THE ROLE OF AXONAL TRANSPORT AND MITOCHONDRIAL DYSFUNCTION IN NEURODEGENERATION - FOCUSING ON HUNTINGTON'S DISEASE

PhD Thesis

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Szeged
2015

Publications directly related to the thesis

I. Braunstein KE*, Eschbach J*, **Róna-Vörös K***, Soylu R, Mikrouli E, Larmet Y, René F, De Aguilar JL, Loeffler JP, Müller HP, Bucher S, Kaulisch T, Niessen HG, Tillmanns J, Fischer K, Schwalenstöcker B, Kassubek J, Pichler B, Stiller D, Petersen A, Ludolph AC, Dupuis L (2010): A point mutation in the dynein heavy chain gene leads to striatal atrophy and compromises neurite outgrowth of striatal neurons. Hum Mol Genet 19: 4385-98 (original paper; **IF: 8,058**)

II. **Róna-Vörös K**, Eschbach J, Vernay A, Wiesner D, Schwalenstocker B, Geniquet P, Mousson De Camaret B, Echaniz-Laguna A, Loeffler JP, Ludolph AC, Weydt P, Dupuis L (2013): Full-length PGC-1α salvages the phenotype of a mouse model of human neuropathy through mitochondrial proliferation. Hum Mol Genet 22: 5096-106 (original paper; **IF: 6,677**)

III. Vámos E, **Vörös K**, Vécsei L, Klivényi P (2010): Neuroprotective effects of L-carnitine in a transgenic animal model of Huntington's disease. Biomed Pharmacother 64: 282-6 (original paper; **IF: 2,208**)

Total impact factor of original papers directly related to the thesis: **16,943**

Publications not directly related to the thesis

I. **Róna-Vörös K**, Weydt P (2010): The role of PGC-1α in the pathogenesis of neurodegenerative disorders. Curr Drug Targets 11: 1262-9 (review; **IF: 3,061**)

II. Vámos E, **Vörös K**, Zádori D, Vécsei L, Klivényi P (2009): Neuroprotective effects of probenecid in a transgenic animal model of Huntington's disease. J Neural Transm 116: 1079-86 (original paper; **IF: 2,259**)

Cumulative impact factor: **22,263**

** The authors wish it to be known that, in their opinion, the first 3 authors should be regarded as joined First Authors and are listed in alphabetical order.*

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List of abbreviations

3-NP	3-nitropropionic acid
AD	Alzheimer's disease
ALC	acetyl-L-carnitine
ALS	amyotrophic lateral sclerosis
BAT	brown adipose tissue
BDNF	brain derived neurotrophic factor
CAG	cytosine-adenine-guanine
CoA	Coenzyme A
Cra	*Cramping* allele of the *Dync1h1* gene (p.Y1055C mutation)
DARPP32	dopamine and cAMP regulated phosphoprotein of a molecular weight of 32 kDa
DYNC1H1	dynein heavy chain gene 1
EM	electron microscopy
EM48	polyclonal antibody recognizing the first 256 amino acids of human huntingtin
FL	full length
GFAP	glial fibrillary acidic protein
HAP1	huntingtin associated protein 1
HD	Huntington’s disease
HSP	heat shock protein
HTT	huntingtin
i.p.	intraperitoneal
IR	immunoreactive
IT	internally truncated
Loa	*Legs at odd angles* allele of the *Dync1h1* gene
LC	L-carnitine
MRI	magnetic resonance imaging
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MSNs	medium sized spiny neurons
mtDNA	mitochondrial DNA
NT	N-terminal truncated
PD	Parkinson's disease
PET	positron emission tomography
PGC-1 α	PPARγ coactivator 1 alpha, encoded by the *PPARGC1A* gene

PPAR	peroxisome proliferator-activated receptor
ROS	reactive oxygen species
SDH	succinate dehydrogenase
SMA-LED	spinal muscular atrophy with lower extremity predominance
TA	tibialis anterior muscle
TCS	Tissue Classification Software
TH	tyrosine hydroxylase
WAT	white adipose tissue

Summary

Neurodegenerative disorders are devastating brain diseases whose importance for the ageing population is steadily increasing. Huntington's disease (HD) is the most common known autosomal dominantly inherited neurodegenerative disorder caused by an expanded polyglutamine tract in the protein called huntingtin. Its characteristic neuropathological changes mainly affect the striatum. Despite continuous research efforts, the exact pathomechanism causing neurodegeneration is not fully known, and there is no specific neuroprotective therapy for Huntington's disease or any other neurodegenerative disease so far.

We investigated some aspects of two pathogenic hallmarks thought to be important in neurodegenerative diseases: disrupted axonal transport and mitochondrial dysfunction. Dynein is a motor protein responsible for intracellular transport of cargoes toward the minus-end of microtubules and mediates retrograde axonal transport in neurons. *Cramping* mice bearing a point mutation in the dynein cytoplasmic heavy chain gene exhibit disturbed dynein function and develop characteristic phenotype including hind limb clasping, twisting the body and progressive loss of muscle tone. This phenotype is partially explained by early-onset sensory neuropathy. Substantial body of evidence suggest the involvement of mitochondrial dysfunction in neurodegeneration, and increased mitochondrial mass is observed in many of these diseases. This mitochondrial proliferation is suggested to be a compensatory mechanism, however the underlying processes are not fully known. Peroxisome proliferator-activated receptor (PPAR) gamma coactivator 1 alpha (PGC-1α) is a transcriptional coactivator considered as master regulator of mitochondrial biogenesis and metabolism, moreover loss of its function was proposed as a key factor during neurodegeneration.

We showed that *Cramping* mice carrying a dynein point mutation have striatal involvement which may be responsible for their behavioural abnormalities, such as hyperactivity, progressive motor incoordination and early muscle weakness. We confirmed the selective down-regulation of D1 dopamine receptors expression, striatal atrophy, accompanied with enlargement of lateral ventricles, decreased binding to either D1 or D2 dopamine receptors and prominent astrocytosis in the striatum of *Cramping* mice. All these findings support the *in vivo* requirement of cytoplasmic dynein in the function of the striatum, and highlight the importance of the dynein motor and axonal transport disruption in the pathogenesis of striatal diseases, notably Huntington's disease.

Others from our workgroup described systemic mitochondrial dysfunction (Eschbach et al., 2013) and compensatory mitochondrial proliferation accompanied by PGC-1α activation

in *Cramping* mice. We demonstrated that the genetic ablation of full length PGC-1α (FL-PGC-1α) in *Cramping* mice completely abolished the previously observed increases in mitochondrial DNA levels in muscles and reverted the mitochondrial phenotype. Moreover FL-PGC-1α ablation significantly worsened the overall and neurological phenotype of *Cramping* mice. Thus, FL-PGC-1α is required for the compensatory maintenance of mitochondrial function in vivo. This observation contributes to a better understanding of the underlying mechanisms in mitochondrial dysfunction related to disease.

Further, we tested the effects of L-carnitine (LC) administration in the N171-82Q transgenic mouse model of HD. LC is an antioxidant nutrient also enhancing mitochondrial function. We demonstrated that L-carnitine administration in higher dose significantly extended the survival and ameliorated the motor symptoms of the N171-82Q transgenic HD mice. Moreover it preserved striatal neuron count and decreased the number of intranuclear huntingtin aggregates. Thus, our data suggest that L-carnitine is neuroprotective and may possibly be beneficial in the treatment of Huntington's disease.

In all, our data highlight the role of the molecular motor dynein and mitochondrial dysfunction in neurodegeneration, notably in Huntington's disease. These findings contribute to better understanding of the pathomechanism of neurodegenerative diseases and offer potential therapeutic ways.

I. Introduction

Neurodegenerative disorders are devastating diseases characterized by progressive, selective loss of specific neuronal systems. Besides the vulnerable neurons, the damage develops also in neighbouring non-neuronal supporting cells, and in multiple cell types in the periphery (Ilieva et al., 2009). Despite recent and continuous research efforts the exact pathomechanism which causes neuronal dysfunction and cell death still remains unknown in the most common sporadic or familial neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), and in the inherited forms such as Huntington's disease (HD), where the disease causing gene and its protein product is known. Despite the heterogeneity of neurodegenerative diseases, there are several common factors well documented for their importance in pathogenesis. Two of these factors, disrupted axonal transport and mitochondrial dysfunction, were investigated during this PhD work, with special focus on Huntington's disease.

I.1. Huntington's disease

Huntington's disease (HD) is the most common known autosomal dominantly inherited neurodegenerative disorder. Clinically it is characterized by motor dysfunction, cognitive and behavioural impairment and psychiatric disturbances, typically starting in mid-life and progressing relentlessly to death after a course of 10-25 years (Walker, 2007). The motor abnormalities of HD derive from dysfunction of brain regions involved in involuntary movement control, in particular the striatum, leading to uncontrollable dance-like movements (''chorea'') as a clinical hallmark. Progressive gait impairment, brady-, and hypokinesia also develop.

HD is caused by expansion of a cytosine-adenine-guanine (CAG) trinucleotide repeat in the protein coding region of the IT15 gene encoding an elongated polyglutamine tract in the huntingtin (HTT) protein (The Huntington's Disease Collaborative Research Group, 1993). The number of CAG repeats in the gene determines HD phenotype. Normal alleles also contain CAG repeats, with 35 or less being non-pathogenic. Incomplete penetrance is observed with 36–39 repeats. Over 40 repeats, the disease is fully penetrant. The length of CAG repeats expansion has been shown to account for about 60% of the variation in age-at-onset, with the remainder represented by modifying genes and environmental factors (Gusella and Macdonald, 2009, Weydt et al., 2009). Neuropathological hallmark of HD is the loss of gamma-aminobutyric acidergic medium-sized spiny neurons (MSNs) in the striatum, neurons in the deeper layers of the cerebral cortex are also affected (Walker, 2007). The N-terminal

fragments of mutant HTT accumulate in the nuclei of the affected neurons and form intranuclear aggregates (DiFiglia et al., 1997, Gutekunst et al., 1999).

A great advantage for investigating the pathomechanism of HD or testing potential protective compounds is the existence of animal models of the disease. Transgenic mice expressing the N-terminal fragment of HTT with 82 CAG repeat, develop a progressive neurological disorder (Schilling et al., 1999). These mice (N171-82Q) fail to gain weight, exhibit an irregular, uncoordinated gait, hypokinesis, tremor and frequent hind limb clasping. In the open field they show decreased spontaneous locomotor activity and reduced explorative behaviour (Klivényi et al., 2006). The animals have shortened lifespan, as they die at an average age of 110-130 days. The brain of these mice is slightly smaller, but grossly normal, and exhibits striatal atrophy and neuronal intranuclear inclusions that are immunopositive for huntingtin and ubiquitin (Schilling et al., 1999). The N171-82Q mouse strain has the advantage of a relatively fast disease progression which provides a good practical use of testing potentially neuroprotective compounds compared with other transgenic (Hodgson et al., 1999) or knock-in (Lin et al., 2001; Menalled et al., 2003) HD mouse models. Still this strain displays slighter phenotype than R6/2 transgenic mice (Mangiarini et al., 1996), which enables the tested compound to exert its supposed beneficial effects and facilitates the detection of potential differences in the onset of symptoms.

I.2. Axonal transport and the molecular motor dynein

Axonal transport is a bidirectional process through which materials and signals are exchanged between the neuronal cell body and the synapse. Neurons are among the largest cells in humans and have extensive processes with large distances separating neuronal cell bodies from axons and synapses which makes them uniquely dependent on axonal transport (Eschbach and Dupuis, 2011; Schiavo et al., 2013; Chevalier-Larsen and Holzbaur, 2006; Morfini et al., 2009). Protein synthesis occurs in the cell body, vesicles and materials are anterogradely transported to the synapses. On the other hand cell body is the site for degradation of misfolded or aggregated proteins, retrograde transport is required for return of degradation products. Further, communication between the cell periphery and the cell center via transport of signalling complexes and neurotrophic factors is also crucial for neuronal maintenance and survival. Axonal transport is mediated primarily by microtubule-based molecular motors, large enzymes that use the energy of ATP hydrolysis to generate movement. Members of the kinesin superfamily are most, but not all, responsible for anterograde transport, while retrograde axonal transport is mainly mediated by the

cytoplasmic dynein (Goldstein and Yang, 2000).

Cytoplasmic dynein is ubiquitously expressed, and is indispensable for the normal development. It is a large motor protein complex composed by different subunits such as the heavy chain, intermediate chains, light-intermediate chains and light chains (Pfister et al., 2006). At the core of the molecule lies a homodimer of heavy chains forming the sites of ATP binding and microtubule binding (Gee et al., 1997; Pfister et al., 2005; Gennerich et al., 2007). At the base of the motor are a number of intermediate, light-intermediate and light chains which are thought to function by maintaining the stability of the complex and cargo attachment (Banks and Fisher, 2008). Dynein functions in association with a multi-protein regulatory complex called dynactin (Schafer et al., 1994; Karki and Holzbaur, 1995) which participates in cargo binding but binds also directly to microtubules probably to increase the efficiency of dynein mediated motility (King and Schroer, 2000). As the motor complex dynein/dynactin is mainly responsible for the retrograde transport, one of the most important roles might be the removal of aggregation-prone proteins from the cell periphery to the place of degradation (Rubinsztein et al., 2005). Of note, besides the role in the retrograde axonal transport, dynein is involved in other basic cellular functions, such as endoplasmic reticulum and Golgi trafficking, mitosis, autophagy and is required for normal development.

Loss of dynein/dynactin function is considered an important factor in the pathogenesis of neurodegenerative diseases (Eschbach and Dupuis, 2011; Schiavo et al., 2013; Chevalier-Larsen and Holzbaur, 2006; Levy and Holzbaur, 2006). Impairment of retrograde axonal transport appears to be one of the earliest pathogenic changes during neurodegeneration (Morfini et al., 2009), and transgenic inhibition of retrograde axonal transport induces the degeneration of motor neurons (LaMonte et al., 2002; Teuling et al., 2008). Mutations in the dynactin subunit $p150^{glued}$ were discovered in familiar forms of motor neuron disease, including ALS and distal spinal and bulbar muscular atrophy (Puls et al., 2003, 2005; Munch et al., 2004), as well as in Perry syndrome, a rare atypical form of Parkinson's disease (Farrer et al., 2009; Wider et al., 2010). Moreover dynein itself is associated with human pathology, mutations in the gene encoding the dynein heavy chain (*DYNC1H1*) were found in Charcot-Marie-Tooth disease axonal type 2 (Weedon et al., 2011), in spinal muscular atrophy with lower extremity predominance (SMA-LED) (Harms et al., 2012; Scoto et al., 2015) and in malformations of cortical development (Poirier et al., 2013).

Several lines of evidence suggest that altered axonal transport and dynein contribute to the pathogenesis of HD. Swollen axons and accumulated vesicular proteins were found in HD patient tissue also in several animal models of HD (Chevalier-Larsen and Holzbaur, 2006;

Morfini et al., 2009). Dynein, as well as $p150^{Glued}$, are binding partners of HTT and of huntingtin associated protein 1 (HAP1) (Li et al., 1995; Li et al., 1998). Moreover, the activity of the dynein complex is positively regulated by wild-type HTT, and strongly decreased by mutant HTT (Caviston et al., 2007; Gauthier et al., 2004). However, the effects of mutant HTT on axonal transport are widespread by affecting both anterograde and retrograde fast axonal transport (Morfini et al., 2009).

The existence of mouse strains bearing mutations in the dynein heavy chain gene (*Dync1h1)* provides an advantage to investigate the role of dynein in neurodegeneration in vivo. There exist currently three mouse strains, *Legs at odd angles* (*Loa*) and *Cramping* (*Cra*) were created by N-ethyl-N-nitrosourea (ENU) mutagenesis, both have a point mutation in *Dync1h1* (Hafezparast et al., 2003), while *Sprawling (Swl)* bears a radiation-induced 9-bp deletion in the gene (Chen et al., 2007). Both mutations are located in the domain involved in homodimerization of the molecular motor. It was shown in *Loa/Loa* embryos that the mutation impairs the ability of dynein to sustain fast retrograde transport (Hafezparast et al., 2003), also leads to decreased retrograde transport in adult dynein mutant motor neurons (Perlson et al., 2009). The three mouse strains have similar phenotypes: homozygous pups die before or within 24 hours of birth, heterozygous mice show unusual twisting of the body, hind limb clasping when held by the tail, develop abnormal gait and have a normal life-span (Hafezparast et al., 2003; Chen et al., 2007). Initially it was suggested that *Cra/+* and *Loa/+* mice display lower motor neuron degeneration, but these findings were not reproduced. On the contrary, a proprioceptive sensory neuropathy was observed in these mice (Ilieva et al., 2008; Dupuis et al., 2009; Chen et al., 2007). However we think that this could not fully explain the mouse phenotype and aimed at a more detailed characterisation.

I.3. Mitochondria in neurodegeneration

Mitochondria are the main energy source of cells and tissues as they provide the production of ATP via oxidative phosphorylation. Besides this major function they play many other roles, such as contributing to cellular stress responses, production of reactive oxygen species (ROS), regulating homeostatic signalling pathways, house parts of the pyrimidine and lipid biosynthesis and modulate Ca^{2+} flux (Nunnari and Suomalainen, 2012). They adopt to the cell's changing energetic needs and protect it from oxidative damage. Therefore mitochondria are key regulators of cell death and survival. They are especially important in tissues with high energy demands like the brain and muscles. Multiple lines of evidence indicate that mitochondrial dysfunction is an early, active, common contributor to all major

neurodegenerative diseases (Lin and Beal, 2006; Nunnari and Suomalainen, 2012). Mutations in mitochondrial DNA (mtDNA) or nuclear-encoded mitochondrial proteins cause a heterogeneous group of different diseases, including neurodegenerative and metabolic disorders (Nunnari and Suomalainen, 2012).

Mitochondrial dysfunction and oxidative stress are also early signs in HD and there is a strong evidence for their causal involvement in the pathogenesis. Toxicological studies with 3-nitropropionic acid (3-NP) and malonate, that selectively inhibit succinate dehydrogenase (complex II), have long established that striatal neurons are exquisitely vulnerable to mitochondrial dysfunction (Beal et al., 1993; Ludolph et al., 1991). Mutant HTT can affect mitochondrial function directly and indirectly (Lin and Beal, 2006). It directly alters the calcium signalling at the mitochondrial membrane leading to a calcium influx into the cytoplasm, which is a potent pro-apoptotic signal postulated to directly lead to cell death (Panov et al., 2002; Tang et al., 2005). Indirectly, mutant HTT could affect mitochondrial function by transcriptional dysregulation (Sugars and Rubinsztein, 2003). It interacts with several transcription factors and coactivators, including CREB-binding protein, Sp1 and TATA binding protein, p53 and peroxisome proliferator-activated receptor (PPAR) gamma coactivator 1 alpha (PGC-1α) (Cha, 2007).

Peroxisome proliferator-activated receptor (PPAR) gamma coactivator 1 alpha (PGC-1α) is a transcriptional coactivator that regulates mitochondrial biogenesis and metabolic pathways. PGC-1α was discovered as a key regulator of adaptive thermogenesis which is a key component of energy expenditure in mammals (Puigserver et al., 1998). PGC-1α orchestrates in a promoter specific manner the activity of a wide range of important transcription factors such as PPARs, estrogen receptor and retinoic acid receptor. Through the regulation of these and other transcription factors, PGC-1α plays a key role in coordinating the expression of a wide range of nuclear encoded mitochondrial proteins. Due to this key position in the metabolic regulatory network PGC-1α is labelled as a “master regulator” of respiration and mitochondrial biogenesis (Canto et al., 2009; Handschin and Spiegelman, 2006; Handschin 2009).

PGC-1α exists in multiple isoforms, that fall into three major families: full length isoforms (FL) that are canonical isoforms including PGC-1α1, N terminal truncated isoforms (NT) that include the recently described NT- PGC-1α and PGC-1α4, and internally truncated (IT) isoforms such as PGC-1α2 and 3, that do not include exons 3-5 (Ruas et al., 2012; Zhang et al., 2009). Interestingly, the different PGC-1α functions are at least partially segregated among the different isoforms with FL-PGC-1α being more specialized in increasing

mitochondrial biogenesis, and NT-PGC-1α being more involved in muscle anabolism (Ruas et al., 2012).

One important step which proposed PGC-1α for neurodegeneration, especially HD was the generation and analysis of animal models with genetically modified PGC-1α expression. Independently, two research teams developed PGC-1α knock-out mouse model (Leone et al., 2005; Lin et al., 2004). Later it turned out that while knock-out mice from Lin and collaborators are complete PGC-1α knock-out (Ruas et al., 2012; Lin et al., 2004), mice from Leone et al. are only knock-out for FL-PGC-1α preserving all IT-isoforms and however NT-isoforms are truncated for the 16 C-terminal amino-acids they show a roughly preserved function (Leone et al., 2005; Chang et al., 2012). These mice will thus be termed as FL-PGC-1α -/- mice. The differences between the two PGC-1α knock-out models are due to different methods used for the PGC-1α gene targeting. Both research groups reported that their respective mouse lines display several metabolic abnormalities, such as cold intolerance and impaired body weight regulation. Moreover they share many phenotypical similarities to transgenic mouse models of HD. Mice show hind limb clasping, dystonic posturing, stimulus-induced myoclonus and an exaggerated startling response. Whereas one strain exhibits profound hyperactivity (Lin et al., 2004), the other shows increased anxiety levels (Leone et al., 2005). At the histopathological level, both mouse strains display spongiform-like vacuolization predominantly in the striatum, and – less consistently – in other brain regions, such as the hippocampus or the pyramidal cells of the cortex (Leone et al., 2005; Lin et al., 2004). In the striatum strong immunoreactivity for glial fibrillary acidic protein (GFAP) was detected, indicating gliosis (Lin et al., 2004). Further, more detailed neuropathological examination revealed that FL-PGC-1α -/- mice do not show immunostaining for a wide range of neurodegeneration-related proteins and rather suggested to model mitochondrial encephalopathies (Szalárdy et al., 2013).

Further investigations provided more direct evidence of a role for PGC-1α function in the pathogenesis of HD (McGill and Beal, 2006). Profound temperature dysregulation was detected in HD transgenic mice and positive regulation of PGC-1α dependent genes, including uncoupling protein 1, was attenuated in brown adipose tissue (BAT) (Weydt et al., 2006). Moreover mutant HTT supresses the expression of PGC-1α by interfering with the formation of a key transcription complex, the CREB/TAF complex (Cui et al., 2006). It was shown that genetic ablation of PGC-1α aggravates the phenotype of a knock-in HD mouse model and *in vivo* transfection with PGC-1α can rescue some aspects of the striatal pathology in the R6/2 transgenic HD mice (Cui et al., 2006). The clinical significance of the PGC-1α

system for HD is demonstrated by that common variants of the *PPARGC1A* gene are associated with a significant delay in onset of motor symptoms in HD patients of several years (Weydt et al., 2009, 2014; Taherzadeh-Fard et al., 2009; Soyal et al., 2012).

Increased mitochondrial mass is frequently observed in human diseases directly or indirectly involving mitochondrial dysfunction (Michel et al., 2012), and is usually called "mitochondrial proliferation". It is considered as a compensatory mechanism mitigating a compromised energy metabolism (Michel et al., 2012). Consistent with this view, muscle overexpression of FL-PGC-1α, leading to remarkable mitochondrial proliferation, is broadly protective for muscle function in mitochondrial myopathies (Wenz et al., 2008; Dillon et al., 2012), also in amyotrophic lateral sclerosis (Da Cruz et al., 2012). Albeit mitochondrial proliferation might also be detrimental through alterations of mitochondrial regulatory functions such as apoptosis, calcium metabolism or oxidative stress. Increased mitochondrial mass was found to be associated with apoptotic features in muscle fibers (Aure et al., 2006) and forced mitochondrial biogenesis leads to muscle atrophy and dilated cardiomyopathy (Miura et al., 2006; Lehman et al., 2000). Indeed, increasing mtDNA through transgenic overexpression of Twinkle and Tfam, two factors that regulate mtDNA replication was deleterious for respiratory chain activities (Ylikkallio et al., 2010). Mechanisms underlying mitochondrial proliferation are unknown. Both increased mitochondrial biogenesis (Lin et al., 2002) or decreased mitochondrial autophagy (Masiero et al., 2009) are sufficient to increase mitochondrial mass. Whether one of these mechanisms, or both, are involved to evoke mitochondrial proliferation in disease conditions remains unknown.

Recently, others from our workgroup showed that the *Cramping* mice develop systemic mitochondrial dysfunction with ragged red fibers (Eschbach et al., 2013). Moreover, increased mitochondrial mass accompanied by PGC-1α activation was detected.

An important opportunity provided by the existence of animal models of neurodegenerative diseases is the test of supposed protective agents. One possible candidate is L-carnitine (LC) which has antioxidant properties and is used to improve mitochondrial function. LC is a nutrient also synthesised in vivo from the amino acids lysine and methionine, however 75% comes from dietary sources (Steiber et al., 2004). The main role of LC is in cellular energy metabolism, it improves mitochondrial energetics and scavenges free radicals (Calabrese et al., 2012). It plays a role in the transport of long-chain fatty acids into the mitochondria for beta-oxidation, providing energy and acetyl-coenzyme A (CoA) formation. On the other hand, it contributes to the removal of short- and medium-chain fatty acids preventing these toxic accumulation in the mitochondria and leading to an increase of

free CoA (Calabrese et al., 2012). Thus, it controls the mitochondrial acetyl-CoA/ CoA ratio which is crucial for mitochondrial metabolism.

Animal experimental data suggested long ago that LC may have antioxidant properties (Koudelova et al., 1994). It can reduce oxidative stress in aged animals (Poon et al., 2006, Long et al., 2009; Rani and Panneerselvam, 2002; Savitha et al., 2005) and acts as a free radical scavenger (Arockia Rani and Panneerselvam, 2001). LC also improves age-related oxidative DNA damage, nucleic acid status and mitochondrial enzymes activity in aged rats (Haripriya et al., 2004; 2005; Juliet et al., 2005). LC was found to be protective against nickel-induced neurotoxicity in Neuro-2a cell line via attenuating the harmful ROS and malondialdehyde level elevation, ATP reduction and disrupted mitochondrial membrane potential (He et al., 2011). Also it prevented peroxynitrite and free radicals production induced by methamphetamine in adult male mice (Virmani et al., 2002). Moreover acetyl-LC modulates endogenous cellular defence mechanisms and stress response by inducing heat-shock proteins (HSPs), heme oxygenase 1 and SOD2 and prevents age-related changes in rats (Calabrese et al., 2006; 2010).

A double-bind placebo controlled human study did not find any significant changes in HD patients as compared with healthy subjects that upon low dose L-carnitine (Goety et al., 1990). We suggested that LC may be effective in higher doses.

II. Aims

We investigated the role of the molecular motor dynein and mitochondrial dysfunction in neurodegeneration using different experimental mouse models.

The aims of our studies were as follows:

(i) to provide a direct genetic evidence linking cytoplasmic dynein mutation to striatal dysfunction by detailed characterisation of *Cramping* mice bearing a point mutation in the dynein heavy chain 1 gene.

(ii) to study whether the recently showed systemic mitochondrial dysfunction (Eschbach et al., 2013) and compensatory mitochondrial proliferation in *Cramping* mice is FL-PGC-1α dependent. We investigated the effect of FL-PGC-1α ablation in *Cramping* mice with extended characterisation of the phenotype using longitudinally performed behavioural tests, muscle histology, electron microscopy.

(iii) to study whether L-carnitine administration, in high dose, exerts beneficial effects on the survival as well as the behavioural and neuropathological phenotype in N171-82Q transgenic mouse model of HD.

III. Materials and methods

III. 1. Animals

We used different experimental mouse models such as *Cramping* mice carrying a point mutation in the dynein heavy chain 1 gene, FL-PGC-1α -/- mice and the N171-82Q transgenic mouse model of HD.

Heterozygous *Cra*/+ mice were obtained from Ingenium Pharmaceuticals AG, Martinsried, Germany. They were identified by tail DNA genotyping as previously described (Hafezparast et al., 2003). Wild-type littermates were used as controls. FL-PGC-1α -/- mice were obtained from Prof. Daniel Kelly (Leone et al., 2005). They were initially published as full PGC-1α -/- mice but newer results of our workgroup and those of others (Chang et al., 2012) show that these mice are only ablated for full length PGC-1α. We created *Cramping* FL-PGC-1α -/- (referred as Cra/FLα -/-) mice in two crossing steps and used F1-generation mice of the four genotypes in the same B6C3He-hybrid background. N171-82Q mice were originally obtained from Jackson Laboratories (Maine, USA) and backcrossed to the B6C3F1 background. The offspring were genotyped by using a PCR assay on the tail DNA at the age of 4 weeks. Animals were maintained in a temperature- and humidity-controlled environment on a 12h light/dark cycle and received food and water *ad libitum*.

For histological analysis, animals were deeply anesthetized with 1 mg/kg body weight ketamine chlorhydrate and 0.5 mg/kg body weight xylazine or with isoflurane (Abott Laboratories Ltd., Queenborough, UK), and transcardially perfused with 4% paraformaldehyde in 0.1 M pH 7.4 phosphate buffer. Tissues were then quickly dissected, post-fixed for 24 hours in 4% paraformaldehyde, and cryoprotected for 48 hours with 30% sucrose or 10% glycerol in PBS before cryostat sectioning. For biochemical analysis, animals were sacrificed and tissues were quickly dissected, snap frozen in liquid nitrogen and stored at -80°C until use.

All animal experiments were performed under the supervision of authorized investigators, followed current EU regulations and were approved by the local animal care committee.

III. 2. Measurement of body temperature and weight

In Cra/FLα -/- and the 3 comparative mouse groups body temperature was monitored 3 times every week from the age of 6 weeks as described previously (Weydt et al., 2006) at noon with a telemetry system using subcutaneously implanted transponders placed in the

interscapular space (Bio Medic Data Systems, Seaford, DE, USA). At the same time body weight was also measured.

III. 3. Testing of motor performance and behaviour

One week before the start of the tests, animals were brought to the behavioural analysis facility and handled every day. Only male mice were used for behavioural tests. A battery of behavioural tests were performed at 3 and 12 months of age in the study comparing *Cra*/+ versus wild-type mice including the following tests: grip strength, rotarod, open field, elevated plus maze, Morris water maze. Whereas the cohorts of the *Cra*/+, FL-PGC-1*α* -/- crossbreeding study were tested longitudinally from 2 to 12 months of age with the range of slightly different tests: modified SHIRPA, grip strength, rotarod, open field, elevated plus maze, string agility.

Modified SHIRPA protocol (Rogers et al., 2001) was used to detect the overall neurological phenotype of the mice.

Muscle grip strength was measured using a Bioseb gripmeter (Vitrolles, France) on forelimbs and all limbs. Each assay was performed in triplicate and measurements were averaged.

The rotarod test was used to assess motor coordination and balance. Mice had to keep their balance on a rotating rod at a continuous acceleration from 4 to 40 rpm in 300 s (Rotarod Version 1.2.0. MED Associates Inc., St. Albans, VT). The time (or latency) it took the mouse to fall off the rod was measured. Each mouse had to perform 3 trials separated by 15 minutes each other, and the 3 trials were averaged.

To identify differences in locomotor activity and exploratory behaviour, mice were tested in the open field. In this test, animals were placed at the border of a square arena (50 cm x 50 cm) and allowed to explore the arena freely. The exploration time was 30 minutes in the study comparing *Cra*/+ versus wild-type mice and 10 minutes in the cohorts of PGC-1α ablation in these mice. Locomotor activity was assessed by the total distance moved and the average velocity. To determine the exploratory behaviour, the number of rearings was measured. Open field was also performed in the N171-82Q transgenic HD mice in a slightly different apparatus as described later.

To assess anxiety, mice were evaluated using an elevated plus maze paradigm. The maze was elevated 92 cm above the floor, and consisted of 4 arms of 30 cm x 5 cm each, including 2 opposite ‘‘closed’’ arms surrounded by dark walls and 2 opposite ‘‘open’’ arms exposed without any walls. The centre of the maze was a 5 cm x 5 cm common area. Each

mouse was placed for a single trial at the centre of the maze facing a closed arm, and allowed to explore the maze freely for a period of 5 min. The amount of time (in seconds) spent in both the open arms and closed arms was recorded.

To measure reference learning (acquisition) and memory (retention), the Morris water maze was performed. The device consisted of a circular pool of 120 cm diameter, filled with water (25-27°C), which was made opaque by adding 2 liter of milk. The pool was divided into 4 equal-sized quadrants and was surrounded by grey curtains covered with various visual cues, which helped the mice to orient their location in the pool. A 10 cm platform was placed in the quadrant A, such that the platform was 1 cm below the water surface and visually indiscernible to the animals. On each trial, mice were allowed to swim for a maximum of 60 s and were released from 4 different defined positions. If the animal failed to discover the location of the platform in 60 s, it was guided to the platform and then allowed to stay for 30 s. After removal, mice were placed under an infrared lamp and allowed to warm up and dry off. The test was divided into 2 phases, an acquisition phase (18 trails, six/day), followed by a reversal phase during which the platform was moved to the opposite quadrant (12 trails, six/day). Escape latency and swum distance were analysed during both acquisition and reversal learning, and 2 successive trials were averaged into one block. All paths were tracked and analysed with an electronic imaging system (Viewer 2.2.0.55, BIOBSERVE GmbH, Bonn, Germany) at a frequency of 15 Hz and a spatial resolution of 720 x 576 pixels.

String agility test was performed to access forepaw grip capacity and agility. Mice were placed in the centre of a 50 cm long string suspended about 33 cm above a padded surface between two platforms. Mice were allowed to grip the string with only their forepaws and then released for a maximum of 60 sec. A rating system, ranging between 0 and 5, was employed to assess string agility for a single 60 sec trial (0=animal unable to remain on string, 1=hangs by two forepaws, 2=attempts to climb onto string, 3=two forepaws and one or both hindpaws around string, 4=four paws and tail around string, with lateral movement, 5=escape to the platform). As both *Cramping* and Cra/FLα -/- mice showed a severe defect in muscle string agility (from early age score 0) we further detected the time spent on the string till falling down giving the maximum of 60 sec for the mice who reached the platform during the trial.

III. 4. Real time (RT) qPCR

Total RNA was extracted using Trizol (Invitrogen, Cergy-Pontoise, France) according to the manufacturer's instructions. cDNA synthesis was performed using 1 µg of total RNA

(iScript cDNA Synthesis kit; Bio-Rad, Marne La Coquette, France). PCR analysis was carried out as described (Dupuis et al., 2009) on a Bio-Rad CFX96 System using iQSYBR Green Supermix. A specific standard curve was performed for each gene in parallel, and each sample was quantified in duplicate. PCR conditions were 3 min at 94°C, followed by 40 cycles of 45 s at 94°C and 10 s at 60°C. Data were analyzed using the iCycler software, and normalized to the reference genes encoding either the 18S ribosomal subunit and the RNA polymerase II mRNA.

III. 5. In vivo brain imaging

Magnetic resonance imaging (MRI) was performed in the In-vivo-Imaging Laboratory of Boehringer Ingelheim Pharma GmbH & Co. KG Biberach, Germany. MRI data were acquired on a Bruker Biospec 47/40 scanner (Bruker BioSpin, Ettlingen, Germany) at 4.7 Tesla. Acquisitions were performed at 5 and 10 months of age, respectively (*Cra*/+, wild-type mice, $n = 20$). Mice were anaesthetized through continuous inhalation of 1.2-1.5% isoflurane (in 70:30 N_2O:O_2) and fixed in a stereotactic head holder. For anatomical analysis of the mouse brain contiguous sets of 6 horizontal T_2-weighted images were acquired using a RARE sequence with slice thickness 600 µm (no gap). Data processing was performed via voxel-based volumetry by the in-house developed software package Tissue Classification Software (TCS). The analysis was blinded, evaluated by the same experienced investigator. The striatum was identified in 4 consecutive horizontal slices as compared with the Mouse Brain Library (http://www.mbl.org/mbl_main/atlas.html) between Bregma: -3,24; Interaural: 6,76 and Bregma: -5,04; Interaural: 4,96. The lateral ventricles were identified in the same horizontal sections by semi-automated region growth.

Positron emission tomography (PET) imaging was performed on a Siemens Inveon small animal PET/CT system (Siemens Preclinical Solutions, Knoxville, TN, USA) using the D1 receptor ligand [^{11}C] SCH-23390 or the D2/3 receptor ligand [^{18}F]Fallypride with slightly different methods (for a detailed description see Appendix I., Braunstein et al., 2010). For [^{11}C] SCH-23390 PET imaging 5 wild-type and 5 *Cra*/+ mice were used, for [^{18}F]Fallypride PET measurements 10 animals of each group (*Cra*/+ and wild-type) were randomly selected at 10 months of age. Mice were anesthetized and a 60 minute emission scan was performed starting with bolus injection of the radioactively labelled specific receptor ligand via a tail vein catheter. The Simplified Reference Tissue Model (SRMT) (Lammertsma et al., 1996) was used to calculate the binding potential. Regions-of-interest were defined on co-registered PET/CT images.

III. 6. Analysis of brain astrocytosis

Brain sections comprising the anterior part of the caudate nucleus and the putamen were cut on a vibratome at a thickness of 50 µm (Leica Microsystems, Wetzlar, Germany) and were stained by indirect immunofluorescence, using an antibody directed against the specific astrocyte marker glial fibrillary acidic protein (GFAP, Santa Cruz Biotechnology, Heidelberg, Germany) following the manufacturer's instructions. Quantitative analysis of immunoreactivity was performed using ImageJ.

III. 7. Stereological analysis of DARPP32 neurons

Coronal sections were cut in six series at a thickness of 35 µm throughout the brains using a freezing microtome. One series of free-floating brain sections were processed for immunohistochemistry with a primary rabbit antibody against DARPP32 (1:1000, Chemicon, AB 1656) (Bode et al., 2008). Stereological estimations of the total number of DARPP32 positive neurons in the striatum were performed unilaterally on blind-coded slides with the Computer Assisted Toolbox Software (New CAST) module in VIS software (Visiopharm, Horsholm, Denmark) by applying the optical fractionator principle (West et al., 1991).

III. 8. mtDNA quantification

Total DNA was extracted from muscle using standard methods. The content of mtDNA was determined using real-time quantitative PCR using 100 ng of purified DNA by measuring the threshold cycle ratio (ΔCt) of a mitochondrial-encoded gene Cox1 versus the nuclear-encoded gene Ppia (cyclophilin A).

III. 9. Muscle histology and electron microscopy

For muscle histology, isopentane frozen samples were cut on a cryostat into slices 16µm thick and processed for succinate dehydrogenase (SDH) staining using standard pathological stainings. For electron microscopy (EM) analysis, 12 months old mice were sacrificed and muscle tissues were quickly dissected and fixed with 2,5% glutaraldehyde, 10% sucrose fixative. Samples were post-fixed, dehydrated, embedded in Epon, and sectioned for EM. EM was performed at the Central Electron Microscopy Department at the University of Ulm.

III. 10. L-carnitine administration in N171-82Q mice

III. 9. 1. Survival

Thirty transgenic N171-82Q mice were used in this experiment. Eleven animals received intraperitoneal (i.p.) injections of L-carnitine (Biocarn, MEDICE, Iserlohn, Germany) at a dose of 250 mg/bodyweight kg (diluted in 0.15 ml, pH 7.4) 5 times a week starting at 6 weeks of age until death; 19 animals received an i.p. injections of the vehicle of L-carnitine in the same volume at the same times.

III. 9. 2. Open-field test

A separate set of 6-week-old transgenic mice were used for behavioural studies. The same experimental protocol and drug administration were used as above (n= 7 per group). The Conducta system and programme (Conducta 1.0; Experimetria Ltd., Hungary) were used to detect and evaluate the changes in spontaneous motor activity and exploration activity in the open-field paradigm. Each mouse was placed in the centre of a square arena (48x48x40 cm) and its behaviour was recorded for 5 minutes with the Conducta software. The ambulation distance, mean velocity, duration of immobility and number and duration of rearings were recorded. Tests were performed once a week for 10 weeks at the same time of the same day in order to avoid alterations due to the diurnal rhythm.

III. 9. 3. Immunohistochemistry

30-microm thick cryostat sections were cut to obtain sections from the entire striatum from 16 weeks old mice. Serial sections were immunostained with a polyclonal antibody recognizing the first 256 amino acids of human huntingtin (EM48, Chemicon International Inc., Temecula, CA, USA) at dilutions of 1:500. The specificity of the immune reactions was controlled by omitting the primary antiserum. An additional series of sections from each case were Nissl-stained with cresyl violet.

III. 11. Statistical analysis

Data are expressed as the mean ± SEM. Statistical analysis was accomplished using non-parametric Student *t*-test or ANOVA followed by Newman-Keuls multiple comparisons test (PRISM version 4.0b; GraphPad, San Diego, CA). Kaplan-Meier analysis and the Mantel-Cox log rank test were used to determine the survival differences between groups in N171-82Q mice. Differences at $P < 0.05$ were considered significant.

IV. Results

The motor phenotype of *Cra*/+ mice is characterized by early muscle weakness, progressive incoordination and hyperactivity

We first performed a battery of motor and behavioural tests in *Cramping* mice (*Cra*/+). *Cra*/+ mice showed reduced total and forelimb muscle grip strength compared with wild-type mice as early as 3 months of age (figure 1A), and suffered from an impairment in motor coordination that mildly increased with aging, as observed using an accelerating rotarod test (figure 1B).

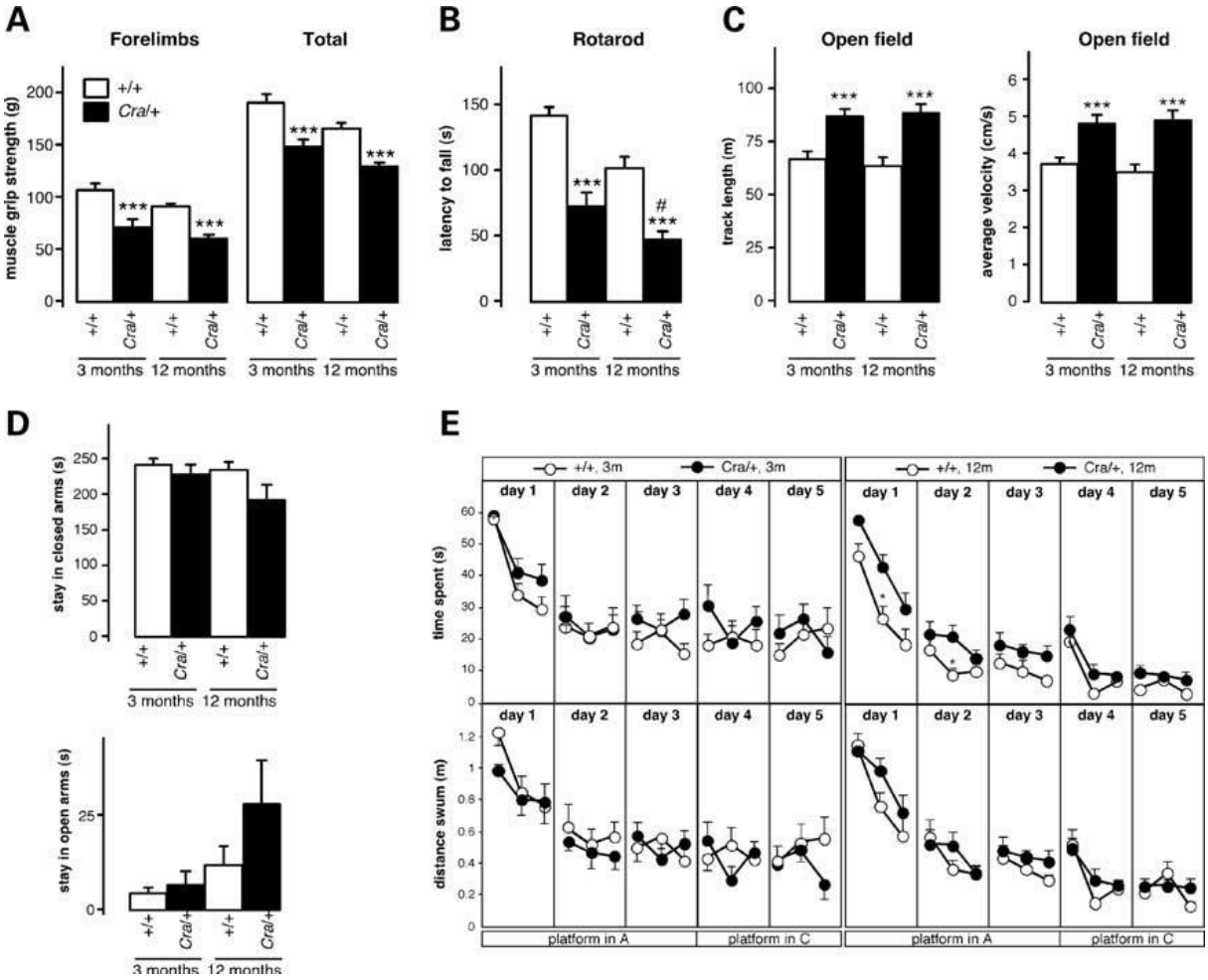

Figure 1. Locomotor and behavioural abnormalities in *Cra*/+ mice compared with wild-type mice (+/+)
A. Grip muscle strength of forelimbs (left panel) and all limbs (right panel)
B. Latency to fall in an accelerating rotarod test
C. Track length (left panel) and average velocity (right panel) in an open field
D. Time spent in closed arms (upper panel) and open arms (lower panel) in an elevated plus maze test
E. Time spent (upper panels) and distance swum (lower panel) to reach the hidden platform in a Morris water maze test at 3 (left panel) and 12 (right panel) months of age. The platform was in position A during the first 3 days of the test, and then moved to position C for the last two days.
***$P<0.001$ *versus* corresponding wild-type, $^{\#}P<0.05$ *versus* 3 months old *Cra*/+ mice (n=12 mice per group), *$P<0.05$ *versus* corresponding wild-type.

Cra/+ mice displayed hyperactivity in the open field arena as revealed by increased track length and average velocity (figure 1C). No differences were observed in the number of rearings, indicating normal vertical behaviour (data not shown). The level of anxiety appeared similar between *Cra/+* mice and their wild-type littermates as assessed using the elevated plus maze paradigm (figure 1D). In the Morris water maze test, *Cra/+* mice tended to spend more time in reaching the platform at 12, but not 3 months of age, as compared to wild-type animals. This was likely due to impaired motor incoordination rather than to spatial memory deficits since the observed difference between genotypes was annulled when considering the distance swum by the mice (figure 1E). Taken together, *Cra*/+ mice display muscle weakness and incoordination with increased open field activity in the absence of anxiety and obvious spatial working memory deficits.

Cra/+ mice present with striatal atrophy and lateral ventricle enlargement

We found that forebrain, but not hindbrain, wet weight was decreased in *Cra*/+ mice (figure 2A), suggestive of atrophy. The striatum and cerebral cortex of *Cra*/+ mice appeared grossly normal using haematoxylin/eosin staining (figure 2B), and the cortical layer organisation was preserved (figure 2C), suggesting that the defect was not due to abnormal cortical development. *In vivo* brain imaging using MRI showed a significant reduction in the volume of the *Cra*/+ mice striata at both 5 and 10 months of age (figure 2D-E), while, concomitantly, the volumes of the lateral ventricles were significantly increased (figure 2D, F). Thus, mutation in dynein leads to striatal atrophy in mice.

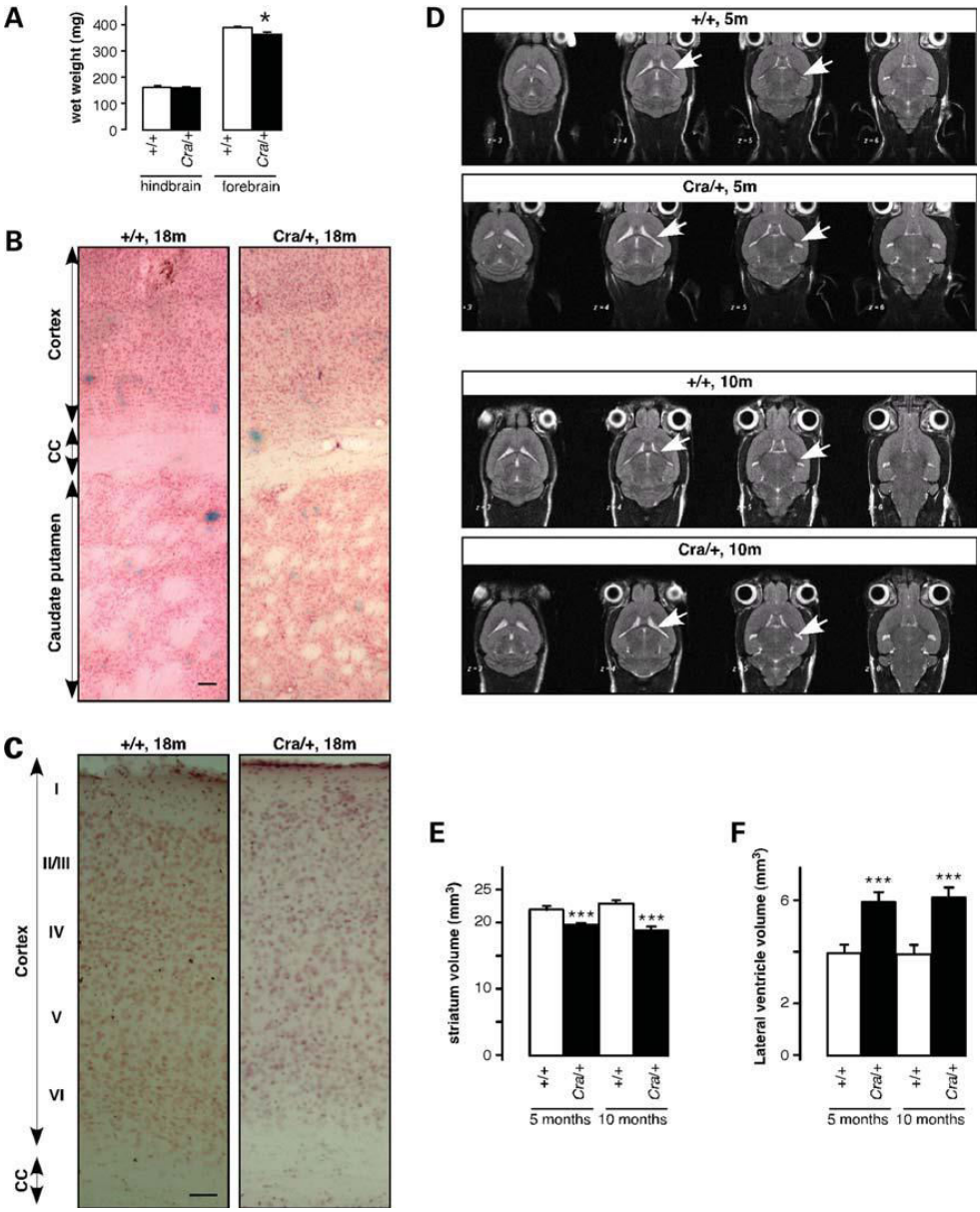

Figure 2. Striatal atrophy in *Cra*/+ mice
Wild-type mice (+/+) are in empty columns, *Cra*/+ mice in black columns
A. Wet weight of hindbrain (left) and forebrain (right) at 12 months of age. **P*<0.05 *versus* corresponding wild-type (n=4 mice per group). **B**. Low magnification photomicrographs of haematoxylin and eosin staining at 18 months of age. CC: corpus callosum. Scale bar = 100 µm. **C.** Higher magnification of B showing the aspect of the six layers of the cortex. Scale bar = 50µm. **D**. Representative horizontal T2-weighted MRI slices at 5 and 10 months of age. Note the enlargement of the lateral ventricles of the *Cra*/+ mouse. **E**. Striatal volume of at 5 (left) and 10 (right) months of age. ****P*<0.001 *versus* corresponding wild-type (n=20 mice per group). **F**. Lateral ventricle volume at 5 (left) and 10 (right) months of age. ****P*<0.001 *versus* corresponding wild-type (n=20 mice per group).

Progressive astrocytosis in the absence of neurodegeneration in the striatum

Reactive astrocytosis represents a typical marker of neuronal stress and is often a sign of an underlying pathology. Interestingly, reactive astrocytosis, as revealed by glial fibrillary acidic protein (GFAP) immunoreactivity (figure 3A), was dramatically increased in the striatum of 8 months old *Cra*/+ mice, and this increase was even higher at 18 months of age

(figure 3B). Consistent with this observation, striatal GFAP mRNA levels as measured using RT-qPCR were higher in *Cra*/+ mice than in wild-type littermates at 8 months, but not at 4 months of age (Figure 3C).

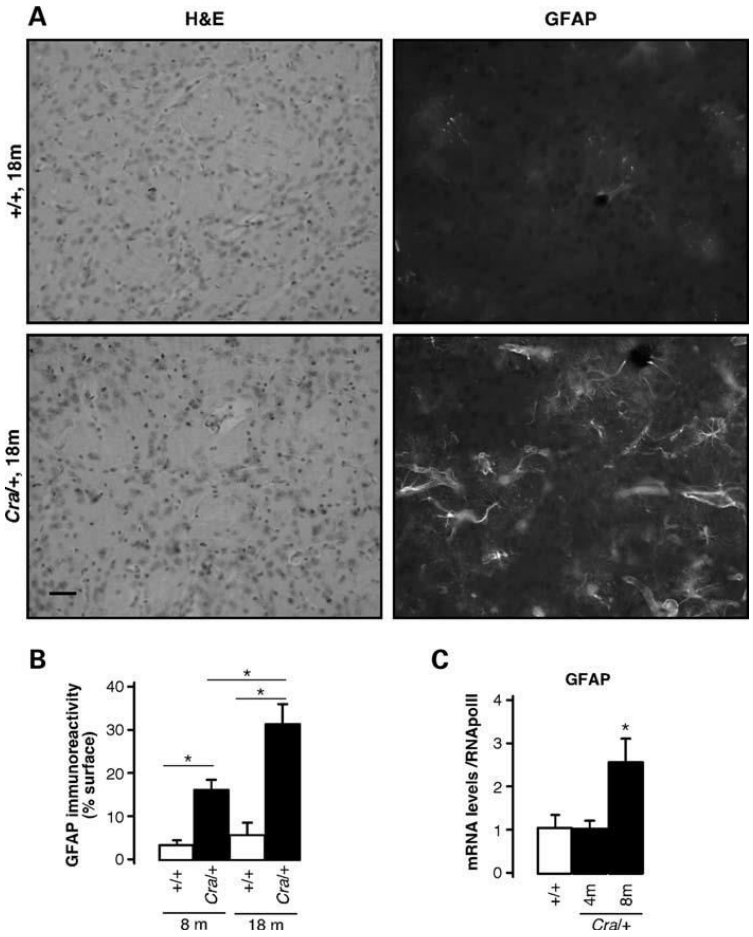

Figure 3: Progressive striatal astrocytosis in *Cra*/+ mice
A. Representative microphotographs showing haematoxylin/eosin staining (left panels) and GFAP immunoreactivity (right panels) in the striatum from wild-type mice (+/+, upper panels) and *Cra*/+ mice (lower panels) at 18 months of age. Scale bar = 25µm.
B. Quantification of the surface occupied by GFAP positive cells in the striatum at 8 and 18 months of age. Data are expressed as percentage of the total surface in the picture. **P*<0.05 *versus* indicated condition (n=5 mice per group).
C. mRNA levels of GFAP in the striatum at 4 and 8 months of age. **P*<0.05 *versus* wild-type (n=5-7 mice per group).

To determine whether astrocytosis was associated to neurodegeneration, we determined the total number of DARPP32 (dopamine and cAMP regulated phosphoprotein of a molecular weight of 32 kDa) positive medium spiny neurons (MSNs), the neuronal population comprising more than 95% of striatal neurons, using stereological analysis. The analysis of DARPP32 positive MSNs showed a non-significant trend towards decreased number at 6 months of age (figure 4). These data show that the phenotype of dynein mutant mice is rather due to neuronal dysfunction than to neurodegeneration in the striatum.

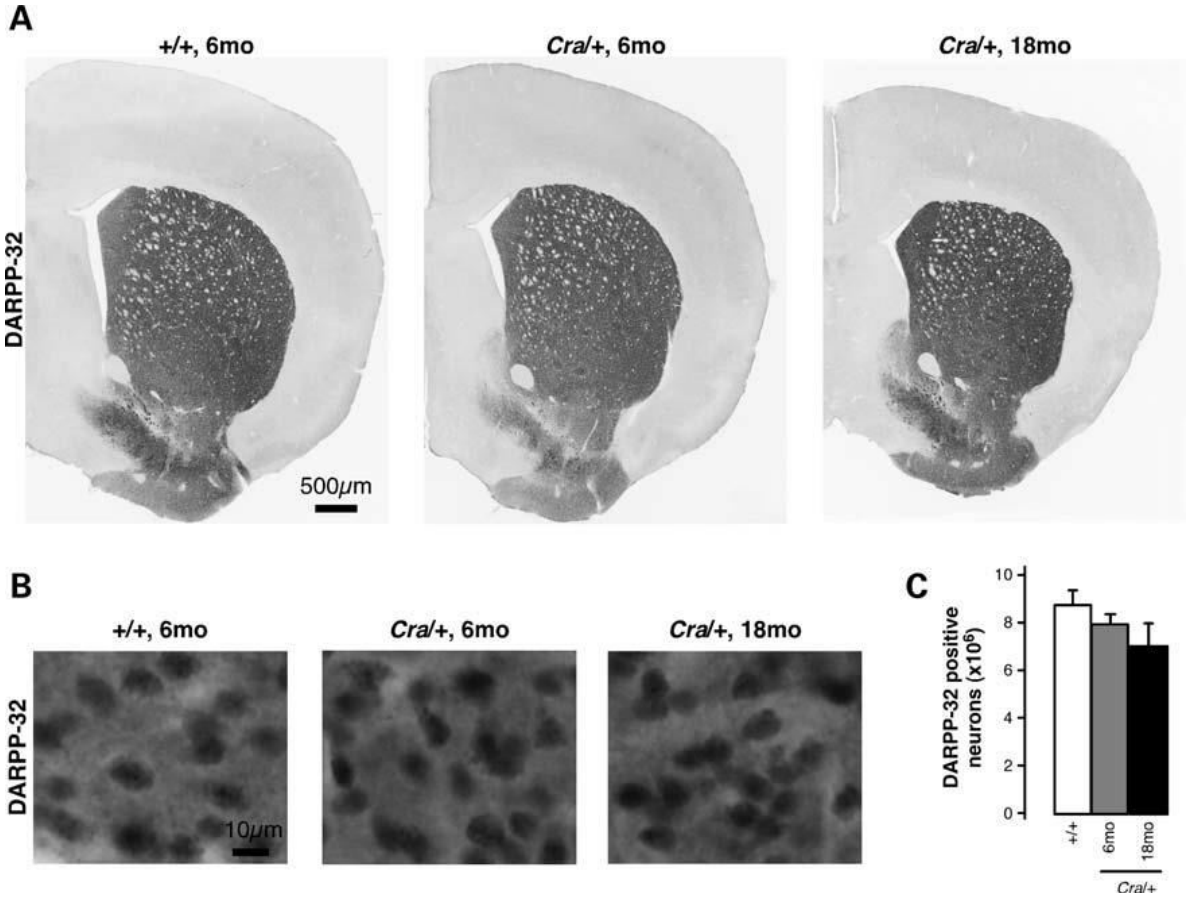

Figure 4: No significant neuronal loss in the striatum of Cra/+ mice.
A and B. Representative photographs of striatal sections processed for DARPP32 immunohistochemistry from wild-type mice (+/+) at 6 months of age as well as Cra/+ mice at 6 and 18 months of age.
C. Stereological estimations of the total number of DARPP32 positive neurons in the unilateral striatum did not reveal any statistically significant differences between the groups (n= 4–6 mice per group)

Altered dopamine signalling and D1 receptor binding in the striatum of *Cra*/+ mice

D1, but not D2, dopamine receptor mRNA levels were decreased in 8 months old *Cra*/+ mice as shown using RT-qPCR (figure 5A). D1 receptor expressing cells synthesize substance P, whereas D2 receptor expressing cells synthesize pre-proenkephalin. We found that substance P, but not pre-proenkephalin, mRNA levels appeared decreased in 8 months old *Cra*/+ mice (figure 5A), which corroborates the selective down-regulation of the expression of D1 dopamine receptors in striatal neurons. In addition, we performed positron emission tomography (PET) analysis of the binding of the D1 receptor selective ligand [^{11}C] SCH-23390 (figure 5B). Quantification of [^{11}C] SCH-23390 showed a decrease of the signal in the brains of *Cra*/+ mice (figure 5C), which further reinforces the presence of striatal dopaminergic impairment. We extended our D1-PET scans by using [^{18}F]Fallypride, a high-affinity selective dopamine D2/3 receptor ligand with the advantage of long half-life compared to [^{11}C]Raclopride (Siessmeier et al., 2005). We observed a significant reduction of

[^{18}F]Fallypride uptake in the striatum of *Cra*/+ mice compared with wild type animals (figure 5D), lending further support for the involvement of the striatal dopaminergic system in the *Cra*/+ pathogenesis.

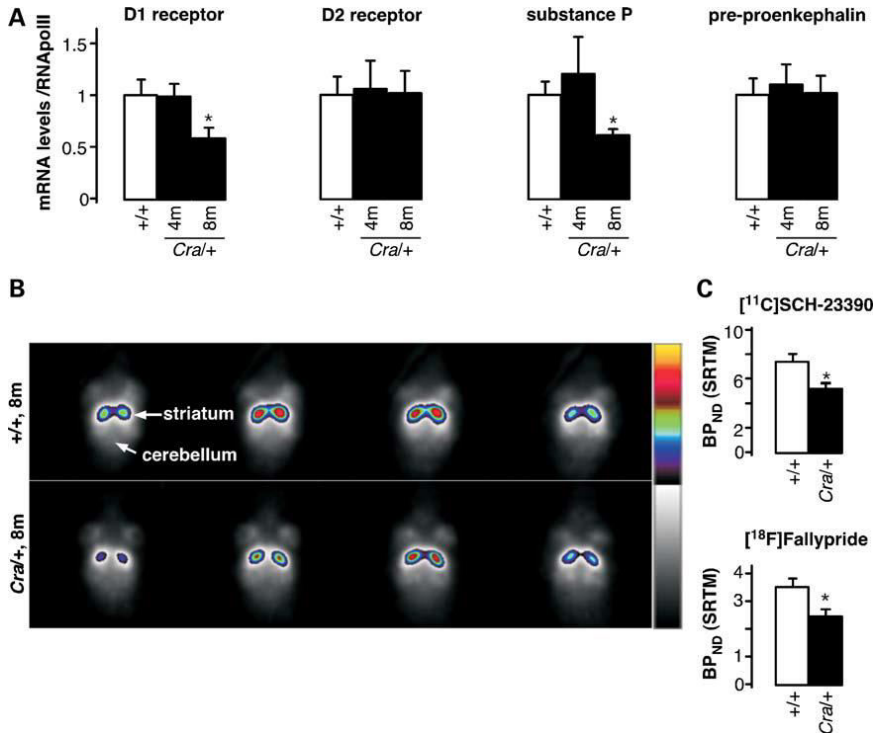

Figure 5. Altered dopamine signalling and binding in the striatum of *Cra*/+ mice
A. mRNA levels of the dopamine D1 and D2 receptor, substance P and pre-proenkephalin in the striatum of wild-type mice (+/+) and *Cra*/+ mice at 4 and 8 months of age. **P*<0.05 *versus* wild-type (n=5-7).
B. Representative [^{11}C]SCH-23390 images (all frames averaged together) through the striatum of a wild-type mouse brain (+/+, upper panels) and a *Cra*/+ mouse brain (lower panels) are shown.
C. Binding potentials (BP_{ND}) of [^{11}C]SCH-23390 (n=5) and [^{18}F]Fallypride (n=10) calculated using SRTM. **P*<0.05 *versus* wild-type.

In all, we showed that *Cramping* mice bearing a point mutation in the molecular motor dynein display striatal dysfunction which can better explain the phenotype observed in these mice. Thus, our findings support the role of dynein and retrograde axonal transport in striatal pathology.

Mitochondrial proliferation in *Cramping* mice is dependent on endogenous FL-PGC-1α

To determine whether PGC-1α is functionally involved in *Cramping* induced mitochondrial proliferation, we ablated FL-PGC-1α in these mice. We chose FL-PGC-1α ablation as this isoform is more specialized in increasing mitochondrial biogenesis and pan-PGC-1α ablation is very toxic *per se* for muscle physiology (Handschin et al., 2007). We crossed FL-PGC-1α -/- mice with *Cramping* mice to generate *Cramping* mice deficient in FL-PGC-1α (termed *Cra*/FLα -/- mice in the rest of the thesis).

The ablation of FL-PGC-1α in *Cramping* mice completely abolished the previously observed increases in mtDNA levels in muscles (figure 6A). At 6 months of age, *i.e.* an age at which mitochondrial dysfunction is not histologically and biochemically evident in *Cramping* mice, we observed a 20% increase in citrate synthase activity in *Cramping* muscle, which was fully reverted by FL-PGC-1α ablation (figure 6B). This was associated with unchanged mitochondrial respiratory complex activities and normal ratios between respiratory chain complex activities (data not shown in the thesis; see Appendix II., Róna-Vörös et al., 2013) suggesting that mitochondrial proliferation maintained close to normal respiratory activity at that age. From an ultrastructural point of view, the *Cramping* mutation leads to giant mitochondria invading sarcomeres (Eschbach et al., 2013). FL-PGC-1α deficiency reverted this mitochondrial proliferation (figure 6C, quantifications in 6D-E).

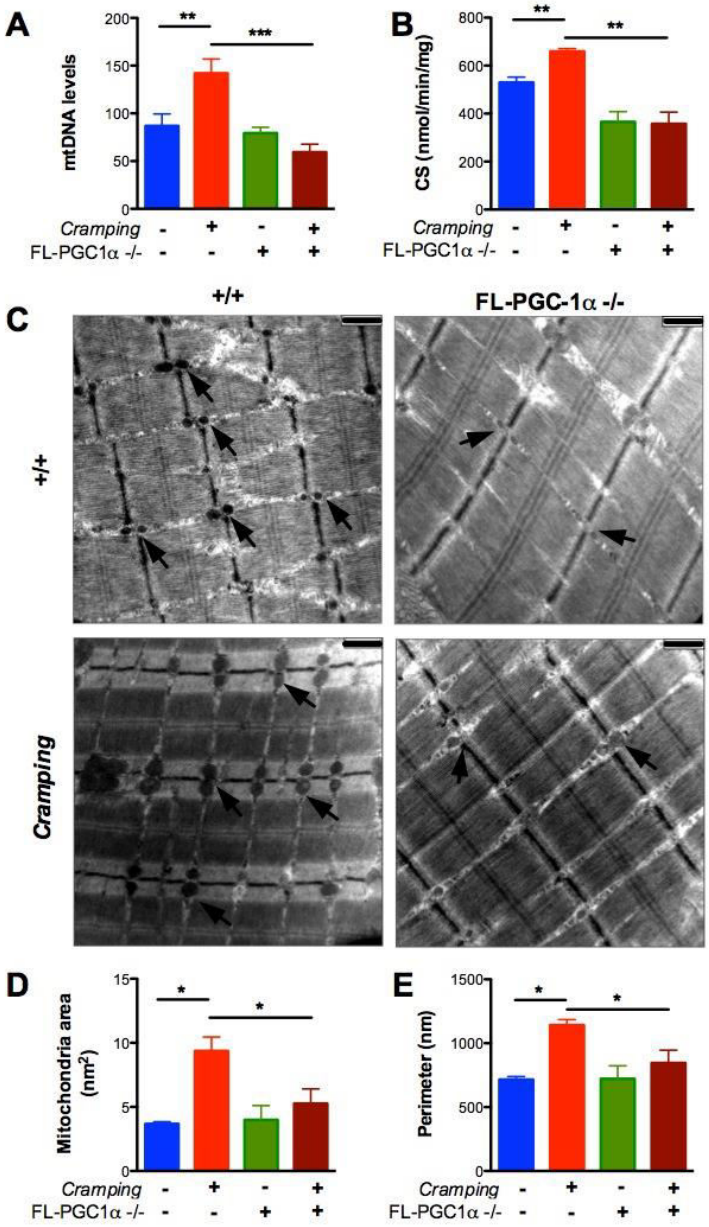

Figure 6. Mitochondrial proliferation in *Cramping* mice is dependent upon FL-PGC-1α

+/+ mice are in blue, *Cramping* mice in red, FL-PGC-1α -/- in green and compound *Cra*/FLα -/-in brown.

A. Mitochondrial DNA (mtDNA) levels in tibialis anterior muscle. **, p<0.01 ; ***, p<0.001, ANOVA followed by Newman-Keuls as compared with the indicated condition. n=8 per group.

B. Citrate synthase activity in nmol/min/mg protein in gastrocnemius muscles. **, p<0.01 ; ***, p<0.001, ANOVA followed by Newman-Keuls as compared with the indicated condition. n=3 per group.

C. Representative electron micrographs of glycolytic gastrocnemius muscle of +/+ (left column) and *Cramping* (right column) mice in either FL-PGC-1α +/+ (upper row) or -/- (lower row) background. Pairs of mitochondria are found in the I-band on both sides of the Z-band in wild type mice. Note the large increase in size in the mitochondria of *Cramping* mice disrupting the alignment of sarcomeres that is reverted by ablation of PGC-1α. As previously observed mitochondria of FL-PGC-1α -/- mice are smaller. Arrows show pairs of mitochondria in each picture. Scale bar: 600 nm.

D-E. Quantification of mitochondrial area (D) and perimeter in experiments presented in C.

Thus, mitochondrial proliferation in *Cramping* mice is fully dependent upon endogenous FL-PGC-1α and cannot be rescued by the roughly normal expression of NT-PGC-1a in FL-PGC-1α -/- mice.

Endogenous FL-PGC-1α mitigates overall phenotype and mitochondrial dysfunction in *Cramping* mice

We next asked whether ablating the increase in mitochondriogenesis in *Cramping* mice, through FL-PGC-1α ablation, modified the phenotype of the mice. *Cra*/FLα -/- mice displayed a much more severe phenotype than single mutations. They showed prominent kyphosis, and abnormal posture as well as progressive hair loss (figure 7A). Both male and female *Cra*/FLα -/- mice displayed body weight loss as compared with the three other genotypes (figure 7B-C). Body temperature of *Cra*/FLα -/- mice became progressively lower in females (figure 7D-E) while in males the defect was also present in single FL-PGC-1α -/- mice.

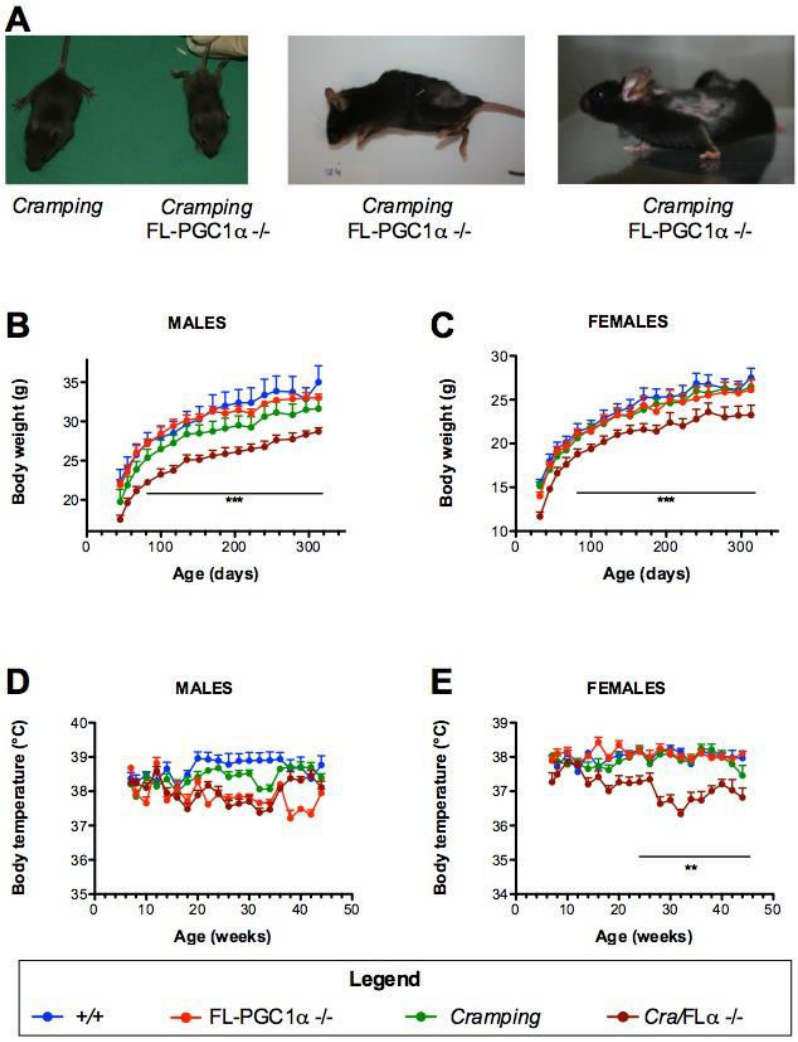

Figure 7. FL-PGC-1α ablation exacerbates global phenotype of *Cramping* mice
+/+ mice are in blue, *Cramping* mice in red, FL-PGC1α -/- in green and compound *Cra*/FLα -/- in brown.
A. Left : representative photographs of 3 weeks old littermate *Cramping* and *Cra*/FLα -/-mice Right : typical kyphosis and hair loss in a 12 months old *Cra/FLα -/-* mouse.
B-E. Body weight (B, C) and body temperature (D-E) of male (B, D) and female (C,E) mice. **, p<0.01 for *Cra*/FLα -/- as compared with the three other groups. N=7-8 per gender per group.

At 12 months of age, both single *Cramping* and FL-PGC-1α -/- muscles showed the expected decrease in SDH activity in both tibialis anterior (TA) and soleus muscles (figure 8). The combination of both mutations potently exacerbated this mitochondrial defect (figure 8).

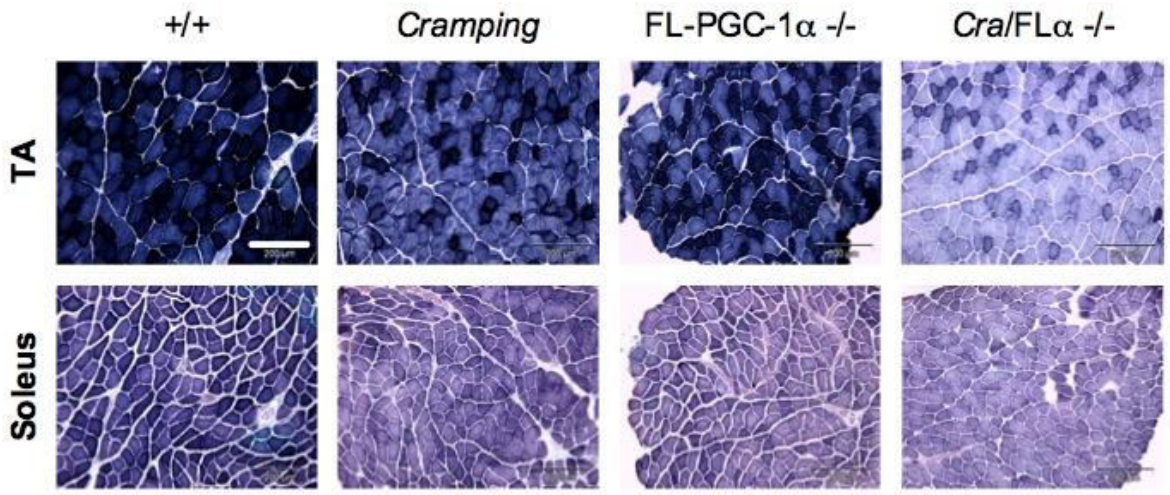

Figure 8. FL-PGC-1α ablation exacerbates mitochondrial dysfunction in *Cramping* mice
Representative photomicrographs showing muscle sections of +/+, *Cramping*, FL-PGC-1α -/- and compound *Cra/*FLα *-/-* tibialis anterior (upper pictures) and soleus muscles from 12 months old mice stained for succinate dehydrogenase activity (SDH). Scale bar : 200 µm. n=4-5 per group.

Endogenous FL-PGC-1α mitigates the neurological phenotype of *Cramping* mice

The *Cramping* mutation leads to a stereotypical neurological phenotype that includes loss of muscle strength and incoordination as prescribed above. As compared with *Cramping* mice, *Cra/*FL*α -/-* mice showed an earlier and stronger loss of grip strength in forelimbs and all limbs (figure 9A-B). Tremor, a phenotype occasionally observed in *Cramping* or FL-PGC-1α -/- mice after 9 months of age, occurred systematically before 6 months of age in *Cra/*FL*α -/-* mice (figure 9C). Indeed, compound transgenic mice were unable to hang on a string as early as 4 months of age, while *Cramping* mice were still able to do so at least 10 seconds until 9 months of age (figure 9D). Further supporting this point, compound transgenic mice showed profoundly impaired rotarod performance as compared with all three other genotypes at 6, 9 and 12 months of age (figure 9E) and decreased rearing activity at 8 and 12 months of age (figure 9F).

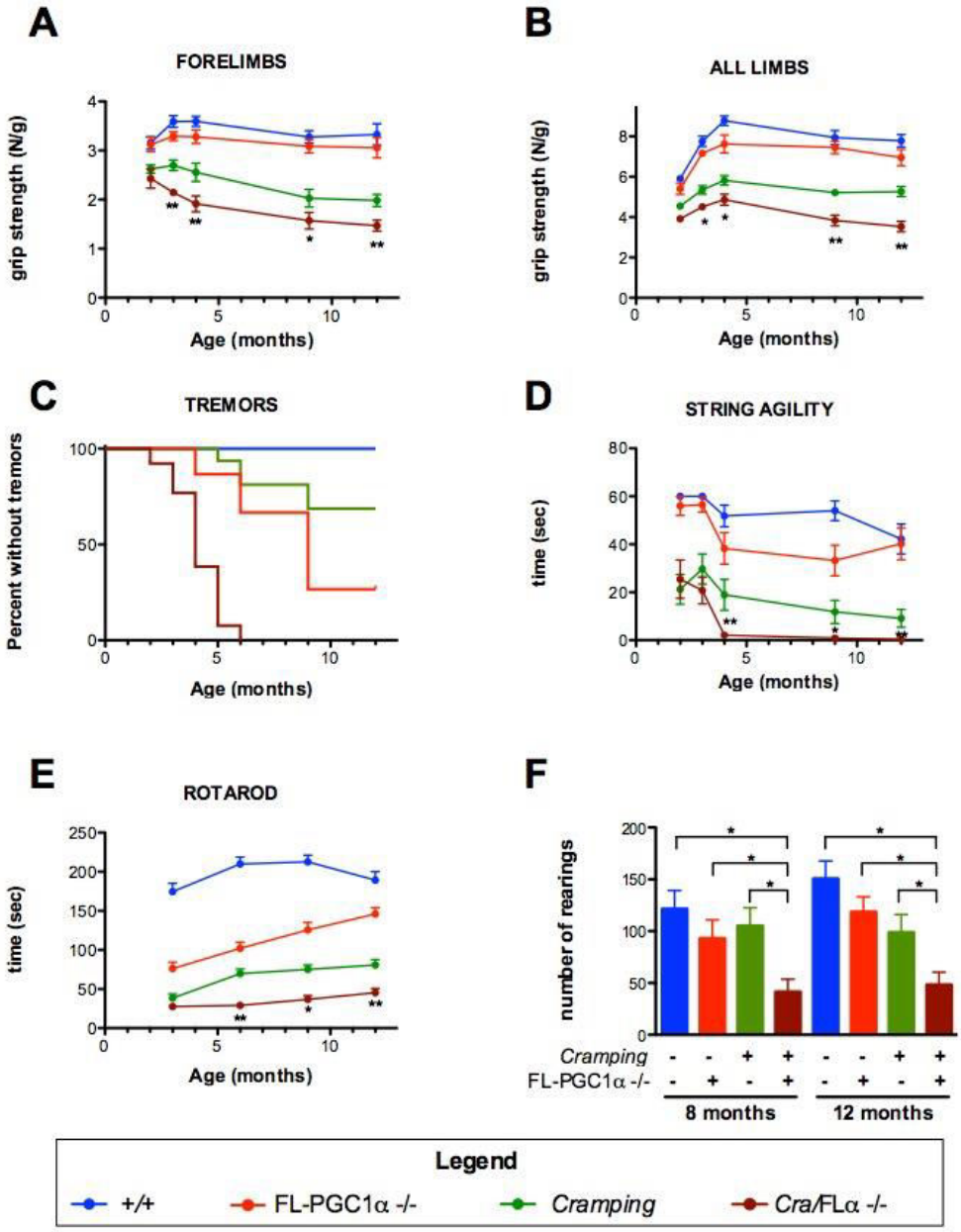

Figure 9. FL-PGC-1α ablation exacerbates neurological phenotype of *Cramping* mice
+/+ mice are in blue, *Cramping* mice in red, FL-PGC-1α -/- in green and compound *Cra*/FLα -/-in brown.
A-B. Forelimb (A) and all limb (B) grip strength. **, p<0.01 for *Cra*/FLα -/- as compared with *Cramping* mice (repeated ANOVA). n= 14-16 per group.
C. Kaplan-Meier plot depicting the onset of tremors. P<0.001 for *Cra*/FLα -/-as compared all three other groups. (log rank test). n= 14-16 per group.
D-E. String agility score (in seconds, D) and rotarod performance (E). **, p<0.01 for *Cra*/FLα -/- as compared with *Cramping* mice (repeated ANOVA). n= 14-16 per group.
F. Number of rearings in a 30 minute open field test for mice at 8 or 12 months of age.*, p<0.05 for *Cra*/FLα -/- as compared with the indicated condition (ANOVA followed by Newman-Keuls). n=7-8 per group.

In all, we demonstrated that FL-PGC-1α is required for mitochondrial proliferation compensatory occurred in *Cramping* mice with overall mitochondriopathy. Moreover ablation of FL-PGC-1α notably worsened the phenotype of *Cramping* mice. These data help for better understanding the pathomechanism of mitochondrial dysfunction in neurodegeneration.

L-carnitine administration significantly improved the survival and ameliorated the motor symptoms of N171-82Q mouse model of HD

The mean of survival of the vehicle-treated transgenic mice was 125.6 days. LC treatment caused a significant increase of 14.91% in the survival time (144.3 days) (figure 10).

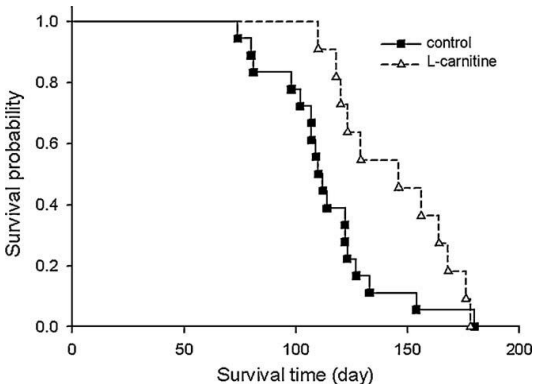

Figure 10. Survival times of the L-carnitine-treated and control transgenic mice. The Kaplan–Meyer survival curve revealed that the L-carnitine-treated animals exhibited an increased duration of survival as compared with the control group (*p<0.05).

From the age of 14 weeks the N171-82Q transgenic HD mice started to move more slowly and less compared with wild-type mice in the open field apparatus. This decreased motility was completely reverted by LC administration (figure 11A-C). LC itself did not have any influence in wild-type mice mobility (figure 11A-C). Further, the frequency of rearing was significantly reduced in transgenic mice at the age of 15 weeks (*p<0.05) not in the L-carnitine treated group (figure 11D).

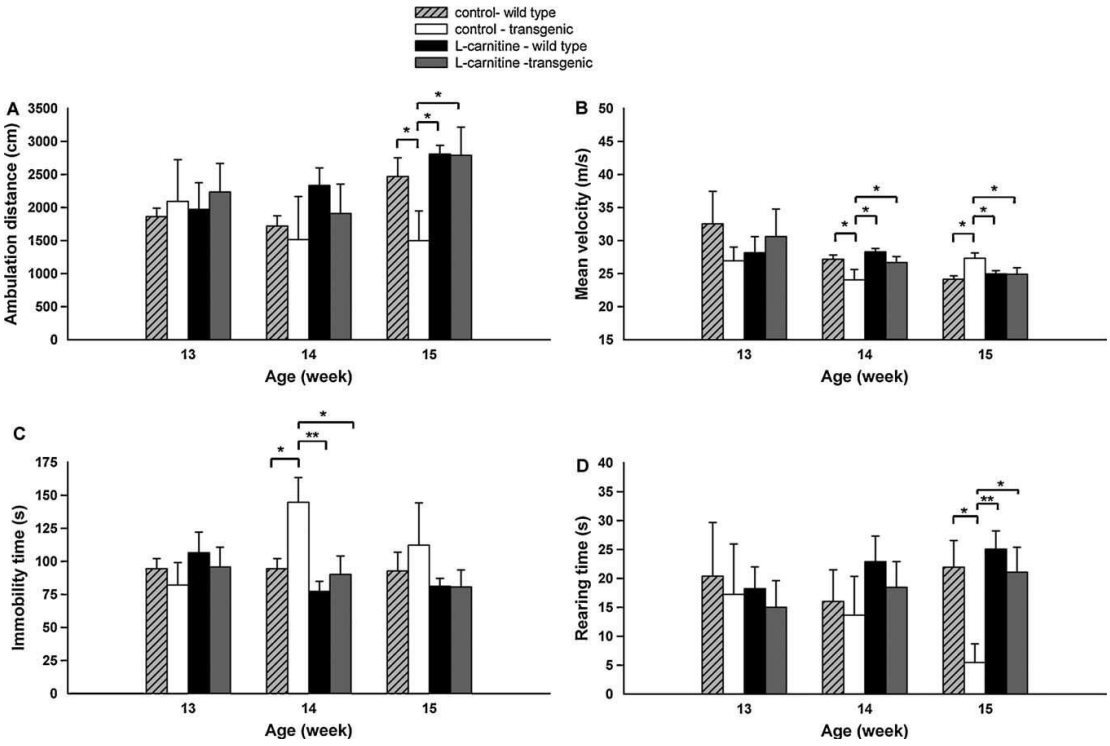

Figure 11. Behavioural assessment of LC treated transgenic mice in open-field tests. (A) Ambulation distance (*p<0.05); (B) mean velocity (*p<0.05); (C) immobility time (**p<0.01, *p<0.05); (D) rearing time (**p<0.01, *p<0.05).

L-carnitine treatment was neuroprotective in N171-82Q HD mice

L-carnitine treatment also ameliorated the striatal neuronal atrophy in transgenic HD mice. Our quantitative analysis demonstrated that the LC-treated transgenic animals had a significantly higher (*p<0.05) number of surviving striatal neurons concerning cresyl violet-staining relative to the vehicle-treated group (figure 12). Moreover we quantified the huntingtin-immunoreactive (IR) aggregates visualized by EM48 polyclonal antibody in the outer lamina of the pyriform cortex (layer II), which is an important area of the N171-82Q transgenic mice and within the lateral striatum. The EM48-IR aggregates were much more prominent within the cortex as compared with the neostriatum. In the L-carnitine-treated group fewer huntingtin aggregates were detected in both areas compared with the vehicle-treated transgenic group. LC treatment significantly (**p<0.01) reduced the number of cortical aggregates. In the lateral striatum LC treatment induced a slight, but not significant decrease of the huntingtin-IR aggregates (figure 13).

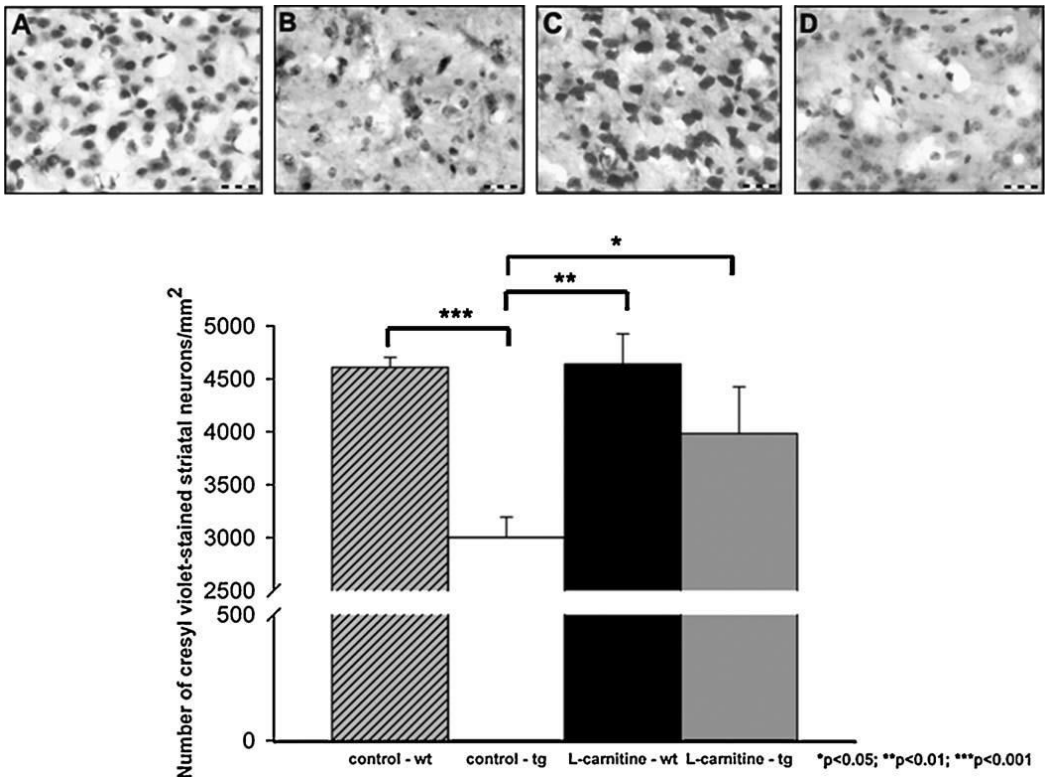

Figure 12. Cresyl violet-stained neurons in the striatum.
Wild-type (wt) (A), control transgenic (tg) (B) L-carnitine-treated wt (C), and the L-carnitine-treated tg (D) groups. Scale bar = 25mm. Diagram shows the mean numbers of cresyl violet-stained neurons in the striatal area of the wild-type mouse (striped grey bar), the control tg mouse (light bar), the control L-carnitine-treated wt mouse (dark bar) and the L-carnitine-treated tg mouse (dark grey bar) at 16 weeks of age.

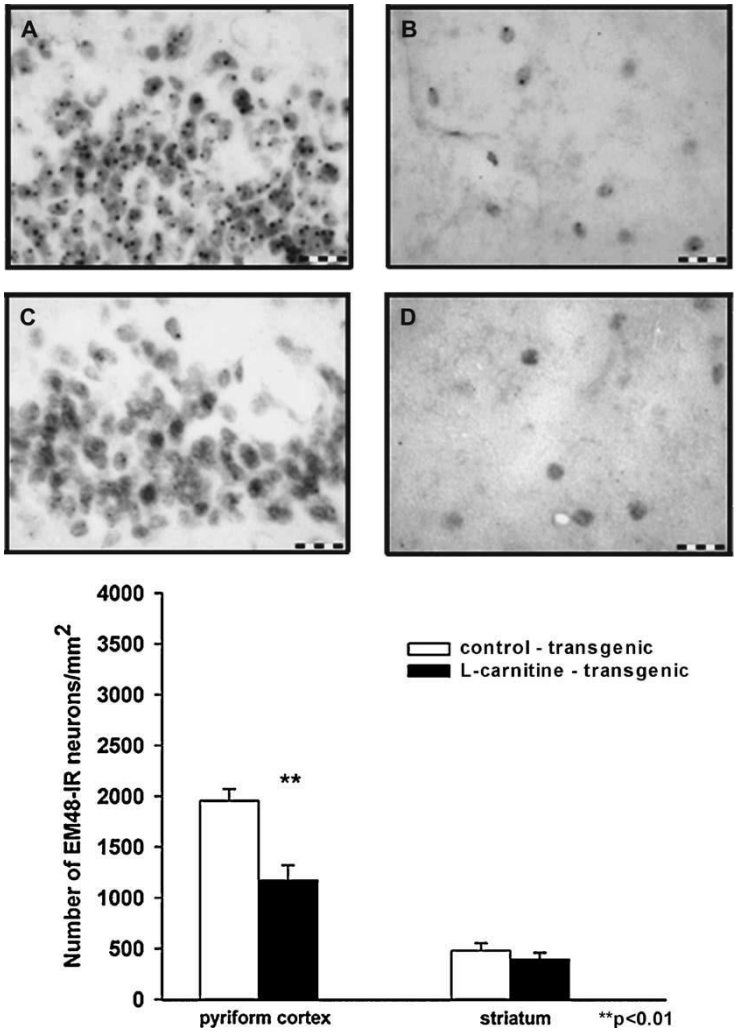

Figure 13. Polyclonal EM48 positivity of cortical sections (A,C) and striatal sections (B,D) of 16-week-old control and L-carnitine-treated transgenic mouse brains. Scale bar = 25 mm. Diagram shows the mean number of EM48-IR cells in the pyriform cortex and in the striatum of L-carnitine-treated (dark bar) and control (light bar) transgenic mice.

In all, we showed that L-carnitine treatment in higher dose significantly increased survival and ameliorated the motor symptoms in HD transgenic mice, moreover it was found to be neuroprotective for striatal neurons.

V. Discussion

In this PhD work we showed additional evidence of the involvement of the molecular motor dynein and mitochondrial dysfunction in the pathogenesis of neurodegenerative diseases.

First, we demonstrated the *in vivo* requirement of cytoplasmic dynein in the function of the striatum. *Cramping* mice display early onset motor and behavioural abnormalities such as abnormal gait, hind limb clasping, motor incoordination, muscle weakness and hyperactivity. A relatively mild proprioceptive neuropathy was proved previously in dynein mutant mice (Dupuis et al., 2009; Chen et al., 2007; Ilieva et al., 2008), but this does not explain the overall behavioural disturbances observed here, rather central involvement is suggested. The phenotype of *Cra/+* mice resembles striatal pathology. Transgenic mice of HD also show abnormal gait and limb clasping when held by the tail, and at the early phase of the disease they have an increased spontaneous locomotor activity (Mangiarini et al., 1996; Schilling et al., 1999; Luesse et al., 2001; Bolivar et al., 2004). A similar phenotype is observed in PGC-1α -/- mice (Lin et al., 2004; Leone et al., 2005) and in transgenic mice lacking cortical BDNF or conditionally ablated for BDNF at adulthood (Baquet et al., 2004; Rauskolb et al., 2010). All of these transgenic animals present clear-cut lesion in the striatum. Besides, the genetic ablation of D1 dopamine receptor expressing cells within the striatum results also in a motor phenotype very similar to that observed in *Cramping* mice (Gantois et al., 2007). In addition others from our workgroup observed that dynein mutant mice exhibit HD-related peripheral phenotypes including increased adiposity and impaired brown adipose tissue thermogenesis (Eschbach et al., 2011), thus strengthening the analogy between HD animal models and dynein mutant mice. Our further histological, in vivo brain imaging findings verified the striatal involvement in *Cramping* mice.

Interestingly, the behavioural phenotype of *Cra/+* mice and striatal atrophy detected by MRI, appeared between 3 and 5 months of age, while astrocytosis, decreased gene expression of D1 receptors and decreased binding potential of D1, D2 receptors were detectable later, after 8 months of age. The fact that behavioural abnormalities forego marked histopathological and biochemical changes in the striatum is not without precedent. For instance, previous studies reported that motor dysfunction in huntingtin knock-in mice occurred long before any clear signs of striatal lesions (Menalled et al., 2002). Of note, conventional research of neurodegeneration focused on neuronal cell death related alterations as earlier the research possibilities were mainly limited to post mortem brain tissues from patients where cell death was obvious. The identification of neurodegeneration related genes

and the occurrence of transgenic animal models permitted to study early pathogenic events (Wong et al., 2002; Price et al., 2000). It turned out that significant abnormalities are detectable before any obvious signs of neuronal loss in most animal models. Moreover, further studies (behavioural, functional imaging, pathological, electrophysiological) in asymptomatic or early symptomatic patients with familial neurodegenerative diseases clearly demonstrated signs of neuronal dysfunction in the absence of overt neuronal cell death (Morfini et al., 2009). We did not detect decreased DARPP32 neuronal counts, showing that the striatal phenotype was not associated with cell death of MSNs. Our data however does not exclude the occurrence of a very slow and subtle process of striatal neurodegeneration that would be difficult to detect. Also, astrocytosis itself might be an astrocyte-autonomous event. Dynein expression and function in astrocytes has been poorly characterized and the elucidation of astrocytic dynein to the phenotype of dynein mutant mice will require the generation of conditional knock out mice.

We found that gene expression of D1 receptor, as well as binding potential of [^{11}C] SCH-23390, a D1 receptor selective radioligand, was decreased in *Cra*/+ striatum. Curiously, despite the binding potential of D2 receptor was decreased, its gene expression was unaffected. There are several potential explanations for this discrepancy. The binding potential calculated from PET analysis is a reflection of both the density of available receptor sides and the apparent ligand affinity (Laruelle, 2000). Since apparent ligand affinity is decreased by competition with the natural ligand, dopamine, an increase in striatal dopamine could in principle explain a decreased binding potential observed with PET. However, this explanation is unlikely in our case as [^{18}F]Fallypride is a high affinity antagonist radioligand, and therefore less sensitive to changes in synaptic dopamine than agonist radioligands (Laruelle, 2000). Thus, the lower D2 binding potential observed in *Cra*/+ mice is most likely due to a reduction of D2 receptor binding sites rather than to increased striatal dopamine. Such a decrease might be explained by defects in the various trafficking events that modulate cell surface expression of D2 receptor (Xiao et al., 2009; Tirotta et al., 2008; Kim et al., 2008). Indeed, given the involvement of dynein in a number of cellular trafficking events, it cannot be excluded that dynein mutation might directly impair D2 receptor trafficking.

Other works also implicated dynein in diseases with striatal involvement, notably HD. The dynein/dynactin motor complex plays an important role in the delivery of autophagosome, in the process of autophagosome-lysosome fusion (Webb et al., 2004, Ravikumar et al., 2002). In neurons, autophagosome formation occurs at the axon tip, autophagosomes are transported retrogradely to perinuclear locations where lysosomes are

concentrated (Rubinsztein et al., 2005; Wong and Holzbaur 2014). Disrupted dynein function (even by inhibitors or genetic manipulations) results increased aggregate formation in cells, flies and mice, in connection with decreased autophagic clearance of aggregate-prone proteins e.g. mutant HTT (Ravikumar et al., 2005; Wiesner et al., 2014). Moreover, the toxicity of mutant HTT is enhanced by dynein dysfunction in neuronal precursor cell lines and the mutant HTT caused phenotype is aggravated in flies and mice by dynein mutation - detected when crossbreeding transgenic HD mice with *Loa*/+ dynein mutant mice (Ravikumar et al., 2005). Huntingtin (HTT) and its associated protein HAP1 bind to dynein directly and indirectly, through dynactin subunit $P150^{Glued}$ (Li et al., 1995; Li et al., 1998). Mutant HTT disrupts these interactions and decreases dynein function (Caviston et al., 2007; Caviston and Holzbaur, 2009), although mutant HTT affects not only retrograde but also anterograde axonal transport (Morfini et al., 2009). On the other hand inhibition of dynein function in HeLa cells causes a significant redistribution of HTT to the cell periphery, suggesting that dynein is required for transport of HTT towards the cell center (Caviston et al., 2007). Moreover live-cell imaging studies proved that HTT and HAP1 colocalize with autophagosomes and control their dynamics via regulating the dynein and kinesin motors (Wong and Holzbaur, 2014). HTT/HAP1 complex enhances retrograde motility and promotes autophagosome transport towards the cell body for degradation. Further, in striatal cells from HD knock-in mice or mutant HTT expressing primary neurons disrupted autophagosome transport was observed causing defects in cargo degradation and ineffective clearance of dysfunctional mitochondria and mutant HTT (Wong and Holzbaur, 2014).

Further to detect a mechanism which could be responsible for the striatal dysfunction we observed in *Cramping* mice we investigated abnormalities in striatal afferentation. The substantia nigra pars compacta dopaminergic neurons were however preserved when performing stereological assessments of tyrosine hydroxylase positive neurons (see Appendix I., Braunstein et al., 2010). We also examined whether deprivation or impaired response of brain-derived neurotrophic factor (BDNF), the major trophic factor for MSNs could be affected. But neither BDNF deprivation appeared to be a cause of dynein mutant striatal phenotype (see Appendix I., Braunstein et al., 2010). Indeed, we found that dynein mutant striatal neurons had dramatically impaired neuritic arborisation in cultured neurons while sparing survival of these cells (see Appendix I., Braunstein et al., 2010).

In summary, our findings provide direct evidence of the involvement of axonal transport machinery, notably dynein in the maintenance of striatal function and may have major implications for our understanding of the pathogenesis of HD.

Besides dynein disruption we investigated the role of mitochondrial dysfunction in neurodegeneration. **We showed that full length PGC-1α is absolutely required for compensatory mitochondrial proliferation occurring in *Cramping* mice, confirmed by the fact that its ablation strongly exacerbates metabolic and neurological phenotype in these mice.**

Increased number of mitochondria in muscle is a hallmark of human mitochondrial diseases although the underlying mechanisms are unclear. Mitochondrial proliferation is also observed in a subset of patients with neuropathies similar to *DYNC1H1* mutations (Yu-Wai-Man et al., 2010; Sitarz et al., 2012). Others from our workgroup previously described decreased mitochondrial respiration in white adipose tissue (WAT) and skeletal muscles along with increased mitochondrial area in muscle of *Cramping* mice (Eschbach et al., 2013). Furthermore, increased and not decreased mtDNA levels was found in tibialis anterior (TA) muscle, gastrocnemius muscle, WAT and striatum of *Cramping* mice (see Appendix II., Róna-Vörös et al., 2013). This increased mitochondrial mass was not accompanied by large-scale deletions of mtDNA suggesting that the mtDNA maintenance is functional. Interestingly, this compensatory response was not observed in cultured embryonic striatal neurons or fibroblasts, even in homozygous *Cramping* cells (see Appendix II., Róna-Vörös et al., 2013). Thus, mtDNA copy number is increased *in vivo* in *Cramping* mice as a possible compensatory response. The activation of PGC-1α, a transcriptional coactivator responsible for mitochondrial biogenesis, was hypothesized to underlie these observations. Overnight fasting followed by 6 hours of re-feeding further increased mtDNA levels in *Cramping* muscles accompanied by increased level of total PGC-1α mRNA (see Appendix II., Róna-Vörös et al., 2013). This increase was due to FL- and NT-PGC-1α isoforms, the alternatively spliced IT-isoforms PGC-1α2 and 3 were unaffected. Moreover the expression of canonical targets of PGC-1α were examined for further support (see Appendix II., Róna-Vörös et al., 2013). Altogether these data showed that mitochondrial proliferation in *Cramping* mice correlates with transcriptional induction of both FL- and NT-PGC-1α.

The widely documented function of FL-PGC-1α in mitochondrial physiology suggested focusing on this specific isoform as a potential key player in mitochondrial proliferation. We showed definitive evidence of FL-PGC-1α involvement by a complete reversal of several indicators of mitochondrial proliferation upon FL-PGC-1α ablation in *Cramping* mice. In the FL-PGC-1α -/- mice we used for our experiments, NT isoforms are functionally preserved, as shown previously by others (Chang et al., 2012) and by our workgroup in skeletal muscle. It follows that NT isoforms are not able to substitute for FL isoforms and induce mitochondrial

proliferation. This function present in FL-PGC-1α but not in NT isoforms might be due to differences in nuclear import. Indeed, NT isoforms accumulate in the cytoplasm, while FL isoforms are exclusively nuclear (Chang et al., 2010; Shen et al., 2012). It is thus possible that a constitutive nuclear presence is necessary for PGC-1α to trigger mitochondrial proliferation. Alternatively, the domains of FL-PGC-1α not present in NT-isoforms include many interaction sites with important transcription factors such as PPARs, FoxO1 or MEFC2 (Zhang et al., 2009). Each of these factors might be critical in this mitochondrial proliferation function. It is also possible that post-transcriptional mechanisms required for triggering mitochondrial proliferation target domains exclusively present in FL-isoforms. In all, FL-PGC-1α is necessary for mitochondrial proliferation, while NT-PGC-1α is not sufficient. NT-PGC-1α, although not sufficient, could however be necessary for mitochondrial proliferation, and answering this question will require specific knock-out mice that are currently not available.

Our results support PGC-1α activation and increased mitochondrial biogenesis underlying the increased mitochondrial mass observed in *Cramping* mice, however it is not excluded that decreased mitochondrial autophagy caused by the dynein mutation may also partially contribute to this phenotype. The above discussed growing evidences for the role of dynein in autophagy stand for this potential (Wong and Holzbaur, 2014). Further investigations are needed to answer this question carefully.

FL-PGC-1α ablation, and subsequent loss of mitochondrial proliferation, strongly exacerbated the previously observed abnormalities of *Cramping* mice, both metabolic (muscle mitochondrial function) and neurological (grip strength, rotarod, tremors). New defects appeared in *Cra/*FL*α -/-* mice that were absent in single transgenic mice, in particular a pronounced kyphosis, profound hair and weight loss and inability to rear. The respective mechanisms underlying these different phenotypes remain unknown, especially whether they are the consequences of worsened mitochondrial dysfunction.

We observed gender differences in body temperature regulation. Others from our workgroup previously observed decreased rectal temperature in aged male *Cramping* mice (Eschbach et al., 2011). This difference in body temperature is also observed in the current study when considering only aged males, although the difference is smaller than reported by Eschbach et al., 2011. There are several explanations for this discrepancy, such as the use of different detection methods (subcutaneous temperature chips in this study, rectal probe in the other), also the different genetic background of the mice due to cross breedings. Our present data show, that the ablation of FL-PGC-1α was on its own sufficient to lead to hypothermia in

male mice, while addition of a *Cramping* allele was necessary to lead to hypothermia in females. This reinforces the observation that dynein and FL-PGC-1α are both important for thermogenesis in mice (Leone et al., 2005; Eschbach et al., 2011; Puigserver et al., 1998). It also illustrates the higher basal thermogenic capacity in female rodents as compared with males (Rodriguez-Cuenca et al., 2002) as well as the gender-dependent effects of FL-PGC-1α which was previously observed in another mouse model of neurodegeneration (Eschbach et al., 2013). The underlying mechanisms for impaired thermogenesis will require further investigation, in particular to determine whether this is due to impaired mitochondrial function and/or impaired beta-adrenergic signalling.

Our cross breeding results indicate that the mitochondrial proliferation elicited by FL-PGC-1α increased activity is able to mitigate the phenotype of *Cramping* mice. This is in line with other experiments that showed that transgenic overexpression of FL-PGC-1α is able to attenuate symptoms of mitochondrial diseases in a tissue specific manner (Wenz et al., 2008, 2009; Dillon et al., 2012; Srivastava et al., 2009). Moreover PGC-1α overexpression selectively in skeletal muscle of transgenic mouse model of ALS improved muscle endurance and locomotor activity at symptomatic stages of the disease (Da Cruz et al., 2012). Lentiviral-mediated expression of PGC-1α in the striatum of R6/2 transgenic mouse model of HD was neuroprotective preventing neuronal atrophy in these mice (Cui et al., 2006). On the other hand crossbreeding PGC-1α knock-out mice with HD knock-in mice worsened significantly the behavioural and neuropathological abnormalities, which deleterious effect was even more profound after 3-NP administration (Cui et al., 2006).

Our data highlight the role of PGC-1α activation in diseases with mitochondrial dysfunction and might provide potential therapeutic targets. Such an approach has already been tested with bezafibrate, a pan-PPAR agonist able to increase PGC-1α activity (Wenz et al., 2008), but the mechanisms involved have recently been challenged (Viscomi et al., 2011; Yatsuga and Suomalainen, 2012). Besides, interventional studies with pioglitazone and rosiglitazone, both able to induce PGC-1α expression and activate the PPAR pathway (Hondares et al., 2006) were found to be beneficial in rodent models of ALS (Kiaei et al., 2004; Schutz et al., 2005) or PD (Breidert et al., 2002; Dehmer et al., 2004). Interestingly, microtubule inhibitors were found to induce PGC-1α expression in primary satellite skeletal muscle cells (Arany et al., 2008). The elucidation of the mechanisms underlying re-feeding induced increase in mtDNA might provide alternative targets. This pathway might also be of high interest for other diseases in which PGC-1α modulates the disease process. This is

especially the case for HD, where specific *PPARGC1A* gene variants are associated with changes in the clinical course (Soyal et al., 2012; Weydt et al., 2014, 2009).

In all, we demonstrated here that the full-length isoform of PGC-1α is required for disease induced mitochondrial proliferation and cannot be substituted by its N-terminally truncated isoforms. We also showed the protective potential of FL-PGC-1α against mitochondrial dysfunction, which has major implications in neurodegeneration and might provide potential therapeutic targets.

An indirect way to support mitochondrial involvement in neurodegeneration is via therapeutic interventions with drugs affecting mitochondrial functions. **We found that in higher doses L-carnitine (LC) produced significant improvement in survival and locomotor activity (including total distance moved, immobility time and velocity) in the N171/82Q transgenic mouse model of HD.** Though in an earlier human study, no significant changes were observed upon low dose LC on the abnormal involuntary movement scale, the mini-mental status, the reaction time and verbal fluency (Goety et al., 1990). During the one week (45 mg/kg/day) treatment period there were no serious side effects detected (Goety et al., 1990). In our experiment, the improvement in survival of 14,91% is nearly equivalent to the effects of other compounds with antioxidant properties, such as BN82451, remacemide and coenzyme Q10, although slightly less than the effects of creatine and cysteamine (Dedeoglu et al., 2002; Ferrante et al., 2002; Klivényi et al., 2003, Schilling et al., 2001).

The neuropathology of HD involves a selective neuronal loss, which occurs most markedly in the striatum and in deeper layers of the cerebral cortex (Ferrante et al., 1997). In the striatum, the loss of MSNs is most prevalent (Walker, 2007). The N171-82Q transgenic mouse model of HD resembles this pathological changes, others demonstrated 25% neuronal cell loss in the striatum of N171-82Q mice at 16 weeks of age and a 20% decrease in striatal cell volume (McBride et al., 2006). We also reproduced this striatal neuronal loss in vehicle-treated transgenic HD mice compared with wild-type animals. This decline was significantly reverted under LC treatment.

The N-terminal fragments of mutant HTT, which are expressed ubiquitously in both the nervous system and the peripheral tissues (Ferrante et al., 1997; Landwehrmeyer et al., 1995; Sharp et al., 1995; Strong et al., 1993) accumulate in the nucleus of affected neurons and form intranuclear aggregates (DiFiglia et al., 1997; Gutekunst et al., 1999). These huntingtin aggregates usually cause transcriptional dysregulation (Cha, 2000; Sugars and Rubinsztein, 2003), which leads to subsequent altered signal cascades among others involved in oxidative stress (Beal and Ferrante 2004). In our results there were significant decreases in EM48

immunoreactivity in the pyriform cortex of LC-treated N171-82Q mice and a slight decrease in the striatum relative to the vehicle-treated group.

LC and its acetyl ester, acetyl-LC (ALC) were found to be neuroprotective in different animal models of neuronal dysfunction / neurodegeneration, such as in spinal cord injury (Karalija et al., 2012; 2014), mitochondrial toxin models induced by MPTP, 3-NP, rotenone (Virmani et al., 2005; Silva-Adaya et al., 2008; Zaitone et al., 2012), and methamphetamine induced neurotoxicity (Virmani et al., 2002). ALC showed neuroprotective properties in rats exposed to global hypoxia via inducing PGC-1α and nuclear respiratory factor-1 mediated mitochondrial biogenesis (Hota et al., 2012). Recent data obtained from patients with inherited neurometabolic diseases confirmed the involvement of L-carnitine in the pathogenesis and supposed LC supplementation as beneficial (Ribas et al., 2014). Several studies were performed in AD and dementia, and however preclinical studies and earlier clinical studies suggested a protective effect of ALC treatment, a Cochrane meta-analysis revealed that there is no evidence of benefit for ALC treatment in dementia and AD (Hudson and Tabet, 2003). Though recently published data of a phase II randomized clinical trial with combinatorial nutritional supplementation (also including ALC) is promising, confirming a significant improvement in dementia rating scale (Remington et al., 2015).

The importance of reactive oxygen species (ROS) and free radicals has an increased attention in the last decade, since these molecules are aggravating factors in cellular injury and aging processes (Halliwell, 2001; Calabrese et al., 2012). A substantial body of evidence suggests a role of excitotoxicity and oxidative damage in the HD pathogenesis (Johri et al., 2013; Chaturvedi and Beal, 2013; Gil-Mohapel et al., 2014). It has been demonstrated that the expression of mutant HTT in neuronal and non-neuronal cells causes increased ROS, which contributes to cell death (Wyttenbach et al., 2002; St-Pierre et al., 2006). They raise the possibility that agents, which have antioxidative activity, may be useful as therapies to slow the progression of neurodegeneration in HD.

We demonstrated that L-carnitine administration to N171-82Q transgenic mice extends the survival, ameliorates the motor performance, preserves striatal neuron count and decreases the number of intranuclear HTT aggregates, these parameters being important in the pathomechanism of HD. We suggest that L-carnitine may develop its effect through decreasing the oxidative damage. While the exact mechanism responsible for the beneficial effects of LC in N171-82Q mice is uncertain, our data suggest that L-carnitine is neuroprotective and may possibly be beneficial in the treatment of HD.

VI. Conclusions

We investigated the role of retrograde axonal transport, notably the molecular motor dynein and mitochondrial dysfunction in neurodegeneration using different mouse models. First, we showed that cytoplasmic dynein is required for the maintenance of striatal function. We demonstrated that the *Cramping* mutation in the dynein heavy chain gene caused a characteristic behavioural phenotype with striatal atrophy and dysfunction in mice confirmed by multimodal approaches. This finding may have major implications for our understanding of the pathogenesis of striatal diseases, notably HD. Second, we proved that the overall mitochondrial proliferation observed in *Cramping* mice is FL-PGC-1α dependent. We showed that genetic ablation of FL-PGC-1α significantly worsened the overall, neurological and mitochondrial phenotype of these mice. It suggests that FL-PGC-1α is required for the compensatory maintenance of mitochondrial function in *Cramping* mice. This observation helps to understand better the underlying mechanisms in mitochondrial dysfunction related to disease. Third, we showed that L-carnitine, a nutrient with antioxidant properties also enhancing mitochondrial function, ameliorates the motor symptoms and increases survival in a HD transgenic mouse model. Moreover it was found to be neuroprotective for striatal neurons, the neuronal population markedly affected in HD. Thus, L-carnitine may be a promising compound in the therapy of Huntington's disease.

In all, our findings highlight the role of the molecular motor dynein and mitochondrial dysfunction in neurodegeneration, notably in Huntington's disease. These data contribute to better understanding of the pathomechanism of neurodegenerative diseases and offer potential therapeutic ways.

VII. Acknowledgements

I would like to express my gratitude to my supervisors, Péter Klivényi, Professor at the Department of Neurology, University of Szeged, and Luc Dupuis, research director at INSERM, University of Strasbourg and Professor at the Department of Neurology, Ulm University, for their excellent scientific guidance, practical advice and continuous support of my research activities. I would like to thank Professor László Vécsei, Head of the Department of Neurology, University of Szeged, and Professor Albert Ludolph, Head of the Department of Neurology, Ulm University, for their scientific supervision and the opportunity to work at their department and for all the support in my research career. Also I would like to thank Patrick Weydt and Anke Witting for their support and shaping my scientific thinking and attitude as well as their friendship. I wish to thank all my colleagues, co-workers and

technical assistants I performed the experiments with for all their help, especially Judith Eschbach, whose friendship I also highly value. Last but not least, I am grateful to my family, particularly my husband for their continuous support and patience during my work and that they let me have all the time necessary for writing the thesis.

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