. Treatment with GM1 ganglioside restores striatal dopamine in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-treated mouse. Journal of neurochemistry. 1988;51(4):1190-6.

435.   Saulino MF, Schengrund CL. Differential accumulation of gangliosides by the brains of MPTP-lesioned mice. J Neurosci Res. 1994;37(3):384-91.

436.   Ghidoni R, Trinchera M, Venerando B, Fiorilli A, Sonnino S, Tettamanti G. Incorporation and metabolism of exogenous GM1 ganglioside in rat liver. Biochem J. 1986;237(1):147-55.

437.   Mitchell MD, Henare K, Balakrishnan B, Lowe E, Fong BY, McJarrow P. Transfer of gangliosides across the human placenta. Placenta. 2012;33(4):312-6.

438.   Palestini P, Masserini M, Fiorilli A, Calappi E, Tettamanti G. Age-related changes in the ceramide composition of the major gangliosides present in rat brain subcellular fractions enriched in plasma membranes of neuronal and myelin origin. Journal of neurochemistry. 1993;61(3):955-60.

439.   Mansson JE, Vanier MT, Svennerholm L. Changes in the fatty acid and sphingosine composition of the major gangliosides of human brain with age. Journal of neurochemistry. 1978;30(1):273-5.

440.   Wiegandt H. The chemical constitution of gangliosides of the vertebrate nervous system. Behavioural brain research. 1995;66(1-2):85-97.

441.   Hussain MM, Jin W, Jiang XC. Mechanisms involved in cellular ceramide homeostasis. Nutrition & metabolism. 2012;9(1):71.

442.   Yu RK, Bieberich E, Xia T, Zeng G. Regulation of ganglioside biosynthesis in the nervous system. Journal of lipid research. 2004;45(5):783-93.

443.   Pohlentz G, Klein D, Schwarzmann G, Schmitz D, Sandhoff K. Both GA2, GM2, and GD2 synthases and GM1b, GD1a, and GT1b synthases are single enzymes in Golgi vesicles from rat liver. Proceedings of the National Academy of Sciences of the United States of America. 1988;85(19):7044-8.

444.   Svennerholm L, Bostrom K, Fredman P, Mansson JE, Rosengren B, Rynmark BM. Human brain gangliosides: developmental changes from early fetal stage to advanced age. Biochimica et biophysica acta. 1989;1005(2):109-17.

445.   Kiarash A, Boyd B, Lingwood CA. Glycosphingolipid receptor function is modified by fatty acid content. Verotoxin 1 and verotoxin 2c preferentially recognize different globotriaosyl ceramide fatty acid homologues. The Journal of biological chemistry. 1994;269(15):11138-46.

446.   Iwabuchi K, Prinetti A, Sonnino S, Mauri L, Kobayashi T, Ishii K, et al. Involvement of very long fatty acid-containing lactosylceramide in lactosylceramide-mediated superoxide generation and migration in neutrophils. Glycoconjugate journal. 2008;25(4):357-74.

447.   Saslowsky DE, te Welscher YM, Chinnapen DJ, Wagner JS, Wan J, Kern E, et al. Ganglioside GM1-mediated transcytosis of cholera toxin bypasses the retrograde pathway and depends on the structure of the ceramide domain. The Journal of biological chemistry. 2013;288(36):25804-9.

448.   Yamashita T, Hashiramoto A, Haluzik M, Mizukami H, Beck S, Norton A, et al. Enhanced insulin sensitivity in mice lacking ganglioside GM3. Proceedings of the National Academy of Sciences of the United States of America. 2003;100(6):3445-9.

449.   Wu G, Lu ZH, Wang J, Wang Y, Xie X, Meyenhofer MF, et al. Enhanced susceptibility to kainate-induced seizures, neuronal apoptosis, and death in mice lacking gangliotetraose gangliosides: protection with LIGA 20, a membrane-permeant analog of GM1. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2005;25(47):11014-22.

450.   Ohmi Y, Tajima O, Ohkawa Y, Yamauchi Y, Sugiura Y, Furukawa K, et al. Gangliosides are essential in the protection of inflammation and neurodegeneration via maintenance of lipid rafts: elucidation by a series of ganglioside-deficient mutant mice. Journal of neurochemistry. 2011;116(5):926-35.

451.   Wang J, Lu ZH, Gabius HJ, Rohowsky-Kochan C, Ledeen RW, Wu G. Cross-linking of GM1 ganglioside by galectin-1 mediates regulatory T cell activity involving TRPC5 channel activation: possible role in suppressing experimental autoimmune encephalomyelitis. Journal of immunology. 2009;182(7):4036-45.

452.   Martinez Z, Zhu M, Han S, Fink AL. GM1 specifically interacts with alpha-synuclein and inhibits fibrillation. Biochemistry. 2007;46(7):1868-77.

453.   Ahn JY. Neuroprotection signaling of nuclear Akt in neuronal cells. Experimental neurobiology. 2014;23(3):200-6.

454.   Polo A, Kirschner G, Guidotti A, Costa E. Brain content of glycosphingolipids after oral administration of monosialogangliosides GM1 and LIGA20 to rats. Molecular and chemical neuropathology / sponsored by the International Society for Neurochemistry and the World Federation of Neurology and research groups on neurochemistry and cerebrospinal fluid. 1994;21(1):41-53.

455.   Manev H, Favaron M, Vicini S, Guidotti A, Costa E. Glutamate-induced neuronal death in primary cultures of cerebellar granule cells: protection by synthetic derivatives of endogenous sphingolipids. The Journal of pharmacology and experimental therapeutics. 1990;252(1):419-27.

456.   de Erausquin GA, Manev H, Guidotti A, Costa E, Brooker G. Gangliosides normalize distorted single-cell intracellular free Ca2+ dynamics after toxic doses of glutamate in cerebellar granule cells. Proceedings of the National Academy of Sciences of the United States of America. 1990;87(20):8017-21.

457.   Lipartiti M, Lazzaro A, Manev H. Ganglioside derivative LIGA20 reduces NMDA neurotoxicity in neonatal rat brain. Neuroreport. 1992;3(10):919-21.

458.   Trettel F, Rigamonti D, Hilditch-Maguire P, Wheeler VC, Sharp AH, Persichetti F, et al. Dominant phenotypes produced by the HD mutation in STHdh(Q111) striatal cells. Human molecular genetics. 2000;9(19):2799-809.

459.	Vermes I, Haanen C, Steffens-Nakken H, Reutelingsperger C. A novel assay for apoptosis. Flow cytometric detection of phosphatidylserine expression on early apoptotic cells using fluorescein labelled Annexin V. Journal of immunological methods. 1995;184(1):39-51.

460.	Prokazova NV, Samovilova NN, Gracheva EV, Golovanova NK. Ganglioside GM3 and its biological functions. Biochemistry Biokhimiia. 2009;74(3):235-49.

461.	Tettamanti G. Ganglioside/glycosphingolipid turnover: new concepts. Glycoconjugate journal. 2004;20(5):301-17.

462.	Zakharova IO, Sokolova TV, Vlasova YA, Furaev VV, Rychkova MP, Avrova NF. GM1 ganglioside activates ERK1/2 and Akt downstream of Trk tyrosine kinase and protects PC12 cells against hydrogen peroxide toxicity. Neurochemical research. 2014;39(11):2262-75.

463.	Atwal RS, Desmond CR, Caron N, Maiuri T, Xia J, Sipione S, et al. Kinase inhibitors modulate huntingtin cell localization and toxicity. Nature chemical biology. 2011;7(7):453-60.

464.	Serb AF, Sisu E, Vukelic Z, Zamfir AD. Profiling and sequencing of gangliosides from human caudate nucleus by chip-nanoelectrospray mass spectrometry. Journal of mass spectrometry : JMS. 2012;47(12):1561-70.

465.	Newburn EN, Duchemin AM, Neff NH, Hadjiconstantinou M. GM1 ganglioside enhances Ret signaling in striatum. Journal of neurochemistry. 2014;130(4):541-54.

466.	Ginzburg L, Li SC, Li YT, Futerman AH. An exposed carboxyl group on sialic acid is essential for gangliosides to inhibit calcium uptake via the sarco/endoplasmic reticulum Ca2+-ATPase: relevance to gangliosidoses. Journal of neurochemistry. 2008;104(1):140-6.

467.	Papini N, Anastasia L, Tringali C, Croci G, Bresciani R, Yamaguchi K, et al. The plasma membrane-associated sialidase MmNEU3 modifies the ganglioside pattern of adjacent cells supporting its involvement in cell-to-cell interactions. The Journal of biological chemistry. 2004;279(17):16989-95.

468.	Zanchetti G, Colombi P, Manzoni M, Anastasia L, Caimi L, Borsani G, et al. Sialidase NEU3 is a peripheral membrane protein localized on the cell surface and in endosomal structures. Biochem J. 2007;408(2):211-9.

469.	Itoh M, Fukumoto S, Iwamoto T, Mizuno A, Rokutanda A, Ishida HK, et al. Specificity of carbohydrate structures of gangliosides in the activity to regenerate the rat axotomized hypoglossal nerve. Glycobiology. 2001;11(2):125-30.

470.	Okada M, Itoh Mi M, Haraguchi M, Okajima T, Inoue M, Oishi H, et al. b-series Ganglioside deficiency exhibits no definite changes in the neurogenesis and the sensitivity to Fas-mediated apoptosis but impairs regeneration of the lesioned hypoglossal nerve. The Journal of biological chemistry. 2002;277(3):1633-6.

471.	Kobayashi T, Goto I. A sensitive assay of lysogangliosides using high-performance liquid chromatography. Biochimica et biophysica acta. 1991;1081(2):159-66.

472.	Numakawa T, Adachi N, Richards M, Chiba S, Kunugi H. Brain-derived neurotrophic factor and glucocorticoids: reciprocal influence on the central nervous system. Neuroscience. 2013;239:157-72.

473.	Ferrari G, Greene LA. Promotion of neuronal survival by GM1 ganglioside. Phenomenology and mechanism of action. Annals of the New York Academy of Sciences. 1998;845:263-73.

474.	Furukawa K, Ohmi Y, Ohkawa Y, Tajima O, Furukawa K. Glycosphingolipids in the regulation of the nervous system. Advances in neurobiology. 2014;9:307-20.

475.   Hadaczek P, Wu G, Sharma N, Ciesielska A, Bankiewicz K, Davidow AL, et al. GDNF signaling implemented by GM1 ganglioside; failure in Parkinson's disease and GM1-deficient murine model. Experimental neurology. 2015;263:177-89.

476.   Mishra R, Hoop CL, Kodali R, Sahoo B, van der Wel PC, Wetzel R. Serine phosphorylation suppresses huntingtin amyloid accumulation by altering protein aggregation properties. Journal of molecular biology. 2012;424(1-2):1-14.

477.   Clayton JA, Collins FS. Policy: NIH to balance sex in cell and animal studies. Nature. 2014;509(7500):282-3.

478.   Rodriguez-Lebron E, Denovan-Wright EM, Nash K, Lewin AS, Mandel RJ. Intrastriatal rAAV-mediated delivery of anti-huntingtin shRNAs induces partial reversal of disease progression in R6/1 Huntington's disease transgenic mice. Molecular therapy : the journal of the American Society of Gene Therapy. 2005;12(4):618-33.

479.   Frank S. Treatment of Huntington's disease. Neurotherapeutics : the journal of the American Society for Experimental NeuroTherapeutics. 2014;11(1):153-60.

480.   Godinho BM, Malhotra M, O'Driscoll CM, Cryan JF. Delivering a disease-modifying treatment for Huntington's disease. Drug discovery today. 2015;20(1):50-64.

481.   Aronin N, DiFiglia M. Huntingtin-lowering strategies in Huntington's disease: antisense oligonucleotides, small RNAs, and gene editing. Movement disorders : official journal of the Movement Disorder Society. 2014;29(11):1455-61.

482.   Wang YL, Liu W, Wada E, Murata M, Wada K, Kanazawa I. Clinico-pathological rescue of a model mouse of Huntington's disease by siRNA. Neuroscience research. 2005;53(3):241-9.

483.   Skotte NH, Southwell AL, Ostergaard ME, Carroll JB, Warby SC, Doty CN, et al. Allele-specific suppression of mutant huntingtin using antisense oligonucleotides: providing a therapeutic option for all Huntington disease patients. PloS one. 2014;9(9):e107434.

484.   Maiuri T, Woloshansky T, Xia J, Truant R. The huntingtin N17 domain is a multifunctional CRM1 and Ran-dependent nuclear and cilial export signal. Human molecular genetics. 2013;22(7):1383-94.