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The Effects Of 27-Hydroxycholesterol And Palmitic Acid On Parkinson's Disease-Like Pathology

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THE EFFECTS OF 27-HYDROXYCHOLESTEROL AND PALMITIC ACID ON PARKINSON'S DISEASE-LIKE PATHOLOGY

by

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Bachelor of Science, University of North Dakota, 2013

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This dissertation, submitted by Jared John Schommer in partial fulfillment of the requirements for the Degree of Doctor of Philosophy from the University of North Dakota, has been read by the Faculty Advisory Committee under whom the work has been done and is hereby approved.

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This dissertation is being submitted by the appointed advisory committee as having met all of the requirements of the School of Graduate Studies at the University of North Dakota and is hereby approved.

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Dean of the Graduate School

June 22, 2018
Date

PERMISSION

Title: The Effects of 27-Hydroxycholesterol and Palmitic Acid on Parkinson's Disease-like Pathology

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Jared John Schommer
August, 2018

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To those suffering with synucleinopathies and their families

To Kirsten and my family

To Adler, Ber, and potential future children:
Life is a gift and what you choose to do with it is up to you. Anything you aspire to do in life is all at your fingertips and can be attained with hard work, drive, and the support of a great family and friend network.

Always believe in yourself and your capabilities.

ABSTRACT

Synucleinopathies is the overarching term used to describe a group of neurodegenerative disorders characterized by aggregates of α-synuclein (α-syn) protein in the cytoplasm of neurons, nerve fibers, and glial cells. The cause(s) for synucleinopathies are likely multi-factorial with genetic predisposition and environmental factors participating in the pathogenesis of the diseases. Dietary factors including dyslipidemia of cholesterol, its metabolites, and fatty acids have shown conflicting results as risk factors in recent years. Our overarching hypothesis is that dietary factors including 27-Hydroxycholesterol (27-OHC) and Palmitic Acid (PA) can affect key proteins involved in synucleinopathies.

Findings regarding risk related specifically to dietary cholesterol have indicated either an increased risk, decreased risk, or no association. We believe the reason for the conflicting association between cholesterol and synucleinopathies lies in the metabolites of cholesterol and not cholesterol *per se*. Supporting our hypothesis many studies have shown increased levels of many different oxysterols, including 27-OHC, within the brains of synucleinopathy patients. However, the extent to which increased 27-OHC levels in the brain causes α-syn deposition and promotes synucleinopathies is yet to be determined. Therefore, in this dissertation we explore the effects of 27-OHC on the accumulation of α-syn and investigate the mechanisms of such involvement.

We demonstrate that 27-OHC induces an increase in α-syn levels in human dopaminergic neurons. The mechanism involved in the α-syn increase does not appear to involve LXRs as we did not observe any significant changes in α-syn mRNA with 27-OHC or LXR agonist and antagonistic treatments. To the best of our knowledge, our results are the first to show that 27-OHC increases α-syn in dopaminergic neurons and that this increase may emanate from inhibition of the proteasomal function. Also, 27-OHC decreases levels of HSP70 protein which is involved in protein folding, and protein degradation through the Ubiquitin-Proteasomal System (UPS). The extent to which a decrease in HSP70 protein levels leads to decreased protein folding and degradation through specific pathways needs to be further elucidated. All-together, our results potentially suggest that restoring proteasomal function and HSP70 protein levels may attenuate the 27-OHC-induced increase in α-syn protein levels *in vitro* and reduce α-syn accumulation that can increase the risk for synucleinopathies.

Additionally, to date, studies focused on the contributions of dietary fat intake to the risk of PD type synucleinopathy have yielded inconsistent results. Epidemiological studies of dietary fat intake and PD have found positive associations, no association, and even protective effects. As implied by all the conflicting studies, the jury is still out on the role(s) of FAs in PD-type synucleinopathy risk.

Palmitic acid (PA) (16:0) is the most abundant saturated fatty acid in the body and the most abundant fatty acid in our diet. It has been shown to increase ER stress proinflammatory cytokine expression in astrocytes and microglia, and

activation of TLRs via NFKβ but its role in pathological hallmark formation of PD-type synucleinopathy remains unknown. Throughout this dissertation we aim to examine the role(s) of PA on various hallmarks of PD-type synucleinopathy pathology in various animal and cellular models. We demonstrate that a PA-enriched diet induces an increase in α-syn and TH protein and mRNA expression in both B6D2 and m-Thy1 mice. We also show that the PA-enriched diet does not affect biogenic amine content in control B6D2 mice but significantly changes dopamine and serotonin levels in m-Thy1 mice relative to control-fed mice. Our results demonstrate that a diet enriched in PA increases the levels of Tyrosine Hydroxylase (TH), and serotonin, an effect that can provide beneficial effects in a variety of conditions.

Additionally, we demonstrate that PA treatment in mouse dopaminergic neurons decreases α-syn protein and mRNA expression as well as it decreases TH protein content. Our study is the first to show that within MPTP-injected C57BL/6 mice a PA-enriched diet preserves motor function, decreases α-syn accumulation, increases TH protein, and increases dopaminergic neuronal survival. Altogether, our results suggest that a diet enriched in PA is protective against MPTP-induced Parkinsonism. Future studies are needed to elucidate the mechanisms by which a PA-enriched diet modulates these proteins. Establishing the effects of a smaller percentage of PA in the diet may reveal beneficial effects of this saturated free fatty acid in neurodegenerative conditions including PD and other synucleinopathies.

CHAPTER I

INTRODUCTION

Synucleinopathies

Synucleinopathies is the overarching term used to describe a group of neurodegenerative disorders characterized by aggregates of alpha-synuclein (α-syn) protein in the cytoplasm of neurons, nerve fibers, and glial cells (Goedert, Jakes, & Spillantini, 2017; Mart�, Tolosa, & Campdelacreu, 2003). Aggregates of α-syn are termed Lewy Bodies after Fritz Heinrich Lewy discovered them in 1912. These disorders include multiple system atrophy (MSA), dementia with Lewy bodies (DLB), and most commonly Parkinson's disease (PD). In the clinic, they are characterized by chronic and progressive decline in motor, cognitive, behavioral, and autonomic functions, depending on the localization of the α-syn aggregates. These disorders have a large clinical overlap that makes distinguishing the diagnosis between the disorders very difficult for physicians.

Multiple Systems Atrophy

MSA is a fatal neurodegenerative disease that is characterized by a combination of parkinsonian features, progressive autonomic failures, and cerebellar and pyramidal features. MSA can be classified as a cerebellar type or parkinsonian type depending on which features are predominately presented (Fanciulli & Wenning, 2015a).

MSA is not very common and is considered an orphan disease (Orpha number, ORPHA102). The estimated mean incidence is 0.6 to 0.7 cases per 100,000/year, with up to 2.4 cases per 100,000 /year (Bower, Maraganore, McDonnell, & Rocca, 1997). Prevalence ranges from 1.9 to 4.9 per 100,000 inhabitants over the age of 50 years (Gregor K Wenning, Colosimo, Geser, & Poewe, 2004). The average age of onset is 60 years and affects males and females equally (Wüllner et al., 2007) for a typical disease duration of 7 to 9 years before death (A. Schrag, Wenning, Quinn, & Ben-Shlomo, 2008).

The causes for MSA are unknown but the following environmental factors have been implicated as risk factors: metal dusts and fumes, plastic additives, organic solvents, pesticides, diet, and physical activity (Alavanja et al., 1996; Frumkin, 1998; Hanna, Jankovic, & Kirkpatrick, 1999; Nagai et al., 2012; Nee et al., 1991) while nicotine use, drinking alcohol, and eating fish and seafood are more common in healthy controls and are considered as being protective against MSA (Chrysostome et al., 2004; Johnsen & Miller, 1986; N Vanacore et al., 2000; Nicola Vanacore et al., 2005; Vidal et al., 2008). MSA is normally considered a sporadic disease but genetic factors play a role in some families. It has been transmitted by an autosomal dominant or autosomal recessive pattern (Itoh et al., 2014; Stemberger, Scholz, Singleton, & Wenning, 2011). Loss of function mutations in COQ2, encoding the coenzyme Q_{10}-synthesizing enzyme (Multiple-System Atrophy Research Collaboration, 2013),single-nucleotide polymorphisms in the SNCA gene (Scholz et al., 2009), and mutations in many inflammatory related genes have been associated with causality of MSA.

Degeneration of the nigrostriatal pathway and olivopontocerebellar atrophy are normally found in postmortem brains of individuals with MSA. These features broadly present the individual with parkinsonism and ataxia (Ahmed et al., 2012). Neurodegenerative changes can also be seen in the hypothalamus, nuclei of noradrenergic and serotonergic connections, dorsal nuclei, and intermediolateral columns of the spinal cord (Benarroch, 1993). The major histological hallmark of MSA is the intracytoplasmic proteinaceous oligodendroglial inclusions (also known as Papp-Lantos bodies). Less commonly, neuronal axonal, neuronal cytoplasmic and nuclear, and oligodendroglial nuclear inclusions are also observed. In addition to cytoplasmic inclusions, reactive astrocytes and microglia are commonly found. The main protein found within the inclusions is misfolded α-syn protein as in other synucleinopathies. The distinguishing factor between MSA and other synucleinopathies is that MSA is the only disease in which the oligodendroglial cells are the cells affected.
Most evidence from animal models and postmortem studies suggest that the pathogenesis of MSA is oligodendrogliopathy (Fig. 1)(Fanciulli & Wenning, 2015b). The redistribution of an important stabilizer of myelin integrity, p25α, into the cell body of oligodendroglial cells happens before α-syn aggregation (Y. J. C. Song et al., 2007) The redistribution of p25α is followed by the swelling of affected cells and the abnormal uptake and/or overexpression of the α-syn protein (Asi et al., 2014; Reyes et al., 2014). The interactions of α-syn and p25α lead to the aggregation of α-syn in oligodendroglial cells. Cytoplasmic inclusions

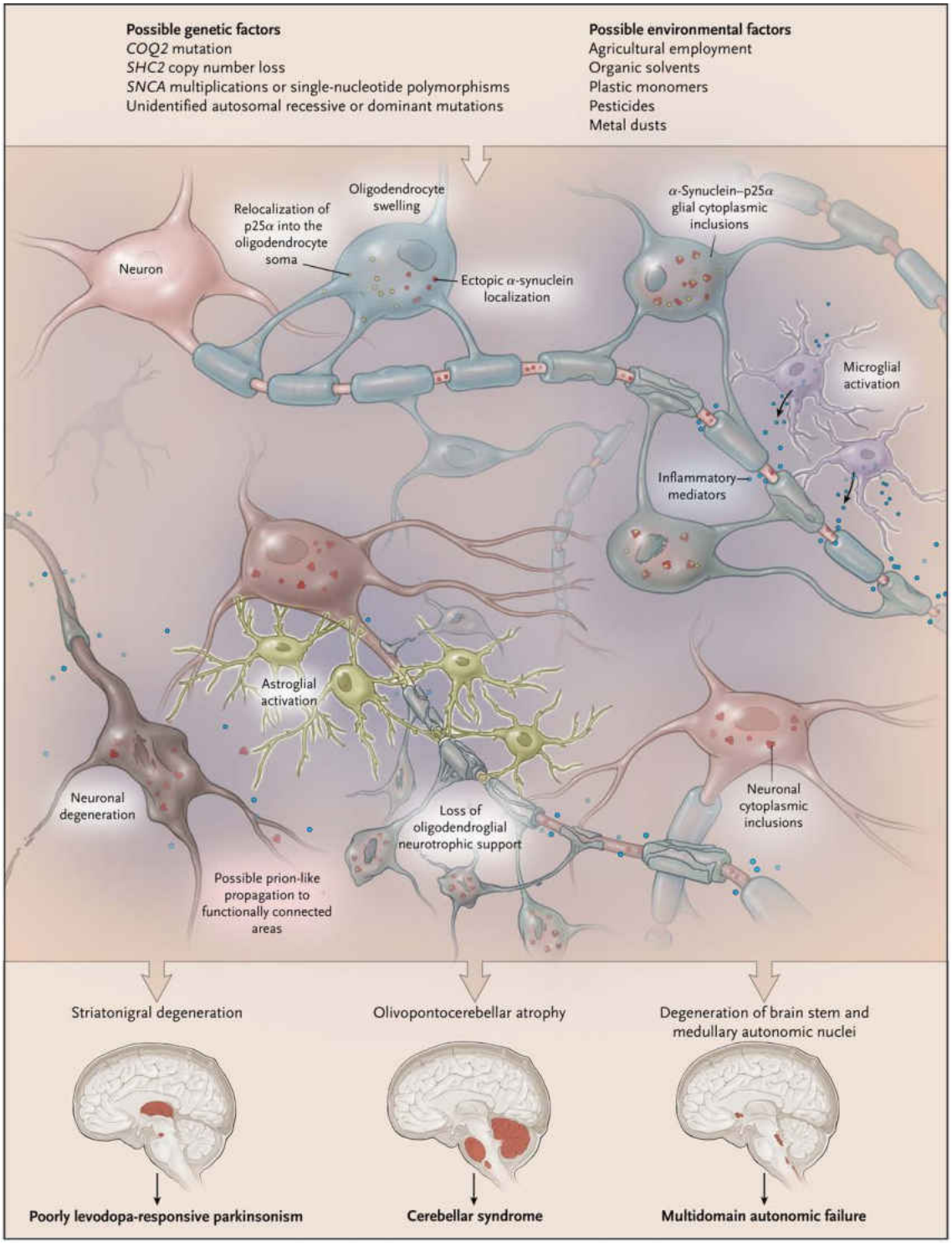

Figure 1. Schematic of the pathogenesis of MSA. Reproduced with permission from (Fanciulli & Wenning, 2015a), Copyright Massachusetts Medical Society.

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cause dysfunctions in these cells and the activation of microglial cells. These events lead to progressive dysfunction in oligodendrocytes and the release of misfolded α-syn into the extracellular space where it is taken up by neighboring neurons and glial cells. This ultimately leads to spreading of the disease to many different brain regions leading to the multisystem dysfunction associated with MSA (Watts et al., 2013).

There is a prodromal premotor phase in 20 to 75% of MSA cases that include urinary urge incontinence or retention, sexual dysfunction, orthostatic hypotension, REM sleep behavior disorder, and inspiratory stridor years before motor symptoms appear (Jecmenica-Lukic, Poewe, Tolosa, & Wenning, 2012). Motor features include Parkinsonism (slow movements, rigidity) while the “pill-rolling” resting tremor is unusual in the parkinsonian subtype of MSA. Levodopa treatments lack a response in MSA (G. K. Wenning, Tison, ben Shlomo, Daniel, & Quinn, 1997). Cerebellar ataxia leads in the motor presentation of the cerebellar subtype of MSA (Bensimon et al., 2009). Features of the cerebellar type consist of uncoordinated limb movements, wide-based gait, action tremors, hyperreflexia, Babinski sign, and spontaneous, gaze-evoked, or positional downbeat nystagmus (Köllensperger et al., 2010). Fig. 2 shows the various presentations of MSA while Fig. 3 shows the disease progression over an average of 10 years.

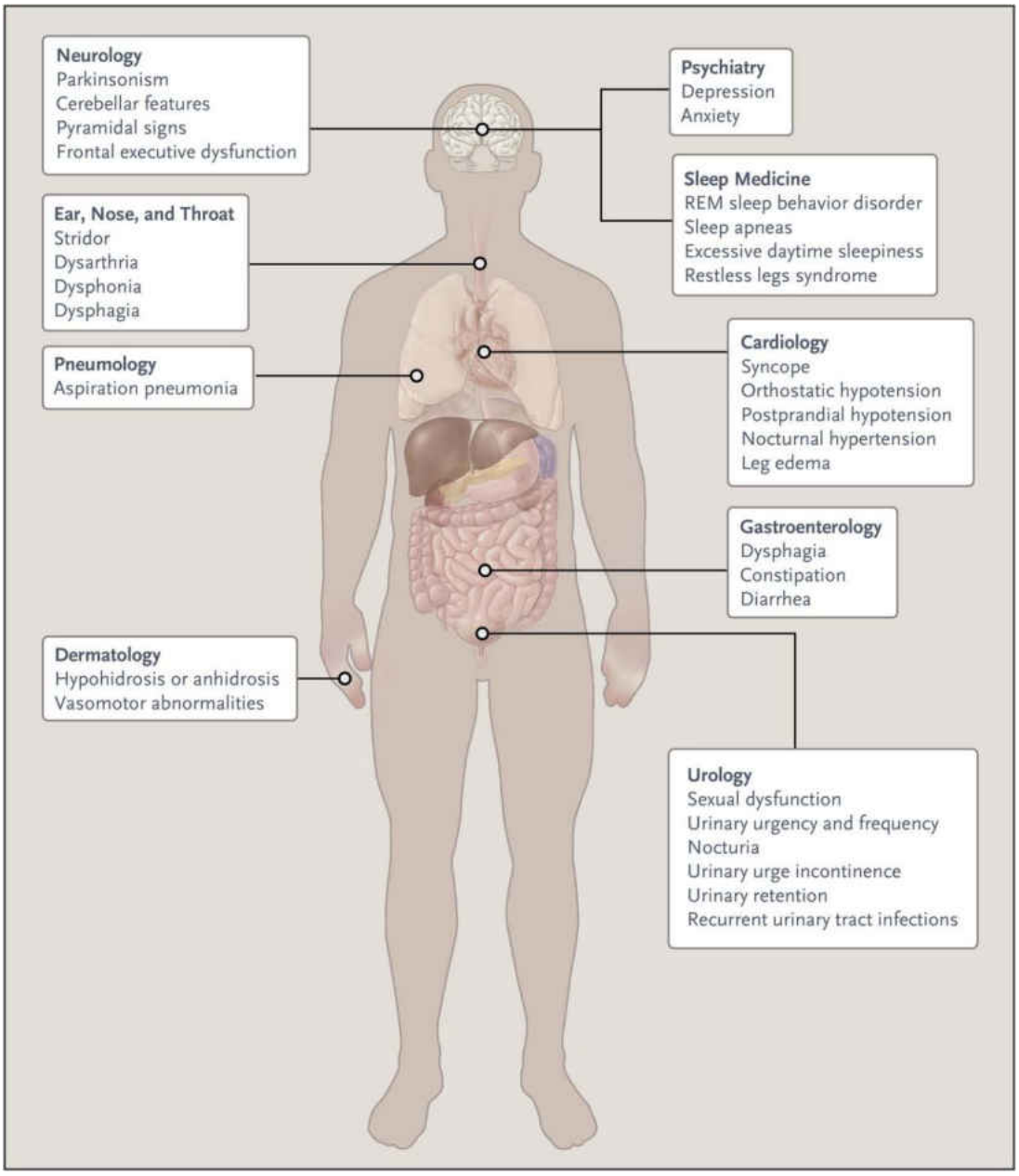


Figure 2. Diagram of the various symptoms associated with MSA. Reproduced with permission from (Fanciulli & Wenning, 2015a), Copyright Massachusetts Medical Society.

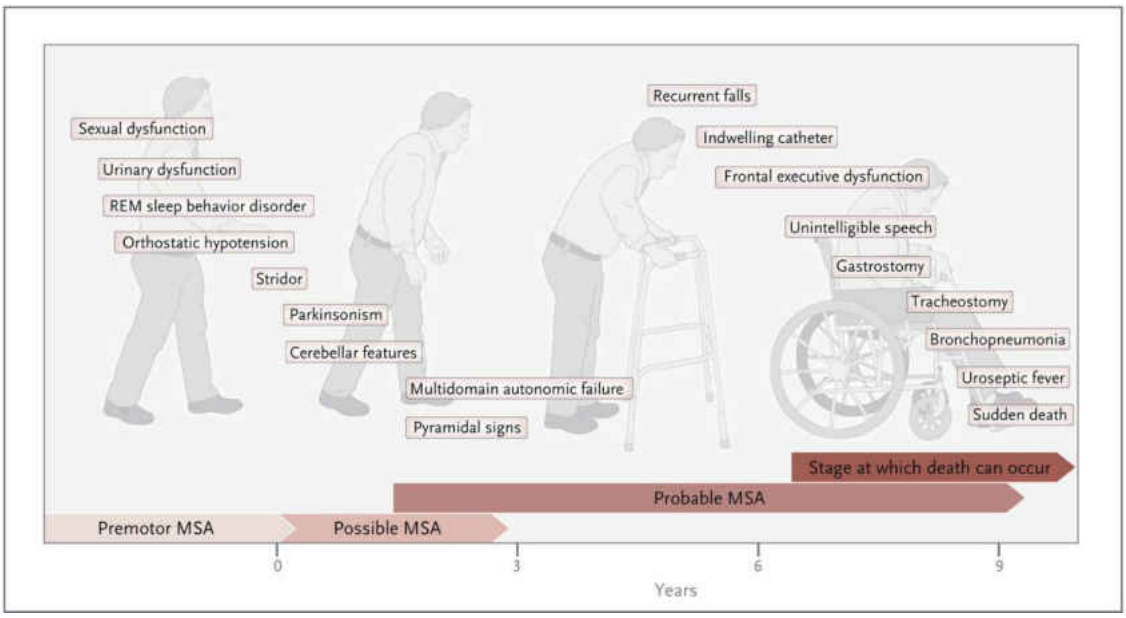


Figure 3. Schematic of MSA progression over 10 years. Reproduced with permission from (Fanciulli & Wenning, 2015a), Copyright Massachusetts Medical Society.

Dementia with Lewy Bodies

Dementia with Lewy Bodies (DLB) also known as Lewy Body Disease, Lewy Body dementia, and diffuse Lewy Body Disease is considered the second most common cause of dementia next to Alzheimer's disease. It is a progressive neurodegenerative disorder that has main features of psychosis, cognitive impairment, and parkinsonism (Fig. 4).

The prevalence of DLB in the United States is estimated to be 1.3 million individuals (Savica et al., 2013). DLB has an incidence of 5.9 cases per 100,000 persons/year, with a higher incidence in men than in women (7.2 vs 4.9) (Savica et al., 2013). The incidence of DLB rises with age from 10.3 cases per 100,000 persons/year for age 60-69 to 44.5 cases per 100,000 persons/year for ages 70-79 and finally remaining high at 30.1 cases per 100,000 persons/year in ages

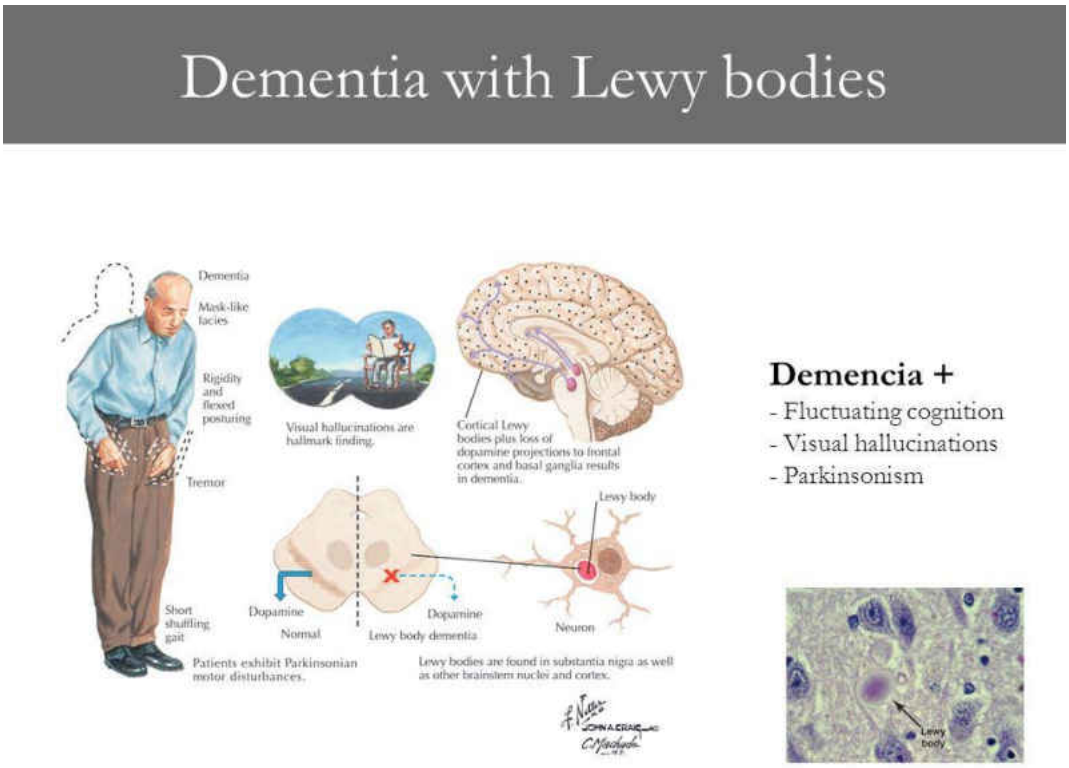


Figure 4. Overview of Dementia with Lewy bodies. Reproduced with permission from (Dra. Carla Abdelnour Ruiz, n.d.).

80-89 (Savica et al., 2013). Average life expectancy is 5-7 years after age of onset (“Dementia with Lewy Bodies | Family Caregiver Alliance,” n.d.).

The causes for DLB are unknown but the following factors have been implicated as risk factors: old age, being male, history of anxiety, family history of dementia, depression, stroke, and a family history of Parkinson’s disease whereas being more educated, history of cancer, smoking, and using caffeine seem to be more protective against DLB (Boot et al., 2013). DLB is normally considered a sporadic disease but genetic factors play a role in some families. Mutations in the GBA gene, a gene normally associated with Gaucher’s disease,

Apolipoprotein E (APOE), and SNCA have been shown in individuals suffering with DLB ("Genetic Variant Increases Risk for Dementia in Lewy Body Diseases | Lewy Body Dementia Association," n.d.; R. Guerreiro et al., 2018).

In DLB, Lewy bodies are found within the cytoplasm of monoaminergic and cholinergic neurons within multiple cortical regions and brainstem nuclei (Dickson, 2002). Lewy bodies are found within neuronal processes called Lewy body neurites in the dorsal motor nucleus of the vagus nerve, hippocampus, amygdala, and throughout temporal lobe structures and the limbic system. Lewy bodies are also found in the peripheral and autonomic nervous systems. The localization of lewy body pathology is very important to specific types of cognitive impairment and clinical symptoms. The presence of Lewy bodies is required for the DLB diagnosis at autopsy but the presence of Lewy bodies is not in itself enough to cause clinical symptoms. An estimated 24 to 55% of healthy elderly adults are found to have a significant amount of Lewy bodies (Jellinger, 2009). Although Lewy bodies are needed in a diagnosis of DLB, 35 to 90% of patients with DLB also have varying degrees of Alzheimer's-like pathology such as neurofibrillary tangles and senile plaques (Hansen & Samuel, 1997). Neurofibrillary tangles are filamentous aggregates, which are composed of the microtubule associated protein TAU within the cell body and dendrites of neurons. These tangles initially start in the limbic structures and progressively move to the multimodal association cortices and finally the primary cortices. Senile plaques are lesions composed of extracellular deposits of beta-amyloid protein (Aβ), which is a 40 to 42 amino acid peptide derived from amyloid

precursor protein (Dickson, 2002) and can also be found throughout much of the brain.

Symptoms of DLB include reoccurring visual hallucinations as one of the first symptoms. Individuals may see people or things that aren't there and hear and smell things that are not present. Parkinsonism (slow movements, tremor, rigidity) may be present along with autonomic nervous system dysfunctions resulting in dizziness, falling, and constipation. Sleep difficulties, fluctuating attention, depression and apathy are also commonly seen in DLB. Finally cognitive problems such as confusion, visual-spatial problems and memory loss are commonly seen in individuals suffering with DLB ("Dementia with Lewy bodies: diagnostic and predictive biomarkers - ppt video online download," n.d.; "Lewy body dementia - Symptoms and causes - Mayo Clinic," n.d.; Dickson, 2002; Javed et al., 2008; Savica et al., 2013). Fig. 5 shows some of the main symptoms of DLB.

Parkinson's Disease

Parkinson's disease (PD) is the second-most common neurodegenerative disorder next to Alzheimer's disease that affects 2-3% of the population ≥ 65 years old (Poewe et al., 2017). It is a progressive disease that consists of dopaminergic neuronal loss in the *substantia nigra*, resulting in deficient striatal dopamine, and Lewy bodies containing α-syn as the neuropathological hallmarks.

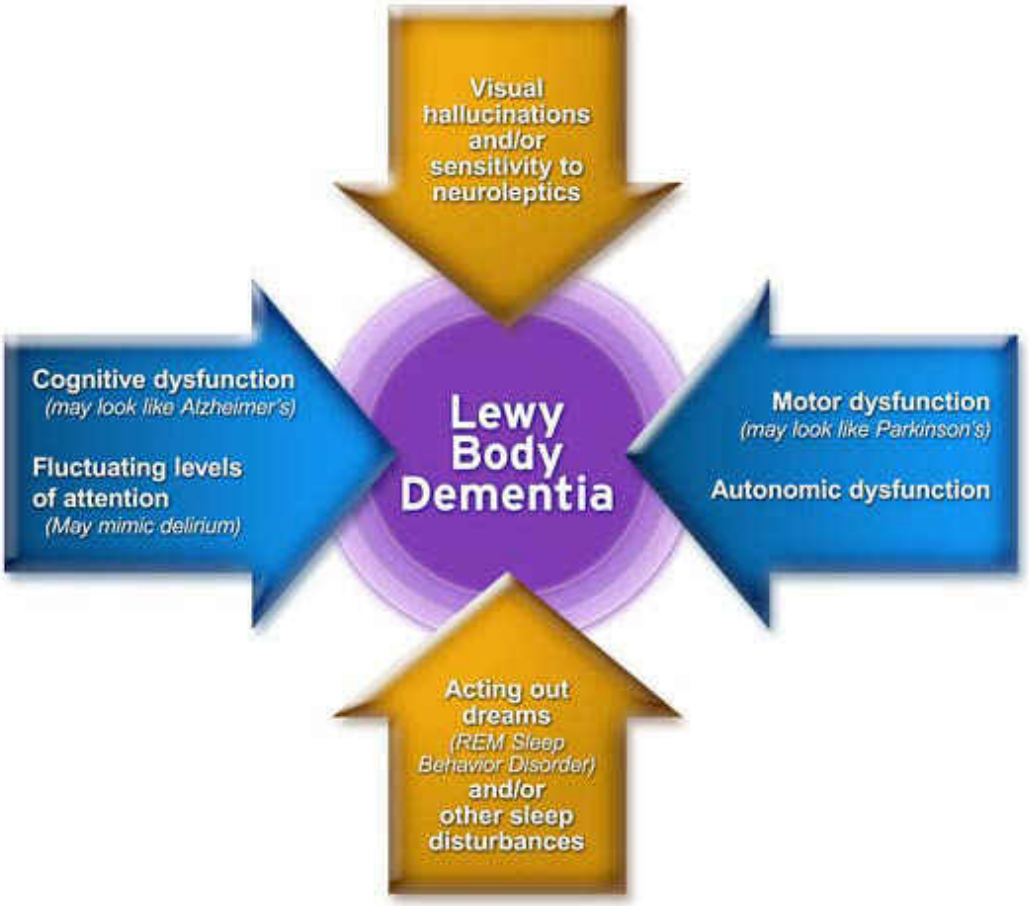


Figure 5. Main symptoms of DLB. Reprinted with permission from ("lewy body dementia going gentle into that good night," n.d.)

.

The worldwide yearly incidence estimates for PD ranges from 5 to >35 new cases per 100,000 people (Twelves, Perkins, & Counsell, 2003). The global prevalence estimated at 0.3% overall, increases sharply with age to >3% in those greater than 80 years of age and is visualized in Fig. 6 (Pringsheim, Jette, Frolkis, & Steeves, 2014). PD is twice as common in men than in women in most populations (Baldereschi et al., 2000; Van Den Eeden et al., 2003). Protective effects of sex-associated genetic mechanisms, female sex hormones, and sex-specific differences in exposures and reactions to environmental risk factors might explain the drastic difference between males and females regarding PD. Mortality does not increase within the first ten years of disease onset, but increases thereafter, eventually being twice as high compared with the general population mortality rate (Pinter et al., 2015).

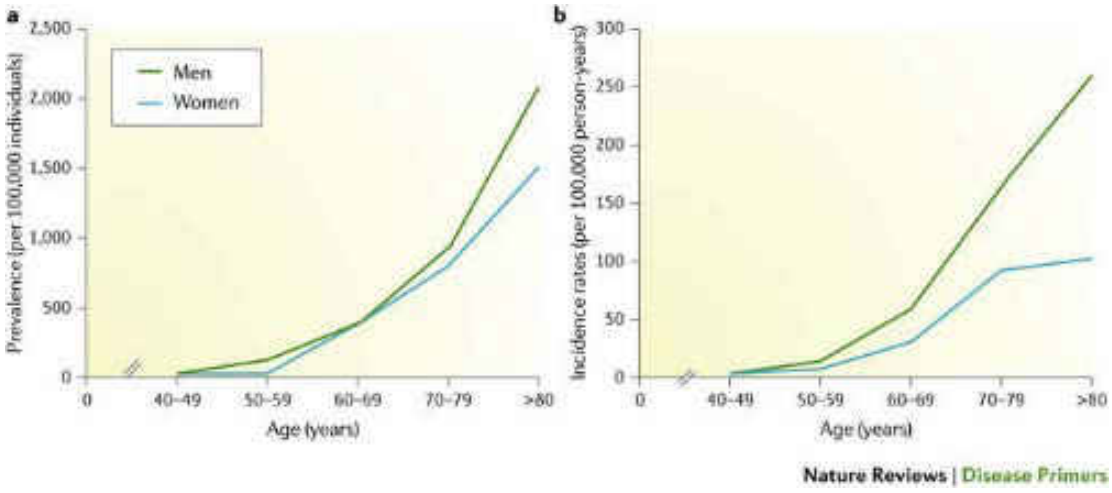


Figure 6. Incidence and prevalence of Parkinson's disease. A. Prevalence of PD in men and women per 100,000 individuals. B. Incidence rate of PD per 100,000 people. Reprinted by permission from RightsLink, Springer Nature, (Poewe et al., 2017), Copyright 2017.

Roughly 90% of PD cases are sporadic with the causes being unknown but the following environmental factors have been implicated as risk factors: dairy products, pesticides, methamphetamine, certain cancers, traumatic brain injury, body-mass index and diabetes, blood cholesterol and hypertention, heavy alcohol consumption, postmenopausal hormones and reproductive factors, and fat and other macronutrients (Abbott, Ross, et al., 2003; Alberto Ascherio et al., 2004, 2004; Callaghan, Cunningham, Sajeev, & Kish, 2010; Honglei Chen, Zhang, Hernán, Willett, & Ascherio, 2003a; Curtin et al., 2015; L M L de Lau et al., 2005a; Driver et al., 2008; Eriksson, Löfving, Callaghan, & Allebeck, 2013; Guilarte, Nihei, McGlothan, & Howard, 2003; G Hu et al., 2006; G Hu, Antikainen, Jousilahti, Kivipelto, & Tuomilehto, 2008; Jiang, Ju, Jiang, & Zhang, 2014; Langston, Ballard, Tetrud, & Irwin, 1983a; R. Liu, Gao, Lu, & Chen, 2011; Marras et al., 2014; Louis C Tan et al., 2008a; Tanner et al., 2011; Tysnes & Storstein, 2017; Qun Xu et al., 2011). Factors considered as protective against PD are as follows: tobacco, coffee and caffeine, green and black tea, urate, physical activity, Non-steroidal anti-inflammatory drugs (NSAIDs), calcium channel blockers, statins in some studies, and flavonoids (A Ascherio et al., 2001; Bakshi et al., 2015; Becker, Jick, & Meier, 2008a; H Chen et al., 2010; H Chen, Zhang, Schwarzschild, Hernán, & Ascherio, 2005; Honglei Chen, Zhang, Hernán, Schwarzschild, et al., 2003; X. Chen et al., 2013; Duan et al., 2002; X Gao, Cassidy, Schwarzschild, Rimm, & Ascherio, 2012; Xiang Gao, Chen, Schwarzschild, & Ascherio, 2011; Xiang Gao, Simon, Schwarzschild, & Ascherio, 2012a, 2012b; Gong et al., 2012; S. Guerreiro et al., 2009; Gang Hu, Bidel,

Jousilahti, Antikainen, & Tuomilehto, 2007; Y.-C. Lee et al., 2014; Lin et al., 2016; R. Liu et al., 2012; G Logroscino, Sesso, Paffenbarger, & Lee, 2006; Morens, Grandinetti, Reed, White, & Ross, 1995; O'Reilly et al., 2005; Pasternak et al., 2012; Ritz, Rhodes, et al., 2010; Ross et al., n.d.; Louis C Tan et al., 2008b; E L Thacker et al., 2007; Evan L Thacker et al., 2008; Undela, Gudala, Malla, & Bansal, 2013; Wolozin et al., 2007; Q Xu et al., 2010; Fei Yang et al., 2015). Though PD is generally of sporadic origin, familial forms do exist. In 1997, the first mutation leading to PD was identified in the gene encoding the vesicular protein α-syn, a missense mutation from alanine to threonine in position 53 (A53T) (Polymeropoulos et al., 1997a) followed by the finding of another missense mutation, alanine 30 to proline (A30P) (Krüger et al., 1998a). Since then, many different genes have become implicated in familial forms of PD (Table 1). These genes code for proteins that normally function as mitochondrial membrane proteins, protein homeostasis and degradation machinery, and oxidative stress sensors.

Neuropathologically, PD includes characteristic features of neuronal loss in the *substantia nigra* and widespread intracellular proteinaceous inclusions consisting of α-syn. The loss of dopaminergic neurons and the deposition of α-syn is not specific to PD but these two neuropathologies in concert are specific for a decisive diagnosis of idiopathic PD (Fig. 7).

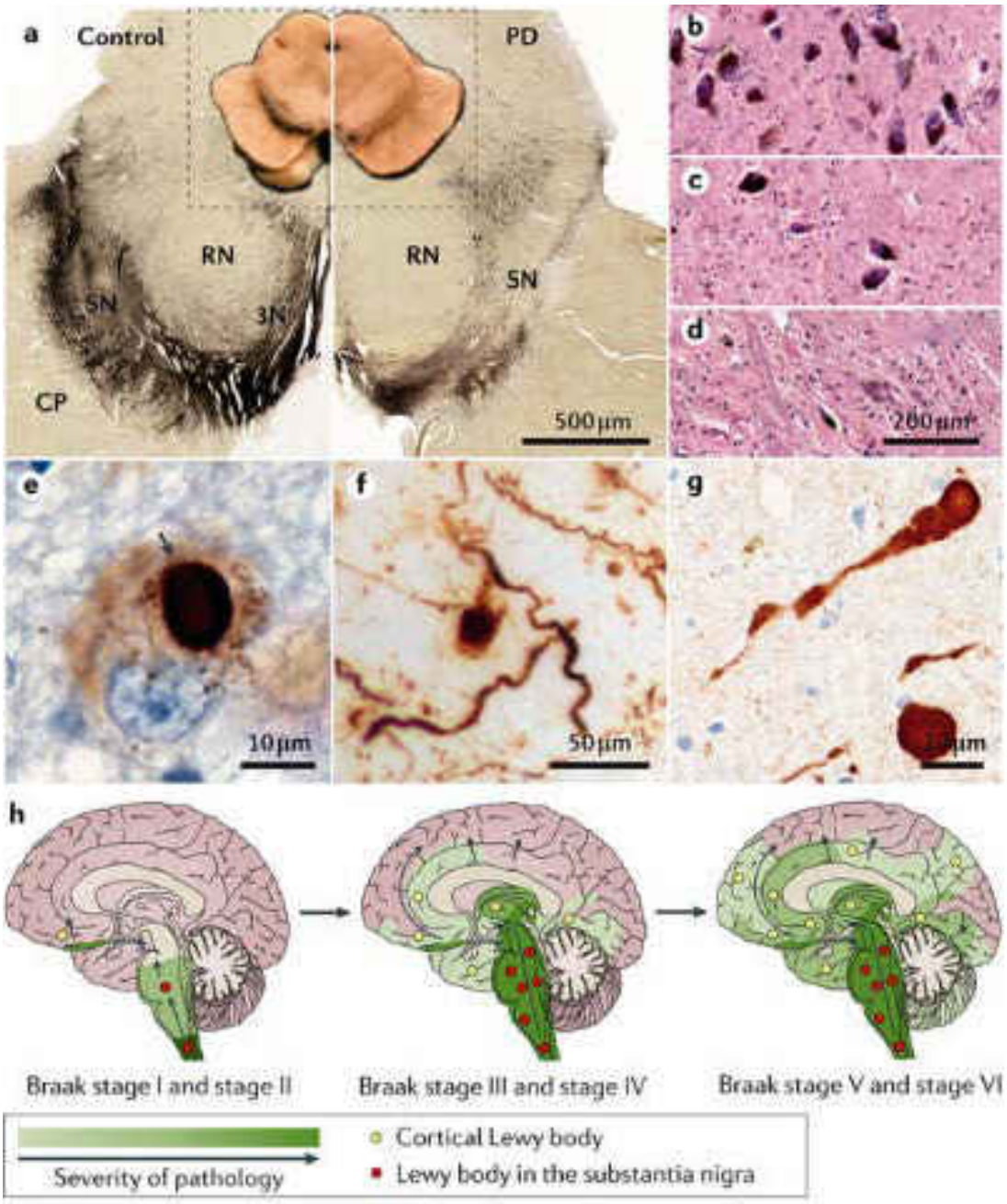
Table 1. Classification of hereditary parkinsonism. Reprinted by permission from RightsLink, Springer Nature, (Poewe et al., 2017), Copyright 2017.

Locus symbol	New designation‡	Gene locus	Gene	OMIM (phenotype MIM number; gene/locus MIM number)	Clinical clues
Autosomal dominant Parkinson disease					
PARK1 or *PARK4*	PARK-*SNCA*	4q22.1	*SNCA*	• 168601; 163890 (*PARK1*) • 605543; 163890 (*PARK4*)	Missense mutations (*PARK1*) cause classic Parkinson disease phenotype. Duplication or triplication of this gene (*PARK4*) causes early-onset Parkinson disease with prominent dementia
PARK8	PARK-*LRRK2*	12q12	*LRRK2*	607060; 609007	Classic Parkinson disease phenotype. Variations in *LRRK2* include risk-conferring variants and disease-causing mutations
PARK17	PARK-*VPS35*	16q11.2	*VPS35*	614203; 601501	Classic Parkinson disease phenotype
Early-onset Parkinson disease (autosomal recessive inheritance)					
PARK2	PARK-*Parkin*	6q26	*PARK2* encoding parkin	600116; 602544	Often presents with lower limb dystonia
PARK6	PARK-*PINK1*	1p36.12	*PINK1*	605909; 608309	Psychiatric features are common
PARK7	PARK-*DJ1*	1p36.23	*PARK7* encoding protein deglycase DJ1	606324; 602533	Early-onset Parkinson disease
PARK19B	PARK-*DNAJC6*	1p31.3	*DNAJC6*	615528; 608375	Onset of parkinsonism between the third and fifth decades of life
Complex genetic forms (autosomal recessive inheritance)					
PARK9	PARK-*ATP13A2*	1p36.13	*ATP13A2*	606693; 610513	Early-onset parkinsonism with a complex phenotype (for example, dystonia, supranuclear gaze palsy, pyramidal signs and cognitive dysfunction); also known as Kufor–Rakeb syndrome

PARK14	PARK-*PLA2G6*	22q13.1	*PLA2G6*	256600; 603604	PLAN (or NBIA2) is characterized by a complex clinical phenotype, which does not include parkinsonism in the majority of cases
PARK15	PARK-*FBXO7*	22q12.3	*FBXO7*	260300; 605648	Early-onset parkinsonism with pyramidal signs and a variable complex phenotype (for example, supranuclear gaze palsy, early postural instability, chorea and dystonia)
PARK19A	PARK-*DNAJC6*	1p31.3	*DNAJC6*	615528; 608375	Juvenile-onset parkinsonism that is occasionally associated with mental retardation and seizures
PARK20	PARK-*SYNJ1*	21q22.11	*SYNJ1*	615530; 604297	Patients may have seizures, cognitive decline, abnormal eye movements and dystonia
PARK23	Not yet assigned	15q22.2	*VPS13C*	616840; 608879	Young-adult-onset parkinsonism associated with progressive cognitive impairment that leads to dementia and dysautonomia

Legend: The locus symbols originate from the Online Mendelian Inheritance in Man (OMIM) catalogue (https://omim.org). Seven loci, which have been assigned a PARK designation, have a yet unconfirmed relationship to disease (that is, PARK3, unknown gene on 2p13; PARK5, *UCHL1* on 4p13; PARK11, *GIGYF25* on 2q37.1; PARK13, *HTRA2* on 2p13.1; PARK18, *ELF4G1* on 3q27.1; PARK21, *DNAJC13* on 3q22; and PARK22, *CHCHD2* on 7p11.2) and three are classified as risk loci (PARK10 on 1p32; PARK12 on Xq21–q25; and PARK16 on 1q32).

Figure 7. Main neuropathologies of PD. (a) PD is defined by depigmentation of the *substantia nigra* (right side) compared to control (left side). Macroscopic and transverse sections of the midbrain following staining with Tyrosine Hydroxylase. Selective loss of the ventrolateral parts of the *substantia nigra* with sparing of the more medial and dorsal regions. (b-d) Haematoxylin and eosin staining of the ventrolateral region of the *substantia nigra* showing a pigmented normal health control (b) and significant moderate (c) and severe (d) pigmeted cell loss. e-g. IHC staining of α-syn shows the round, intracytoplasmic Lewy bodies (e), more diffuse, granular deposits of α-syn (e and f), deposits of α-syn in neuronal cell processes (f.), extracellular dot-like α-syn structures (f) and α-syn spheroids in axons (g). (h) The theorized progression of α-syn aggregation in PD. α-syn inclusions occur in monoaminergic and cholinergic lower brainstem neurons in asymptomatic cases (Braak stage I and stage II), infiltrate similar neurons in the midbrain and basal forebrain in those with the motor symptoms of PD (Braak stage III and stage IV), and then are found later in limbic and neocortical brain regions with disease progression (Braak stage V and stage VI) 3N, 3rd nerve fibres; CP, cerebral peduncle; RN, red nucleus. Reprinted by permission from RightsLink, Springer Nature, (Poewe et al., 2017), Copyright 2017.



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PD does not feature global macroscopic atrophy of the brain, rather neuronal degeneration occurs in certain areas and mainly in dopaminergic neurons. In early-stage PD, loss of these neurons is restricted to the ventrolateral *substantia nigra* with relative conservation of other dopaminergic neurons of the midbrain (Damier, Hirsch, Agid, & Graybiel, 1999; Fearnley & Lees, 1991) but spreads to the rest of the midbrain by end-stage PD. The loss of these dopaminergic neurons at such an early time in the disease progression suggests that the deterioration in the *substantia nigra* starts earlier than motor symptoms present, which is supported by several studies (Dijkstra et al., 2014; Iacono et al., 2015).

The abnormal accumulation of α-syn protein in the cytoplasm of certain neurons in several different brain regions is the other required neuropathology for a definitive PD diagnosis (Braak et al., 2003). Lewy bodies, mainly consisting of α-syn protein were the first to be described. Since then, a wider range of α-syn aggregates have been described (Fig. 7 e-g). The Lewy type pathology initially occurs in monoaminergic and cholinergic neurons of the brainstem and in neurons of the olfactory system but also is found in neocortical and limbic regions as PD progresses (Fig. 7 h).

Heritable forms of PD only represent about 5-10% of cases but have provided invaluable clues to the mechanisms underlying the neuropathology of PD. Many of the proteins associated with PD are involved in molecular pathways that, when disturbed, can lead to pathology that resembles sporadic PD. Examples of these pathways are: proteostasis of α-syn, oxidative stress,

mitochondrial function, neuroinflammation, transport via axons, and calcium homeostasis (Fig. 8).

PD diagnosis consists of 2 steps. Step 1 includes the presence of bradykinesia as a slowness of movement and a decrease in amplitude and/or speed as movements are started and continued along with the combination of at least one of: resting tremor and/or rigidity. Step 2 involves determining PD as the cause of parkinsonism with two levels of diagnostic certainty. The diagnosis of PD requires all three of the below parameters:

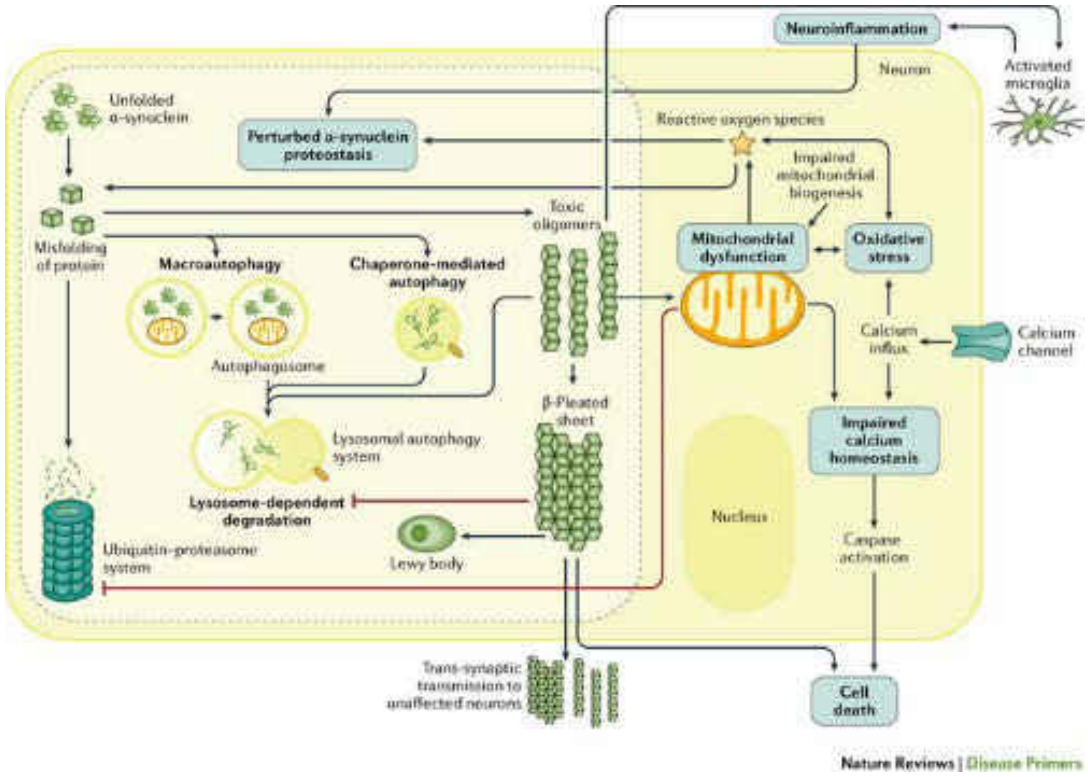

Figure 8. Schematic of molecular mechanisms of Parkinson's Disease. Reprinted by permission from RightsLink, Springer Nature, (Poewe et al., 2017), Copyright 2017.

- Absence of absolute exclusion criteria. This includes evidence for alternative diagnoses of parkinsonism like atypical parkinsonism, drug-induced parkinsonism, or essential tremor.
- Two or more supportive criteria. Included in supportive criteria are L-DOPA responsiveness, presence of L-DOPA-induced dyskinesia, presence of olfactory loss, and presence of classic rest tremor.
- No red flags, which refers to the features that are uncommon but not exclusionary for PD. For example, the development of severe autonomic failure within 5 years of disease onset or the quick progression of gait impairment that calls for wheelchair use.

The typical onset of motor symptoms is usually unilateral and remains asymmetrical throughout the disease. The average age of onset is in the late fifties with a general range between forty and eighty years. There is a young-onset form of PD that is commonly defined by an age of onset less than forty-five years. More than 10% of these young-onset forms have a genetic source, while in individuals that develop the disease before thirty years of age have greater than a 40% chance of having a genetic origin of PD (Alcalay et al., 2010; Marder et al., 2010).

In addition to the main motor symptoms observed in PD patients, a number of individuals also have non-motor symptoms (Chaudhuri & Schapira, 2009). Non-motor symptoms involve many different systems and functions including sleep-wake cycle regulation, cognitive impairments, autonomic dysfunction, disorders of mood and affect, as well as sensory problems (Fig. 9).

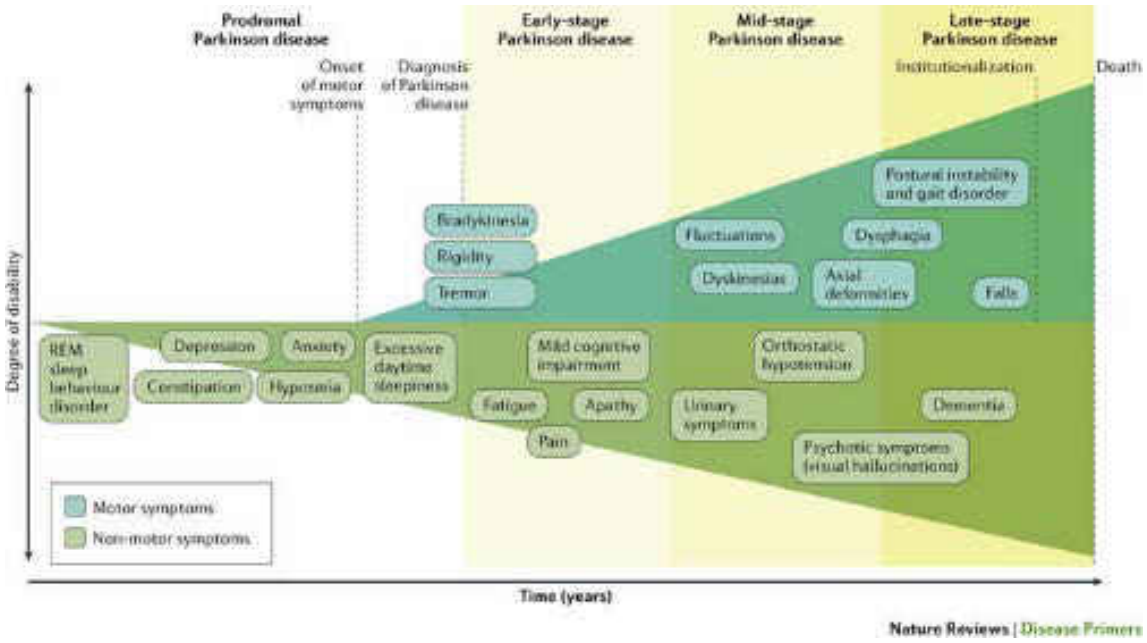

Figure 9. Clinical symptoms and timeline associated with PD. Reprinted by permission from RightsLink, Springer Nature, (Poewe et al., 2017), Copyright 2017.

Many of these nonmotor symptoms can appear years before the classical motor symptoms. Non-motor symptoms become more prevalent as the disease progresses and become major determinants in quality of life and progression of overall disabilities.

Parkinson's disease sufferers can also develop dementia. Dementia associated with PD has been reported as ranging from 2% in early-onset cases (Hietanen & Teräväinen, 1988) all the way up to 81% in a patient population (Martin, Loewenson, Resch, & Baker, 1973). Additionally, in a review of nearly 30 studies, it was found that 40% of individuals with PD have some sort of dementia (Cummings, 1988). There was a large association with age: no individuals with PD under the age of 50 had dementia while 69% of patients above the age of 80 met the criteria (Mayeux et al., 1992). The incidence of dementia has been

shown to be six times higher in patients with PD than in controls (Aarsland et al., 2001).

There are many risk factors that have been reported to be associated with dementia in PD. See Fig. 10. These factors include age at onset of PD, duration of PD, depression, and atypical neurological features (Aarsland, Tandberg, Larsen, & Cummings, 1996; Rajput, Offord, Beard, & Kurland, 1987; Stern, Marder, Tang, & Mayeux, 1993). The main features of the dementia associated with PD in addition to the symptoms already present from PD are impairments in executive functioning (Litvan, Mohr, Williams, Gomez, & Chase, 1991; Pillon, Deweer, Agid, & Dubois, 1993; Pillon, Dubois, Lhermitte, & Agid, 1986; Pillon, Dubois, Ploska, & Agid, 1991) and can be found in Fig. 11.

Panel 1. Risk factors for dementia in patients with PD

Advanced age
Advanced age at onset of motor symptoms
Early occurrence of levodopa related confusion or pscyhosis
Presence of speech and axial involvement
Severe motor symptoms, especially bradykinesia
Poor cognitive (especially verbal fluency) test scores
Depression
Smoking

Figure 10. Risk factors for dementia in patients with PD. Reprinted from The Lancet, Volume 361, (Emre, 2003), Dementia associated with Parkinson's disease, 229-237, Copyright 2003, with permission from Elsevier.

Panel 2. Clinical features of dementia associated with PD

Impaired attention with fluctuations

Impaired executive functions

- Concept formation
- Problem solving
- Set elaboration, shifting, and maintenance
- Internally cued behaviour; benefit from external cues

Impaired memory

- Impaired free recall; benefit from external cues
- Well preserved recognition

Impaired visuospatial functions

Language largely preserved, except for verbal fluency

Praxis largely preserved

Personality changes

Multiple behavioural symptoms

Figure 11. Clinical features of dementia associated with PD. Reprinted from The Lancet, Volume 361, (Emre, 2003), Dementia associated with Parkinson's disease, 229-237, Copyright 2003, with permission from Elsevier.

Tyrosine Hydroxylase

Tyrosine hydroxylase (TH), also known as tyrosine 3-monooxygenase, is the rate-limiting enzyme in the synthesis of the catecholamines dopamine, epinephrine, and norepinephrine that are important neurotransmitters and hormones in the peripheral and central nervous system. It is a cytoplasmic protein found in the brain, gut and retina, the sympathetic nervous system and the adrenal medulla (Haycock, George, & Waymire, 1985). A deficiency in TH in the *substantia nigra* leading to a reduction of striatal dopamine is a hallmark of PD (Adams, Chang, & Klaidman, 2001). TH is part of the aromatic amino acid

hydroxylases (AAAHs) family of enzymes that also include phenylalanine hydroxylase and tryptophan hydroxylase. All three enzymes participate in hydroxylation of aromatic rings of amino acids. TH catalyzes the conversion of the amino acid L-tyrosine to L-3,4-dihydroxyphenylalanine (L-DOPA) (George & Yang, 2013). It does so by using molecular oxygen (O_2), in addition to iron (Fe^{2+}) and tetrahydropiopterin as cofactors. TH is a tetramer of four identical subunits that have multi-domain structures with an amino-terminal of 150 amino acids acting as the regulatory domain followed by a catalytic domain of roughly 300 amino acids and a coiled-coil domain consisting of 20 amino acids at the carboxyl terminus. TH is coded for by a single gene, the TH gene, and there are multiple isoforms due to multiple mRNAs formed by generating slice variants of the gene (Brigitte Grima et al., 1987; Kappock & Caradonna, 1996; Kumer & Vrana, 1996; Le Bourdellès et al., 1988; Nagatsu, 1995).In humans, there are four different variations of the regulatory domain and therefore four variants of the enzyme due to alternative splicing(Kobayashi et al., 1988).

Regulation.

The activity of TH can be modulated by two mechanisms: longer term regulation of gene expression (enzyme stability, transcriptional regulation, RNA stability, alternative RNA splicing and translational regulation) and more transient regulation of enzyme activity by feedback inhibition, allosteric regulation and phosphorylation (Kumer & Vrana, 1996).

TH can be phosphorylated by cAMP-dependent mechanisms that will be elaborated upon in the forthcoming paragraphs. Although phosphorylation leads

to an increase in enzymatic activity, it can also decrease the stability of the enzyme (Lazar, Truscott, Raese, & Barchas, 1981; Vrana, Allhiser, & Roskoski, 1981; Vrana & Roskoski, 1983).

The TH gene contains many binding sites in its promotor for transcription factors to increase its mRNA expression. An AP-1 site has been identified where c-fos and Jun transcription factors can take action to increase transcription of the gene (Goc & Stachowiak, 1994). Many studies have been undertaken toward the characterization of a CRE binding site in the promotor (Carroll, Kim, Kim, Goodman, & Joh, 1991; Fader & Lewis, 1990; K. S. Kim, Lee, Carroll, & Joh, 1993; K. S. Kim, Tinti, Song, Cubells, & Joh, 1994). Additionally, many labs have shown that estrogen can bind an ERE in the TH promotor and modulate levels of TH mRNA (Ivanova & Beyer, 2003; Maharjan, Serova, & Sabban, 2005; Raab, Pilgrim, & Reisert, 1995) and the Ghribi lab has shown that 27-OHC can negatively regulate TH mRNA expression by binding to the ERE in the TH promotor (Marwarha, Rhen, Schommer, & Ghribi, 2011a).

Alternative RNA splicing results in four different forms of human TH mRNA and protein (B Grima et al., 1987; Haycock, 1991; Kaneda et al., 1987; O'Malley et al., 1987). The variants are made from differential splicing of the single gene copy of TH through the use of two splice donor sites in the first exon and the inclusion/exclusion of the second exon (O'Malley et al., 1987). The protein products differ at most by 71 amino acids in the amino-terminal regulatory domain. A functional role for the various TH isoforms has yet to be established.

TH is subject to feedback inhibition by all of the catecholamines by competing for the binding site of TH with the pterin cofactor ("Psychopharmacology - 4th Generation of Progress - ACNP," n.d.; Zigmond, Schwarzschild, & Rittenhouse, 1989). It is also subject to allosteric regulation. Allosteric regulation involves the modulation of enzyme activity at a site that is not the active site of the protein. Phospholipids (Lloyd, 1979; Lloyd & Kaufman, 1974; Raese, Patrick, & Barchas, 1976), heparin (Kuczenski & Mandell, 1972), and polyanions (Katz, Yamauchi, & Kaufman, 1976) have all been shown to allosterically regulate TH.

Activation by phosphorylation is the primary mechanism responsible for the maintenance of catecholamine levels in tissues after secretion of catecholamines. TH can be phosphorylated at serine residues 8, 19, 31, and 40 by a variety of protein kinases (Dunkley, Bobrovskaya, Graham, Von Nagy-Felsobuki, & Dickson, 2004a). The phosphorylation at Ser40 increases the activity of TH *in vitro, in vivo* and, *in situ* (Dunkley et al., 2004a; Le Bourdellès et al., 1991). Phosphorylation at Ser31 also increases the activity but to a much lesser extent than for Ser40. The phosphorylation at Ser19 and Ser8 has no direct effect on TH activity (Dunkley et al., 2004a). TH can be phosphorylated at Ser40 by protein kinase A (PKA) (Campbell, Hardie, & Vulliet, 1986a; Edelman, Raese, Lazar, & Barchas, 1978; Joh, Park, & Reis, 1978). TH can also be phosphorylated at Ser40 by a range of other protein kinases, including protein kinase C (PKC) (Albert et al., 1984), calcium and calmodulin stimulated protein kinase (CaMPK) II (Vulliet, Woodgett, & Cohen, 1984), protein kinase G (PKG)

(Roskoski, Vulliet, & Glass, 1987), MAPK-activated protein kinases (MAPKAPKs) 1 and 2 (Sutherland et al., 1993a), p38-regulated/activated kinase (PRAK) and mitogen and stress-activated protein kinase (MSK) 1 (Toska et al., 2002a). TH that has been phosphorylated at Ser40 can be dephosphorylated by protein phosphatases PP2A and PP2C (Berresheim & Kuhn, 1994; Bevilaqua, Cammarota, Dickson, Sim, & Dunkley, 2003; Haavik et al., 1989a). Bradykinin and nerve growth factor have been shown to phosphorylate TH at Ser31 by an extracellular regulated kinase (ERK) 1 and 2 dependent mechanism (Haycock, Ahn, Cobb, & Krebs, 1992) while PP2A has been shown to dephosphorylate this site (Leal, Sim, Gonçalves, & Dunkley, 2002). TH can be phosphorylated at Ser19 by CaMPKII (Campbell, Hardie, & Vulliet, 1986b), MAPKAPK2 (Sutherland et al., 1993b), and PRAK (Toska et al., 2002b). PP2A and PP2C have the capability of dephosphorylating TH at Ser19 (Haavik et al., 1989b). ERK has been shown to be able to phosphorylate TH at Ser8 (Royo, Daubner, & Fitzpatrick, 2004) while no pathways have been identified that dephosphorylate this site.

Alpha-Synuclein

Alpha-synuclein (α-syn) is a protein product of the SNCA gene that is located on chromosome 4 (Shibasaki, Baillie, St. Clair, & Brookes, 1995). The accumulation, oligomerization, and aggregation of α-syn protein have been implicated as contributing factors in the development of synucleinopathies including MSA, DLB, and PD. It is a small (140 amino acids) cytosolic protein that is abundantly expressed in neurons and also found in astrocytes (Castagnet,

Golovko, Barceló-Coblijn, Nussbaum, & Murphy, 2005a) and microglial cells (Austin, Floden, Murphy, & Combs, 2006). Its primary sequence can be divided into three main domains: 1. the N-terminal domain (1-60) contains multiple repeats of the consensus sequence (KTKEGV) and has alpha-helical propensity upon binding membranes; 2. the central domain (61-95), also known as the non-amyloid-beta component (NAC) because it was found to aggregate in Beta-amyloid plaques in Alzheimer's disease. It is highly hydrophobic and is involved in α-syn aggregation when it acquires a beta-sheet structure; and 3. the C-terminal domain (96-140), enriched in negative charged and proline residues, providing flexibility to the protein. This region is intrinsically disordered in structure. Remarkably, all of the mutations associated with familial forms of PD i.e. A53T, A30P, E46K, G51D, and H50Q (Appel-Cresswell et al., 2013; Krüger et al., 1998b; Lesage et al., 2013; Polymeropoulos et al., 1997b; Proukakis et al., 2013; Zarranz et al., 2004) reside within the N-terminal domain.

Function.

The function of α-syn is perhaps the most controversial subject in the field. Many hypothetical functions have been ascribed over the past 20 years but none is fully consensual. This has limited our ability to fully assess the protein and to create effective therapies. One of the most prominent lines of work suggests that α-syn functions at the pre-synaptic terminal and regulates synaptic transmission. In support of this idea many studies have shown that α-syn is highly enriched at the synapse (Maroteaux, Campanelli, & Scheller, 1988; Withers, George, Banker, & Clayton, 1997) and co-localizes with reserve pools of synaptic vesicles

(S.-J. Lee, Jeon, & Kandror, 2008; Zhang et al., 2008). α-syn may be involved in the cycling of synaptic vesicles, modulating the vesicle pool size, mobilization and endocytosis (Bendor, Logan, & Edwards, 2013; Vargas et al., 2014). Direct evidence for the role of α-syn in synaptic transmission didn't arise until (Burre et al., 2010) showed that the C-terminus of α-syn and synaptobrevin-2 (VAMP2), a key protein in synaptic exocytosis (Schoch et al., 2001), interact. Additionally, it was shown that the N-terminus can bind to phospholipids and promote SNARE complex assembly *in vivo* and *in vitro* (Burre et al., 2010). α-syn has been shown to compensate for the loss of cysteine-string protein-alpha (CSPα), a presynaptic chaperone, (Chandra, Gallardo, Fernández-Chacón, Schlüter, & Südhof, 2005) suggesting that α-syn might have a similar role to CSPα in maintaining the nerve terminal, rather than transmitter release. α-syn multimers have been reported to affect synaptic transmission by enhancing vesicle clustering without changing the efficiency or kinetics of vesicle fusion upon calcium triggering (Diao et al., 2013). This enhancement may delay vesicle trafficking. Although an alternative mechanism has been proposed where high levels of monomeric α-syn can inhibit vesicle docking, a SNARE-independent pathway, via interaction with acidic lipids (Lai et al., 2014). This brings the N-terminus of α-syn into the spotlight because of its known lipid-binding properties. Since all known PD-related mutations in α-syn are located in the lipid-binding domains, it is possible that they change the homeostasis needed for membrane-protein interactions and subsequent oligomerization, impairing the physiological functionality of α-syn.

α-syn also functions in the nucleus and was initially found in the nuclear compartment (Z. Huang, Xu, Wu, & Zhou, 2011; John Goers et al., 2003). The N- and C- termini have been implicated in nuclear translocation, while familial mutations, post-translational modifications, and oxidative stress can increase its nuclear localization (Gonçalves & Outeiro, 2013; Kontopoulos, Parvin, & Feany, 2006a; X. Liu et al., 2011; Schell, Hasegawa, Neumann, & Kahle, 2009; S. Xu et al., 2006). The mechanisms of nuclear import are elusive but once inside the nucleus α-syn may play a role on transcriptional regulation. It is possible that α-syn either interacts directly with DNA or it regulates players involved in transcription. It has been shown that α-syn can bind to the GC1α promotor, a crucial mitochondrial transcription factor, leading to negative impacts on mitochondria homeostasis (Desplats et al., 2012; Siddiqui et al., 2012). α-syn has been reported to interact with histones and may affect histone function via acetylation-deacetylation cycles, a process that might be strongly dependent on α-syn levels (Kontopoulos, Parvin, & Feany, 2006b; X. Liu et al., 2011).

α-syn has been reported to interact with a large number of proteins which might regulate its activity. One of the first identified was synphilin, which appears to promote the aggregation of α-syn (Engelender et al., 1999; P. J. McLean, Kawamata, & Hyman, 2001; Ribeiro, Carneiro, Ross, Menezes, & Engelender, 2002). Tubulin appears to interact with a form of α-syn, and this can influence the microtubule cytoskeleton (H.-J. Lee, Khoshaghideh, Lee, & Lee, 2006). However, this interaction seems to be more relevant for the toxicity associated with α-syn and not for its normal function (Alim et al., 2002; Leo Chen et al., 2007; M. Kim et

al., 2008). α-syn has been shown to interact with dopamine transporter (DAT) where it colocalizes to the plasma membrane and increases dopamine efflux and enhances DAT localization to cholesterol-rich membrane microdomains (Butler et al., 2015). α-syn has also been shown to activate protein phosphatase 2A which effects the phosphorylation status of TH (Peng et al., 2005). Additionally, α-syn has been shown to bind fatty acids and polyunsaturated fatty acids increase the oligomerization of α-syn while monounsaturated and saturated fatty acids do not affect its propensity to form oligomers (Karube et al., 2008).

Clearance and spreading.

In order for cells to maintain intracellular homeostasis, proper protein degradation is crucial and is ensured by two independent, but complementary, systems that work in concert, the Ubiquitin Proteasomal System (UPS) and the Autophagy-Lysosomal Pathway (ALP). Monomeric α-syn can be actively degraded by both pathways (Cuervo, Stefanis, Fredenburg, Lansbury, & Sulzer, 2004; C.-W. Liu, Corboy, DeMartino, & Thomas, 2003) that compensate each other when one fails (Fang Yang et al., 2013). Higher molecular species of α-syn, including oligomers and aggregates, are mainly degraded by pathways involving the lysosome (H.-J. Lee, Khoshaghideh, Patel, & Lee, 2004). α-syn was originally thought to be degraded by the proteasome without a requirement for ubiquitination (Bennett et al., 1999a; Hardy J Rideout & Stefanis, 2002; G K Tofaris, Layfield, & Spillantini, 2001). However, it was later found that monoubiquitination apparently promotes the degradation of α-syn by the proteasome, and this can be controlled by the ubiquitin ligase (SIAH-2) and

deubiquitinase (USP9X) (Liani et al., 2004; Rott et al., 2011). Additionally, much evidence has accumulated suggesting the ALP can clear α-syn. Initially thought to only clear α-syn aggregates by macroautophagy, the lysosome also clears monomers and oligomers of α-syn (H.-J. Lee et al., 2004; Mak, McCormack, Manning-Boğ, Cuervo, & Di Monte, 2010; Hardy J Rideout, Lang-Rollin, & Stefanis, 2004). Indeed, chaperone-mediated autophagy (CMA), a process that targets individual, soluble proteins to the lysosome for proteolysis via HSC70, contributes to the clearance of α-syn, and α-syn can disrupt CMA, altering the turnover of CMA-dependent proteins (Cuervo et al., 2004; Vogiatzi, Xilouri, Vekrellis, & Stefanis, 2008). Ubiquitination by the E3 ligase Nedd4 has been shown to target α-syn for degradation by the lysosome, rather than the proteasome (George K Tofaris et al., 2011).

Heat-shock protein 70 (HSP70) is capable of being involved in various degradation pathways by the presence of specific chaperones and co-chaperones that aid in guiding the targeted protein to a specific degradation pathway (see Fernández-Fernández *et al.* 2017). HSP70 uses its ATP hydrolysis-powered conformational changes to assist protein folding, disaggregation and degradation, and is a key contributor in cellular proteostasis. α-syn has been shown to bind HSP70 (Aprile et al., 2017; Dedmon, Christodoulou, Wilson, & Dobson, 2005; Luk, Mills, Trojanowski, & Lee, 2008) and be degraded by the UPS and autophagy (Webb, Ravikumar, Atkins, Skepper, & Rubinsztein, 2003). In this dissertation we show that 27-OHC

decreases levels of HSP70 and may lead to aberrant clearance of α-syn through the UPS (Schommer et al., 2018).

In addition to clearance, α-syn has been proposed to act as a prion-like protein and has the ability to spread intercellularly between neurons, astrocytes, and microglia by cellular release, movement and uptake, including exocytosis, exosomes, tunneling nanotubes, glymphatic flow, and endocytosis (Valdinocci, Radford, Siow, Chung, & Pountney, 2017).

Regulation.

Transcriptional and post-translational mechanisms regulate α-syn gene expression and may play important roles in the development of synucleinopathies (Tagliafierro & Chiba-Falek, 2016). Many groups have investigated the transcriptional regulation of SNCA and have identified few putative transcription factors that mediate its expression. PARP-1, binds to Rep1 and has been shown to regulate SNCA via this interaction (Chiba-Falek, Kowalak, Smulson, & Nussbaum, 2005). LXRs have been shown to regulate its expression in SHSY5Y neuroblastoma cells (Cheng, Kim, & Garner, 2008a; Marwarha, Rhen, Schommer, & Ghribi, 2011b). Transcription factors of the ZSCAN21 (Richard Lee Clough, Dermentzaki, & Stefanis, 2009) and GATA family (Scherzer et al., 2008) bind to regions in intron 1 and the promotor region (Brenner, Wersinger, & Gasser, 2015) of SNCA to induce transcription. A signaling pathway involving ERK/PI3 mediated ZSCAN induced SNCA transcriptional activation has been suggested (R. Lee Clough & Stefanis, 2007; Richard Lee Clough, Dermentzaki, Haritou, Petsakou, & Stefanis, 2011; Richard

Lee Clough et al., 2009). Additionally, five other factors (PITX3, OTX2, NR3C1, AR, TBP) have been shown to interact with the SNCA promotor (Sterling, Walter, Ting, & Schüle, 2014).

The modulation of SNCA mRNA levels by endogenous microRNAs (miRNAs) has been proposed as a post-transcriptional mechanism of regulation. miR-7 and miR-153 are abundantly expressed in the brain and have been shown to regulate SNCA mRNA levels. In mouse primary neurons, both miRNAs downregulated SNCA levels (Doxakis, 2010). Within PD brains, miR-34b and miR-34c are downregulated (Miñones-Moyano et al., 2011; Villar-Menéndez et al., 2014). Within SHSY5Y cells miR-34b and miR-34c decrease mRNA levels of SNCA (Kabaria, Choi, Chaudhuri, Mouradian, & Junn, 2015).

Another form of post-transcriptional regulation of SNCA is alternative splicing. At least six different transcript variants have been described for the SNCA gene (SNCA 140, SNCA 126, SNCA 115, SNCA 112, SNCA 98, SNCA 67) and SNCA 112, SNCA 126, and SNCA 98 arise from alternative splicing (J. R. McLean, Hallett, Cooper, Stanley, & Isacson, 2012; W. Xu, Tan, & Yu, 2015). No biological or pathological significance of the different variants have been explained yet. However, isoforms are differently expressed in human synucleinopathies (Beyer et al., 2008) and have been associated with intracellular aggregation (Kalivendi, Yedlapudi, Hillard, & Kalyanaraman, 2010).

α-syn is vulnerable to many types of post-translational modifications (Lopes da Fonseca, Villar-Piqué, & Outeiro, 2015). Modifications such as ubiquitination (Rott et al., 2008; Shin, Klucken, Patterson, Hyman, & McLean,

2005; George K Tofaris, Razzaq, Ghetti, Lilley, & Spillantini, 2003), sumoylation (Y. M. Kim et al., 2011; Krumova et al., 2011; Shahpasandzadeh et al., 2014), and N-terminal acetylation (Bartels, Kim, Luth, & Selkoe, 2014; Dikiy & Eliezer, 2014; Maltsev, Ying, & Bax, 2012) have been described. Additionally, α-syn can be phosphorylated at two serine residues (S129 and S87) and three tyrosine residues (Y125, Y133, and Y135) (Li Chen et al., 2009; Y. Xu, Deng, & Qing, 2015). Approximately 90% of the α-syn in Lewy Bodies is phosphorylated at S129 (Fujiwara et al., 2002; Sato, Kato, & Arawaka, 2013b). However, S129 phosphorylation can inhibit α-syn fibrillization (Li Chen & Feany, 2005; Tenreiro, Reimão-Pinto, et al., 2014) which has been similarly shown for S87 phosphorylation (Paleologou et al., 2010). Unfortunately, the functional relevance of phosphorylation of α-syn is elusive (Oueslati, Fournier, & Lashuel, 2010; Sato, Kato, & Arawaka, 2013a; Tenreiro, Eckermann, & Outeiro, 2014). Many kinases, including casein kinases (CKs), polo-like kinases (PLKs), and G protein-coupled receptor kinases (GRKs) are capable of phosphorylating α-syn (Arawaka et al., 2006; Basso et al., 2013; Inglis et al., 2009; Ishii et al., 2007; Mbefo et al., 2010; Okochi et al., 2000; Pronin, Morris, Surguchov, & Benovic, 2000).

In contrast, when α-syn is nitrated on tyrosine residues (Y39, Y125, Y133, Y136) it is known to produce toxic effects. Nitrated α-syn is present in Lewy Bodies (Giasson et al., 2000) and nitrated α-syn multimers have been shown to promote mitochondrial impairment and cell death (Y. Liu, Qiang, Wei, & He, 2011) Nitration on Y39 has been shown to block α-syn fibril formation and reduces monomer degradation via the UPS (Hodara et al., 2004).

Epigenetic regulation, particularly DNA methylation, of SNCA has been suggested to play a key role in expression levels of α-syn (Ammal Kaidery, Tarannum, & Thomas, 2013). In PD and DLB human brains reduced DNA methylation has been reported which leads to increased α-syn expression (Desplats et al., 2011; Matsumoto et al., 2010).

Acetyl-CoA and Its Numerous Fates

Acetyl-Coenzyme A (acetyl-CoA) is a very important molecule that is involved in many biochemical processes including protein, carbohydrate, cholesterol, and fatty acid metabolism (Fig. 12). It is vital to energy generation from the degradation of carbohydrates, fatty acids, and proteins and is also heavily involved in the production of cholesterol and fatty acids. Acetyl-CoA consists of a two-carbon activated acetyl unit attached to coenzyme A via a thioester linkage (Fig. 13). The thioester linkage is a high energy bond that is broken during oxidation and the acetyl group is donated to be oxidized for energy production. The acetyl unit is also the initial building block of fatty acids and cholesterol.

Acetyl-CoA is produced via three types of oxidative pathways (Fig. 14): the activation of acetate, the thiolytic cleavage of β-ketoacyl-CoAs and β-hydroxy acids, and the oxidative decarboxylation of pyruvate. In mammalian cells, acetate is the end product of threonine degradation and ethanol metabolism. Acetate is converted to acetyl-CoA by the enzyme acetyl-CoA synthetase. Thiolytic cleavage of β-ketoacyl or β-hydroxy acyl-CoA derivatives to acetyl-CoA occurs in the pathways for oxidation of fatty acids, synthesis of ketone bodies, and

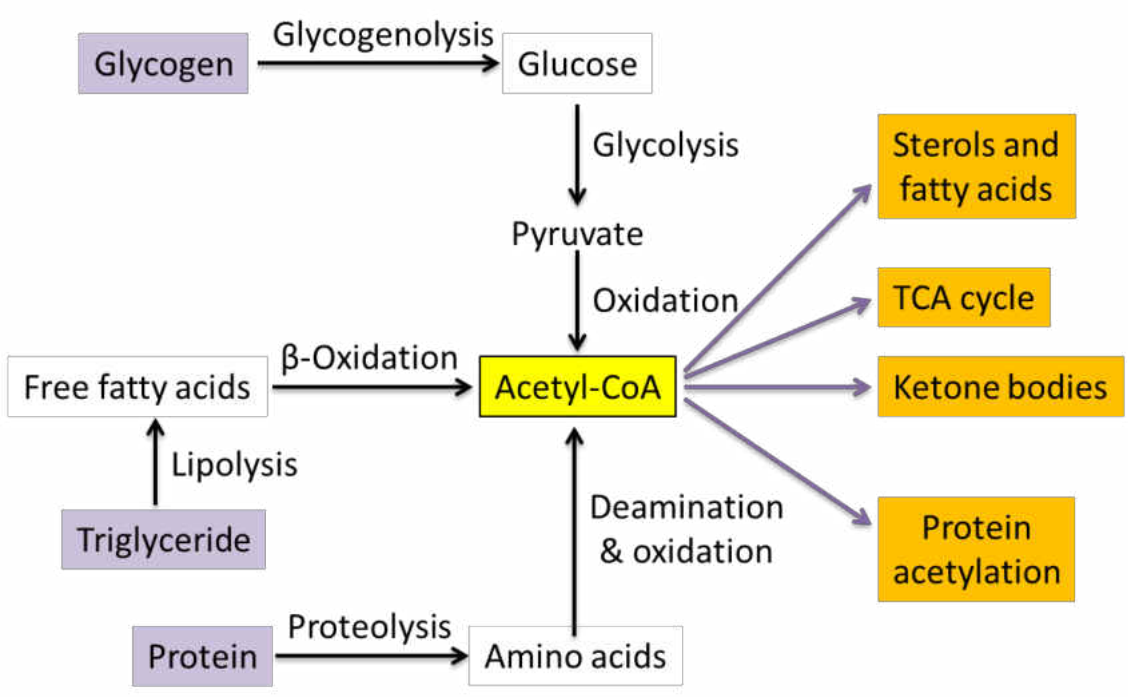

Figure 12. Sources and fates of acetyl-CoA. Acetyl-CoA is mainly generated by burning of glucose, fatty acid, and proteins. When abundant, it can be used to make sterols and fatty acids, and can also bind to proteins, forming acetylated protein. In long term fasting or starvation, ketone bodies can be formed from acetyl-CoA that can be utilized by the brain. Under normal conditions, acetyl-CoA is metabolized to provide energy via TCA cycle and oxidative phosphorylation inside mitochondria. Reprinted with permission from (Luo, Wu, Jing, & Yan, 2016).

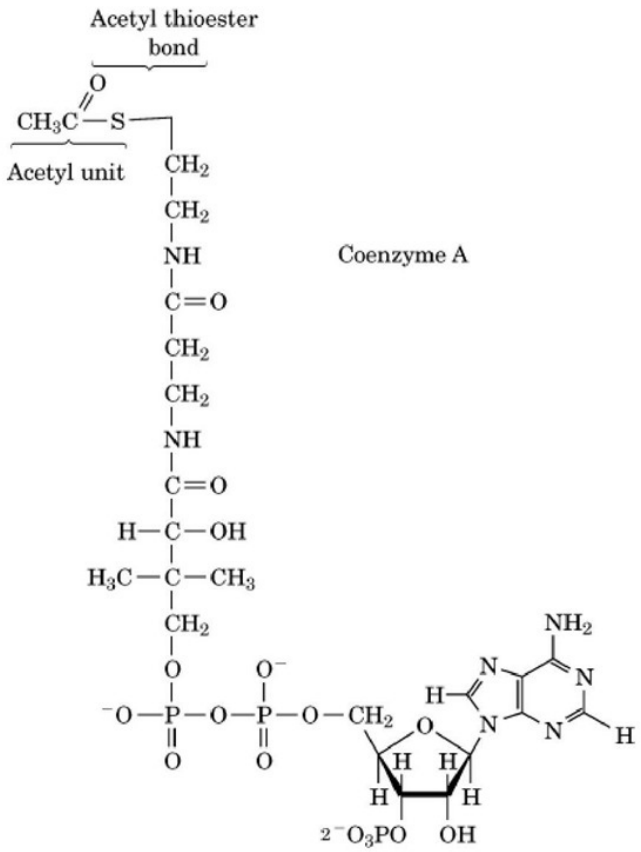

Figure 13. Structure of acetyl CoA. Reprinted with permission from ("Acetyl Coenzyme A (Molecular Biology)," n.d.).

oxidative degradation of various amino acids. In β-oxidation of fatty acids, one molecule of palmitate is metabolized into eight molecules of acetyl-CoA. The last type involves the oxidative decarboxylation of pyruvate formed in glycolysis to produce acetyl-CoA to feed into the Kreb's cycle.

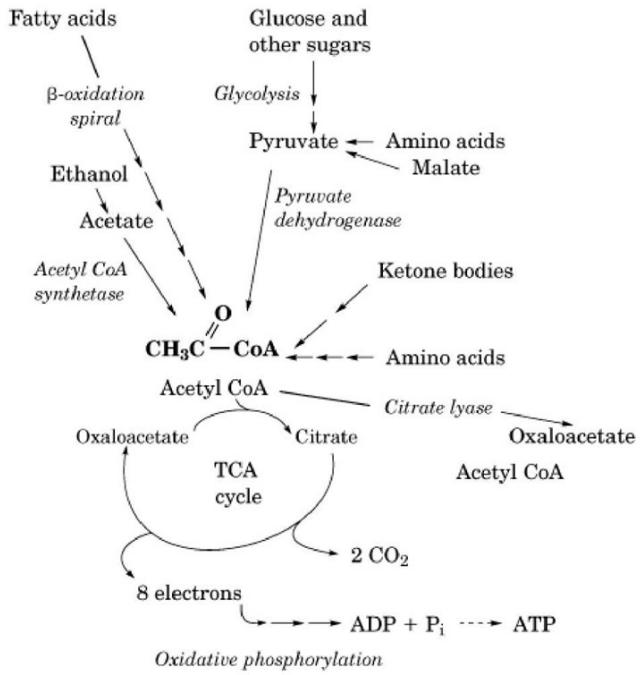

Figure 14. Oxidative pathways leading to Acetyl-CoA production. Reprinted with permission from ("Acetyl Coenzyme A (Molecular Biology)," n.d.).

Cholesterol Metabolism

Acetyl-CoA has a two-carbon acetyl unit that is the basic building block of cholesterol, fatty acids, and other compounds derived from the five-carbon isoprenoid unit (Fig. 15). In the production of cholesterol (Fig. 16), acetyl-CoA formed by glycolysis of sugars and β-oxidation of fatty acids is first transported out of the mitochondrion as citrate. Citrate, produced by the condensation of acetyl-CoA with oxaloacetate, is removed from the Kreb's cycle and is carried across the mitochondrial membrane by the citrate shuttle. Citrate is then cleaved by ATP citrate lyase into acetyl-CoA and oxaloacetate. Once in the cytosol,

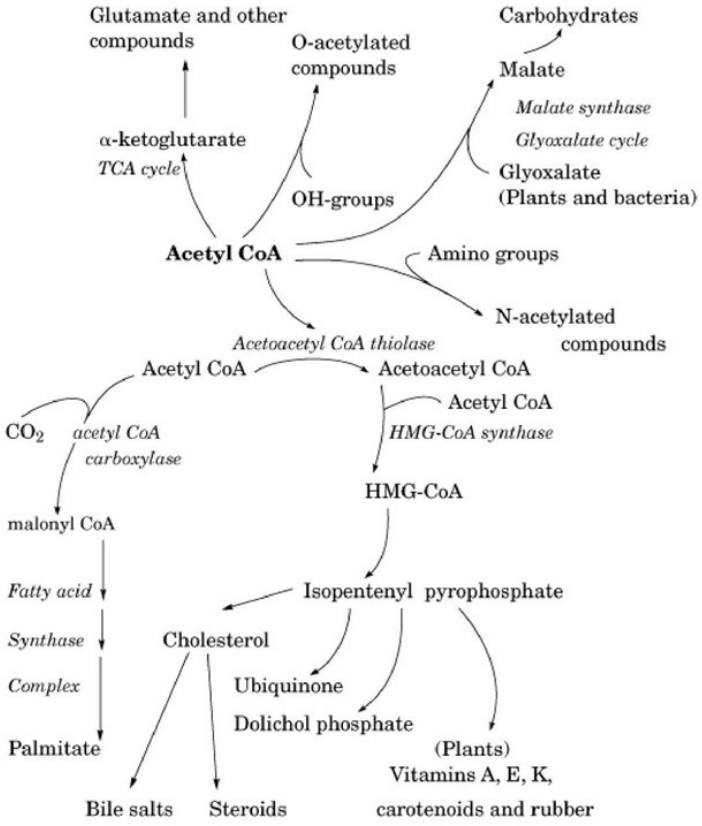

Figure 15. Acetyl-CoA is a building block for many compounds. Reprinted with permission from ("Acetyl Coenzyme A (Molecular Biology)," n.d.)

acetyl-CoA undergoes a series of reactions to form hydroxymethyglutaryl-CoA (HMG-CoA). This reaction requires thiolase and HMG-CoA synthase enzymes. HMG-CoA is then converted into mevalonate by HMG-CoA reductase. This is the rate limiting step of cholesterol biosynthesis. Mevalonate undergoes pyrophosphorylation by two consecutive reactions with ATP followed by a decarboxylation step to form isopentenyl pyrophosphate (IPP). IPP is then isomerized into dimethylallyl pyrophosphate (DPP) by the enzyme isopentenyl pyrophosphate isomerase. This leaves an equilibrium between IPP and DPP.

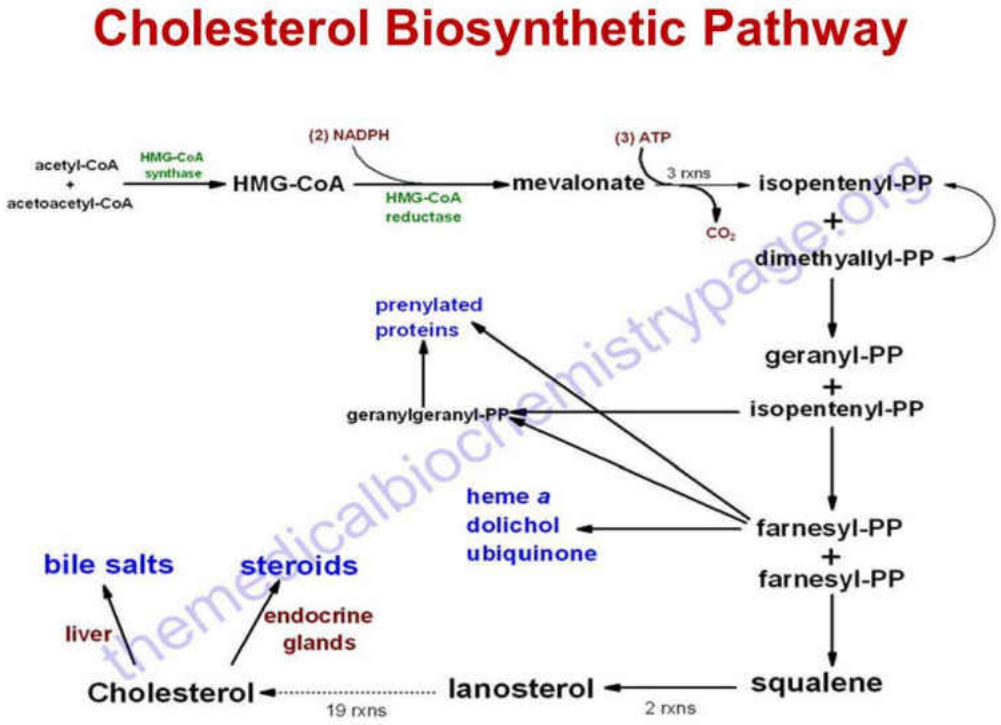

Figure 16. Cholesterol Biosynthetic Pathway. Reproduced with permission from ("Cholesterol: Synthesis, Metabolism, Regulation," n.d.) themedicalbiochemistrypage, LLC.

Squalene is then formed by the condensation of isoprene units. Four IPP and two DPP molecules condense to form the cholesterol precursor squalene by a series of three reactions. Prenyltransferase (farnesyl pyrophosphate synthase) catalyzes the condensation of DPP and IPP to yield geranyl pyrophosphate. Prenyltransferase then catalyzes a second condensation of geranyl pyrophosphate and IPP to yield farnesyl pyrophosphate (FPP). Squalene synthase then catalyzes the condensation of two farnesyl pyrophosphate molecules to form squalene. Squalene is then cyclized into lanosterol by a series of two reactions involving squalene epoxidase and oxidosqualene cyclase. Finally, lanosterol undergoes a series of nineteen reactions to form cholesterol

(Fig. 17). Cholesterol synthesized in the liver is then either converted into bile acids or esterified by acyl-CoA:cholesterol acyltransferase (ACAT) to form cholesterol esters which are secreted into the bloodstream in lipoproteins to be destined for target cells.

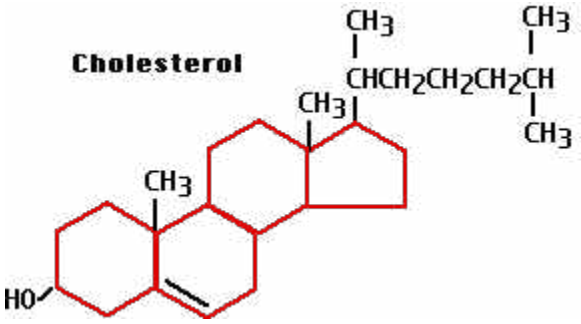

Figure 17. Structure of Cholesterol. Reproduced with permission from ("Cholesterol: Synthesis, Metabolism, Regulation," n.d.) themedicalbiochemistrypage, LLC.

Fatty Acid Metabolism

In the production of the fatty acid palmitate, pyruvate from glycolysis is converted into acetyl-CoA in the mitochondrion where it can be used in the Kreb's cycle to produce energy. In order for acetyl-CoA to be used for fatty acid synthesis it needs to be moved into the cytosol (Fig 18). Citrate, produced by the condensation of acetyl-CoA with oxaloacetate, is removed from the Kreb's cycle and is carried across the mitochondrial membrane by the citrate shuttle. Citrate is then cleaved by ATP citrate lyase into acetyl-CoA and oxaloacetate. Oxaloacetate can be used by the liver for gluconeogenesis or can be returned to the mitochondrion as malate. The cytosolic acetyl-CoA is carboxylated by acetyl-CoA carboxylase into malonyl-CoA. This is the first committed step in the

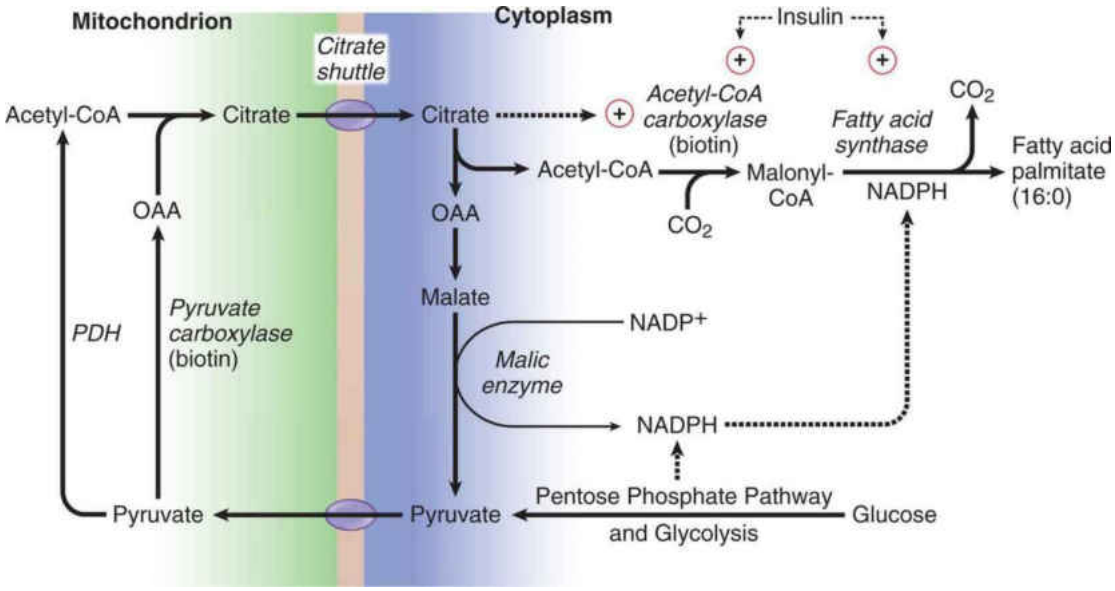

Figure 18. Shuttling of acetyl-CoA for palmitic acid production. Reprinted with permission from ("Fatty Acids and Triacylglycerols - Lipid and Amino Acid Metabolism," n.d.)

synthesis of fatty acids. Fatty acid synthase complex then adds malonyl-CoA units to the growing chain until palmitic acid (16:0) is formed (Fig. 19). Palmitic acid can be further modified by elongases and desaturates to form other saturated and unsaturated fatty acids within the body.

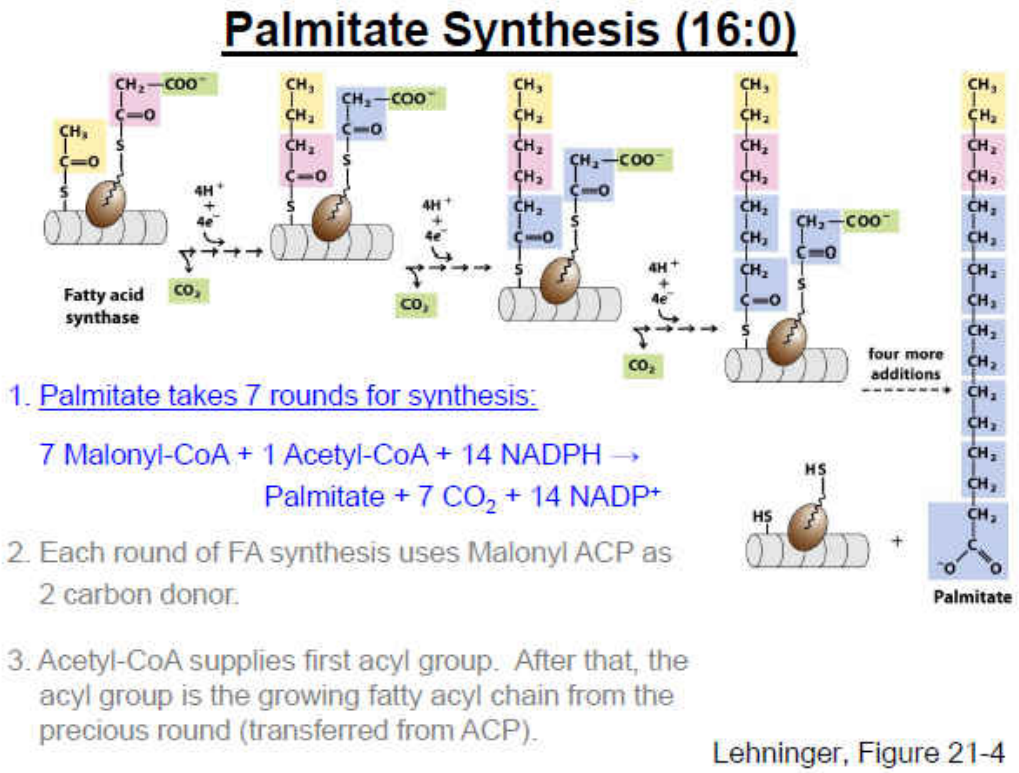

Figure 19. The formation of palmitic acid. Reprinted with permission from (Lehninger, n.d.).

Cholesterol, Oxysterol Biosynthesis, and 27-OHC

Cholesterol is a vital molecule that has many different roles. It plays a role in membrane structure and fluidity and is a precursor for the synthesis of the steroid hormones, the bile acids, and vitamin D.(Berg, Tymoczko, & Stryer, 2002; Michael W King, 2017; Norlin & Wikvall, 2007) Only about 1/3 of the cholesterol in a human body is consumed through diet while the other 2/3 is synthesized *de novo*. (Dietschy, 1984) Both dietary cholesterol and that synthesized *de novo*, are transported through the circulation in lipoprotein particles. Cholesteryl esters, the form in which cholesterol is stored in cells is also circulated in lipoprotein

particles. Due to its important role in membrane function, all cells express the enzymes of cholesterol biosynthesis.

Cholesterol is necessary for life but its overabundance and deposition in arteries has been associated with cardiovascular disease and stroke.(Huxley, Lewington, & Clarke, 2002) Therefore, cholesterol regulation is important to cell survival. Regulation of the rate limiting enzyme, HMG-CoA reductase, in cholesterol production is one method. Another is the regulation of excess intracellular free cholesterol by the activity of sterol O-acyltransferases, SOAT1 and SOAT2. Finally, regulation of plasma cholesterol levels via low density lipoprotein (LDL) receptor-mediated uptake and high density lipoprotein (HDL) mediated reverse cholesterol transport. (Michael W King, 2017)

Cholesterol is also a precursor to a host of active derivatives called oxysterols. Oxysterols are oxidized cholesterol metabolites that are intermediates or even end products in cholesterol excretion pathways. They are 27-carbon compounds with a cholesterol like backbone containing either an epoxide or ketone or an additional hydroxyl group in the sterol center and/or hydroxyl group in the side chain (Fig. 20) (Vurusaner, Leonarduzzi, Gamba, Poli, & Basaga, 2016). They are formed directly by autoxidation, the action of monooxygenases, or may come from enzymatic or nonenzymatic lipid peroxidation. (Fig. 21, 22) In many cases, the introduction of an oxygen moiety in the cholesterol molecule drastically reduces the half-life of the molecule and directs it for excretion or to further oxidation to water-soluble bile acids. This rapid degradation and excretion

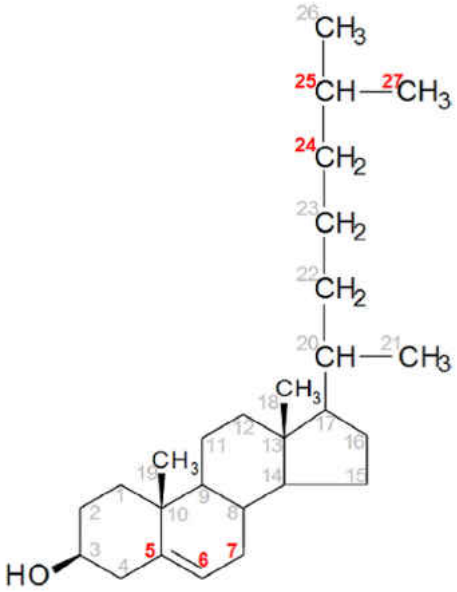

Figure 20. Main oxidation sites (in red) of cholesterol. Reprinted from Oxysterols and mechanisms of survival signaling, Volume 49 June 2016, (Vurusaner et al., 2016), 8-22, Copyright 2016, with permission from Elsevier.

Origin of plasma and tissue oxysterols.

Oxysterols of not enzymatic origin
Deriving from the diet Mainly formed by autoxidation of foodstuff containing cholesterol (meat, cheese, milk, dairy products, etc.) as induced by heat, light exposure, refrigeration, freeze-drying. *Generated in the body by:* Oxidation driven by reactive oxygen species and the leukocyte/ H2O2/HOCl system, the most frequent source being the inflammatory processes; attack by peroxyl and alkoxyl radicals. The quantitatively most represented compounds of this subgroup are: 7ketocholesterol, 7β-hydroxycholesterol, 5α,6α-epoxycholesterol, 5β,6β-epoxycholesterol, 3β,5α,6β- trihydroxycholestane
Oxysterols of enzymatic origin
Generated in the body by: Cholesterol 27-hydroxylase (CYP27A1) (various tissues) Cholesterol 25-hydroxylase (various tissues) Cholesterol 24-hydroxylase (CYP46A1) (mainly brain) Cholesterol 7α-hydroxylase (CYP7A1) (liver, prostate) ***27-hydroxycholesterol, 25-hydroxycholesterol, 24-hydroxycholesterol, 7α-hydroxycholesterol***

Figure 21. Origins of oxysterols. Reprinted from (Vurusaner et al., 2016).

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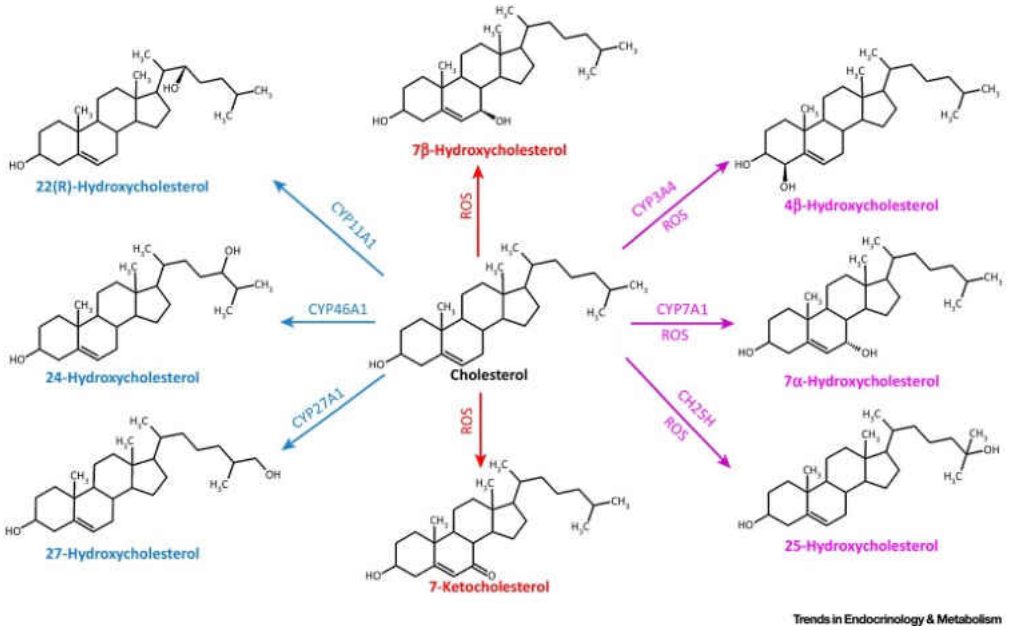


Figure 22. Structure of Common Oxysterols. Side-chain oxysterols of enzymatic origin are in blue. Ring oxysterols of non-enzymatic origin mediated by reactive oxygen species (ROS) in red. Oxysterols generated by both mechanisms are in purple. Reprinted from Oxysterols and mechanisms of survival signaling, Volume 49 June 2016, (Vurusaner et al., 2016), 8-22, Copyright 2016, with permission from Elsevier.

of oxysterols are facilitated by their physical properties, allowing them to pass lipophilic membranes and to be rearranged in cells at a much faster rate than cholesterol. (Björkhem & Diczfalusy, 2002) In light of their ability to readily pass membranes and the blood-brain barrier at faster rates than cholesterol, oxysterols are also a very important form of cholesterol transport. (Björkhem & Diczfalusy, 2002) In addition to their role in cholesterol transport, oxysterols play important roles in cholesterol turnover, apoptosis, atherosclerosis, inflammation, differentiation, and immunosuppression (Fig. 23) (Björkhem & Diczfalusy, 2002; Kha et al., 2004; Kosmider, Loader, Murphy, & Mason, 2010; Lordan, Mackrill, & O'Brien, 2009; Lütjohann, Lizard, & Iuliano, 2017; Moog et al., 1991; Murdolo et

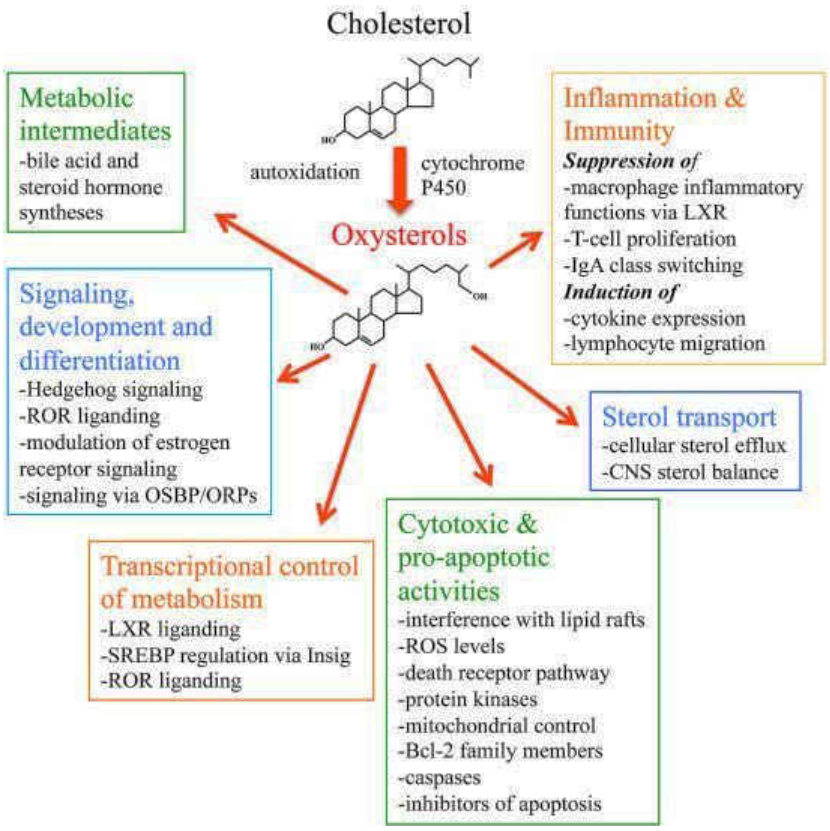


Figure 23. A diagram of the major functions of oxysterols. Reprinted with permission from (Olkkonen et al., 2012).

al., 2016; Olkkonen, Béaslas, & Nissilä, 2012; Ryan, O'Callaghan, & O'Brien, 2004; Sottero, Rossin, Poli, & Biasi, 2017; Spann & Glass, 2013; Vurusaner et al., 2016; Zmysłowski & Szterk, 2017).

27-Hydroxycholesterol (27-OHC) is made in the mitochondria of most cells by the enzyme CYP27A1 and it is the most abundant oxysterol in the periphery (Björkhem, 2013; Burkard, von Eckardstein, Waeber, Vollenweider, & Rentsch, 2007; Hirayama et al., 2009). It has been shown to cross into the brain readily via direct diffusion because it isn't bound to similar carrier proteins as cholesterol (Hughes, Rosano, Evans, & Kuller, 2013; Lee, Marszalek, & Cleveland, 1994; Leoni & Caccia, 2011a, 2011b) (Heverin et al., 2005b) (Marjan Shafaati et al.,

2011). Males on average have 0.6 uM 27-OHC in plasma circulation while females have 0.4 uM (Karuna et al., 2011). Within the CSF and brains of PD and Alzheimer's patients levels of 27-OHC have been shown to be elevated 3-6 fold from control samples (Cheng et al., 2011a; Heverin et al., 2005c; Leoni et al., 2004a; Leoni & Caccia, 2011a; M. Shafaati et al., 2011). 27-OHC has been implicated in numerous diseases including cancers and neurodegenerative diseases. It has been shown to promote atherosclerosis via activation of proinflammatory processes (Umetani et al., 2014) and promotes breast and prostate cancer (Marwarha, Raza, Hammer, & Ghribi, 2017). Throughout the next section of this dissertation, we will make the case for increased 27-OHC and not cholesterol *per se* as a potential risk factor for synucleinopathies.

The Role of Cholesterol and 27-OHC in Synucleinopathies

The cause(s) for synucleinopathies are likely multi-factorial with genetic predisposition and environmental factors participating in the pathogenesis of the diseases. Dyslipidemia has shown conflicting results as a risk factor in recent years.(Xiang Gao, Simon, Schwarzschild, & Ascherio, 2012c; Marwarha & Ghribi, 2015; Mutez et al., 2009) Findings regarding risk related specifically to dietary cholesterol have indicated either an increased risk,(Bosco et al., 2006; G. Hu, Antikainen, Jousilahti, Kivipelto, & Tuomilehto, 2008; Johnson, Gorell, Rybicki, Sanders, & Peterson, 1999a) decreased risk (Miyake, Tanaka, et al., 2010; Karen M. Powers et al., 2009a; Simon, Chen, Schwarzschild, & Ascherio, 2007), or no association.(Abbott, Webster Ross, et al., 2003a; L M L de Lau et al., 2005b).

Statins are widely utilized medications used to lower cholesterol levels. Statins have strong immune modulating and anti-inflammatory effects that could potentially be valuable in PD, but they also lower levels of coenzyme Q_{10} (Human et al., 1997), an important part of the cellular respiratory chain and a potent antioxidant that has been hypothesized to protect against PD (Shults et al., 2002) although high doses of coenzyme Q_{10} have not benefited individuals with PD (Parkinson Study Group QE3 Investigators et al., 2014).

The effects of statin use in epidemiological studies of PD have been contradictory (Becker, Jick, & Meier, 2008b; Lonneke M L de Lau, Stricker, & Breteler, 2007; Xiang Gao et al., 2012a; Lin et al., 2016; Ritz, Manthripragada, et al., 2010; Samii, Carleton, & Etminan, 2008; Undela et al., 2013; Wolozin et al., 2007). No association between statins and PD risk has been reported in some studies, (Becker et al., 2008b; Lonneke M L de Lau et al., 2007; Ritz, Manthripragada, et al., 2010; Samii et al., 2008) while others have shown a protective effect (Xiang Gao et al., 2012a; Lin et al., 2016; Undela et al., 2013; Wolozin et al., 2007) and even increased risk of PD with statin use (X. Huang et al., 2015). It is clear that no definitive answer has been reached regarding the use of statins and the risk of developing PD. Interestingly, after 12 weeks of simvastatin use, levels of 27-OHC are not changed in human plasma or CSF (Serrano-Pozo et al., 2010). The inconsistencies observed in studies utilizing statins and no change in 27-OHC levels lead us to believe that cholesterol itself may not be the guilty culprit.

We believe the reason for the conflicting association between cholesterol and synucleinopathies lies in the metabolites of cholesterol and not cholesterol *per se*. Supporting our hypothesis many studies have shown increased levels of many different oxysterols, including 27-OHC, within the brains of synucleinopathy patients.(Bosco et al., 2006; Cheng et al., 2011b; Leoni et al., 2004b; Leoni & Caccia, 2011a; Marwarha, Rhen, et al., 2011b; Rantham Prabhakara et al., 2008) Oxysterol levels have also been shown to increase in the circulation of hypercholesterolemic individuals,(Bertolotti et al., 2012; van Doormaal et al., 1989) in the ageing(Marwarha & Ghribi, 2015; Sottero, Gamba, Gargiulo, Leonarduzzi, & Poli, 2009) and have also been shown to increase with oxidative stress (Thanan et al., 2015) all of which are risk factors for PD type synucleinopathy. Another interesting fact is that oxysterols can cross the blood brain barrier while cholesterol cannot (Björkhem, Cedazo-Minguez, Leoni, & Meaney, 2009; Heverin et al., 2015; Leoni et al., 2004b; Leoni & Caccia, 2011a). This data shows an association between accumulation of 27-OHC and synucleinopathies. However, the extent to which increased 27-OHC levels in the brain causes α-syn deposition and promotes synucleinopathies is yet to be determined.

Oxysterols are active products of cholesterol metabolism that have a variety of biological functions. One of their main functions is to act as ligands of the LXRs (Gabbi, Warner, & Gustafsson, 2014; Olkkonen, 2008). LXRs, by means of gene transcription, regulate several metabolic pathways including lipid metabolism, glucose homeostasis, and inflammation (Gabbi et al., 2014). LXRs

regulate gene transcription by binding to promotors of genes and recruiting co-activators or co-repressors to encourage or repress the expression of target genes.(Gabbi et al., 2014) Since oxysterols act as ligands of LXRs they can influence the expression of numerous target genes. In previously published work, (Cheng, Kim, & Garner, 2008b; Marwarha, Rhen, et al., 2011b; Rantham Prabhakara et al., 2008) 27-OHC has been shown to increase levels of α-syn through an LXR dependent mechanism in human neuroblastoma SHSY5Y cells. In this dissertation we aim to examine the roles of 27-OHC on LXR in human dopaminergic neurons.

Altered protein homeostasis from folding to dysfunction in the two major degradation systems, the UPS and the autophagy-lysosomal pathway (ALP), is prevalent in many neurodegenerative diseases attributed to the presence of aggregates of ubiquitinylated proteins in Lewy Bodies in neurons and glial cells. α-syn has been frequently shown to be degraded by the proteasome (Alvarez-Castelao, Goethals, Vandekerckhove, & Castaño, 2014; Bennett et al., 1999b; Webb et al., 2003) and proteasomal dysfunction has often been implicated in PD (McNaught, Jackson, JnoBaptiste, Kapustin, & Olanow, 2006; McNaught, Olanow, Halliwell, Isacson, & Jenner, 2001). Impairment of the UPS has been shown to cause dopaminergic cell death and inclusion bodies in ventral mesencephalic cultures (McNaught, Mytilineou, et al., 2002). Proteasome inhibition causes nigral degeneration with inclusion bodies in rats (McNaught, Björklund, et al., 2002) and the formation of ubiquitin/α-syn-immunoreactive inclusions in PC12 cells (H J Rideout, Larsen, Sulzer, & Stefanis, 2001). Also,

injections of the proteasomal inhibitor, lactacystin, directly into the nigral tissue of C57Bl/6 mice results in PD-like motor symptoms and α-syn accumulation (Savolainen, Albert, Airavaara, & Myöhänen, 2017). The UPS is a highly regulated system that controls the degradation of proteins involved in signal transduction, apoptosis, cell cycle progression and differentiation (Cook & Petrucelli, 2009). It is the chief pathway involved in the removal of damaged, misfolded and short-lived proteins within the cytoplasm and nucleus of cells. It functions by sequentially ubiquitinylating and degrading target proteins (Dantuma & Bott, 2014; Hershko & Ciechanover, 1998; Kleiger & Mayor, 2014). To this date, oxysterols have not been implicated in proteasomal dysfunctions but 27-OHC has been shown to increase oxidative stress in astrocytes (Ma et al., 2015) and prolonged oxidative stress has been shown to decrease proteasomal function (Shang & Taylor, 2011). Here, we look to provide evidence that 27-OHC inhibits proteasomal function.

Heat shock proteins (HSPs) play a pivotal role in preventing protein misfolding and inhibiting apoptotic activity, and represent a class of proteins potentially involved in the pathogenesis of PD (Aridon et al., 2011). HSPs are ubiquitous and usually expressed at relatively low levels under normal circumstances but are dramatically increased in response to cellular stressors as well as many normal processes such as cell growth, differentiation, development and aging (Multhoff, 2007). This response represents a naturally occurring mechanism to protect cells against environmental and physiological stresses. HSPs operate primarily as molecular chaperones that support protein folding,

preventing protein aggregation, and targeting misfolded proteins for degradation in the UPS and ALP (Aridon et al., 2011; Ebrahimi-Fakhari, Saidi, & Wahlster, 2013; Ebrahimi-Fakhari, Wahlster, & McLean, 2011, 2012).

HSP70 is one of the most structurally and functionally conserved proteins in evolution. In addition to its role in cellular stress, it is involved in the disassembly of protein aggregates and targeting of proteins for degradation. HSP70 uses its ATP hydrolysis-powered conformational changes to assist protein folding, disaggregation and degradation. It is a key contributor in cellular proteostasis. To date, four major protein degradation pathways have been implicated in mammalian systems including UPS and three major types of autophagy: macroautophagy, microautophagy, and chaperone-mediated autophagy (Fernández-Fernández et al., 2017). HSP70 is able to be involved in these various degradation pathways by the presence of specific chaperones and co-chaperones that aid in guiding the targeted protein to a specific degradation pathway (Fernández-Fernández et al., 2017). In PD, the first evidence of the involvement of molecular chaperones was provided by pathological studies that identified many heat shock proteins, including HSP70, as components of Lewy bodies (Namba, Tomonaga, Ohtsuka, Oda, & Ikeda, 1991). Increasing HSP70 has been shown to inhibit α-syn accumulation in PC12 cells (H. Wang et al., 2017). It has been shown that oxysterols are cytotoxic but fail to induce hsp70 expression in endothelial cells (Pirillo et al., 1999) so in this dissertation we aim to investigate the effects of 27-OHC on HSP70 protein levels.

In summary, throughout this dissertation, we will discuss the role of 27-OHC on the accumulation of α-syn. We will investigate the mechanism(s) by which 27-OHC elicits its effects to alter levels of α-syn protein.

Global Fatty Acid and Palmitic Acid Function and Biosynthesis

Fatty acids (FAs) have roles in many different cellular properties resulting in altered gene expression, metabolism, responsiveness to hormones, and production of bio-active substances. Through these many actions, FAs affect health, physiology, and disease risk (Calder, 2015).

A FA molecule, such as palmitic acid, has 2 chemically distinct regions. A long hydrocarbon chain, which is hydrophobic and not very reactive chemically (doesn't form hydrogen bonds with H_2O very readily) The other is a carboxyl (-COOH) group, which behaves as an acid (carboxylic acid): it is ionized in solution ($-COO^-$), extremely hydrophilic, and chemically reactive. Almost all fatty acid molecules in a cell are covalently linked to other molecules by their carboxylic acid group. FAs can either be saturated (have all single bonds between carbon atoms), be monounsaturated (have one double bond), or polyunsaturated (more than one double bond between carbons). The degree of saturation and the length of the carbon chain affects the function of specific FAs.

FAs are major components of phospholipids, triacylclycerols (TAGs), and other complex lipids. These compounds are widely dispersed in nature and the foods we eat. Different foods contain varying amounts of fat and different types of fatty acids. Hence, diets will vary drastically in FA content from day to day and meal to meal. Individual fatty acids have been shown to have their own specific

functions regardless of if they are in the same general FA class. In healthy individuals roughly 95% of the FAs consumed are available in the bloodstream (Calder, 2015) and they can also be synthesized in the human body as previously mentioned. Table 2 shows % by weight of total FAs in plasma, whole blood, red blood cells, lipoproteins and platelets. Table 3 shows % of total FA composition in phospholipids and neutral lipids extracted from temporal cortex sample from controls and PD patients.

Table 2. Fatty acid profiles of lipids in whole blood, plasma, red blood cells (RBCs), lipoproteins and platelets (PLTs).[a.] Reprinted from Fatty acid composition of plasma, blood cells and whole blood: relevance for the assessment of the fatty acid status in humans, Volume 76 Issue 6, June 2007, (Risé, Eligini, Ghezzi, Colli, & Galli, 2007) 363-369, Copyright 2007, with permission from Elsevier.

% by weight of total fatty acids						
FA	**WB**	**Plasma**	**LDL**	**HDL**	**RBCs**	**PLTs**
16:0	26.01±4.80	22.66±1.72	18.75±1.68[b,c]	24.04±0.67[b,d]	24.22±3.10[d]	13.90±1.07[b,c,d,e,f,]
18:0	11.01±2.09	8.11±0.73[b]	7.25±0.41[b,c]	9.45±0.31[b,c,d]	20.63±2.10[b,c,d,e]	19.38±0.67[c,d,e]
16:1	0.59±0.21	1.51±0.37	1.11±0.15[c]	1.17±0.12[c]	0.22±0.07[c,d,e]	0.26±0.04[c,d,e]
18:1	17.97±1.52	19.38±2.61	17.95±0.92	15.67±0.68[b,c,d]	15.63±1.93[b,c,d]	17.18±0.54[c,e,f]
18:2n-6	19.36±2.73	28.95±2.71[b]	34.94±1.61[b,c]	29.48±1.03[b,d]	9.77±1.37[b,c,d,e]	9.31±0.35[b,c,d,e]
18:3n-6	0.23±0.10	0.36±0.12	0.47±0.07	0.34±0.03[d]	n.d.	0.06±0.01[c,d,e]
20:3n-6	1.41±0.15	1.70±0.22	1.51±0.07	1.97±0.10[c,d]	1.38±0.19[c,e]	1.29±0.08[c,d,e]
20:4n-6	9.01±0.82	8.59±1.01	8.94±0.53	9.59±0.38[c]	12.88±2.09[b,c,d,e]	21.23±0.80[b,c,d,e,f]
22:4n-6	1.06±0.06	0.25±0.04	0.21±0.03	0.26±0.02	2.22±0.83[c,d,e]	2.03±0.17[c,d,e]
22:5n-6	0.26±0.05	0.30±0.19	0.23±0.07	0.24±0.03	0.59±0.46[c,d,e]	1.81±0.28[c,d,e,f]
18:3n-3	0.24±0.07	0.26±0.06	0.27±0.02	0.24±0.03	n.d.	0.13±0.04[b,c,d,e,f]
20:5n-3	0.69±0.46	0.80±0.24	0.98±0.13	0.92±0.10	0.42±0.12[c,d,e]	1.44±0.67
22:5n-3	0.96±0.20	0.47±0.11[b]	0.34±0.04[b,c]	0.55±0.04[b,d]	1.13±0.59[c,d,e]	1.09±0.08[c,d,e]
22:6n-3	2.69±0.66	2.68±0.60	2.25±0.20	2.91±0.18[d]	3.24±1.19[d]	2.21±0.11[b,c,d,e,f]

% by weight of total fatty acids						
FA	**WB**	**Plasma**	**LDL**	**HDL**	**RBCs**	**PLTs**
SFA	41.83±3.20	32.26±1.64[b]	29.04±1.84[b,c]	35.52±0.87[b,c,d]	49.36±3.02[b,c,d,e]	39.03±0.79[b,c,d,e,f]
MUFA	22.20±2.70	22.25±2.77	20.72±1.02	17.87±0.76[b,c,d]	18.92±1.32[c]	20.19±0.64[b,c,e]
PUFA	35.97±4.99	44.49±2.89[b]	50.24±1.49[b,c]	46.62±1.11[b,d]	31.71±3.76[c,b,d,e]	40.78±0.78[b,c,d,e,f]
U.I.	131.07±11.07	146.78±6.34[b]	155.52±4.41[b,c]	150.23±2.93[b]	133.43±18.05[c,d,e]	171.79±3.02[b,c,d,e,f]
n-3 HUFA Index	27.60±7.50	26.65±4.07	24.60±4.17	26.62±3.32	21.31±4.36[b,e]	15.21±6.76[b,c,d,e,f]

[a]Values are the mean±SD (*n*=10). The ANOVA analysis (SPSS 12.0.1 for Windows) and Tukey's post hoc test was used for the comparisons: [b]*P*<0.05 compared with WB. [c]*P*<0.05 compared with plasma. [d]*P*<0.05 compared with LDL. [e]*P*<0.05 compared with HDL. [f]*P*<0.05 compared with RBCs. FA, fatty acids; WB, whole blood; SFA, saturated FA; MUFA, monounsaturated FA; PUFA, polyunsaturated FA; U.I. unsaturation index; n-3 HUFA (high unsaturated FA) index: ratio between n-3 FA (with 20 and more carbon atoms) and n-3 plus n-6 FA (with 20 and more carbon atoms).

Table 3. Fatty acid (FA) composition in phospholipids and neutral lipids extracted from temporal cortex sample from controls (n = 9) and Parkinson disease (PD) patients (n = 12) expressed in percent of fatty acids. Reprinted from Postmortem brain fatty acid profile of levodopa-treated Parkinson disease patients and parkinsonian monkeys, Volume 48 Issue 5 April 2006, (Julien et al., 2006), 404-414, Copyright 2006, with permission from Elsevier.

Fatty acid	Controls phospholipids (%FA/total FA)	PD patients phospholipids (%FA/total FA)	Controls neutral lipids (%FA/total FA)	PD patients neutral lipids (%FA/total FA)
14:0 (Myristic)	0.38 ± 0.01	0.39 ± 0.01	0.99 ± 0.06	1.14 ± 0.09
14:1*n* − 5	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
16:0 (Palmitic)	18.97 ± 0.67	20.17 ± 0.24	19.66 ± 0.33	19.17 ± 0.33
16:1*n* − 7	0.61 ± 0.04	0.56 ± 0.04	1.08 ± 0.11	1.00 ± 0.06
18:0 (Stearic)	22.35 ± 0.39	22.55 ± 0.39	24.71 ± 0.45	25.44 ± 0.55
18:1*n* − 9 (Oleic)	17.56 ± 0.92	15.85 ± 0.61	14.87 ± 0.45	14.25 ± 0.27
18:1*n* − 7	4.11 ± 0.16	3.90 ± 0.20	5.11 ± 0.33	4.44 ± 0.26
18:2*n* − 6 (LA)	0.65 ± 0.06	0.72 ± 0.05	1.45 ± 0.11	1.78 ± 0.20
18:3*n* − 6	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
18:3*n* − 3 (LNA)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
18:4*n* − 3	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
20:0	0.22 ± 0.03	0.21 ± 0.03	0.00 ± 0.00	0.00 ± 0.00
20:1*n* − 12	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
20:1*n* − 9	0.88 ± 0.14	0.63 ± 0.08	0.41 ± 0.09	0.24 ± 0.07
20:2*n* − 6	0.43 ± 0.05	0.37 ± 0.04	0.16 ± 0.08	0.38 ± 0.14
20:3*n* − 6	0.96 ± 0.08	0.92 ± 0.04	1.50 ± 0.15	1.41 ± 0.05
20:4*n* − 6 (ARA)	8.56 ± 0.36	9.32 ± 0.29	16.24 ± 0.49	16.89 ± 0.45
20:3*n* − 3	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
20:4*n* − 3	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
20:5*n* − 3 (EPA)	0.08 ± 0.03	0.09 ± 0.06	0.00 ± 0.00	0.13 ± 0.13
22:0	0.19 ± 0.04	0.22 ± 0.10	0.04 ± 0.04	0.00 ± 0.00
22:1*n* − 11	0.02 ± 0.01	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00
22:1*n* − 9	0.02 ± 0.01	0.02 ± 0.01	0.00 ± 0.00	0.00 ± 0.00
22:2*n* − 6	0.14 ± 0.14	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
22:4*n* − 6 (DTA)	4.40 ± 0.85	5.00 ± 0.47	3.22 ± 0.15	2.71 ± 0.29
22:3*n* − 3	1.26 ± 0.83	0.48 ± 0.48	0.00 ± 0.00	0.00 ± 0.00
22:5*n* − 6 (DPA)	1.21 ± 0.12	1.38 ± 0.06	0.62 ± 0.10	0.57 ± 0.08

24:0 (Tetracosanoic)	0.30 ± 0.14	0.04 ± 0.03*	0.22 ± 0.12	0.12 ± 0.09
22:5*n* − 3	0.79 ± 0.27	0.82 ± 0.07	0.47 ± 0.11	0.61 ± 0.11
22:6*n*-3 (DHA)	12.49 ± 0.97	13.90 ± 0.67	7.51 ± 0.37	7.02 ± 0.35
24:1*n* − 9 (Tetracosenoic)	2.49 ± 0.63	1.61 ± 0.32	0.37 ± 0.13	0.36 ± 0.14
Saturated FA (total)	42.92 ± 0.92	43.98 ± 0.37	46.81 ± 0.87	47.26 ± 0.80
Monounsaturated FA (total)	25.70 ± 1.78	22.57 ± 1.18	21.84 ± 0.94	20.35 ± 0.54
Polyunsaturated FA (total)	30.94 ± 0.86	32.97 ± 0.82	31.31 ± 0.78	32.25 ± 0.78
n − 6 total	16.34 ± 0.93	17.69 ± 0.57	23.21 ± 0.55	23.74 ± 0.57
n − 3 total	14.61 ± 1.21	15.29 ± 0.81	8.11 ± 0.45	8.51 ± 0.50
n − 9 total	20.96 ± 1.67	18.11 ± 0.99	15.65 ± 0.59	14.91 ± 0.40
n − 3:*n* − 6 ratio	0.95 ± 0.14	0.88 ± 0.08	0.35 ± 0.02	0.36 ± 0.02
SFA/MUFA	1.72 ± 0.12	1.99 ± 0.10	2.13 ± 0.11	2.27 ± 0.08
PUFA/MUFA	1.25 ± 0.09	1.52 ± 0.10	1.46 ± 0.08	1.60 ± 0.07
n − 6/MUFA	0.66 ± 0.06	0.81 ± 0.05	1.08 ± 0.05	1.18 ± 0.04
n − 3/MUFA	0.60 ± 0.06	0.71 ± 0.06	0.38 ± 0.03	0.42 ± 0.03
	mg/100 g of tissue	**mg/100 g of tissue**	**mg/100 g of tissue**	**mg/100 g of tissue**
Total FA	2116.57 ± 155.34	1821.21 ± 99.26	111.18 ± 6.53	86.53 ± 4.20
mg lipids/100 g (wet tissue)	10494.69 ± 4434.31	10006.60 ± 4037.09	10494.69 ± 4434.31	10006.60 ± 4037.09

Values are expressed as the mean ± S.E.M. Abbreviations—ARA: arachidonic acid (20:4*n* − 6); DHA: docosahexaenoic acid (22:6*n*-3); DPA: docosapentaenoic acid (22:5*n* − 6); DTA: docosatetraenoic acid (22:4*n* − 6); EPA: eicosapentaenoic acid (20:5*n* − 3); FA: fatty acids; LA: linoleic acid (18:2*n* − 6); LNA: linolenic acid (18:3*n* − 3); PD: Parkinson disease.

* $P < 0.05$ vs. Controls.

FAs are transported in the blood as parts of more complex lipids (ex. TAGs, phospholipids, cholesteryl esters) within lipoproteins, although some “free” non-esterified FAs also circulate in the blood. Lipoproteins and non-esterified FAs act as means for transporting FAs throughout the body where they can serve metabolic, functional, and storage roles. One of the major metabolic roles is to be used as a source of energy via β-oxidation. An important functional role is to act as components of cell membrane phospholipids. The wide variety of cell types, membranes, and phospholipids consist of very differential FA compositions. The composition of FAs within cell membranes affects the fluidity of the membrane which in turn affects the function of membrane proteins and the movement of proteins within the membrane. Membrane lipids are also precursors to diacylglycerols, ceramides, lyso-phospholipids, and endocannabinoids which are important in cellular signaling (Calder, 2015). As previously mentioned, individual FAs have specific roles. For example, the saturated FAs myristic (14:0) and palmitic (16:0) have specific roles in acylation of membrane proteins that are important for anchoring those proteins to the membrane and also used for cellular localization signaling. The ω-6 polyunsaturated fatty acid arachidonic acid (20:4ω-6) is the main precursor for prostaglandins, thromboxanes, and leukotrienes that have many important roles in inflammatory signaling. Many FAs are able to control expression and activity of transcription factors meaning that FAs can control gene expression and protein production throughout the body. This allows FAs to regulate cellular processes like fatty acid synthesis and oxidation, lipoprotein assembly, inflammation, and insulin sensitivity. With the

plethora of biological effects of FAs it is no question that FAs can influence health, well-being and disease risk. Throughout this dissertation we aim to more fully explore the roles of FAs and more specifically, palmitic acid, the most abundant saturated FA in the body, in the context of PD type-synucleinopathy risk.

The Role of Palmitic Acid in Synucleinopathies

To date, studies focused on the contributions of dietary fat intake to the risk of PD type synucleinopathy have yielded inconsistent results (White et al., 2009). Epidemiological studies of dietary fat intake and PD have found positive associations (Anderson et al., 1999a; Johnson, Gorell, Rybicki, Sanders, & Peterson, 1999b; Giancarlo Logroscino et al., 1996a; Miyake, Sasaki, et al., 2010), no association (Hellenbrand et al., 1996a; L. C. Tan et al., 2007), and even protective effects (Abbott, Webster Ross, et al., 2003b; Honglei Chen, Zhang, Hernán, Willett, & Ascherio, 2003b; L M L de Lau et al., 2005c; Kamel et al., 2014; Kyrozis et al., 2013; Karen M. Powers et al., 2009b). Studies focused on specific groups of fatty acids have provided little clarity. Indeed, PUFAs and MUFAs have been shown to be protective in some studies (Abbott, Webster Ross, et al., 2003c; L.M.L. de Lau et al., 2005) and detrimental in another (Dong et al., 2014), while studies of saturated fatty acids have shown positive associations (Anderson et al., 1999b; Honglei Chen, Zhang, Hernán, Willett, & Ascherio, 2003c; Johnson, Gorell, Rybicki, Sanders, & Peterson, 1999c; Giancarlo Logroscino et al., 1996b) and no significant relationship with PD risk (Honglei Chen, Zhang, Hernán, Willett, & Ascherio, 2002; Honglei Chen, Zhang,

Hernán, Willett, et al., 2003c; Hellenbrand et al., 1996b; K M Powers et al., 2003). Additionally, *in vitro* studies have shown that PUFAs increase α-syn oligomerization and insoluble aggregate formation while saturated fatty acids did not (Assayag, Yakunin, Loeb, Selkoe, & Sharon, 2007; Ronit Sharon, Bar-Joseph, Mirick, Serhan, & Selkoe, 2003). With all of the conflicting studies, the role(s) of FAs in PD-type synucleinopathy risk are still yet to be determined.

Palmitic acid (16:0) is the most abundant saturated fatty acid in the body and the most abundant fatty acid in meats, cheeses, and dairy products. It is synthesized *de novo* in the body and makes up 24-26% of total fatty acids in our blood and 28% of total fatty acids in our CSF (Guest, Garg, Bilgin, & Grant, 2013; Risé et al., 2007). Numerous *in vitro* studies have focused on various roles of palmitic acid. It has been shown to increase ER stress (Marwarha, Claycombe, Schommer, Collins, & Ghribi, 2016b), proinflammatory cytokine expression in astrocytes and microglia (Gupta, Knight, Gupta, Keller, & Bruce-Keller, 2012; Tracy, Bergqvist, Ivanova, Jacobsen, & Iverfeldt, 2013), and activation of TLRs via NFKβ (Oberbach et al., 2012) but its role in pathological hallmark formation of PD-type synucleinopathy remains unknown. In the frontal cortex of normal human brains PA makes up roughly 21% of total FAs, while in the frontal cortex of PD patient brains it makes up roughly 22% of total FAs (Ronit Sharon et al., 2003) while PD brains have been shown to have significantly higher levels of polyunsaturated fatty acids than control brains which potentially leads to more α-syn oligomerization (Assayag et al., 2007; Ronit Sharon et al., 2003).

Throughout this dissertation we aim to examine the role(s) of Palmitic acid on various hallmarks of PD-type synucleinopathy pathology in various animal and cellular models of PD.

CHAPTER II

METHOD FOR ORGANOTYPIC TISSUE CULTURE IN THE AGED ANIMAL

Abstract

Organotypic slicing of brain tissue from young rodents has been used as a powerful model system for biomedical research (Gähwiler, Capogna, Debanne, McKinney, & Thompson, 1997; Humpel, 2015; Stoppini, Buchs, & Muller, 1991). Organotypic slicing complements cell culture and *in vivo* studies in multiple facets. This system can be useful for investigating manipulation of cellular signaling pathways without the hindrance of the blood-brain barrier while sacrificing fewer animals in the process. It also allows for preserved cellular connectivity and local intact circuitry which is a drawback of isolated cell cultures. Studies on age-related diseases have mainly used embryonic or early postnatal organotypic slice tissue. Excluding synaptic plasticity studies that are usually carried-out over a few hours and use adult mice or rats, a handful of studies performed on adult animals have had success for survival of slices (H. Kim, Kim, Park, Lee, & Namkoong, 2013; Mewes et al., 2012). Here we describe a method for culturing organotypic slices with high viability from hippocampus of aged mice and rabbits. See Figure 24 for a graphical representation of our method.

- Our method permits slices from mice as old as 16 months and rabbits as old as years of age to survive *ex vivo* up to 8 weeks (Marwarha, Dasari,

- Prasanthi, Schommer, & Ghribi, 2010b; Marwarha, Prasanthi, Schommer, Dasari, & Ghribi, 2011a; Prasanthi, Larson, Schommer, & Ghribi, 2011; M. Schrag, Sharma, Brown-Borg, & Ghribi, 2008). Such a slice system may be relevant to investigating age-related brain diseases.

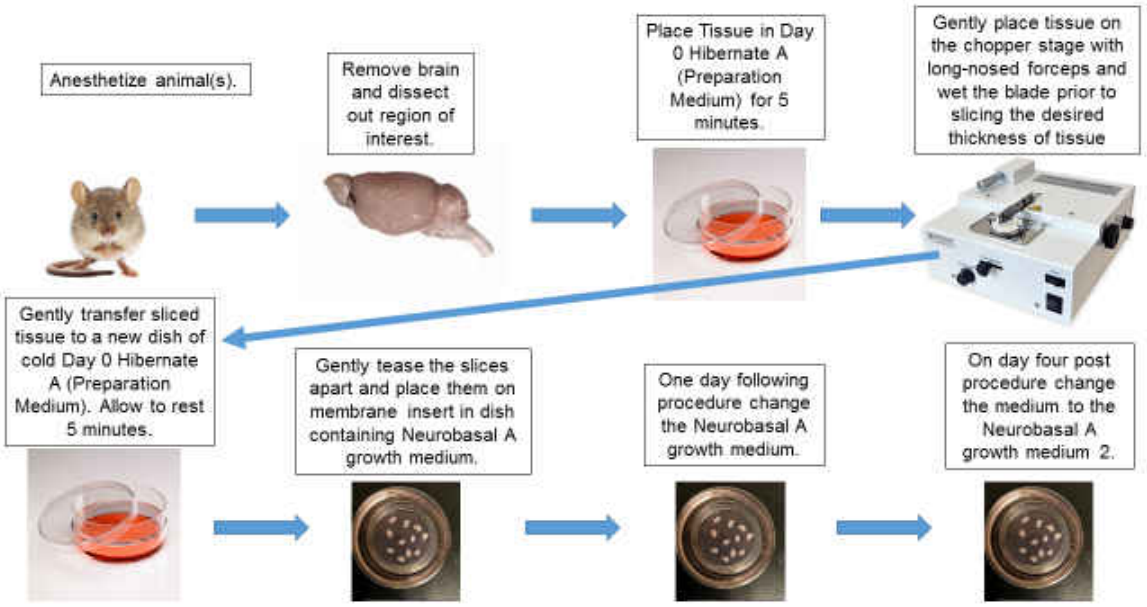


Figure 24. Graphical Abstract of method for organotypic tissue culture in the aged animal. Reprinted from (Schommer, Schrag, Nackenoff, Marwarha, & Ghribi, 2017)

Materials

Table 4. Materials needed for Organotypic tissue culture in the aged animal. Reprinted from (Schommer et al., 2017)

Material	**Company**	**Catalog Number**
McIlwain Tissue Chopper	The Mickle Laboratory Engineering Co. LTD	Model MTC/2
Teflon insert	The Mickle Laboratory Engineering Co. LTD	
Grade 50 hardened filter paper	Whatman	1450-055
35x15mm tissue culture treated dishes	Santa Cruz	Sc-200284
100x20mm cell culture dishes	Greiner Bio-One	664-160
Size 2 oil paint brushes	Silver Fox	
Long-nosed forceps		
Premium Sterile Stainless Steel Scalpel Blades - #22	Havel's	FHS22
0.4µm, 30mm cell culture inserts	Millipore	PICMORG50
Hibernate A	Brain Bits	Hibernate A
L-Glutamine 200mM (100x)	Gibco	25030-081
Horse Serum	Gibco	16050-122
Antibiotic/Antimycotic (100x)	Gibco	15240-062
Neurobasal-A Medium	Gibco	10888-022
2% B27 Supplement (50x)	Gibco	17504-044

Method

Preparation—Prior to animal sacrifice.

Day 0 Medium Preparation

Hibernate A (preparation medium):

To a sterile 50 mL centrifuge tube add:

- 0.5mM Glutamine (250µL of stock solution)
- 10mL Horse Serum
- 40mL standard Hibernate A Medium

Prepare 2-3 batches if you desire extra medium and/or to change out when medium containing the slices starts to discolor.

Neurobasal A (growth medium):

To a sterile 50 mL centrifuge tube add:

- 20% Horse Serum (8mL)
- 400µL standard antibiotic mixture (Antibiotic/Antimycotic)
- 40mL Neurobasal A Medium

Prepare Day 1 and Day 4-Treatment Day Medium fresh on the day of use

Day 1: Neurobasal A (growth medium 1):

To a sterile 50mL centrifuge tube add:

- 20% Horse Serum (8mL)
- 400µL standard antibiotic mixture (Antibiotic/Antimycotic)
- 40mL Neurobasal A Medium

Day 4 through Treatment Day: Neurobasal A (growth medium 2):

To a sterile 50mL centrifuge tube add:

- 2% B27 supplement (800µL)
- 400µL standard antibiotic mixture (Antibiotic/Antimycotic)
- 40mL Neurobasal A Medium

McIlwain chopper preparation.

- Prepare the chopper by adjusting the dial for the desired slice thickness (we have used 250µM and 300µM slices).
- Install a sharp double-sided razor and loosely attach the clamp.
- Thoroughly clean the stage of the chopper and blade with 70% ethanol.
- Place a sterile Teflon insert surrounded by 2 filter paper disks on the stage.
- Turn the dial on the chopper to allow the arm to drop onto the stage containing the Teflon insert and filter papers. Once the arm has dropped make sure the blade is resting flush on top of the stage, then tighten the clamp.
- Just prior to use, wet the top filter paper with a few drops of Hibernate A preparation medium and wet the blade using the paintbrush to ensure that the tissue will stick to the filter paper but not the blade.

Insert preparation.

- Place 1.1 mL of growth medium 1 into the desired number of 35 mm tissue culture dishes. For hippocampal slices from mice, you can expect to use 3 dishes per mouse (8-10 slices per dish). For hippocampal slices from rabbit you can expect to use 12-15 dishes per rabbit (4-6 slices per dish).

- Place one Millicell insert in each dish trying to avoid trapping air bubbles underneath the membrane to allow the tissue to contact the medium.
- Store the prepared dishes in the incubator (35ºC, 5% CO_2) for at least 1 hour prior to use.

Procedure.

- Anesthetize animal with Euthasol diluted 1:1 with dH_2O and rapidly decapitate. Other forms of anesthesia also work including CO_2 and Ketamine/Xylazine.
- Dissect area of interest and place in chilled preparation medium in a 100 mm tissue culture dish. Store on ice for 5 minutes or less.
- Transfer the tissue to the stage of the McIlwain chopper and proceed to chop the tissue.
- Gently move the sliced tissue from the stage into a new 100mm tissue culture dish containing chilled preparation medium and allow the slices to sit in the solution for 5 minutes.
- Transfer to a new 100mm tissue dish containing 4 mL of chilled preparation medium. Less medium in the dish allows for easier handling and separation of slices.
- Gently tease the slices apart using a small size 2 oil paint brush and scalpel. Once separated, pull the slices from their outer extremity onto the scalpel blade using the paint brush while being careful not to damage the slices integrity. Transfer the slices from the scalpel blade to the membrane of the dishes that were prior placed in the incubator using the paint brush

again on the outer extremity of the slice to minimize damage to the slice. Each membrane can hold 8-10 mouse hippocampal slices or 4-6 rabbit hippocampal slices.

- Change the medium on Day 1 and on every third day. Do this as quick as possible, if necessary only change media on 2-4 dishes at a time.

The sections attach to the culture membranes in a few days and become fully attached to the membrane after ten days. One half of the growth medium should be replaced every 3-4 days. Sections plated at lower density (i.e. 3-5 sections of mouse hippocampus per membrane) will require media exchange every 7-10 days.

Though infection is rare (roughly 1 in 50 dishes) and user dependent, standard antibiotic mixture is used to minimize infection throughout the duration of culture. If desired, user may exclude standard antibiotic mixture following day 4 with similar infection rate. Figures 25–27 show that organotypic slices in the aged animal do indeed survive. Figures 28–29 show the effects of treatment of slices with 27-OHC and PA respectively on important proteins in PD.
In organotypic slices, 27-OHC increases α-syn protein content (Fig. 28A,B), decreases TH protein levels (Fig. 28C,D), and decreases $pS^{40}TH$ protein levels (Fig. 28E,F) in concordance with our forthcoming data in human dopaminergic neurons. PA treated of organotypic slices at 200 μM concentration decreases α-syn protein levels (Fig. 29A,B), increases TH protein levels (Fig.29C,D) and increases $pS^{40}TH$ protein levels. Suggested mechanisms of action for these effects will be highlighted in the following chapters.

Figure 25. Hippocampal slices from 1 year old C57BL6 mice. A. Healthy dish of mouse hippocampal slices 10 days post tissue sectioning. B. Dead/Dying infected mouse hippocampal slices 10 days post tissue sectioning. Reprinted from (Schommer et al., 2017).

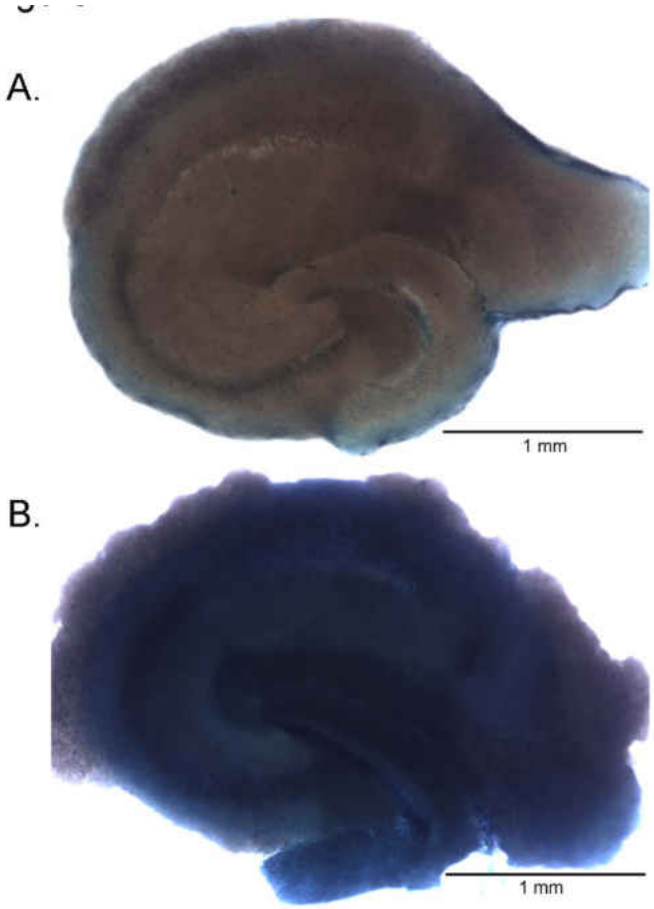


Figure 26. Hippocampal slices from 10.5 month old B6129SF2/J mice. A. Healthy hippocampal slice 7 days post tissue sectioning exposed to Trypan Blue staining. B. Medium deprived dead hippocampal slice 7 days post tissue sectioning exposed to Trypan Blue staining. Reprinted from (Schommer et al., 2017)

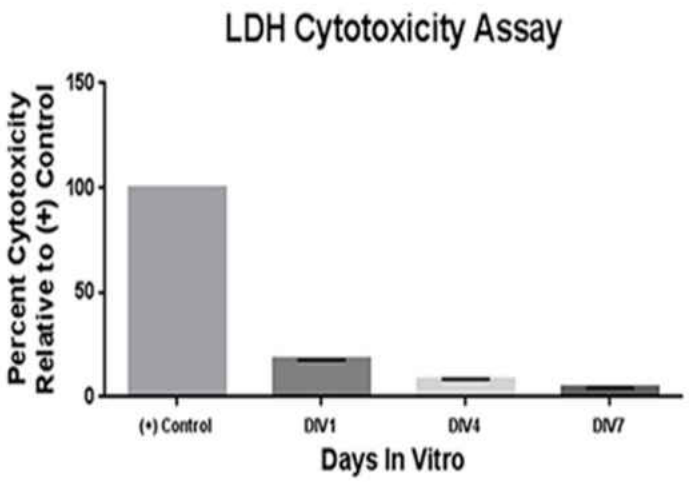


Figure 27. LDH Assay on the medium of culture dishes containing 4 hippocampal slices of 1 year old C57BL6 mice at sequential days In Vitro. Reprinted from (Schommer et al., 2017)

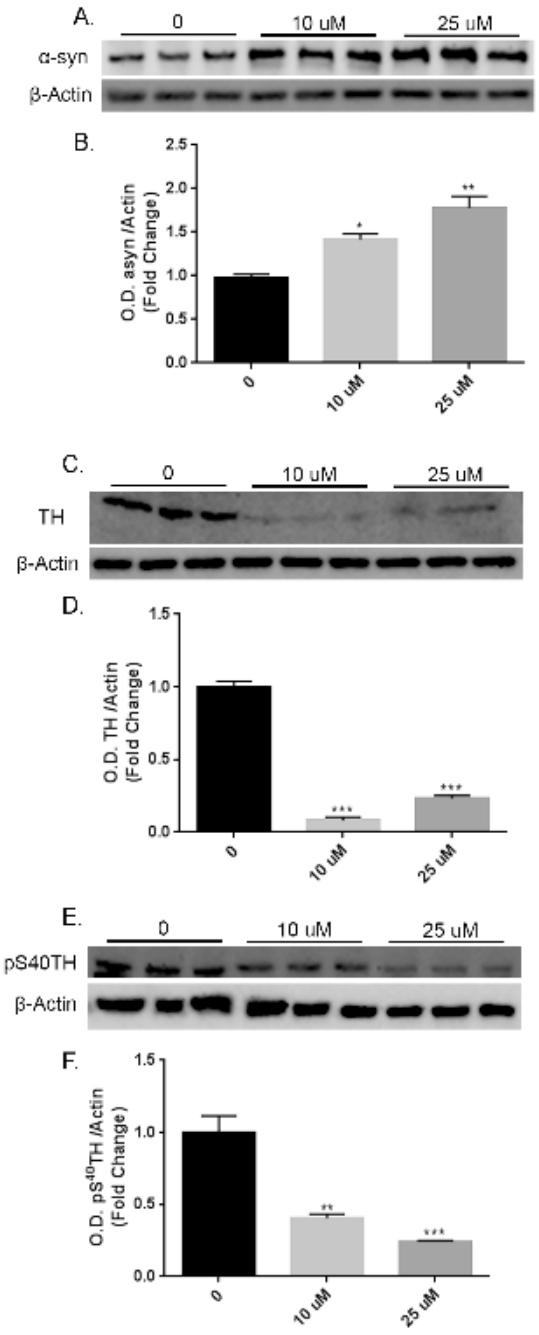


Figure 28. 27-OHC treatments in C57BL/6 *substantia nigra* slices. A,B Western Blot and optical density of alpha-synuclein. C,D Western Blot and optical density of Tyrosine Hydroxylase. E,F Western Blot and optical density of pS^{40} Tyrosine Hydroxylase.

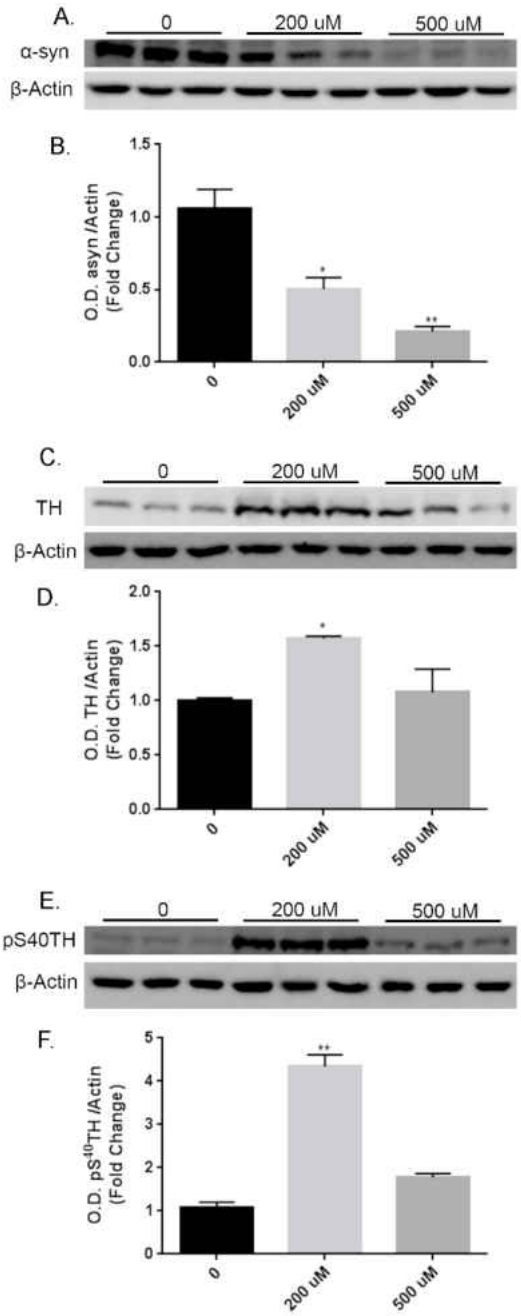


Figure 29. PA treatments in C57BL/6 *substantia nigra* slices. A,B Western Blot and optical density of alpha-synuclein. C,D Western Blot and optical density of Tyrosine Hydroxylase. E,F Western Blot and optical density of pS40 Tyrosine Hydroxylase.

CHAPTER III

27-HYDROXYCHOLESTEROL INCREASES α-SYNUCLEIN PROTEIN LEVELS THROUGH PROTEASOMAL INHIBITION IN HUMAN DOPAMINERGIC NEURONS

Abstract

Background. Accumulation of the α-synuclein (α-syn) protein is a hallmark of a group of brain disorders collectively known as synucleinopathies. The mechanisms responsible for α-syn accumulation are not well understood. Several studies suggest a link between synucleinopathies and the cholesterol metabolite 27-hydroxycholesterol (27-OHC). 27-OHC is the major cholesterol metabolite in the blood that crosses the blood brain barrier, and its levels can increase following hypercholesterolemia, aging, and oxidative stress, which are all factors for increased synucleinopathy risk. In this study, we determined the extent to which 27-OHC regulates α-syn levels in human dopaminergic neurons, the cell type in which α-syn accumulates in PD, a major synucleinopathy disorder.

Results. Our results show that 27-OHC significantly increases the protein levels, not the mRNA expression of α-syn. The effects of 27-OHC appear to be independent of an action through liver X receptors (LXR), its cognate receptors, as the LXR agonist, GW3965, or the LXR antagonist ECHS did not affect α-syn protein or mRNA levels. Furthermore, our data strongly suggest that the

27-OHC-induced increase in α-syn protein levels emanates from inhibition of the proteasomal degradation of this protein and a decrease in the heat shock protein 70 (HSP70).

Conclusions. Identifying 27-OHC as a factor that can increase α-syn levels and the inhibition of the proteasomal function and reduction in HSP70 levels as potential cellular mechanisms involved in regulation of α-syn. This may help in targeting the correct degradation of α-syn as a potential avenue to preclude α-syn accumulation.

Introduction

Synucleinopathies are pathologically characterized by the abnormal accumulation of α-syn protein in intracellular inclusions known as Lewy bodies. The role of α-syn in the pathogenesis of synucleinopathies is not well understood but extensive experimental data points to a neurotoxic role of high levels of the protein in its soluble and aggregated forms (Adamczyk, Kaźmierczak, & Strosznajder, 2006; Brown, 2010; Halbach, Schober, & Krieglstein, 2004; Snyder & Wolozin, 2004). For the last decade, hyperlipidemia has been under scrutiny as a risk factor for synucleinopathy of Parkinson's disease (PD) type (Xiang Gao et al., 2012c; Marwarha & Ghribi, 2015; Mutez et al., 2009). However, while various studies showed an increased risk (Bosco et al., 2006; G. Hu et al., 2008; Johnson et al., 1999a), other studies reported a decreased risk (Miyake, Tanaka, et al., 2010; Karen M. Powers et al., 2009a; Simon et al., 2007), or no association with high cholesterol levels (Abbott, Webster Ross, et al., 2003a; L M L de Lau et al., 2005b). It may be possible that the conflicting results are

indicative of the disturbances in the cholesterol oxidation derivative 27-OHC, not cholesterol *per se*, as the risk factor for PD. In addition to being a cholesterol oxidation product (oxysterol), 27-OHC is an active product that has a variety of biological functions. One of its main functions is to bind to liver X receptors (LXRs), thus affecting genes and proteins that are regulated by these receptors (Gabbi et al., 2014; Olkkonen, 2008). LXRs, by means of gene transcription, regulate several metabolic pathways including lipid metabolism, glucose homeostasis, and inflammation (Gabbi et al., 2014). LXRs regulate gene transcription by binding to promotors of genes and recruiting co-activators or co-repressors to enhance or repress the expression of target genes(Gabbi et al., 2014). In support of our speculation of a link between synucleinopathies and 27-OHC are studies showing increased levels of a variety of cholesterol oxidation products (oxysterols), including 27-OHC, within the brains of patients with synucleinopathies (Bosco et al., 2006; Cheng et al., 2011b; Leoni et al., 2004b; Leoni & Caccia, 2011a; Marwarha, Rhen, et al., 2011b; Rantham Prabhakara et al., 2008). Oxysterol levels have also been shown to be increased in the circulation of hypercholesterolemic individuals (Bertolotti et al., 2012; van Doormaal et al., 1989), with aging (Marwarha & Ghribi, 2015; Sottero et al., 2009), and with oxidative stress (Thanan et al., 2015), all of which are risk factors for PD. Another interesting observation in support of a role of 27-OHC in brain neurodegeneration is that 27-OHC can cross the blood brain barrier while cholesterol cannot (Björkhem et al., 2009; Heverin et al., 2015; Leoni et al., 2004b; Leoni & Caccia, 2011a). This data points to a potential association

between accumulation of the oxysterol 27-OHC and synucleinopathies. However, the potential mechanisms by which 27-OHC may affect α-syn levels and increase the risk for synucleinopathies remains to be determined. Ours (Marwarha, Rhen, et al., 2011b; Rantham Prabhakara et al., 2008) and others (Cheng et al., 2008a) published data showed that 27-OHC increases the transcription of α-syn through activation of LXRs in human neuroblastoma SHSY-5Y cells. However, whether 27-OHC can also affect α-syn transcription in human dopaminergic neurons is yet to be demonstrated. Furthermore, whether the accumulation of α-syn involves the inhibition of its degradation by 27-OHC is not known.

α-syn has been shown to be degraded by the proteasome (Alvarez-Castelao et al., 2014; Bennett et al., 1999b; Webb et al., 2003), and proteasomal dysfunction has often been implicated in PD (McNaught et al., 2006, 2001). Currently, the extent to which 27-OHC inhibits the UPS to increase α-syn accumulation remains to be shown. Heat shock proteins (HSPs) are one of the most structurally and functionally conserved proteins in evolution. In addition to their role in cellular stress, they are involved in the disassembly of protein aggregates and targeting of proteins for degradation. Increasing HSP70 has been shown to inhibit α-syn accumulation in PC12 cells (H. Wang et al., 2017). In this study we aimed to investigate the effects of 27-OHC on both UPS and HSP70 protein levels. We found that 27-OHC increases α-syn protein levels independently of LXR, through proteasomal inhibition and HSP70 reduction in normal human dopaminergic neurons.

Methods

Materials.

27-OHC (Cat. # 3907), the LXR agonist GW3965 (Cat. # G6295), and the proteasome inhibitor MG132 (Cat. # 1748) were purchased from Tocris (Minneapolis, MN, USA). The LXR antagonist Cholestan-5α, 6α-EPOXY-3β-OL sulfate sodium salt (ECHS, Cat. # C4136-000) was purchased from Steraloids Inc. (Newport, RI, USA). All cell culture reagents, with the exception of fetal bovine serum (Cat. # S11150H, Atlanta Biologicals, Lawrenceville, GA, USA), dibutyryl cAMP (Cat. # sc-201567 Santa-Cruz Biotechnology, Inc. Dallas, TX, USA), antibiotic/antimycotic mix (Cat. # 15240-062, Sigma Aldrich) and Poly L-Lysine (Cat. # P4707, Sigma Aldrich) were purchased from Applied Biological Materials (Richmond, BC, Canada). Human primary Dopaminergic Neuronal Precursor cells (Cat. # T4034), PriGrow IV medium (Cat. # TM004), Fibroblast Growth Factor 2 (Cat. # Z101455), Glial-Derived Neurotrophic Factor (Cat. # Z101055), and Epidermal Growth Factor (Cat. #Z100135) were purchased from Applied Biological Materials (Richmond, BC, Canada).

Cell culture and treatments.

Human primary dopaminergic neuronal precursor cells were grown in PriGrow IV medium containing 5% fetal bovine serum, 10ng/mL Fibroblast Growth Factor 2, 10ng/mL Glial-Derived Neurotrophic Factor, and 1% Penicillin-Streptomycin. Cells were maintained at 37°C in a saturated humidity atmosphere containing 95% air and 5% CO_2. Cells were cultured and passaged for thirty days prior to differentiation. Plates were coated with Poly L-Lysine and the cells were

plated at a density of 10^4 cells per cm^2 with differentiation media consisting of PriGrow IV, 5% fetal bovine serum, 10ng/mL fibroblast growth factor 2, 10ng/mL epidermal growth factor, and 100µM dibutyryl cAMP for twenty-five days to allow for differentiation. Following differentiation, cells were incubated with ethanol vehicle (control), 0.5 µM 27-OHC (physiological concentration), and 1 or 10µM 27-OHC (high concentrations) for twenty-four hours for the 27-OHC alone experiments. For experiments involving LXR agonist and antagonist cells were incubated with ethanol and DMSO vehicle (control), 10 µM 27-OHC, 10 µM GW3965, 10 µM ECHS, 10 µM 27-OHC + 10 µM ECHS for twenty-four hours. The concentrations we used are based on our previously published data in SHSY-5Y cells (Marwarha, Rhen, et al., 2011b). For experiments involving the proteasomal inhibitor MG132 cells were incubated with ethanol vehicle (control), 10µM 27-OHC, and 1µM MG132 for twenty-four hours. The half-life of α-synuclein has been estimated by pulse-chase experiments to be 26.5 hours (Kirik et al., 2002). We chose MG132 for 24hrs for this reason and because others have inhibited the proteasome for 24hrs with MG132 prior to experimentation involving α-syn (Kirik et al., 2002). In all above mentioned treatments, three biological replicates were assigned to plates and were all utilized in the subsequent experiments including technical replicates. Cells were authenticated by Applied Biological Materials and tested negative for Mycoplasma contamination.

LDH assay.

The effect of 27-OHC, GW3965, ECHS, and 27-OHC + ECHS on cell toxicity was quantitatively determined by the measurement of lactate dehydrogenase (LDH) released from the cells into the medium 24 hours post treatments using an LDH Assay (Promega, Madison, WI, USA) according to the manufacturer's recommendations. Data were analyzed by comparison of the intensity of the absorbance in vehicle-treated cells to the treatments and subjected to one-way ANOVA. Data are expressed as individual values with mean ± SEM (n=3 wells per one sample from three separate samples).

Western blotting.

Cultured human dopaminergic neurons were treated for 24 hours with ethanol vehicle control, 0.5 µM, 1 µM, 10 µM 27-OHC in the initial experiments. In the second set of experiments cultured human dopaminergic neurons were treated for 24 hours with ethanol and DMSO vehicle (control), 10 µM 27-OHC, 10 µM GW3965, 10 µM ECHS, 10 µM 27-OHC + 10 µM ECHS. For experiments involving the proteasomal inhibitor MG132 cells were incubated with ethanol vehicle (control), 10µM 27-OHC, and 1µM MG132 for twenty-four hours. Treated cells were washed with phosphate-buffered saline (PBS), followed by protein extraction with RIPA buffer. Protein concentrations were determined with the BCA protein assay reagent by standard protocol. Proteins (10µg) were separated on sodium dodecyl sulfate-polyacrylamide gel electrophoresis gels, transferred to a polyvinylidene difluoride membrane (Bio-Rad, Hercules, CA, USA), and incubated overnight at 4ºC with the following antibodies: anti-α-synuclein rabbit

antibody (Cat. # 2642S, RRID: AB_10695412) (1:500; Cell Signaling Danvers, MA, USA), anti-ATP-binding cassette transporter (ABCA1) (Cat. # Mo13101, RRID: AB_2220136) (1:500; Neuromics Minneapolis, MN USA), anti-HSP70 antibody (Cat. # PA5-28003, RRID: AB_2545479) (1:1000; Thermo Fisher Scientific Waltham, MA USA), and anti-Dopamine Transporter (Cat. #MAB369, RRID: AB_2190413) (1:1000; EMD Millipore Temecula, CA USA). Antibodies have been extensively validated by the companies of origin. β-Actin was used as a gel loading control. The blots were developed with Clarity Western ECL Substrate (Biorad, Hercules, CA). Bands were visualized on a polyvinylidene difluoride membrane on an Aplegen Omega Lum G System (Pleasanton, CA, USA) and analyzed by ImageJ (NIH, USA). The results were quantified by densitometry and represented as total integrated densitometric values. Data are expressed as individual values with mean ± SEM and includes determinations made in two separate experiments containing (n=3) and technical replicates for all proteins except DAT which was one experiment (n=2).

Immunofluorescence.

Human primary Dopaminergic neuronal precursor cells were grown in PriGrow IV medium containing 5% fetal bovine serum, 10ng/mL Fibroblast Growth Factor 2, 10ng/mL Glial-Derived Neurotrophic Factor, and 1% Penicillin-Streptomycin. Cells were maintained at 37°C in a saturated humidity atmosphere containing 95% air and 5% CO_2. Cells were cultured and passaged for thirty days prior to differentiation. Coverslips were coated with Poly L-Lysine and the cells were plated at a density of 10^4 cells per cm^2 with differentiation media consisting

of PriGrow IV, 5% fetal bovine serum, 10ng/mL Fibroblast Growth Factor 2, 10ng/mL Epidermal Growth Factor, and 100µM dibutyryl cAMP for twenty-five days to allow for differentiation. Cells were either used immediately for Fig. 30 and 31 or were treated for 24 hours with 10 µM 27-OHC and 1 µM MG132 (Fig. 35). Cells were rinsed briefly with PBS, fixed in ice-cold acetone for 5 minutes, washed twice with PBS and incubated for 1 hour with PBS containing 10% normal goat serum before applying PBS containing 5% normal goat serum and the following antibodies for the various figures: anti-TH mouse antibody (Cat. # MAB7566) (8µg/mL R&D Systems, Minneapolis, MN, USA), anti-Neuron specific β-III Tubulin (Cat. # ab18207, RRID AB_444319) (Abcam, Cambridge, MA, USA), anti-α-synuclein rabbit antibody (Cat. # 2642S, RRID: AB_10695412) (1:500; Cell Signaling Danvers, MA, USA), anti-HSP70 antibody (Cat. # PA5-28003, RRID: AB_2545479) (1:1000; Thermo Fisher Scientific Waltham, MA USA), and anti-Dopamine Transporter (Cat. #MAB369, RRID: AB_2190413) (1:1000; EMD Millipore Temecula, CA USA) overnight at 4ºC. Cells were then washed three times with PBS (5 mins each) and reacted to AlexaFluor 594 goat-anti-rabbit (Cat. # A11037, RRID AB_2534095) (Life Technologies, Carlsbad, CA, USA) and AlexaFluor 488 goat-anti-mouse antibody (Cat. # A11001, RRID AB_2534069) (Life Technologies, Carlsbad, CA, USA) in PBS containing 5% normal goat serum for 1 hour at room temperature in the dark. Cells were washed three times with PBS for five minutes in the dark and mounted with Vectashield containing 4',6-diamidino-2-pheylindole (DAPI) (Cat. # H-1500, RRID AB-2336788) (Vector Labs, Burlingame, CA USA), and visualized with a Leica

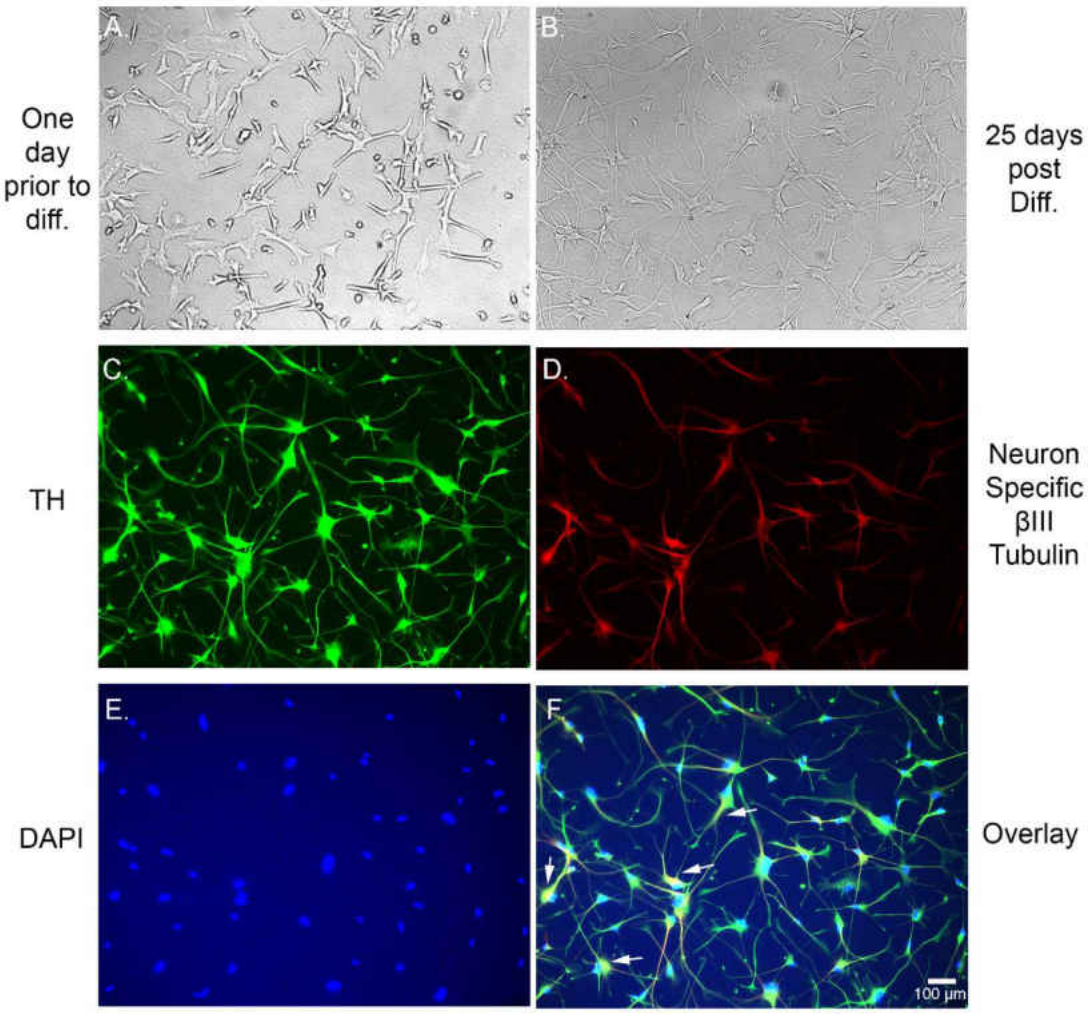


Figure 30. Human dopaminergic neurons express Tyrosine Hydroxylase. Bright field microscopy of human dopaminergic neuronal precursor cells one day prior to the start of differentiation (A) and 25-day post differentiation (B). Immunofluorescence staining showing showing that the neurons express tyrosine hydroxylase, the rate limiting enzyme in dopamine synthesis (C; green) suggesting that these neurons are predominantly of dopaminergic origin. (D) is immunostaining with the neuron specific β-III Tubulin (red), and (E) is nuclear counterstain with DAPI (blue). F. Overlay of tyrosine hydroxylase, neuron specific β-III Tubulin, and DAPI staining showing multiple neurons with nuclear and axonal/dendritic staining for tyrosine hydroxylase (arrows).Reprinted from (Schommer et al., 2018).

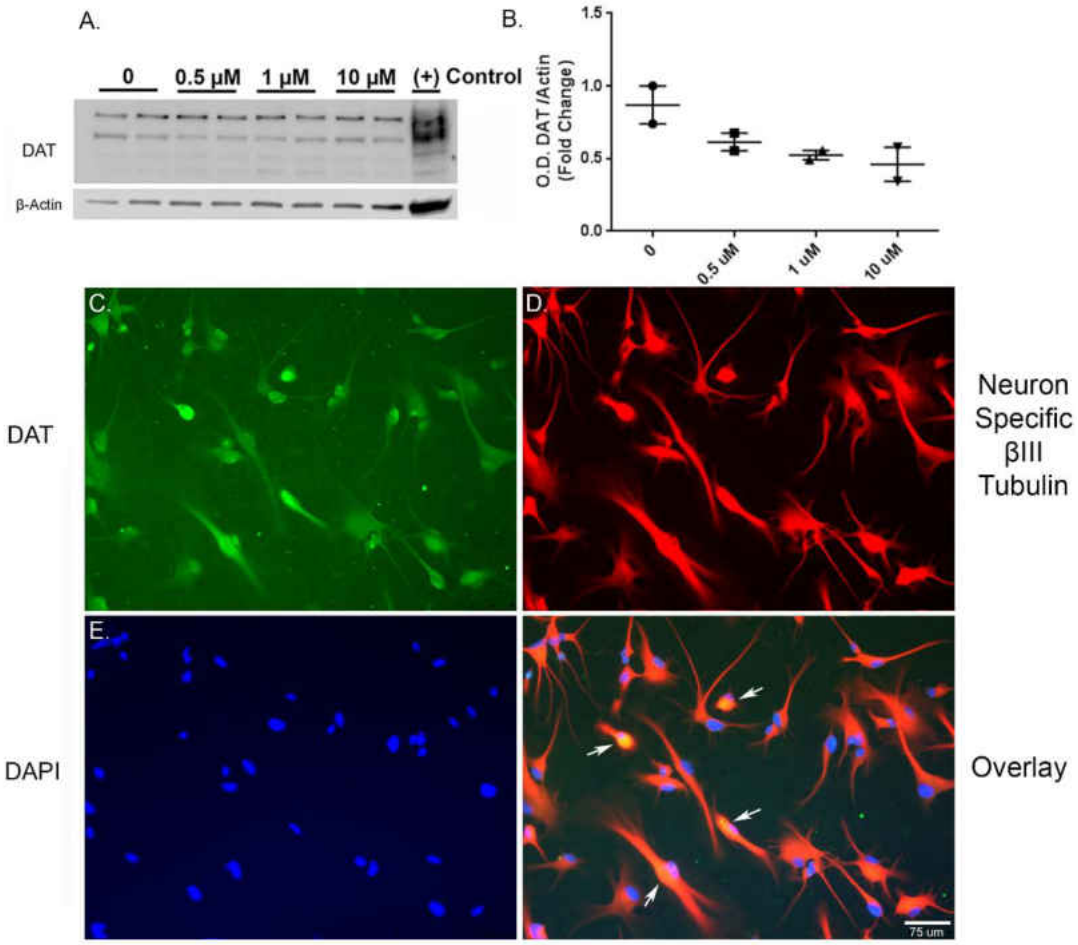


Figure 31. Human dopaminergic neurons express Dopamine Transporter. A representative western blot (A) and optical density (B) of dopamine transporter (DAT) show the presence of DAT in the lysates from vehicle and 27-OHC-treated neurons. Immunofluorescence imaging shows immunopositive staining for DAT in untreated neurons (C; green). Immunofluorescence for the neuron specific β-III Tubulin marker (D; red) and for nuclear counterstaining with DAPI (E; blue). F. Overlay of dopamine transporter, neuron specific β-III Tubulin, and DAPI showing both nuclear and cytoplasmic localization of DAT (arrows). (Schommer et al., 2018).

DMI6000B microscope with a Leica DFC350 FX camera (Buffalo Grove, IL USA). Imaging was performed with a 10x (Fig. 30) and 20x (Fig. 31, 35) objective.

Real time-rtPCR.

Total RNA was extracted with the QuickGene RNA cultured cell HC kit S (Autogen, Holliston, MA). 1µg of total RNA was reverse transcribed into cDNA with qScript cDNA SuperMix (Quanta Biosciences, Gaithersburg, MD). Real-time rtPCR was performed on the cDNA with taqman probes for the SNCA (Hs01103383_m1) gene (Applied Biosystems, Foster City, CA) and normalized to 18S rRNA. The data were quantified and expressed as fold-change compared to the control by using the $\Delta\Delta C_T$ method. Data are expressed as individual values with mean ± SEM and includes determinations made with (n=3) and three technical replicates.

Proteasome-Glo™ caspase-like, chymotrypsin-like, and trypsin-like cell-based assays.

Human primary dopaminergic neuronal precursor cells were grown in PriGrow IV medium containing 5% fetal bovine serum, 10ng/mL Fibroblast Growth Factor 2, 10ng/mL Glial-Derived Neurotrophic Factor, and 1% Penicillin-Streptomycin. Cells were maintained at 37°C in a saturated humidity atmosphere containing 95% air and 5% CO_2. Cells were cultured and passaged for thirty days prior to differentiation. Plates were coated with Poly L-Lysine and the cells were plated at a density of 10^4 cells per cm^2 with differentiation media consisting of PriGrow IV, 5% fetal bovine serum, 10ng/mL Fibroblast Growth Factor 2, 10ng/mL Epidermal Growth Factor, and 100µM dibutyryl cAMP for twenty-five days to allow for differentiation. 96-well plates were then coated with Poly L-

Lysine and differentiated cells were plated at a density of 10^3 cells per well. Cells were treated in triplicate for 24 hours with ethanol vehicle (control), 10 µM 27-OHC, and 1 µM MG132. Respective Proteasome-Glo™ substrates were added to cells for the three different types of proteasomal activity. The plates were placed on a plate shaker for 2 minutes at 700 rpm and incubated at room temperature for 15 minutes. The luminescence was measured using a luminometer and is expressed as Relative Luminescence Units (RLU) minus no cell media and reagent only blank wells. Data are expressed as individual values with mean ± SEM and includes determinations made in (n=3).

Statistical analysis.

One-way analysis of variance (one-way ANOVA) was used to assess the significance of differences among the samples including more than two groups assuming the data was of parametric nature followed by Tukey's post hoc test. Unpaired student's t-test was used to assess the significance of difference among the samples for the HSP70 western blots. Statistical analysis was performed with GraphPad Prism software 6.07. Quantitative data for western blotting analysis are presented as individual values with mean ± SEM with unit value assigned to control and the extent of differences among the samples being expressed relative to the unit value of control. Quantitative data for Real Time-rtPCR analysis are presented as individual values with mean ± SEM and expressed as fold-change from control.

Results

Human dopaminergic neurons express tyrosine hydroxylase and dopamine transporter.

Human primary dopaminergic neuronal precursor cells were cultured and differentiated into human dopaminergic neurons according to the protocol provided by the vender. We acquired light microscopy images of pre (Fig. 30A) and post-differentiation (Fig. 30B). Twenty-five-day post differentiation, immunofluorescence imaging shows that the neurons express TH, the rate limiting enzyme in dopamine synthesis (Fig. 30C-F). We also performed western blotting to determine whether the cells express the dopamine transporter protein. Our results show that the differentiated neurons express dopamine transporter (DAT) and the DAT bands are present in absence or presence of the various concentrations of 27-OHC we used (Fig. 31 A-B). There were no significant differences in the protein levels of DAT between the used concentrations of 27-OHC. Immunofluorescence assay corroborates the western blot results and shows that the untreated cells express DAT, (Fig. 31C-F).

27-OHC increases protein but not mRNA levels of α-synuclein.

We determined the effects of increasing concentration of 27-OHC on the viability of dopaminergic neurons and found that there was no significant cell death with any of the chosen treatment concentrations (Fig. 32A). We then investigated the effects of 27-OHC on α-syn protein levels in human dopaminergic neurons. We found that 27-OHC significantly increases the levels of α-syn protein with 0.5 μM 27-OHC ($p<0.01$), 1 μM 27-OHC ($p<0.001$), and 10 μM 27-OHC ($p<0.001$) (Fig. 32B-C). Real-time RT-PCR analysis demonstrates that 27-OHC does not affect the α-syn mRNA levels (Fig. 32D). This data suggests the mechanism by which 27-OHC increases α-syn protein levels in human dopaminergic neurons is through post-translational modifications or processing of the α-syn protein because 27-OHC does not appear to be modulating α-syn at a transcriptional level.

The LXR agonist, GW3965, and the LXR antagonist, ECHS, do not affect α-synuclein mRNA or protein levels.

We determined the extent to which 27-OHC increases α-syn through its cognate receptors LXRs as we have previously shown in human neuroblastoma SHSY5Y cells (Marwarha, Rhen, et al., 2011b). We utilized the LXR agonist GW3965 (Collins et al., 2002; Joseph et al., 2002; Naik et al., 2005) and the LXR antagonist ECHS (Marwarha, Rhen, et al., 2011b; C. Song, Hiipakka, & Liao, 2001) in these experiments. Fig. 33A shows that 10 μM 27-OHC, 10 μM GW3965, 10 μM ECHS, and 10 μM 27-OHC + 10 μM ECHS did not kill the dopaminergic neurons relative to control as determined with the LDH assay. In order to test whether 27-OHC is activating LXRs in normal human dopaminergic

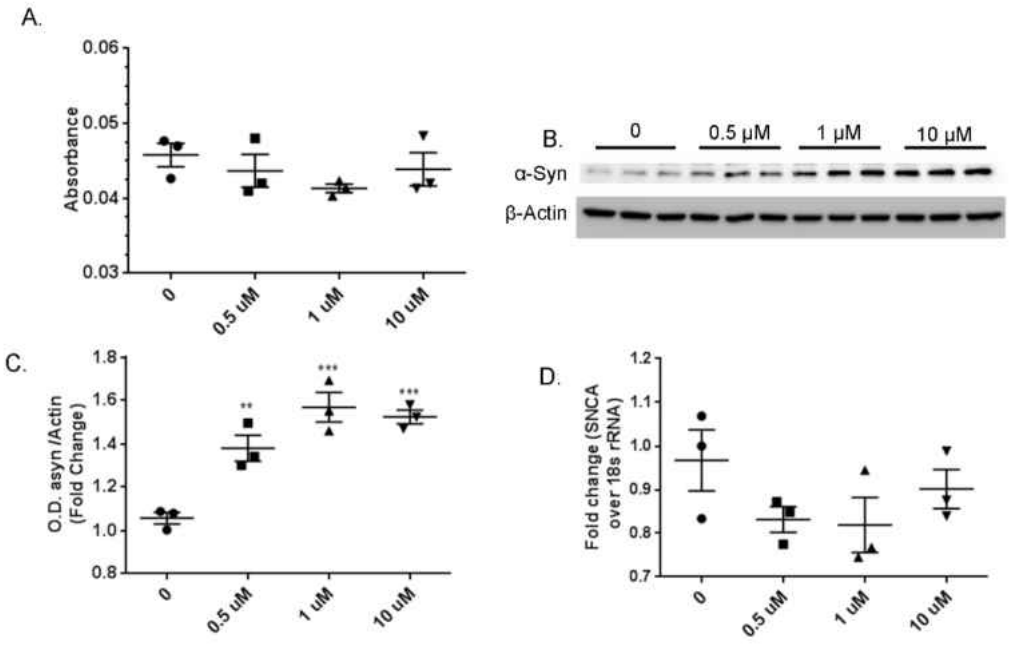
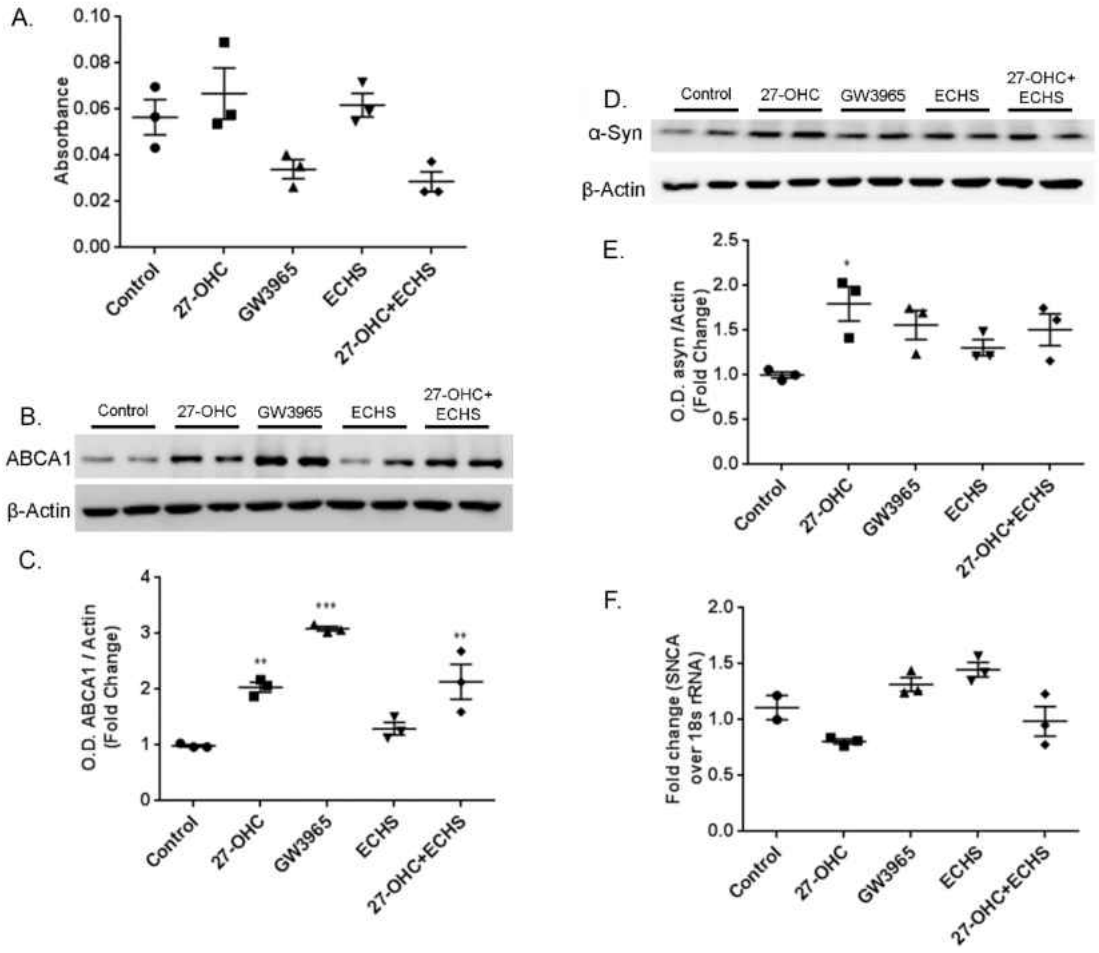


Figure 32. 27-OHC does not kill cells and increases α-synuclein protein levels while mRNA levels remain unchanged. A. Lactate Dehydrogenase assay shows that varying concentrations of 27-OHC do not elicit cell death relative to control untreated cells. Representative western blot (B) and optical density (C) of α-syn. Western blots are expressed as fold change over β-Actin. The amount of α-syn significantly increases with increased 27-OHC concentrations. (D) Real-time rt-PCR shows that 27-OHC does not increase *SNCA* mRNA. Data are expressed as individual values with mean ± SEM and includes determinations made in one experiment with (n=3) and three technical replicates for LDH assay, two separate experiments with (n=3) and three technical replicates for western blots, and one experiment with (n=3) and three technical replicates for Real Time- rtPCR. $^{**}p < 0.01$, $^{***}p < 0.001$ versus control. Reprinted from (Schommer et al., 2018).

Figure 33. The LXR agonist, GW3965, and the LXR antagonist, ECHS, do not affect α-synuclein protein or mRNA levels. (A) Lactate Dehydrogenase assay shows that 27-OHC, GW3965, ECHS, and 27-OHC + ECHS do not elicit significant cell death relative to control untreated cells. Representative western blot (B) and optical density (C) of ABCA1 expressed as fold change over β-Actin. 27-OHC and GW3965 both significantly increase the amount of ABCA1 protein levels. ECHS does not significantly alter the protein levels of ABCA1 while 27-OHC + ECHS significantly increases the amount of ABCA1. Representative western blot (D) and optical density (E) of α-syn. Western blots expressed as fold change over β-Actin. 27-OHC significantly increased the amount of α-syn protein levels while the LXR agonist GW3965 and LXR antagonist ECHS had no effects on α-syn protein levels. F. Real-time rt-PCR shows that 27-OHC, GW3965, ECHS, and 27-OHC+ECHS do not significantly affect *SNCA* mRNA levels. Data are expressed as individual values with mean ± SEM and includes determinations made in one experiment with (n=3) and three technical replicates for LDH assay, two separate experiments with (n=3) and technical replicates for western blots, and one experiment with (n=3) and three technical replicates for PCR. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ versus control. Reprinted from (Schommer et al., 2018).


A.
Absorbance
0.10
0.08
0.06
0.04
0.02
0.00
Control
27-OHC
GW3965
ECHS
27-OHC+ECHS
B.
Control
27-OHC
GW3965
ECHS
27-OHC+ ECHS
ABCA1
β-Actin
C.
O.D. ABCA1 / Actin (Fold Change)
4
3
2
1
0
**
***
**
Control
27-OHC
GW3965
ECHS
27-OHC+ECHS
D.
Control
27-OHC
GW3965
ECHS
27-OHC+ ECHS
α-Syn
β-Actin
E.
O.D. asyn /Actin (Fold Change)
2.5
2.0
1.5
1.0
0.5
0.0
*
Control
27-OHC
GW3965
ECHS
27-OHC+ECHS
F.
Fold change (SNCA over 18s rRNA)
2.0
1.5
1.0
0.5
0.0
Control
27-OHC
GW3965
ECHS
27-OHC+ECHS

neurons we performed western blots on ABCA1, a downstream protein of LXR activation. 27-OHC ($p<0.01$) and GW3965 ($p<0.01$) significantly increased the amount of ABCA1 protein while ECHS kept the levels near control. ECHS in combination with 27-OHC was not able to rescue ABCA1 levels back to baseline as this treatment also exhibited a significant increase in ABCA1 ($p<0.01$) (Fig. 33B-C). These blots strongly suggest that 27-OHC is able to activate LXRs in normal human dopaminergic neurons. When probing for α-syn in western blotting we observed that only 27-OHC significantly increased α-syn protein levels while GW3965, ECHS, and 27-OHC + ECHS had no significant effect on α-syn protein content (Fig. 33D-E). To test the hypothesis that the effects of 27-OHC on α-syn are transcriptional through LXRs, we performed a real-time RT-PCR analysis in the presence of 27-OHC, GW3965, ECHS, and 27-OHC + ECHS and discovered that no treatments significantly affected α-syn mRNA content (Fig. 33F). This data strongly suggests that 27-OHC increases α-syn protein levels through a mechanism independent of transcriptional control by LXRs.

27-OHC impairs proteasomal function and decreases HSP70 protein levels leading to increased α-syn protein levels.

As the LXR transcriptional activity appears to not be involved in the 27-OHC-induced increase in α-syn protein levels we determined the potential role of 27-OHC in inhibiting the degradation of α-syn protein using ExPASy PeptideCutter, a tool that predicts potential cleavage sites cleaved by proteases in a given protein sequence. Our data shows that both 27-OHC and the proteasomal inhibitor MG132 reduce Caspase-like activity (Fig. 34A). The

chymotrypsin-like and trypsin-like, the two proteasomal modes of α-syn degradation, are significantly inhibited by 27-OHC (Fig. 34B, C) as well as MG132. Our data demonstrates that MG132 and 27-OHC treatments both significantly increase α-syn protein levels versus vehicle-treated cells as shown with western blotting (Fig. 34D-E) and immunofluorescence imaging (Fig. 35A-L). This data strongly suggests that the 27-OHC-induced proteasomal inhibition plays a key role in the accumulation of α-syn protein. Our data also shows that 27-OHC significantly decreases while MG132 significantly increases HSP70 protein levels versus vehicle-treated cells as determined with western blotting (Fig. 34 F,G). A significant decrease in HSP70 protein content could lead to abnormal cellular proteostasis as HSP70 is involved in protein folding and numerous degradation pathways, including the UPS, depending on which co-chaperones are involved. The significant decrease in HSP70 protein level caused by 27-OHC could possibly be the cause of proteasomal inhibition or another compromising event to cellular protein maintenance machinery involved in folding and degradation of proteins which is yet to be determined.

Discussion

Abnormal accumulation of α-syn protein is a characteristic of PD and other disorders collectively referred to as synucleinopathies. The causes of the accumulation of α-syn remain unknown, but genetic predisposition together with environmental factors are likely to contribute to the pathogenesis of synucleinopathies. 27-OHC is an active product of cholesterol metabolism made in the mitochondria of most cells by the enzyme CYP27A1 and serves many

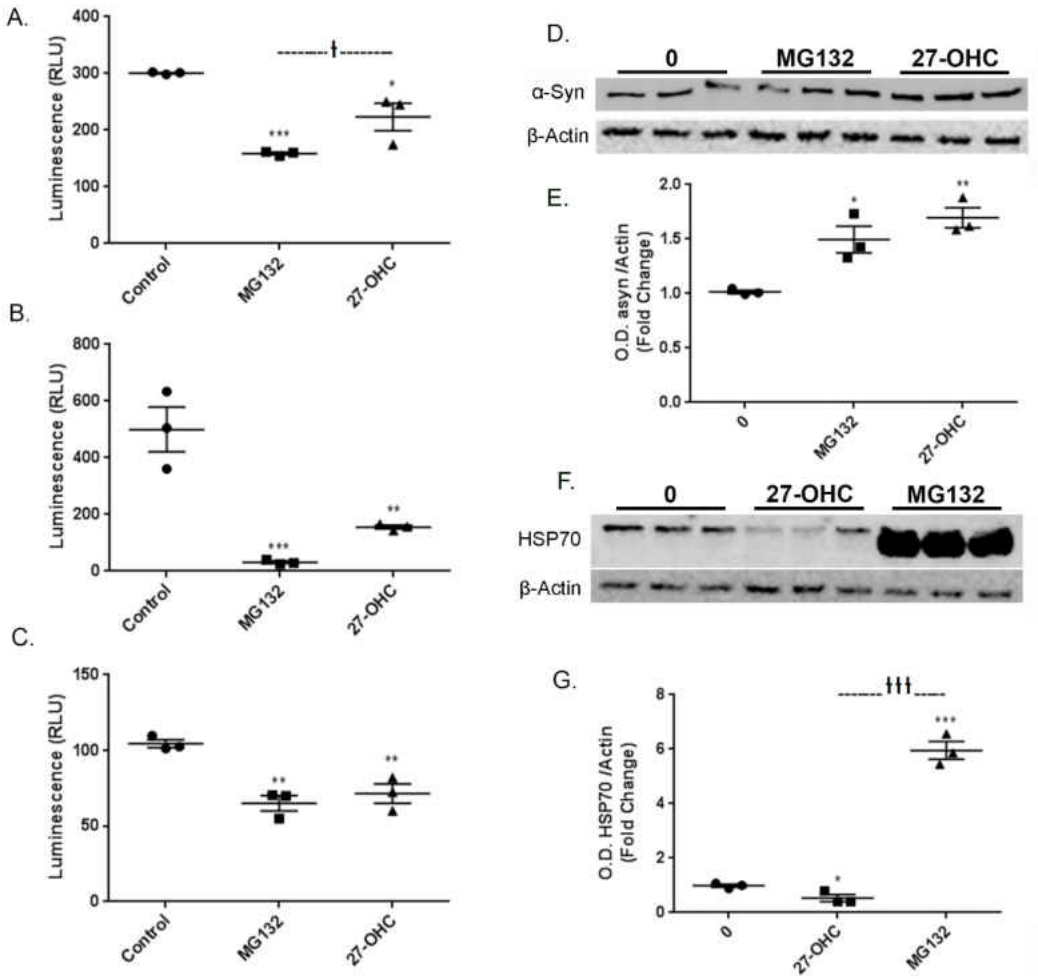


Figure 34. 27-OHC inhibits proteasomal function and reduces HSP70 levels. 27-OHC and the proteasomal inhibitor MG132 significantly decrease Caspase-Like Proteasomal Activity (A), Chymotrypsin-Like Proteasomal Activity (B), and Trypsin-Like Proteasomal Activity (C). Representative western blot (D) and optical density (E) of α-syn. Western blots are expressed as fold change over β-Actin. 27-OHC and MG132 significantly increase the amount of α-syn protein. Representative western blot (F) and optical density (G) of HSP70 showing that while 27-OHC reduces HSP70 levels, treatment with MG132 dramatically increases HSP70 protein levels. Data are expressed as individual values with mean ± SEM and includes determinations made in one experiment with (n=3) for the proteasomal assays and two separate experiments including (n=3) and three technical replicates for the western blots. *$p < 0.05$, **$p < 0.01$, ***$p<0.001$ versus control. †$p < 0.05$, †††$p < 0.001$ MG132 versus 27-OHC.Reprinted from (Schommer et al., 2018).

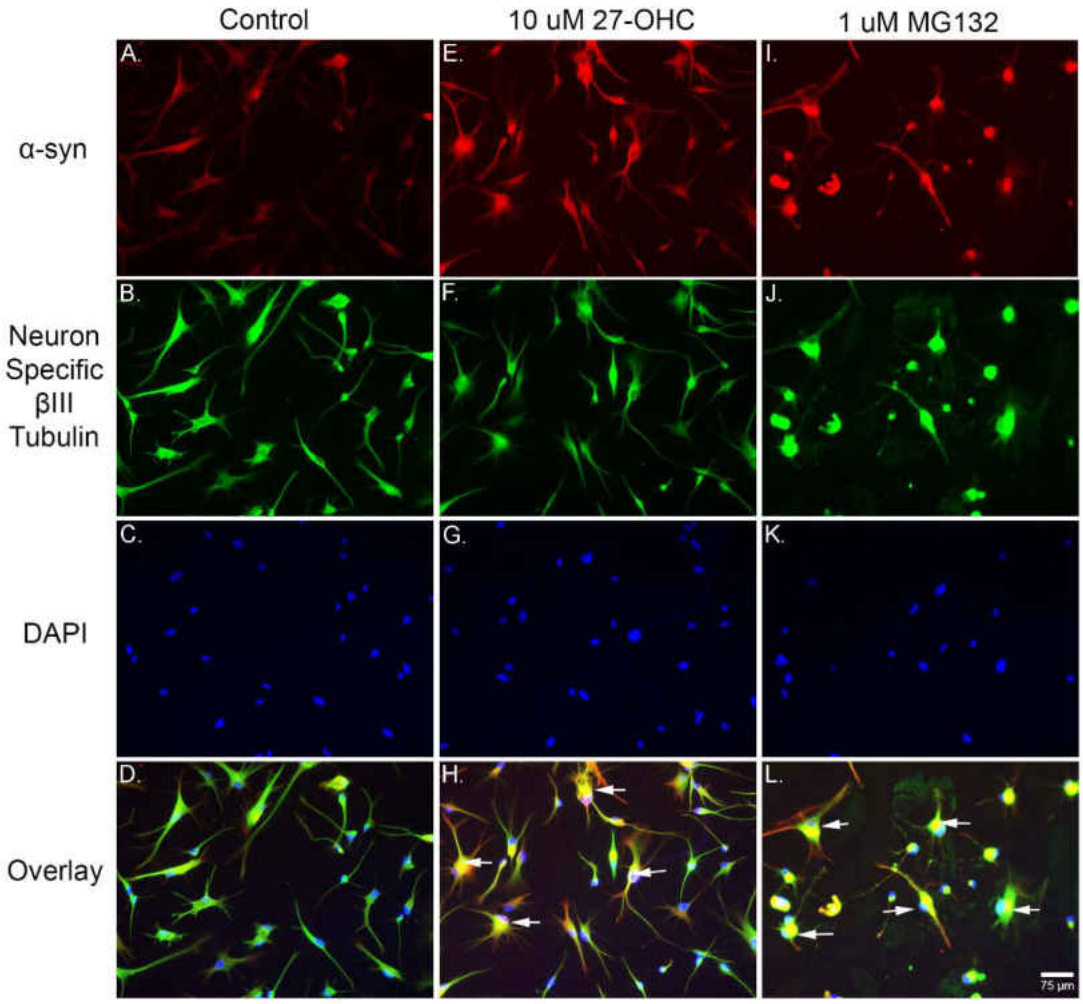


Figure 35. Both 27-OHC and the proteasomal inhibitor MG-132 increase α-syn protein levels. Immunofluorescene imaging shows that both 27-OHC (E) and MG132 (I) increase the immunostaining of α-syn compared to control untreated cells (A). Staining with the Neuron Specific βIII-Tubulin marker in control (B), 27-OHC-treated (F) and MG132-treated (J) neurons. Staining with the nuclear counterstain DAPI in control (C), 27-OHC-treated (G) and MG132-treated (K) neurons. The overlay shows multiple neurons exhibiting nuclear α-syn staining (arrows) in 27-OHC (H) and MG132 (L) treated neurons compared to untreated neurons (D). Reprinted from (Schommer et al., 2018).

biological roles. This oxysterol has been shown to promote atherosclerosis via activation of proinflammatory processes (Umetani et al., 2014), promotes breast and prostate cancers (Marwarha, Raza, et al., 2017) and functions as a ligand of the LXRs (Gabbi et al., 2014). LXRs, by means of gene transcription, regulate several metabolic pathways including lipid metabolism, glucose homeostasis, and inflammation (Gabbi et al., 2014). We (Marwarha, Rhen, et al., 2011b; Rantham Prabhakara et al., 2008) and other laboratories (Cheng et al., 2008a) have previously shown that the oxysterol 27-OHC evokes an increase in α-syn expression by mechanisms involving LXR activation in human neuroblastoma SHSY5Y cells. In this study, we determined the extent to which 27-OHC can regulate α-syn expression levels in human dopaminergic neurons, an *in vitro* model system that recapitulates synucleinopathies of PD type. We found that 27-OHC increases α-syn protein levels, activates LXR as shown by an increase in ABCA1, but fails to elicit a change in α-syn mRNA. GW3965, an LXR agonist and ECHS, an LXR antagonist also failed to elicit any change in α-syn protein or mRNA content suggesting there is no link between LXR and α-syn level increase in the dopaminergic neuronal model. We took our investigation further to study proteasomal inhibition as a potential post-translational event that could contribute to the 27-OHC induced increase in α-syn protein levels. Proteasomes are large intracellular protein complexes whose main function is to degrade short-lived, damaged, and misfolded proteins by proteolysis. Proteasomes help control for the amount of proteins necessary for normal cellular functioning. In higher organisms, proteasomes are located both in the cytoplasm and nucleus. The

most common form is the 26S proteasome, which contains one 20S core catalytic particle and normally one 19S regulatory particle at each side of the 20S core particle. The 20S core, which is concealed inside the 19S particles, is the active site of the proteasome which is responsible for its caspase-like, chymotrypsin-like, and trypsin-like activities. Initially, proteins targeted for degradation are tagged with several molecules of ubiquitin. Ubiquitin is covalently attached to target proteins by three sequential enzymatic steps: ubiquitin activation by E1 enzymes, ubiquitin conjugation by E2 enzymes, and ubiquitin ligation to target proteins by E3 enzymes. Ubiquitin is normally conjugated via its carboxy-terminal glycine to an internal lysine residue (Pickart, 2001). Following many rounds of ubiquitinylation a polyubiquitin chain is formed. This chain can function as a signal for degradation by the proteasome. The proteasome unfolds substrates and threads the polypeptide chains through the inner channel, where they are cleaved into short peptides (Bhattacharyya, Yu, Mim, & Matouschek, 2014). After release from the proteasome, peptides are quickly processed into amino acids and recycled (Reits et al., 2003). One approach to determine the functionality of the UPS is to assess the individual enzymatic activities involved in ubiquitin-dependent proteasomal degradation (Dantuma & Bott, 2014; Lindsten & Dantuma, 2003). Examining proteasomal function is the final stop of all ubiquitinylated proteins to be degraded and creates a bottleneck in the UPS pathway (Dantuma & Bott, 2014). Therefore, it isn't surprising that most studies focusing on the functionality of the UPS examine proteasomal function and not the individual enzymatic reactions leading up to it. However, this correlation of

proteasomal activity and overall UPS impairment is complicated because it is presently unknown to what extent altered proteasomal activity affects the overall changes in degradation of ubiquitinylated proteins (Dantuma & Bott, 2014). The UPS is a highly regulated system that controls the degradation of proteins involved in signal transduction, apoptosis, cell cycle progression and differentiation (Cook & Petrucelli, 2009). It is the chief pathway involved in the removal of damaged, misfolded and short-lived proteins within the cytoplasm and nucleus of cells. It functions by sequentially ubiquitinylating and degrading target proteins (Dantuma & Bott, 2014; Hershko & Ciechanover, 1998; Kleiger & Mayor, 2014). We show that 27-OHC increases α-syn protein levels through proteasomal inhibition in normal dopaminergic neurons that could potentially have implications in protein folding, UPS function and autophagy-lysosomal pathways of degradation. 27-OHC may potentially affect the UPS as this oxysterol has been shown to increase oxidative stress in astrocytes (Ma et al., 2015)**,** and prolonged oxidative stress has been shown to decrease proteasomal function (Shang & Taylor, 2011). HSP70 uses its ATP hydrolysis-powered conformational changes to assist protein folding, disaggregation and degradation, and is a key contributor in cellular proteostasis. The decrease in HSP70 protein levels we observed could have numerous effects on cellular proteostasis. HSP70 is capable of being involved in various degradation pathways by the presence of specific chaperones and co-chaperones that aid in guiding the targeted protein to a specific degradation pathway (see Fernández-Fernández *et al.* 2017 for a review). α-syn has been shown to bind HSP70 (Aprile

et al., 2017; Dedmon et al., 2005; Luk et al., 2008) and be degraded by the UPS and autophagy (Webb et al., 2003). The extent to which a decrease in HSP70 affects proteasomal and/or autophagy is yet to be determined in future studies.

In summary, we demonstrate that 27-OHC induces an increase in α-syn levels in human dopaminergic neurons. The mechanism involved in the α-syn increase does not appear to involve LXRs as we did not observe any significant changes in α-syn mRNA with 27-OHC or LXR agonist and antagonistic treatments. To the best of our knowledge, our results are the first to show that 27-OHC increases α-syn in dopaminergic neurons and that this increase may emanate from inhibition of the proteasomal function. Also, 27-OHC decreases levels of HSP70 protein which is involved in protein folding, and protein degradation through the UPS (Fernández-Fernández et al., 2017). The extent to which a decrease in HSP70 protein levels leads to decreased protein folding and degradation through specific pathways needs to be further elucidated. All-together, our results potentially suggest that restoring proteasomal function and HSP70 protein levels may attenuate the 27-OHC-induced increase in α-syn protein levels *in vitro* and reduce α-syn accumulation that can increase the risk for synucleinopathies.

CHAPTER IV

PALMITIC ACID-ENRICHED DIET INCREASES α-SYNUCLEIN AND TYROSINE HYDROXYLASE EXPRESSION LEVELS IN THE MOUSE BRAIN

Abstract

Background: Accumulation of the α-synuclein (α-syn) protein and depletion of dopaminergic neurons in the *substantia nigra* are hallmarks of Parkinson's disease (PD). Currently, α-syn is under scrutiny as a potential pathogenic factor that may contribute to dopaminergic neuronal death in PD. However, there is a significant gap in our knowledge on what causes α-syn to accumulate and dopaminergic neurons to die. It is now strongly suggested that the nature of our dietary intake influences both epigenetic changes and disease-related genes and may thus potentially increase or reduce our risk of developing PD.

***Objective*:** In this study, we determined the extent to which a three month diet enriched in the saturated free fatty acid palmitate (PA) influences levels of α-syn and tyrosine hydroxylase, the rate limiting enzyme in dopamine synthesis in mice brains.

***Methods*:** We fed the m-Thy1-αSyn (m-Thy1) mouse model for synucleinopathy and its matched control, the B6D2F1/J (B6D2) mouse a PA-enriched diet or a normal diet for three months. Levels of α-syn, tyrosine

hydroxylase, and the biogenic amines dopamine and dopamine metabolites, serotonin and noradrenaline were determined.

***Results*:** We found that the PA-enriched diet induces an increase in α-syn and TH protein and mRNA expression levels in m-Thy1 transgenic mice. We also show that, while it didn't affect levels of biogenic amine content in the B6D2 mice, the PA-enriched diet significantly reduces dopamine metabolites and increases the level of serotonin in m-Thy1 mice.

***Conclusions*:** Altogether, our results demonstrate that a diet rich in the saturated fatty acid palmitate can modulate levels of α-syn, TH, dopamine and serotonin which are proteins and neurochemicals that play key roles in increasing or reducing the risk for many neurodegenerative diseases including PD.

Introduction

Hallmarks of Parkinson's disease (PD) include the loss of dopaminergic neurons containing-tyrosine hydroxylase (TH) in the *substantia nigra pars compacta* and the abnormal accumulation of α-syn protein in Lewy bodies (Crowther, Daniel, & Goedert, 2000; Schapira, 1997; Spillantini et al., 1997). The role of α-syn in the pathogenesis of PD is not well understood but extensive experimental data points to a neurotoxic role of high levels of the protein in its soluble and aggregated forms (Adamczyk et al., 2006; Brown, 2010; Halbach et al., 2004; Snyder & Wolozin, 2004). The causes of PD are likely multi-factorial with genetic predisposition and environmental factors contributing to the pathogenesis of the disease. To date, studies focused on the contributions of dietary fat intake to the risk of PD have yielded inconsistent results (White et al.,

2009). Epidemiological studies of dietary fat intake and PD have found either a positive association (Anderson et al., 1999a; Johnson et al., 1999b; Giancarlo Logroscino et al., 1996a; Miyake, Sasaki, et al., 2010), no association (Hellenbrand et al., 1996a; L. C. Tan et al., 2007), or protective effects (Abbott, Webster Ross, et al., 2003b; Honglei Chen, Zhang, Hernán, Willett, et al., 2003b; L M L de Lau et al., 2005c; Kamel et al., 2014; Kyrozis et al., 2013; Karen M. Powers et al., 2009b). Studies focused on specific groups of fatty acids have provided little clarity. Indeed, while poly-unsaturated fatty acids (PUFAs) and mono-unsaturated fatty acids (MUFAs) have been shown to be protective in some studies (Abbott, Webster Ross, et al., 2003c; L.M.L. de Lau et al., 2005) and detrimental in another (Dong et al., 2014), saturated free fatty acids (sFFAs) have shown positive associations (Anderson et al., 1999b; Honglei Chen, Zhang, Hernán, Willett, et al., 2003c; Johnson et al., 1999c; Giancarlo Logroscino et al., 1996b) or no significant relationship with PD risk (Honglei Chen et al., 2002; Honglei Chen, Zhang, Hernán, Willett, et al., 2003c; Hellenbrand et al., 1996b; K M Powers et al., 2003). Additionally, *in vitro* studies have shown that while PUFAs increase α-syn oligomerization and insoluble aggregate formation, sFFAs did not (Assayag et al., 2007; Ronit Sharon et al., 2003). Many of these epidemiological studies utilized food frequency questionnaires without clarifying the specific role of each sFFAs. In studies carried out in mice, the n-3 poly unsaturated fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have been shown to provide neuroprotective effects in animal models of PD (M Bousquet et al., 2008; Mélanie Bousquet, Calon, & Cicchetti,

2011; Dyall, 2015; Seidl, Santiago, Bilyk, & Potashkin, 2014). Another study in which m-Thy1 mice were fed a diet enriched in DHA over a 10-month span showed improved survival but no major impact on the dopaminergic system, motor impairments, or brain α-syn levels (M Bousquet et al., 2008; Mélanie Bousquet et al., 2011; Dyall, 2015; Seidl et al., 2014). Several other animal studies utilized high fat diets that aren't isocaloric and contain high levels of cholesterol; however these studies didn't determine the contribution of specific fatty acids (M Bousquet et al., 2012; Choi, Jang, Park, & Kang, 2005a). Therefore, the role of dietary fat in PD risk requires a more precise examination of the contributions of individual fatty acids including saturated fats to elucidate the effects of each fat on PD risk.

In this study, we determined the specific effects of a diet rich in palmitic acid (PA), the most abundant SFFA in the body, on key proteins involved in PD risk. We fed m-Thy1 mice and their controls, the B6D2 mice, an isocaloric diet enriched in PA and examined the effects on the levels of two major hallmarks of PD, α-syn protein and TH, the rate limiting enzyme in dopamine synthesis in the *substantia nigra*. We chose the m-Thy1 mouse model that exhibits many similarities with PD (Rockenstein et al., 2002a) (see Table 5 for details). The m-Thy1 mouse model overexpresses full-length human wild-type α-synuclein under the murine Thy-1 promoter. They have been extensively characterized and exhibit 3-fold increase in α-syn protein at 5 months, and decreased TH at 8 months (Chesselet et al., 2012c; S M Fleming et al., 2006; Sheila M Fleming et al., 2004, 2008; Rabl et al., 2017; Rockenstein et al., 2002b).Our data shows that

Table 5. Classification of the m-Thy1 Mouse

Age	Experiment/ Behavioral Test Used	Pathology/Behavioral Deficits	Reference
1, 2, 3, 6 months	Wire hanging	Significant decrease in performance of mice	(Rabl et al., 2017)
1 month in striatum 6 months in SN	mRNA and protein levels of proinflammatory cytokines	Microglia are activated in mice. TNF alpha was elevated in striatum at 1 month and at 5-6 months in SN. Microglial activation persisted through 14 months in both areas of the brain.	(Pradhan & Andreasson, 2013)
1 month in striatum and 5-6 months in SN	IHC, rt PCR, flow cytometry for t cells, ELISAs	Increased numbers of activated microglia and increased tnf-alpha in striatum at 1 month. and in *substantia nigra* at 5-6 months. but not in cortex or cerebellum. TLR4, and TLR8 were increased at 5-6 months in SN. and TLR2 in SN at 14 months MHCII staining was not detected in the regions and ages examined at 14 months. peripheral CD4 and CD8 T-cells were increased in the blood only at 22 months of age suggesting later involvement of the adaptive immune response	(Watson et al., 2012)
1, 3, 10 months		Mice displayed significant decreases in the frequency of spontaneous excitatory postsynaptic currents (EPSCs). altered corticostriatal plasticity; abnormal corticostriatal transmission	(Wu, Joshi, Cepeda, Masliah, & Levine, 2010)
2, 4, 6, 8 months	Scale	At all ages mice were significantly less weight than controls	(Sheila M Fleming et al., 2004)
2, 4, 6, 8 months	Challenging beam (motor performance and coordination)	At all ages mice were significantly worse than controls and progressively got more dysfunctional at 6 and 8 months	(Sheila M Fleming et al., 2004)
Multiple ages see description	Spontaneous activity in a cylinder	Spontaneous rearing, forelimb and hindlimb steps and grooming were measured for 3 minutes. at 2 months mice reared significantly less. for forelimb stepping significantly less steps made by mice at 2 and 6 months. for hindlimb steps at all ages 2, 4, 6, 8 mice made less steps significantly. at 8 months of age mice groom significantly less than controls	(Sheila M Fleming et al., 2004)
2.5-3 months And 7-8 months	Defecation, gastric emptying	Reduction in fecal pellet output in novel environment, mice 8-10 months had increased asyn in myenteric plexuses	(L Wang et al., 2012)

2-3 months 6-7 months 9 months		Vocalization deficits at all ages and progressively got worse	(Grant et al., 2014)
2, 3, 6 months	Rotarod	Significantly decreased performance of mice	(Rabl et al., 2017)
2 months progressively worse to 14 montsh	Beam overlaid with grid, pole test, limb movement in cylinder	Deficits as early as 2 months and progressively gets worse to 8 months. very severe at 14 months	(Chesselet et al., 2012a)
3 to 4 months	Pole test	Deficits on pole test	(S M Fleming et al., 2006; Sheila M Fleming et al., 2004)
3 to 4 months	Inverted wire screen hanging test (grip strength)	Deficits on ability to stay on screen	(Rabl et al., 2017)
3-4 months and again at 9 months	Block test	Mice spent less time sniffing the new block	(Sheila M Fleming et al., 2008)
3-4 months And again at 9 months	Habituation/dishabituation	Mice spent less time sniffing the novel scent	(Sheila M Fleming et al., 2008)
3 months	Challenging Beam, spontaneous activity, pole test, gait	Mice injected with L-DOPA performed even more significantly poorly on challenging beam, spontaneous activity, pole test and gait than transgenic mice alone versus control.	(S M Fleming et al., 2006)
3-4 months	Pole test (motor coordination)	Mice took significantly more time to turn and descend the pole than controls	(Sheila M Fleming et al., 2004)
3-4 months	Inverted grid	T test showed that mice had a significant difference in step distance than controls. forelimb faults were not significantly different but increased in transgenic mice	(Sheila M Fleming et al., 2004)
3-4 months and progressively worsened	Wheel-running activity	Selective deficits in the expression of circadian rhythms of locomotor activity, including lower night-time activity and greater fragmentation in the wheel-running activity.	(Kudo, Loh, Truong, Wu, & Colwell, 2011)
3 months	IHC, Western Blotting	Inclusions in olfactory bulb, *substantia nigra*, locus coeruleus and other brain regions. Inclusions are bigger with aging and paraquat alpha-synuclein accumulated in synapses and neurons throughout the brain, including the thalamus, basal ganglia, *substantia nigra*, and brainstem. Human asyn does not accumulate in glial cells in this mouse model but does in the PDGF line M glial cells	(Rockenstein et al., 2002b)

4-6 months	y-maze, novel object recognition, object-place recognition, operant reversal learning	Exhibit deficits in cholinergic systems involved in cognition, and cognitive deficits in domains affected in early PD. Together with an increase in extracellular dopamine and a decrease in cortical acetylcholine (30%) at 4-6 months of age. these mice made fewer spontaneous alternations in the y-maze and showed deficits in tests of novel object recognition, object-place recognition, and operant reversal learning compared to controls	(Magen et al., 2012)
4 months	Grabbing bin cotton (fine motor skills)	Mice used significantly less cotton to build nests	(Sheila M Fleming et al., 2004)
4-5 months		Mice treated with paraquat did not have worsened behavioral deficits. there was also no added cell loss from the paraquat in these mice. paraquat did increase the amount of proteinase K resistant a syn aggregates in SN of mice.	(Fernagut et al., 2007)
4 to 5 months 7 to 8 months	Open field	Hyperactive in open field, displaying increase in move time, distance traveled, movement velocity	(Lam et al., 2011; L Wang et al., 2012)
4 months	y-maze, novel object recognition, object place recognition, operant reversal learning	Deficits as early as 4 months	(Chesselet et al., 2012
5 months	Western Blotting	3 fold increase in asyn in most brain regions	(Chesselet et al., 2012d)
5 months	IHC	Aggregates of asyn	(Chesselet et al., 2012d)
5 months	Western Blotting	Increased pS129 in SN	(Chesselet et al., 2012d)
5-6 months	Buried pellet test	Mice took significantly longer to find the buried pellet	(Sheila M Fleming et al., 2008)
5 months	IHC	Proteinase K resistant aggregates of asyn in locus coeruleus, which may be related to neuronal dysfunction and sleep disorders in these mice	(Chesselet et al., 2012a)
6 months	Adhesive removal	Mice took significantly longer to make contact and remove adhesive at 6 months. at 2, 4, 8 months there was no significant difference	(Sheila M Fleming et al., 2004)
6 months	Transcriptome analysis of striatum	Alterations in multiple biological processes in the striatum including synaptic plasticity, signaling, transcription, apoptosis, neurogenesis	(Cabeza-Arvelaiz et al., 2011)

6 months		Gut microbiota are required for motor deficits, microglia activation and alpha synuclein pathology	(Sampson et al., 2016)
6 and 9 months	IF	GFAP and IBA1-immunofluorescence in the cortex and hippocampus of 6 and 9 months old animals revealed no significant alterations in astrogliosis or activated microglia levels in mice compared to non-transgenic littermates	(Rabl et al., 2017)
6 months	Beam walk	Significant increase in number of slips	(Rabl et al., 2017)
6 months	Block test	Catalepsy in block test	(Chesselet et al., 2012a)
8 months	IHC	The time when loss of nigrostriatal dopamine neurons commences	(Sheila M Fleming et al., 2008)
8 months	Fear conditioning task	Less freezing in fear conditioning task at 8 months	(Rabl et al., 2017)
9 months	IHC	No obvious neuronal loss or changes in myelination	(Rabl et al., 2017)
9-12 months	Morphometry, hemodynamic assessment, dobutamine challenge, telemetric assessment	Impaired baroreflex	(Sheila M Fleming et al., 2013)
10 months	EEG, electromyograms	Sleep disturbances and lower frequencies in EEG (show increased Non REM sleep during quiescent phase, increased active wake during their active phase, and decreased REM over a 24hr period. also EEG power spectra shifts towards lower frequencies with a significant decrease in gamma power during wakefulness	(McDowell, Shin, Roos, & Chesselet, 2014)
11-12 months		Basal fecal output was significantly lower in mice	(Lixin Wang, Fleming, Chesselet, & Taché, 2008)
11-13 month old mice	TEM microscopy	Mice treated with MPTP had extensive mitochondrial alterations, increases in mitochondrial size, filamentous neuritic aggregations, axonal degeneration, and formation of electron dense perinuclear cytoplasmic inclusions in the SN that did not occur in the hippocampus or neocortex compared to controls	(D. D. Song, Shults, Sisk, Rockenstein, & Masliah, 2004)
14 months	Mass Spec.	Loss of striatal dopamine	(Chesselet et al., 2012b)

14 months	IHC	No decrease in TH positive neurons or fibers until 14 months of age	(Lam et al., 2011)
14 months	Open field	Decreased locomotion at 14 months	(Chesselet et al., 2012a)
22 months	IHC	At 22 months # of TH positive neurons was not significantly different	(Chesselet et al., 2012b)

the PA diet regulated the expression levels of α-syn, TH, dopamine and serotonin, which are all key proteins and neurochemicals involved in the pathogenesis of neurodegenerative diseases.

Methods

Feeding regimens.

Mice overexpressing full-length human wild-type α-syn under the murine Thy-1 promoter on the X chromosome were procured from the Chesselet laboratory at the University of California, Los Angeles (UCLA). The corresponding background control B6D2F1/J mice (Stock # 100006) were procured from The Jackson Laboratory (Bar Harbor, ME). We used male animals in these studies because female mice have the ability to inactivate the X chromosome, which may contain the inserted human α-syn gene under the m-Thy1 promoter. All animal procedures were carried out in accordance with the U.S. Public Health Service Policy on the Humane Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee at the University of North Dakota (Protocol 1506-2). All animal experiments complied with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1978). The mice were housed individually in ventilated cages at an ambient room temperature (23-25ºC) and ambient relative humidity ranging between 50-70%. The mice were

maintained on 12:12 hour light:dark cycle and allowed access to food and water *ad libitum*. Both genotypes of 3-month-old male mice, m-Thy1 and their backcrossed wild-type B6D2 mice (n=8-9 per group), were fed either a PA-enriched diet (custom-made, TD 1106162, Harlan Teklad, 2.2% w/w palmitic acid) or a control diet (custom made, TD 85172, Harlan Teklad, 0.8% w/w palmitic acid) for three months. The diets were isocaloric in relation to each other with the exception of palmitate and linoleate content and based on the NIH-07 open formula. The respective composition of the diets is shown in Table 6. Necropsy was performed at six (6) months of age. The genotype of all mice was verified with PCR analysis of tail snip DNA via general endpoint PCR. The HPRT gene was used as the internal control with a forward primer of GAAGAGCTACTGTAATGATCAGTCAACGG and a reverse primer of GAGAGGTCCTTTTCACCAGCAAGC. The forward primer used for the human SNCA gene was GCTACTGCTGTCACACCCGTC and the reverse primer was GATGATGGCATGCAGCACTGG.

Western blotting analysis.

Substantia nigra-enriched fractions were prepared as previously described (Marwarha, Dasari, Prasanthi, Schommer, & Ghribi, 2010a; Marwarha, Prasanthi, Schommer, Dasari, & Ghribi, 2011a) and as follows. *Substantia nigra*-enriched tissues (20 mg) were dounce homogenized in RIPA tissue lysis buffer (50 mM Tris, 150 mM Nacl, 0.1% SDS, 0.5% sodium deoxycholate, 1% Triton X, pH 7.4) supplemented with protease and phosphatase inhibitors. The samples were centrifuged at 5000 x g for 15 min and the supernatant harvested. Protein

Table 6. Composition of the control chow diet and palmitate-enriched diet

Components	**Control chow diet**	**Palmitic acid-enriched diet**
Proteins	23.6 % w/w	23.6 % w/w
Carbohydrates	65.8 % w/w	65.8 % w/w
Total Fat	5.6 % w/w	5.6 % w/w
Total Energy	4.08 Kcal/gram	4.08 Kcal/gram
Myristic acid (14:0)	0.1 % w/w	0.1 % w/w
Palmitic acid (16:0)	**0.8 % w/w**	**2.2 % w/w**
Stearic acid (18:0)	0.2 % w/w	0.2 % w/w
Palmitoleic acid (16:1)	Trace	Trace
Oleic acid (18:1)	1.2 % w/w	1.2 % w/w
Gadoleic acid (20:1)	Trace	Trace
Linoleic acid (18:2 n6)	**2.2 % w/w**	**0.8 % w/w**
Linolenic acid (18:3 n3)	0.2 % w/w	0.2 % w/w
Arachadonic acid (20:4 n6)	Trace	Trace
EPA (20:5 n3)	0.1 % w/w	0.1 % w/w
DHA (22:6 n3)	0.3 % w/w	0.3 % w/w

concentrations were determined by the Bradford protein assay method. Proteins (10 µg) were resolved on SDS-PAGE gels followed by transfer to a polyvinylidene difluoride (PVDF) membrane (BioRad, Hercules, CA) and incubation with the antibodies listed in Table 7. The origin, source, and dilutions of the respective antibodies used for this study are compiled in Table 7. β-actin was used as a gel loading control. The blots were developed with enhanced chemiluminescence (Clarity™ Western ECL blotting substrate, Bio-Rad, Hercules, CA) and imaged using an Aplegen Omega Lum G System (Pleasanton, CA, USA). The analysis was performed using ImageJ (NIH, USA) software. The results were quantified by densitometry and represented as total

Table 7. List of antibodies used in the study

Antibody	Dilution	Host	Manufacturer	Catalog #	RRID
α-syn	1:500	Rabbit	Cell Signaling	2642S	AB_10695412
TH	1:500	Rabbit	Cell Signaling	2792S	AB_10691683
$pS^{40}TH$	1:500	Rabbit	Sigma Aldrich	T9573	AB_261823

integrated densitometric values. Data were analyzed using the nonparametric, unpaired student's t-test with the Mann-Whitney post hoc test. Western blots are expressed as fold change over β-Actin (n=4 for B6D2 mice, n=3-4 for m-Thy1 mice) including 3 technical replicates.

Real time-rtPCR.

Total RNA was extracted from *substantia nigra*-enriched tissue with the QuickGene RNA cultured cell HC kit S (Autogen, Holliston, MA). Total RNA (0.5 µg) was reverse transcribed into cDNA with qScript cDNA SuperMix (Quanta Biosciences, Gaithersburg, MD). Real time-rtPCR was then performed on the cDNA with taqman probes for the SNCA (Mm01188700_m1) and TH (Mm00447557_m1) genes (Applied Biosystems, Foster City, CA) and normalized to 18S rRNA. Data were analyzed using the nonparametric, unpaired student's t-test with the Mann-Whitney post hoc test. Real time-rtPCR is expressed as fold change over 18s rRNA using the $\Delta\Delta C_T$ method (n=4-5) including 2 technical replicates.

Immunohistochemistry.

The right cerebral hemispheres of m-Thy1 and B6D2 mice were sectioned using a freezing microtome. As previously described (Manocha et al., 2017),

multiple paraformaldehyde-fixed and sucrose-equilibrated tissues were embedded in a 15% gelatin (in 0.1 M phosphate buffer, pH 7.4) matrix to form sample blocks for simultaneous processing. The blocks were immersed in a 4% paraformaldehyde solution for 3-4 days to harden the gelatin matrix, followed by a 30% sucrose solution that was replaced every 2 days until the blocks were utilized. The blocks were then flash frozen using dry-ice/isomethylpentane, and 40 µm serial sections were cut using a freezing microtome. Serial sections (960 µm apart) were then immunostained using an anti-TH antibody (1:500 dilution) and an anti α-syn antibody (1:500 dilution, see Table 7 for detailed descriptions of antibodies). The antigens were visualized using a Vector ABC kit and DAB as the chromogen (Vector Laboratories, Inc., Burlingame, CA) according to the manufacturer's protocols. The slides were dehydrated through a series of ethanol concentrations and Histo-Clear (National Diagnostics, Atlanta, GA) before being coverslipped using Permount. Photomicrographs were taken using an upright Leica DM1000 microscope and a Leica DF320 digital camera system (n=2).

Biogenic amine analysis using HPLC-ECD.

Substantia nigra-enriched tissues were shipped to the Neurochemistry Core at Vanderbilt University where biogenic amine analysis was performed. Briefly, tissue samples were homogenized using a tissue dismembrator in 100-750 ul of 0.1M TCA, which contains 10^{-2} M sodium acetate, 10^{-4} M EDTA, and 10.5 % methanol (pH 3.8). Ten microliters of homogenates were used for the protein assay. The samples were then spun in a microcentrifuge at 10,000 x g for 20 minutes, and the supernatant was removed for biogenic monoamine analysis.

Protein concentrations were determined using a BCA Protein Assay Kit (Thermo Scientific). Ten microliters of tissue homogenate was distributed into a 96-well plate, and 200 μl of mixed BCA reagent (25 ml of Protein Reagent A mixed with 500 μl of Protein Reagent B) was added. The plate was then incubated at room temperature for two hours for color development. A BSA standard curve was run at the same time. Absorbance was measured using a plate reader (POLARstar Omega) purchased from BMG LABTECH Company.

Biogenic amine concentrations were determined using an Antec Decade II (oxidation: 0.65) electrochemical detector operated at 33°C. Twenty microliter samples of the supernatant were injected using a Water 2707 autosampler onto a Phenomenex Kintex C18 HPLC column (100 x 4.60 mm, 2.6 um). Biogenic amines were eluted with a mobile phase consisting of 89.5% 0.1M TCA, 10^{-2} M sodium acetate, 10^{-4} M EDTA, and 10.5 % methanol (pH 3.8). Solvent was delivered at 0.6 ml/min using a Waters 515 HPLC pump. Using this HPLC solvent, the biogenic amines were eluted in the following order: Noradrenaline, Adrenaline, DOPAC, Dopamine, 5-HIAA, HVA, 5-HT, and 3-MT. HPLC control and data acquisition were managed using Empower software. Isoproterenol (5 ng/mL) was included in the homogenization buffer for use as a standard to quantify the biogenic amines. Data were analyzed using the nonparametric, unpaired student's t-test with the Mann-Whitney post hoc test and are expressed as ng/mg protein (n=3) including 3 technical replicates.

Statistical analysis.

Data were analyzed using the nonparametric, unpaired student's t-test with the Mann-Whitney post hoc test. Statistical analysis was performed with GraphPad Prism software 6.07. Western blots are expressed as fold change over β-Actin (n=4) including 3 technical replicates. Quantitative data from the western blotting analysis are presented as mean ± SEM with unit value assigned to control diet and the extent of differences among the samples being expressed relative to the unit value of control diet. Quantitative data for Real time-rtPCR analysis are presented as mean ± SEM and expressed as fold-change from control diet. Real-Time RT-PCR for SNCA and TH is expressed as fold change over 18s rRNA using the $\Delta\Delta C_T$ method (n=4-5) including 2 technical replicates.

Results

PA-enriched diet exhibit increased α-syn expression levels.

We examined the effects of a PA-enriched diet on α-syn protein levels and mRNA expression in the *substantia nigra*-enriched fractions from the B6D2 mice and found that three months of feeding with a PA-enriched diet significantly increased ($p<0.05$) α-syn protein levels compared to the control diet (Fig. 36A-B). To determine whether the PA-enriched diet affected α-syn gene expression via transcription, we performed Real time rt-PCR and found that the SNCA gene was significantly increased ($p<0.01$) in mice fed PA-enriched chow (Fig. 36C). We then performed immunohistochemistry and found that the PA-enriched diet that confirms increased positive staining of α-syn (Fig. 36E) than the control diet

(Fig. 36D). This data suggests that a PA-enriched diet is capable of regulating α-syn at a transcriptional level in B6D2 mice.

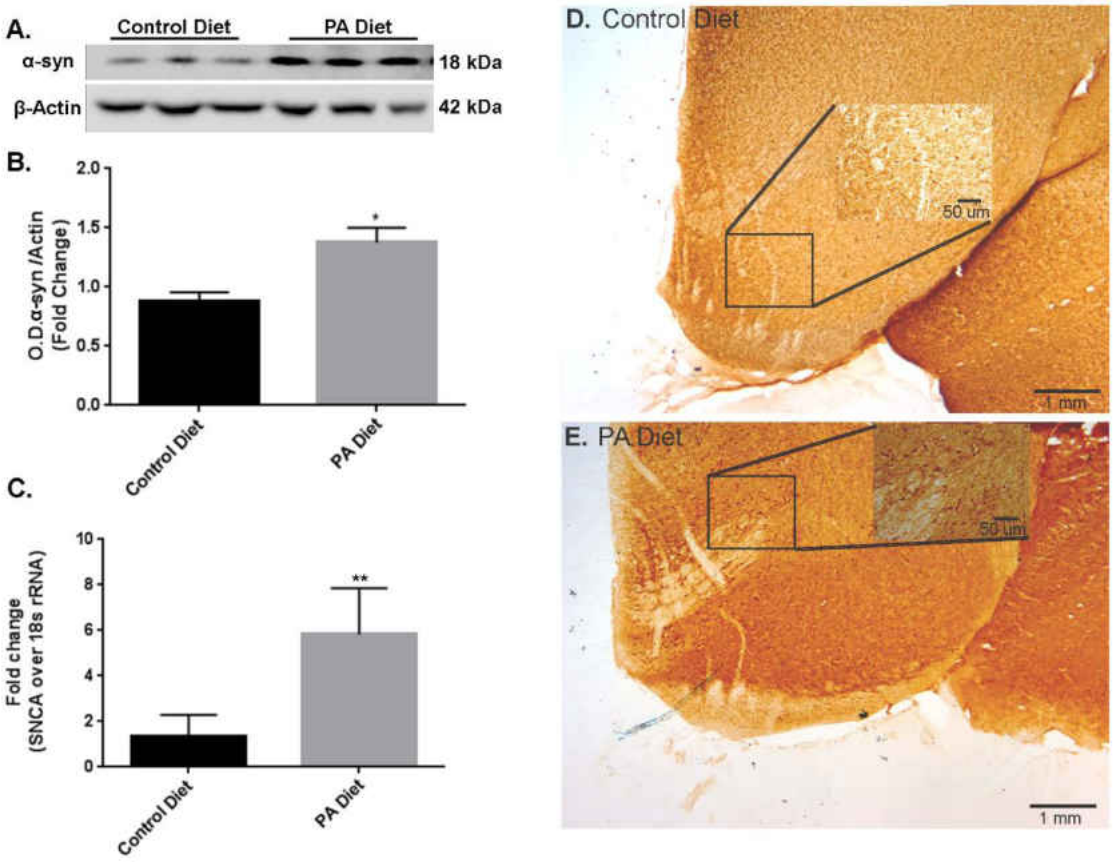


Figure 36. PA-enriched diet exhibit increased α-syn expression in B6D2 mice. Representative western blot (A) and optical density (B) of α-syn in the *substantia nigra*-enriched fraction of brains from B6D2 mice showing that the PA diet significantly increases α-syn protein levels. C. Real-time RT-PCR shows that the PA diet increases SNCA mRNA. Immunocytochemistry of the *substantia nigra* shows that the PA diet-fed mice exhibit increased α-syn immunoreactivity (E) compared to the control diet (D). *p<0.05, **p<0.01 versus control diet.

.

We also examined the effects of a PA-enriched diet on α-syn protein and mRNA expression levels in the *substantia nigra*-enriched fractions from m-Thy1 mice and found that three months of a PA-enriched diet feeding significantly increased ($p<0.05$) α-syn protein levels as demonstrated by western blotting (Fig. 37A-B). To determine whether the PA-enriched diet also affects α-syn gene expression, we performed Real time-rtPCR and found that the SNCA gene was significantly increased ($p<0.05$) in mice fed PA-enriched diet (Fig. 37C). We then performed immunohistochemistry and found that the PA-enriched diet resulted in increased α-syn immunostaining (Fig. 37E) compared to the control diet-fed mice (Fig. 37D). This data suggests that a PA-enriched diet is capable of regulating α-syn expression levels in m-Thy1 mice.

PA-enriched diet increases TH expression levels.

We examined the effects of a PA-enriched diet on TH protein and mRNA expression levels in the *substantia nigra*-enriched fractions of brains from B6D2 mice and found that three months of feeding with a PA-enriched diet significantly increased ($p<0.05$) TH protein levels compared to the control diet (Fig. 38A-B). In addition, levels of phospho S^{40}TH, the active form of TH (Dunkley, Bobrovskaya, Graham, Von Nagy-Felsobuki, & Dickson, 2004b) were also shown to be significantly increased ($p<0.05$) following PA-enriched diet feeding (Fig. 38C-D). To assess whether the PA-enriched diet affects TH gene expression via transcription, we performed Real time-rtPCR and found that the TH gene expression was significantly increased ($p<0.05$) in mice fed the PA-enriched diet compared to control diet (Fig. 38E). Immunohistochemistry shows that the PA-

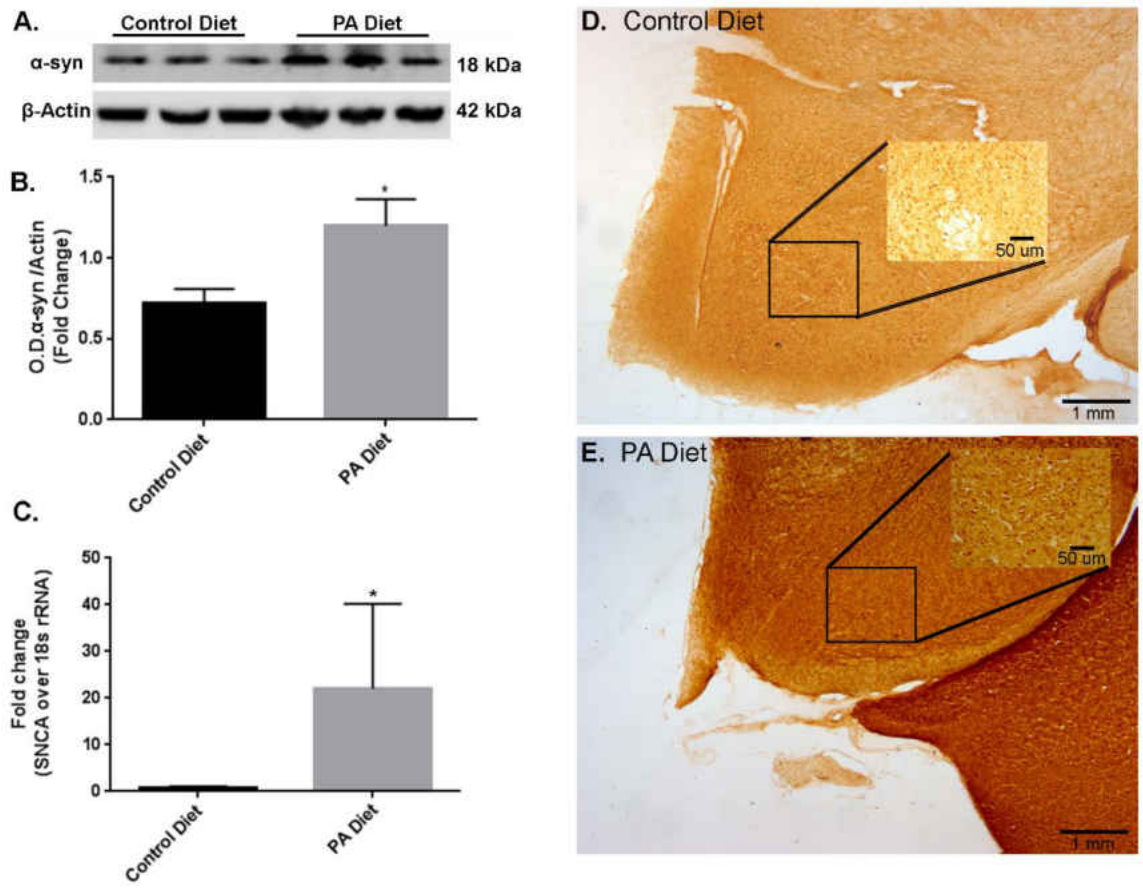
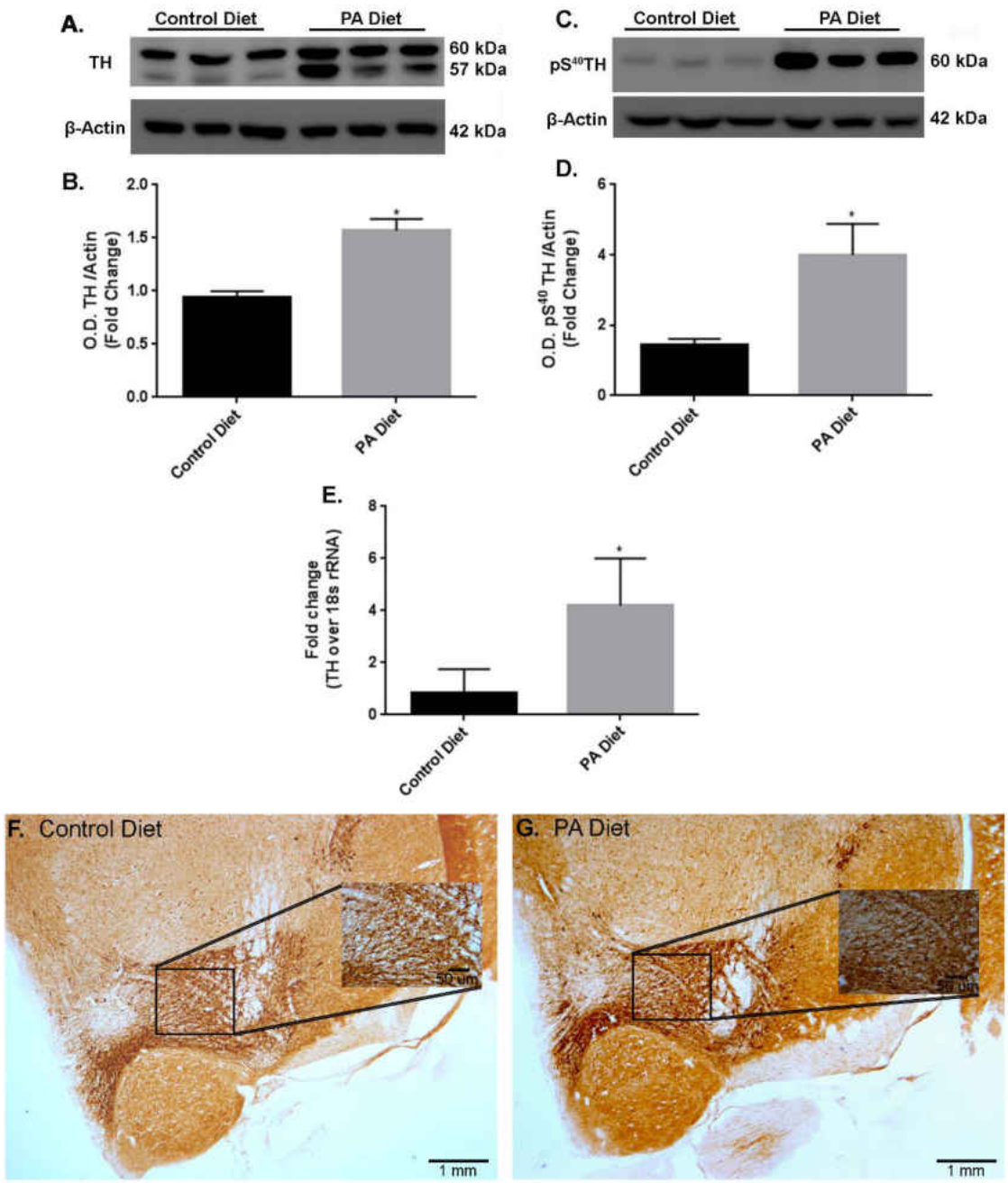


Figure 37. PA-enriched diet increases α-syn expression levels in m-Thy1-mice. Representative western blot (A) and optical density (B) of α-syn in the *substantia nigra*-enriched fraction of brains from m-Thy1-αsyn mice showing that the PA diet significantly increases α-syn. (C) Real time-rtPCR shows that the PA diet also increases SNCA mRNA. Immunocytochemistry of the *substantia nigra* shows that PA diet-fed mice (E) exhibit more immunoreactivity to α-syn antibody than control diet fed mice (D). *$p<0.05$ versus control diet.

Figure 38. B6D2 mice on a PA diet exhibit increased TH and pS40TH expression. Representative western blot (A,C) and optical density (B,D) showing the PA diet significantly increases levels of TH and $pS^{40}TH$ respectively in the *substantia nigra*-enriched fraction of brains from B6D2 mice . Real time-rtPCR shows that the PA diet significantly increases TH mRNA (E). Immunocytochemistry shows that PA diet-fed mice exhibit increased immunoreactivity to TH antibody (F) compared to control diet-fed (G). *$p<0.05$ versus control diet.


A.
Control Diet
PA Diet
TH
60 kDa
57 kDa
β-Actin
42 kDa
C.
Control Diet
PA Diet
pS40TH
60 kDa
β-Actin
42 kDa
B.
O.D. TH /Actin (Fold Change)
2.0
1.5
1.0
0.5
0.0
Control Diet
PA Diet
D.
O.D. pS40 TH /Actin (Fold Change)
6
4
2
0
Control Diet
PA Diet
E.
Fold change (TH over 18s rRNA)
8
6
4
2
0
Control Diet
PA Diet
F. Control Diet
50 um
1 mm
G. PA Diet
1 mm

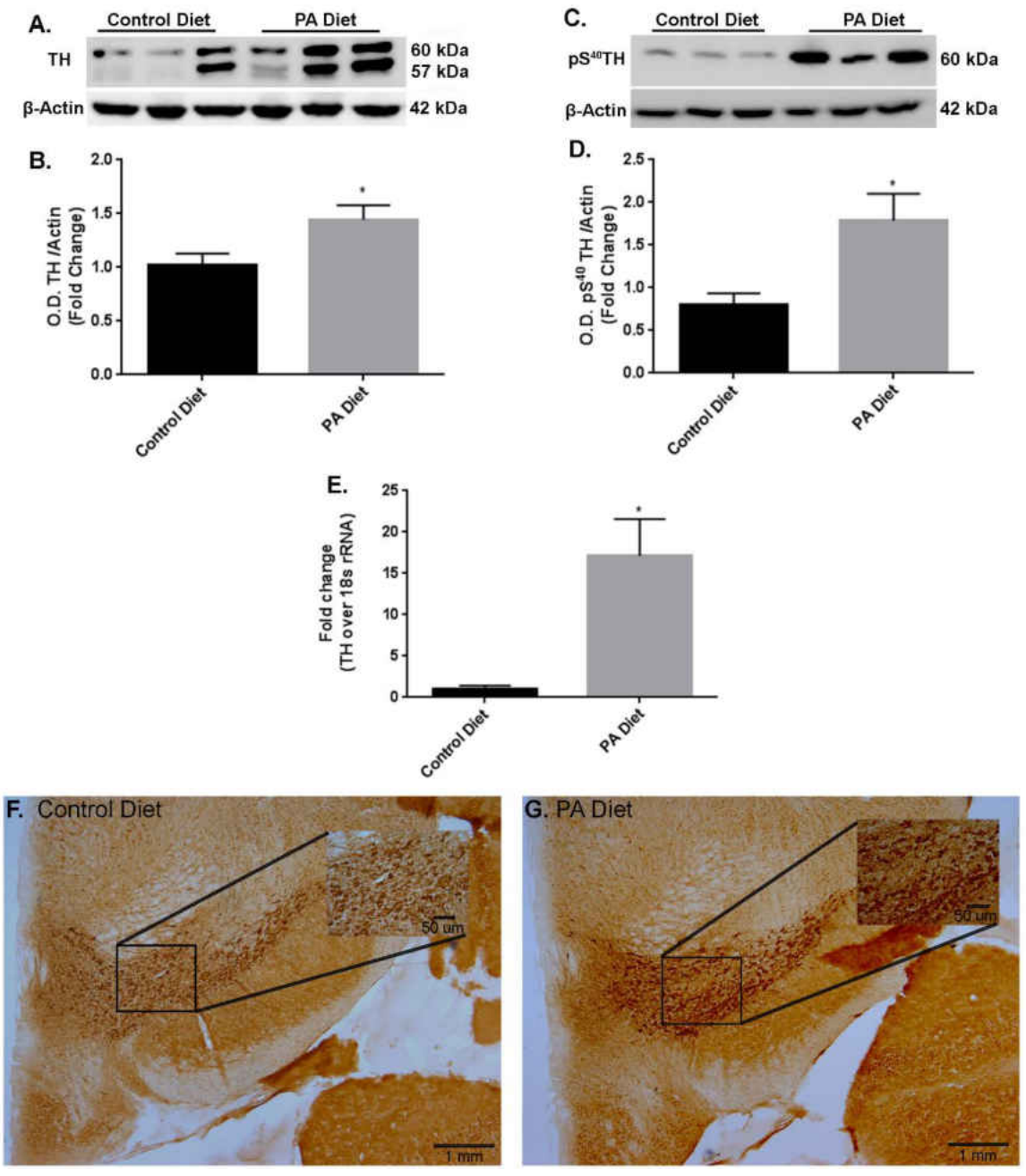
enriched diet increases TH staining (Fig. 38G) compared with the control diet-fed mice (Fig. 38F). This data suggests that in B6D2 mice, a PA-enriched diet is capable of regulating TH at a transcriptional and translational level.

In the *substantia nigra*-enriched fraction of m-Thy1 mice, the three months of feeding with a PA-enriched diet significantly increases ($p<0.05$) TH protein levels (Fig. 39A-B). In addition, levels of $pS^{40}TH$ were also shown to be significantly increased ($p<0.05$) with the PA-enriched diet (Fig. 39C-D). Real time-rtPCR shows that the TH gene was significantly increased ($p<0.05$) in mice fed the PA-enriched chow (Fig. 39E). We then performed immunohistochemistry in the *substantia nigra* and found that the PA-enriched diet resulted in increased TH staining (Fig. 39G) compared with the control diet-fed mice (Fig. 39F). This data suggests that in m-Thy1 mice a PA-enriched diet can regulate TH at a transcriptional and translational level. Not only did the PA diet increase TH but it also increased $pS^{40}TH$ in both B6D2 and m-Thy1 mouse models.

PA diet differently affects biogenic amines.

We assessed the levels of various biogenic amines in the *substantia nigra*-enriched fraction of B6D2 mice fed the control or the PA diets and observed no significant differences in dopamine (DA) content (Fig. 40A) or its metabolites DOPAC (Fig. 40B) and HVA (Fig. 40C) between the two feeding regimens. Also, no significant differences in noradrenaline (Fig. 40D), serotonin (Fig. 40E), and the serotonin metabolite 5-HIAA (Fig. 40F) were observed.

Figure 39. m-Thy1-αSyn mice on a PA diet exhibit increased TH and pS40TH expression. Representative western blot (A,C) and optical density (B,D) showing the PA diet significantly increases levels of TH and $pS^{40}TH$ respectively in the *substantia nigra*-enriched fraction of brains from m-Thy1-αSyn mice. Real time-rtPCR shows that the PA diet significantly increases TH mRNA (E). Immunocytochemistry shows that PA diet-fed mice exhibit increased immunoreactivity to TH antibody (G) compared to control diet (F). *$p<0.05$ versus control diet.


A.
Control Diet
PA Diet
TH
60 kDa
57 kDa
β-Actin
42 kDa
C.
Control Diet
PA Diet
pS40TH
60 kDa
β-Actin
42 kDa
B.
O.D. TH /Actin (Fold Change)
2.0
1.5
1.0
0.5
0.0
*
Control Diet
PA Diet
D.
O.D. pS40 TH /Actin (Fold Change)
2.5
2.0
1.5
1.0
0.5
0.0
*
Control Diet
PA Diet
E.
Fold change (TH over 18s rRNA)
25
20
15
10
5
0
*
Control Diet
PA Diet
F. Control Diet
50 um
1 mm
G. PA Diet
50 um
1 mm

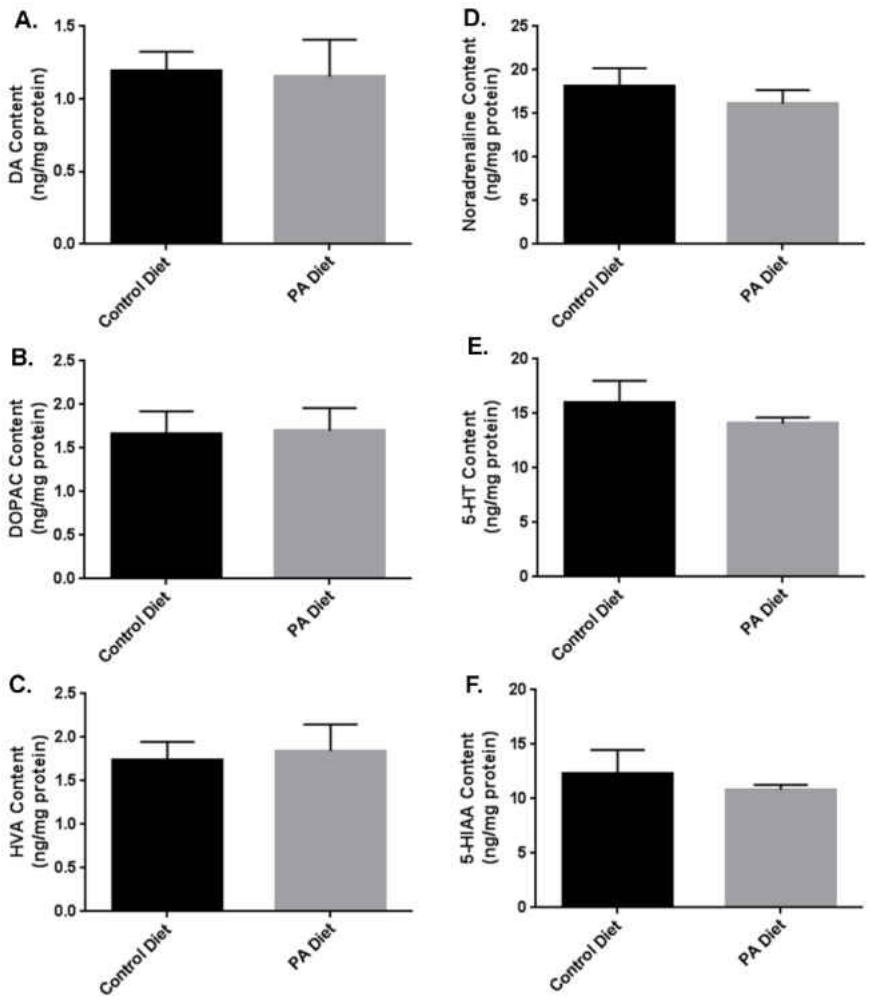


Figure 40. Biogenic amine analysis revealed no significant differences in biogenic amines between B6D2 mice on a control or PA diet. PA-enriched diet doesn't affect DA (A), DOPAC (B), HVA (C), Noradrenaline (D), Serotonin (5-HT) (E), or 5-HIAA (F) content compared to control diet. Data is expressed as ng/mg protein.

Although the PA-enriched diet increased the expression of α-syn and TH, this data suggests the diet does not alter normal biogenic amine content in B6D2 mice. In the *substantia nigra*-enriched fractions of m-Thy1 mice we observed reduced levels of DA (Fig. 41A) and DOPAC (Fig. 41B), and increased levels of serotonin (Fig. 41E) in mice fed a PA-enriched diet, while HVA (Fig. 41C), Noradrenaline (Fig. 41D), and 5-HIAA (Fig. 41F) levels were unchanged between PA-enriched and control diets.

Discussion

In this study, we determined the specific contribution of the fatty acid PA in regulating expression levels of α-syn and TH, two proteins that are tightly linked to PD, in the m-Thy1 mouse model of PD and its matched control the B6D2 mouse. We found that the PA-enriched diet increased α-syn protein levels and mRNA content in both strains of mice. We also found that in both strains of mice the PA-enriched diet increases the protein and mRNA levels of TH, the rate limiting enzyme in the synthesis of dopamine (Sjoerdsma, Engelman, Spector, & Udenfriend, 1965). Our results suggest that the PA diet may have both protective and destructive effects by increasing TH and α-syn expression levels respectively.

PA (16:0) is the most abundant sFFA acid in the body and the most abundant sFFA in certain foods including meats, cheeses, and dairy products. It is also synthesized *de novo* in the body and makes up 24% of total fatty acids in our blood and 28% of total fatty acids in our CSF (Guest et al., 2013). Human

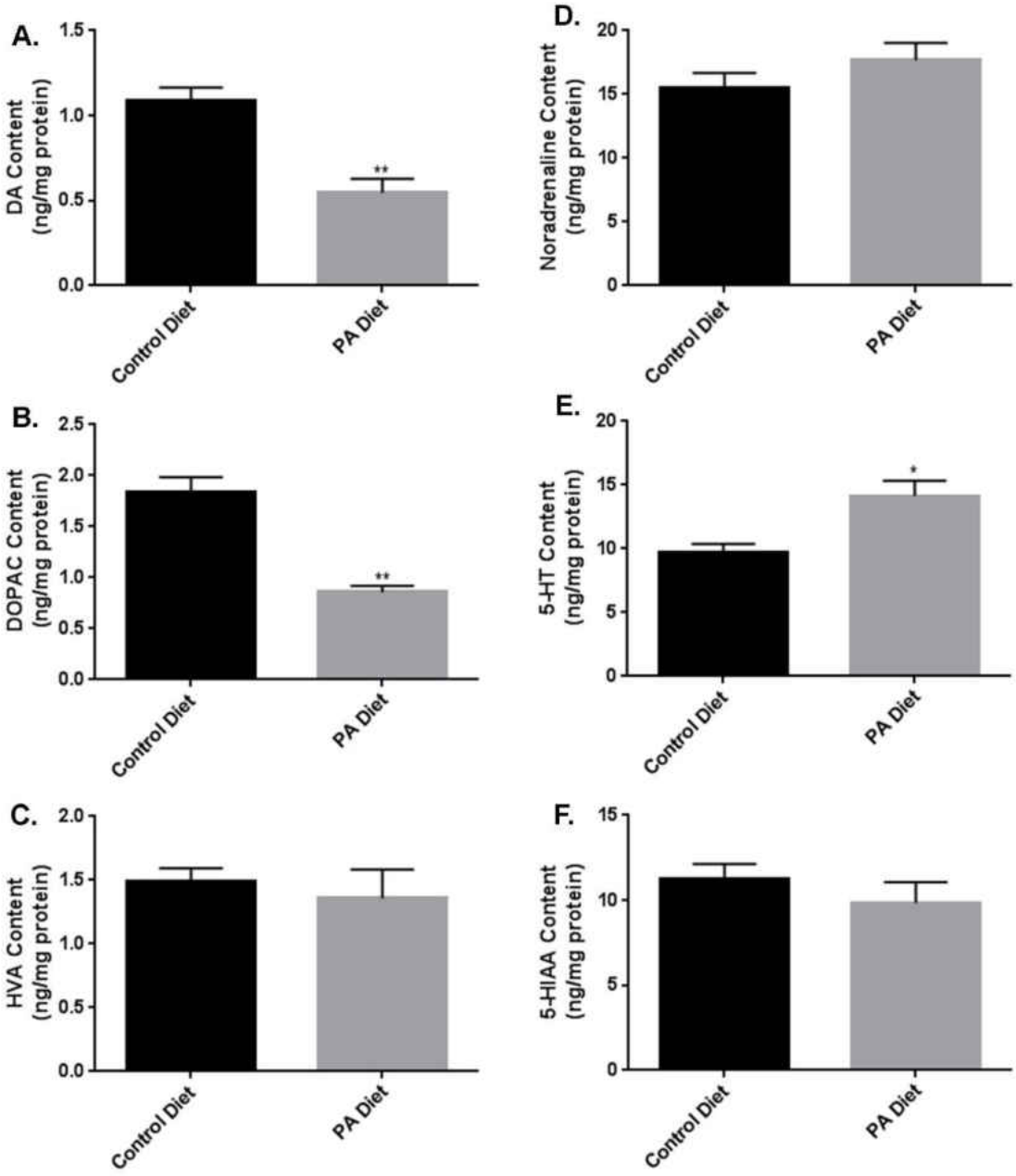


Figure 41. Biogenic amine analysis shows significant differences in biogenic amine levels between control and PA diet-fed m-Thy1 mice. While PA-enriched diet reduced DA (A) and DOPAC (B), it increased 5-HT content (E) and didn't significantly affect Noradrenaline (D), HVA (C), or 5-HIAA (F) levels. Data were analyzed using the nonparametric, unpaired student's t test with the Mann-Whitney post hoc test. Data is expressed as ng/mg protein. **$p<0.01$ versus control diet.

research studies of sFFA have found positive associations (Anderson et al., 1999b; Honglei Chen, Zhang, Hernán, Willett, et al., 2003c; Johnson et al., 1999c; Giancarlo Logroscino et al., 1996b) or no significant relationship (Honglei Chen et al., 2002; Honglei Chen, Zhang, Hernán, Willett, et al., 2003c; Hellenbrand et al., 1996b; K M Powers et al., 2003) with PD risk. While these studies provide important information, they utilized food frequency questionnaires in which individuals described what they have consumed. This type of survey can be difficult to interpret because individuals may not accurately report what, when, and how much they consumed.

Abnormal accumulation of α-syn protein is a characteristic of PD and other synucleinopathies. While the cause of the accumulation remains unknown, genetic predisposition along with environmental factors are likely to contribute to the pathogenesis of PD. *In vitro* studies have shown that PUFAs increase α-syn oligomerization and insoluble aggregate formation while sFFAs do not (Assayag et al., 2007; Ronit Sharon et al., 2003). Additionally, α-syn has been proposed to act as a lipid carrier to shuttle fatty acids around the cell (George & Yang, 2013) and a previous study showed that when α-syn is ablated in primary astrocytes, PA incorporation into membranes is decreased (Castagnet, Golovko, Barceló-Coblijn, Nussbaum, & Murphy, 2005b). The increase in PA content in our study may therefore lead to increased α-syn expression, which could function to properly traffic the excess PA to lipid membranes or to the mitochondria for β-oxidation and might therefore be functioning as a protective measure to maintain normal lipid homeostasis.

TH is a very important enzyme in the synthesis of dopamine (Sjoerdsma et al., 1965) and has been shown to decrease in PD (Tabrez et al., 2012; Zhu, Zhang, & Zeng, 2012). Short chained fatty acids have been shown to upregulate TH expression through a cAMP dependent mechanism (DeCastro et al., 2005; Mally et al., 2004) while the role of long chain saturated and unsaturated fatty acids remains to be determined. In this study, we found that a PA-enriched diet increases TH protein and mRNA expression in both strains of mice. cAMP response element binding protein (CREB) has been shown to be the mediator by which cAMP upregulates TH expression in PC12 cells (Piech-Dumas & Tank, 1999). It is possible that longer chain fatty acids like palmitic acid may have the same effect on CREB activation and are yet to be determined in future studies. To the best of our knowledge, we are the first to show that a PA enriched diet can induce TH protein and mRNA expression. Elucidation of the mechanism by which PA enriched diet induces TH expression is of great importance in the search for disease altering therapies in PD.

We did not observe any significant difference in the levels of biogenic amines in the control B6D2 mice fed the PA-enriched diet compared to the control diet. However, dopamine and serotonin levels were significantly modulated by the PA-enriched diet in the m-Thy1 mice. Our findings demonstrating reduced dopamine content in m-Thy1 mice are intriguing as we show that the PA diet increases TH expression. It is possible that the PA diet increases TH expression levels as a protective strategy to compensate the loss of dopamine content in the m-Thy1 mice. As well, our findings showing that PA

increases serotonin levels are of extreme significance as such an increase is desirable in many conditions such as depression that is frequently associated with PD presentation. The significant increase in serotonin could be explained by the fact that sFFAs have been shown to decrease the binding of serotonin to transporters (du Bois, Deng, Bell, & Huang, 2006). The PA-enriched diet may thus result in less serotonin binding, which could lead to an increase in serotonin availability.

Conclusions

In summary, we demonstrate that the PA-enriched diet induces an increase in α-syn and TH protein and mRNA expression in both B6D2 and m-Thy1 mice. To the best of our knowledge, our study is the first to show that a diet enriched in PA increases the levels of TH protein and mRNA in these mouse models. We also show that the PA-enriched diet does not affect biogenic amine content in control B6D2 mice but significantly changes dopamine and serotonin levels in m-Thy1 mice relative to control-fed mice. Altogether, our results demonstrate that a diet enriched in PA increases the levels of TH, and serotonin, an effect that can provide beneficial effects in a variety of conditions. Future studies are needed to elucidate the mechanisms by which a PA-enriched diet modulates these proteins.

CHAPTER V

PALMITIC ACID ENRICHED DIET RESCUES MOTOR FUNCTION, TYROSINE HYDROXYLASE, AND DOPAMINERGIC NEURONS FROM MPTP IN C57BL/6 MICE

Abstract

α-synuclein (α-syn) protein accumulation is a hallmark of a group of brain disorders known collectively as synucleinopathies. These include Parkinson disease (PD), dementia with Lewy bodies (DLB), and multiple system atrophy (MSA). Currently, α-syn is under scrutiny as a potential pathogenic factor in the progression of synucleinopathies. However, there is a significant gap in our knowledge on what causes α-syn to accumulate. It is now becoming evident that the nature of our dietary intake influences disease-related genes and may thus potentially increase or reduce our risks of developing synucleinopathies. In this study, we determined the extent to which a two month diet enriched in the saturated free fatty acid palmitate (PA) influences motor function, along with levels of α-syn and tyrosine hydroxylase (TH), the rate limiting enzyme in dopamine synthesis, and finally dopaminergic neuronal survival in the MPTP-induced C57BL/6 mouse model for synucleinopathy. We demonstrate that a PA-enriched diet rescues motor function. We show that it induces a decrease in α-syn and an increase in TH protein content in the *substantia nigra* of MPTP-treated animals. We also show that the number of surviving dopaminergic neurons is significantly rescued in PA fed MPTP injected animals in comparison

to control fed MPTP animals. Altogether, our results demonstrate that a diet rich in the saturated fatty acid PA may be protective against the MPTP-induced perturbation in motor function, α-syn and TH protein level fluctuations, and survival of dopaminergic neurons in the *substantia nigra* of mice.

Introduction

Synucleinopathies, a group of neurodegenerative disorders, are pathologically characterized by the abnormal accumulation of α-synuclein (α-syn) protein in intracellular neuronal and glial inclusions known as Lewy bodies. The role of α-syn in the pathogenesis of synucleinopathies is not well understood but extensive experimental data points to a neurotoxic role of high levels of the protein in its soluble and aggregated forms (Adamczyk et al., 2006; Brown, 2010; Halbach et al., 2004; Snyder & Wolozin, 2004). The cause(s) of synucleinopathies are likely multi-factorial with genetic predisposition and environmental factors contributing to the pathogenesis of the diseases. To date, the intake of dietary fats have shown many inconsistencies in relation to PD type synucleinopathy risk (White et al., 2009). Epidemiological studies on dietary intake of fats and PD have shown positive associations (Anderson et al., 1999a; Johnson et al., 1999b; Giancarlo Logroscino et al., 1996a; Miyake, Sasaki, et al., 2010), no association (Hellenbrand et al., 1996a; L. C. Tan et al., 2007), and even protective effects (Abbott, Webster Ross, et al., 2003b; Honglei Chen, Zhang, Hernán, Willett, et al., 2003b; L M L de Lau et al., 2005c; Kamel et al., 2014; Kyrozis et al., 2013; Karen M. Powers et al., 2009b). Examining specific groups of fatty acids does not allow for much more clarity as polyunsaturated

fatty acids (PUFAs) and monounsaturated fatty acids (MUFAs) have been shown to be protective in some studies (Abbott, Webster Ross, et al., 2003c; L.M.L. de Lau et al., 2005) and detrimental in another (Dong et al., 2014), while saturated fatty acids have been shown to be associated with (Anderson et al., 1999b; Honglei Chen, Zhang, Hernán, Willett, et al., 2003c; Johnson et al., 1999c; Giancarlo Logroscino et al., 1996b) and have no significant relationship with PD risk (Honglei Chen et al., 2002; Honglei Chen, Zhang, Hernán, Willett, et al., 2003c; Hellenbrand et al., 1996b; K M Powers et al., 2003).

Hallmarks of PD type synucleinopathy include the loss of dopaminergic neurons in the *substantia nigra pars compacta* and the abnormal accumulation of α-syn protein aggregates in Lewy bodies. The reduction in TH, the rate limiting component of dopamine synthesis, and the accumulation of α-syn in Lewy bodies are key components observed in PD brains (Crowther et al., 2000; Schapira, 1997; Spillantini et al., 1997) and are hallmarks of late stage disease.

A variety of model systems have been utilized to investigate the mechanisms of neuronal damage in PD that link cell loss with characteristic motor deficits associated with PD pathology. A commonly utilized drug in *in vitro* and *in vivo* studies of PD is 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Meredith & Rademacher, 2011; Notter, Irwin, Langston, & Gash, 1988; Sriram, Pai, Boyd, & Ravindranath, 1997) that has been used to model PD based on its ability to phenocopy the pathology and behavioral outcomes of PD. MPTP, first synthesized in 1947 (ZIERING & LEE, 1947), was not implicated as a potent drug that induces PD until the 1980s (Langston, Ballard, Tetrud, & Irwin, 1983b).

MPTP, itself, is not toxic but is lipid-soluble and readily crosses the blood-brain barrier (Langston et al., 1983b). Once within the brain, it is taken up by astrocytes that contain monoamine oxygenase B enzymes that convert MPTP into the toxic cation 1-methyl-4-phenylpyridinium (MPP^+) (Chiba, Trevor, & Castagnoli, 1984; Langston, Irwin, Langston, & Forno, 1984; Ransom, Kunis, Irwin, & Langston, 1987). MPP^+ is then released by astrocytes and selectively taken up by dopaminergic neurons via the dopamine transporter (Shen, Abell, Gessner, & Brossi, 1985). Once inside dopaminergic neurons, it becomes concentrated in mitochondria and inhibits Complex I of the respiratory chain ultimately leading to their selective death (Ramsay, Salach, & Singer, 1986).

Mouse studies involving the administration of omega-3 polyunsaturated fatty acids (PUFAs) have shown beneficial against MPTP-induced effects (M. Bousquet et al., 2009; M Bousquet et al., 2008; Melanie Bousquet et al., 2011; Mélanie Bousquet et al., 2011; Coulombe et al., 2018), while diets high in fat either exacerbate the progression of parkinsonism by exhibiting enhanced dopamine depletion in the *substantia nigra*, striatum, and nigrostriatal pathway (M. Bousquet et al., 2012; Choi, Jang, Park, & Kang, 2005b; Morris, Bomhoff, Stanford, & Geiger, 2010; White et al., 2009) or have shown to to be protective against MPTP induced motor dysfunction and TH neuronal loss (X. Yang & Cheng, 2010a).

However, some of these studies examined the effects of PUFAs and others utilized diets high in fat and were not isocaloric to the control diet. Additionally, many of the aforementioned epidemiological studies utilized food

frequency questionnaires and were unable to clarify the roles of specific fatty acids. The use of a high fat diet does not pinpoint the precise contributions of individual fatty acids because they contain elevated levels of cholesterol and various fatty acids in combination. Another confounding factor is that many of the high fat diets used were not isocaloric to the control diet, which adds another layer of complexity to interpreting whether changes were caused by one of many specific fatty acids, cholesterol, or the increased caloric intake that can lead to obesity and a plethora of metabolic diseases. Therefore, the role of dietary interventions in PD risk requires a more precise examination of the contributions of individual fatty acids including palmitate to more thoroughly elucidate the effects of saturated fats on PD risk.

Here we describe the effects of palmitate on cultured mouse dopaminergic neurons and the effects of an isocaloric diet enriched in PA in an MPTP mouse model of PD type synucleinopathy. We show that palmitate treatment decreases α-syn and TH protein content in cultured mouse dopaminergic neurons. We also show that the effects of a diet enriched in PA differentially affects animals. We show that a PA enriched diet rescues motor function, decreases α-syn, and increases TH protein levels, in addition to preventing dopaminergic neuron loss in an MPTP mouse model of PD. Taken together, our results suggest that a diet enriched in PA may protect against the development of PD pathology and symptoms.

Methods

Materials.

The cells and the respective kits and reagents used to grow and differentiate them were obtained by companies highlighted in Table 8. Palmitic acid (CAS 57-10-3, Catalog # sc-203175) and MPTP-HCl (CAS 23007-85-4, Catalog # sc-206178) were purchased from Santa-Cruz Biotechnology, Inc. Dallas, TX, USA.

Mouse dopaminergic neuronal differentiation from pluripotent stem cells.

Mouse pluripotent stem cells were differentiated into mature dopaminergic neurons following an optimized protocol provided by the R & D Systems Stem Cell Kit Human/Mouse Dopaminergic Neuron Differentiation Kit. Briefly, irradiated mouse embryonic fibroblasts were plated on gelatin coated plates as a feeder layer for PSCs at a density of approximately 3 x 10^6 cells/100 mm plate and incubated for 24 hours at 37˚C and 5% CO_2. PSCs were then seeded on top of the feeder layer and allowed to increase colony sizes for a few days before moving to stage I of differentiation. Stage I of differentiation involved the expansion of undifferentiated PSCs for four days. Stage II consisted of seeding PSCs on nonadherent plates to allow for the formation of embryoid bodies for four days. In stage III, the embryoid bodies were transferred to a tissue culture plate and subjected to a selection media to allow for the enrichment of nestin-positive cells for six to eight days. The nestin-positive cells were further expanded in another media for four to six days in stage IV. Once cells were

Table 8. Materials list for differentiation of PSCs into dopaminergic neurons.

Product	Catalog #	Company
Stem Cell Kit Human/Mouse Dopaminergic Neuron Differentiation Kit	SC001B	R & D Systems
Mouse Embryonic Stem cells	CRL-11632	ATCC
Irradiated Mouse Embryonic Fibroblasts (iMEFs) feeder cells	PSC001	R & D Systems
10 cm tissue culture dishes Falcon 353003 (case of 200)	08-772-E	ThermoFisher
10 cm bacterial culture dishes Falcon 351029 (case of 500)	08-757-100D	ThermoFisher
12mm coverslips glass (pack of 100)	633009	www.carolina.com
24 well flat well bottom TC treated (case of 50)	09-761-146	ThermoFisher
15ml and 50ml centrifuge tubes	C-3394-1 and C-3394-3	Bioexpress
0.2um syringe filter (pack of 50)	SLGP033RS	Millipore
0.2um, 500ml filter units (pack of 12)	SCGV05RE	Millipore
Cryotubes 2ml corning 431386	89089-764	VWR
Serological pipettes	P-2827-10, P-2837-25, P-2834-5	Bioexpress
Pipettes and pipette tips		Bioexpress and USA Scientific
10ml syringes	305482	BD ET eccentric tip
Dulbecco's Modified Eagle Medium (DMEM High Glucose) (500mL)	11965-092	Invitrogen
DMEM/F12 no HEPES powder (10x1L)	12500-062	Invitrogen
FBS ES cell qualified (500mL)	SH30071.03HI	Hyclone ThermoFisher
Phosphate Buffered Saline (PBS) (500mL)	10010-023	Invitrogen
0.05% Trypsin/EDTA	25300-054	Invitrogen
Gelatin from porcine skin	G2500-100G	Sigma
ESGRO (recombinant mouse LIF)	ESG1106	Millipore
Knockout DMEM (500mL)	10829-018	Invitrogen
MEM Non-essential AA Solution	11140-050	Invitrogen
Pen-Strep-Glutamine (100X) (100mL?)	10378-016	Invitrogen
Pen-Strep (100X) (20mL)	15140-148	Invitrogen
2-Mercaptoethanol	21985-023	Invitrogen
D-(+)-Glucose	G6152-100G	Sigma
L-Glutamine	G-8540-25G	Sigma
Sodium Bicarbonate $NaHCO_3$	S-5761-500G	Sigma
Poly-L-ornithine Hydrobromide (50mg)	P3655-50MG	Sigma
L-Ascorbic Acid (Tocris 4055) (50mg)	40-555-0	ThermoFisher
Sterile, deionized water		
BSA-very low endotoxin	81-068-3	Millipore
Acetic Acid (J.T. Baker 9508-03)	JT9508-2	VWR
Anti-Nestin Antibody	AF2736	R & D Systems
Anti-Tyrosine Hydroxylase Antibody	T1299	Sigma
Anti-Neuron Specific Beta-III Tubulin Antibody	MAB1195	R & D Systems

expanded, another medium change occurred and cells could differentiate into mature dopaminergic neurons in stage V remaining viable for two weeks or more.

Cell culture treatments.

Following differentiation, cells were treated as previously described (Marwarha, Claycombe, Schommer, Collins, & Ghribi, 2016a; Marwarha, Schommer, Lund, Schommer, & Ghribi, 2018a) with modifications as indicated. Dopaminergic neurons were treated with different concentrations of BSA (bovine serum albumin) – conjugated palmitic acid. Palmitic acid stock solution 100 mM was prepared in 100% ethanol. A 5 mM BSA stock solution was prepared in endotoxin-free milliQ water (18 MΩ). Both, the palmitic acid and BSA stock solutions were sterile filtered using a 0.2 µm filter. The necessary amounts of palmitic acid and BSA were added to the medium to yield the desired palmitic acid concentrations with the ratio of palmitic acid and BSA being 6:1. The combined mediums were incubated for 1.5 hours to allow for palmitic acid/BSA conjugation. The cells were then treated with the designated concentrations of palmitic acid/BSA for 24 hours.

Mouse husbandry and diet.

Male C57BL/6 mice were obtained from The Jackson Laboratory (Stock # 000664) for this study. All animal procedures were carried out in accordance with the U.S. Public Health Service Policy on the Humane Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee at the University of North Dakota (Protocol 1506-2 and 1407-1). All animal experiments complied with the National Institutes of Health Guide for the

Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1978). The mice were housed individually in ventilated cages at an ambient room temperature (23-25ºC) and ambient relative humidity ranging between 50-70%. The mice were maintained on 12:12 hour light:dark cycle and allowed access to food and water *ad libitum*. Male mice, four (4) months of age (n=10-12) were fed either a palmitate-enriched diet (custom-made, TD 1106162, Harlan Teklad, 2.2% w/w palmitic acid) or a control diet (custom made, TD 85172, Harlan Teklad, 0.8% w/w palmitic acid) for two months. The diets were isocaloric in relation to each other with the exception of palmitate and linoleate content and based on the NIH-07 open formula. The respective composition of the diets is shown in Table 9. Following the two months of feeding, 5-6 animals on each of the diets were subjected to either saline vehicle or MPTP intraperitoneal injections according to previously published work (Manocha et al., 2017). Behavioral testing and necropsy were performed five days post-injection at six (6) months of age.

MPTP-based PD model.

The six-month-old male C57BL/6 mice were given 3 intraperitoneal (i.p.) injections of saline vehicle or MPTP-HCl (18 mg/kg of free base) at 2 hour intervals for a total of 3 injections. C57Bl/6 mice have proven to be a good model for the study of MPTP induced Parkinson's disease for they exhibit a greater reduction in dopaminergic neurons compared to other strains of mice subjected

Table 9. Composition of the control chow diet and palmitate-enriched diet.

	Control chow diet	**Palmitic acid-enriched diet**
Proteins	23.6 % w/w	23.6 % w/w
Carbohydrates	65.8 % w/w	65.8 % w/w
Total Fat	5.6 % w/w	5.6 % w/w
Total Energy	4.08 Kcal/gram	4.08 Kcal/gram
Myristic acid (14:0)	0.1 % w/w	0.1 % w/w
Palmitic acid (16:0)	**0.8 % w/w**	**2.2 % w/w**
Stearic acid (18:0)	0.2 % w/w	0.2 % w/w
Palmitoleic acid (16:1)	Trace	Trace
Oleic acid (18:1)	1.2 % w/w	1.2 % w/w
Gadoleic acid (20:1)	Trace	Trace
Linoleic acid (18:2 n6)	**2.2 % w/w**	**0.8 % w/w**
Linolenic acid (18:3 n3)	0.2 % w/w	0.2 % w/w
Arachadonic acid (20:4 n6)	Trace	Trace
EPA (20:5 n3)	0.1 % w/w	0.1 % w/w
DHA (22:6 n3)	0.3 % w/w	0.3 % w/w

to similar doses of MPTP (Meredith & Rademacher, 2011; Muthane et al., 1994; Sonsalla & Heikkila, 1986). The MPTP dosages utilized in our study were based on the concentrations established in the following publications (Manocha et al., 2017; Sonsalla & Heikkila, 1986). Within these studies, neither Lewy bodies nor inclusions that resemble these bodies were observed when mice were acutely or subchronically (subacutely) treated with MPTP although α-syn is increased in the dopaminergic neurons of the *substantia nigra* and correlated with TH neuron loss (Sonsalla & Heikkila, 1986; Vila et al., 2000a).

Bright-field microscopy.

Bright-field images of the mouse PSCs differentiation procedure were obtained on an EVOS® cell imaging system with a 10x objective.

Immunofluorescence.

Mouse PSCs were first differentiated via an optimized human/mouse dopaminergic neuron differentiation kit into nestin-positive cells that were further differentiated into dopaminergic neurons as confirmed by TH-positive immunolabeling. Cells were rinsed briefly with phosphate buffered saline (PBS), fixed in ice-cold acetone for 5 minutes, washed twice with PBS and incubated for 1 hour with PBS containing 10% normal goat serum before applying PBS containing 5% normal goat serum and the following antibodies which are further classified in table 3: anti-nestin antibody, anti-neuron specific β-III tubulin, anti-TH antibody overnight at 4ºC. Cells were then washed three times with PBS (5 mins each) and double-labeled with AlexaFluor 594 goat-anti-rabbit and AlexaFluor 488 goat-anti-mouse antibody in PBS containing 5% normal goat serum for 1 hour at room temperature in the dark. Cells were washed three times with PBS for five minutes in the dark and mounted with Vectashield containing 4',6-diamidino-2-pheylindole (DAPI), and visualized with a Leica DMI6000B microscope with a Leica DFC350 FX camera (Buffalo Grove, IL USA). Imaging was performed with 10x and 20x objectives and composites were generated using Photoshop Software (San Jose, CA).

Western blotting analysis.

Substantia nigra-enriched fractions were prepared as previously described (Marwarha et al., 2010a; Marwarha, Prasanthi, et al., 2011a) and as follows. *Substantia nigra*-enriched tissues (20 mg) were dounce homogenized in RIPA tissue lysis buffer (50 mM Tris, 150 mM Nacl, 0.1% SDS, 0.5% sodium

deoxycholate, 1% Triton X, pH 7.4) supplemented with protease and phosphatase inhibitors. The samples were centrifuged at 5000 x g for 15 min and the supernatant harvested. Protein concentrations were determined by the Bradford protein assay method. Proteins (10 µg/lane) were resolved on SDS-PAGE gels followed by transfer to a polyvinylidene difluoride (PVDF) membrane (BioRad, Hercules, CA) and incubation with the antibodies listed in Table 3. The origin, source, and dilutions of the respective antibodies used for this study are compiled in Table 10. β-actin was used as a gel loading control. The blots were developed with enhanced chemiluminescence (Clarity™ Western ECL blotting substrate, Bio-Rad, Hercules, CA) and imaged using an Aplegen Omega Lum G System (Pleasanton, CA, USA). The densitometric analysis was performed using ImageJ (NIH, USA) software with the results represented as total integrated densitometric values for fold change over β-Actin (n=5 for each condition) including 3 technical replicates. Data were analyzed using the nonparametric, unpaired student's t-test with the Mann-Whitney post-hoc test (GraphPad Prism Software, San Diego, CA).

Real time-rtPCR.

Total RNA was extracted from mouse dopaminergic neurons with the QuickGene RNA cultured cell HC kit S (Autogen, Holliston, MA). Total RNA (0.5 µg) was reverse transcribed into cDNA with qScript cDNA SuperMix (Quanta Biosciences, Gaithersburg, MD). Real time-rtPCR was then performed on the cDNA with taqman probes for the SNCA (Mm01188700_m1) and TH (Mm00447557_m1) genes (Applied Biosystems, Foster City, CA) and

Table 10. List of antibodies used in the study					
Antibody	**Dilution**	**Host**	**Manufacturer**	**Catalog #**	**RRID**
α-syn	1:500	Rabbit	Cell Signaling	2642S	AB_10695412
TH	1:500	Rabbit	Cell Signaling	2792S	AB_10691683
$pS^{40}TH$	1:500	Rabbit	Sigma Aldrich	T9573	AB_261823
TH	1:500	Mouse	Sigma Aldrich	T1299	AB_477560
Nestin	1:500	Mouse	R & D Systems	AF2736	AB_416673
Neuron specific β-III tubulin	1:500	Rabbit	Abcam	Ab18207	AB_444319
AlexaFluor 594 goat-anti-rabbit	1:250	Goat	Life Technologies	A11037	AB_2534095
AlexaFluor 488 goat-anti-mouse	1:250	Goat	Life Technologies	A11001	AB_2534069
Vectashield containing DAPI			Vector Labs	H-1500	AB_2336788

normalized to 18S rRNA. The data were quantified and expressed as fold-change compared to the control by using the $\Delta\Delta C_T$ method. Data are expressed as mean ± SEM and includes determinations made with (n=3) and three technical replicates.

Immunohistochemistry.

The left cerebral hemispheres of C57BL/6 mice in the four different conditions were sectioned using a freezing microtome. As previously described (Manocha et al., 2017), multiple paraformaldehyde-fixed and sucrose-equilibrated tissues were embedded in a 15% gelatin (in 0.1 M phosphate buffer, pH 7.4) matrix to form sample blocks for simultaneous processing. The blocks were immersed in a 4% paraformaldehyde solution for 3-4 days to harden the

gelatin matrix, followed by a 30% sucrose solution that was replaced every 2 days until the blocks were utilized. The blocks were then flash frozen using dry-ice/isomethylpentane, and 40 µm serial sections were cut using a freezing microtome. Serial sections (960 µm apart) were then immunolabeled using an anti-TH antibody (1:500 dilution) or an anti α-synuclein antibody (1:500 dilution, see Table 10 for detailed descriptions of antibodies). The antigens were visualized using a Vector ABC kit and DAB as the chromogen (Vector Laboratories, Inc., Burlingame, CA) according to the manufacturer's protocols. The slides were dehydrated through a series of ethanol concentrations and Histo-Clear (National Diagnostics, Atlanta, GA) before being coverslipped using Permount. Images were collected using an upright Leica DM1000 microscope and a Leica DF320 digital camera system (n=4-5) with exposure times consistent across the sample set.

Locomotor activity assessment via the Pole test.

Following the saline and MPTP injections, mice were housed for an additional 4 days and behaviorally tested on day 5. Each animal was trialed on the pole test to assess locomotor activity as a measure of dopaminergic neuron function following the MPTP injections (Matsuura, Kabuto, Makino, & Ogawa, 1997). Briefly, mice were placed head-upward on the top of a vertical rough-surfaced pole (diameter 8 mm, height 55 cm) with a base that was positioned on a flat surface. The time until the mouse descended to the bottom of the pole floor was recorded with a maximum of 120 seconds. Mice were returned to their home

cages after testing and the pole was wiped clean with 70% ethanol in between mice trials and was allowed to dry before the next trial.

Grip strength test via Kondziela's inverted screen test.

To test gross strength of the forelimb muscles in mice, mice were challenged with the inverted screen test as previously described (Deacon, 2013). A 43 x 43 cm^2 square wire mesh frame was utilized in this test. The mesh was 12 x 12 mm^2 square formed by 1 mm diameter wires. The frame was 4 cm deep wooden beading to prevent mice from climbing to the other side of the mesh. After a 30-minute rest following the pole test, each mouse was placed onto the center of the mesh square frame and the screen was rotated over the course of 2 seconds to an inverted position with the mouse's head declining first. The screen was held at 40-50 cm over a clean foam surface, and the time it took each mouse to let go of the screen was measured until the maximum of 120 seconds was reached. After testing, mice were returned to their home cages.

Quantification of TH-positive neurons.

The number of TH-positive neurons in the *substantia nigra* of saline- and MPTP-injected mice on the control or PA diet were determined using design-based stereology. The mouse brains were cryosectioned and a subset immunolabeled for TH as described above with the sectioned brains processed in parallel blocks. The section number start point was random for each brain based on the key anatomical features of the *substantia nigra* positioned ventral and caudolateral to the hippocampus. Non-biased quantification of TH-positive cells was performed using the optical fractionator approach (Hattiangady, Rao, &

Shetty, 2008; Rao, Hattiangady, & Shetty, 2006; West & Gundersen, 1990) and the optical fractionator workflow in StereoInvestigator 11.06 (Microbrightfield Inc., Williston, VT). Low power tracings of the *substantia nigra* were generated at 2X magnification on an Olympus BX51WI with a motorized *x*, *y* and *z* stage and a minimum of four sections per brain were counted at 120 µm intervals encompassing the entirety of the structure. A range of randomly and systematically selected frames, 250 – 1500 depending on the *substantia nigra* representation, were counted at 40X magnification with each frame measuring 200 µm X 200 µm and the z-frame threshold distance measured at each counting site. The number and location of counting frames and the counting depth for that section were determined by entering parameters for the sample grid size (250 X 250 µm), the thickness of the guard zones (5 µm) and the optical dissector height (30 µm). The TH-positive cells were counted if the immunolabeled cell body was fully within the counting frame. The values for total numbers of TH-positive neurons were determined using the optical fractionator formula ($N=1/ssf.1/asf.1/hsf.\Sigma Q^-$) to quantify the estimated population number using mean section thickness with counts. For the calculations, ssf=section sampling fraction, which was 12 in our study as every 12th section was sampled; asf=area sampling fraction, which is calculated by dividing the area sampled with total area of the *substantia nigra*; hsf=height sampling fraction, which was calculated by dividing the height of the counting frame with the section thickness at the time of analysis (40 µm as the block advance value), and ΣQ^- denotes the sum of the marks counted for the *substantia nigra*. The sampling was optimized for maximal

efficiency, with a final mean coefficient of error (CE) of less than 0.01 for each set of sections counted per brain based on preliminary overcounting to determine optimal counting parameters.

Statistical analysis.

One-way analysis of variance (one-way ANOVA) was used to assess the significance of differences among the mouse dopaminergic neuronal samples including more than two groups assuming the data was of parametric nature followed by Tukey's post hoc test. Quantitative data for Real time-rtPCR analysis are presented as mean ± SEM and expressed as fold-change from control. Animal data for western blots were analyzed using the nonparametric, unpaired student's t-test with the Mann-Whitney post hoc test. Quantitative data from the western blotting analysis are presented as mean ± SEM with unit value assigned to control diet and the extent of differences among the samples being expressed relative to the unit value of control diet. Behavioral and quantification of TH-positive neuron data were analyzed via an ordinary Two-way ANOVA with multiple comparisons across cell means using a Sidak post-hoc test to account for multiple comparisons and compute confidence intervals (CI) and significance. Data are expressed as mean ± SEM with the n reported in the figure legend for each experiment. +**.** Statistical analysis was performed with GraphPad Prism software 6.07.

Results

Mouse dopaminergic neurons express tyrosine hydroxylase.

Mouse pluripotent stem cells were cultured and differentiated into dopaminergic neurons according to the protocol provided with the StemXVivo differentiation kit. We acquired light microscopy images of the differentiation procedure starting with the plating of iMEF cells as a feeder layer for the PSCs (Fig. 42A). Once a uniform monolayer of iMEFs was formed, PSCs were plated on top and formed colonies (Fig. 42B). PSCs were then plated and expanded in stage I of differentiation (Fig. 42C), followed by the formation of embryoid bodies in stage II (Fig. 42D), selection of nestin-positive cells in stage III (Fig. 42E), and expansion of nestin-positive cells in stage IV (Fig. 42F). Nestin-positive cells were then immunofluorescently labeled for nestin (Fig. 42G) and overlaid with DAPI (Fig. 42H). Once nestin-positive cells were confirmed we moved the cells into stage V of differentiation to derive mature dopaminergic neurons (Fig. 42I). Following stage V of differentiation dopaminergic neurons were immunofluorescently labeled for TH (Fig. 42J), neuron specific β-III tubulin (Fig. 42K), DAPI (Fig. 42L), and overlay (Fig. 42M) to confirm cells are of dopaminergic origin.

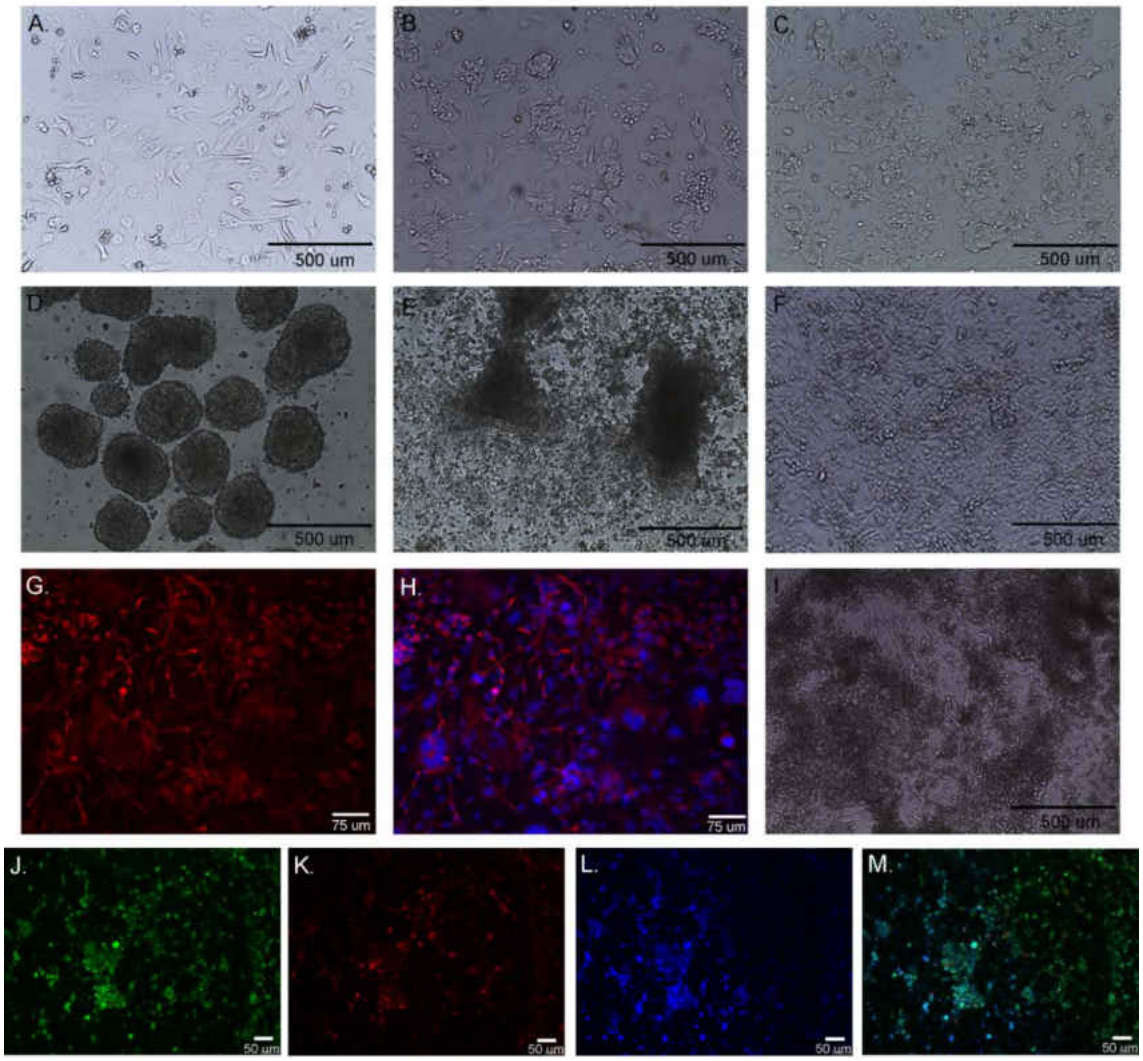


Figure 42. Mouse dopaminergic neurons express Tyrosine Hydroxylase. Bright field microscopy of iMEFs used as a feeder layer for PSCs (A). (B) shows colonies of PSCs attached to the feeder layer of iMEFs. Bright field images of stage I of differentiation (C), stage II of differentiation (D), stage III of differentiation (E), and stage IV of differentiation (F) into dopaminergic neurons. (G) is immunostaining of nestin positive cells of neuronal lineage and (H) is an overlay of nestin and DAPI. Bright field microscopy following stage V of differentiation shows mature dopaminergic neurons (I). Post differentiation immunofluorescent labeling shows cells contain TH (J), Neuron specific β-III Tubulin (K), DAPI (L), and overlay (M).

Palmitic Acid decreases α-syn and TH in mouse dopaminergic neurons.

We determined the effects of increasing concentrations of palmitic acid on the protein and mRNA expression levels of key proteins involved in synucleinopathies in mouse dopaminergic neurons. We found that palmitic acid significantly reduced the amount of α-syn protein ($p<0.05$) (Fig. 43 A,B) and mRNA content ($p<0.05$) (Fig. 43C) suggesting that palmitic acid is able to regulate α-syn at a transcriptional level. We also found that palmitic acid significantly reduces the level of TH protein ($p<0.01$) (Fig. 43D, E) while it significantly increases TH mRNA content at 150 uM ($p<0.05$) (Fig. 43F). In addition, levels of $pS^{40}TH$, the active form of TH (Dunkley et al., 2004b) are unchanged (Fig. 43G) by palmitic acid in mouse dopaminergic neurons. This suggests that palmitic acid is capable of regulating TH at a transcriptional and translational level in mouse dopaminergic neurons.

PA diet is protective against MPTP-induced motor strength deficits in C57BL/6 mice.

Following the administration of a two-month control or PA enriched diet, mice were subjected to either acute saline or MPTP injections and motor functions of the mice were assessed using the grip strength and pole tests. As expected, animals injected with MPTP performed significantly ($p<0.001$) worse than saline injected animals on both tests (Fig. 44 A, B) regardless of diet consumed. Interestingly, the PA-enriched diet significantly ($p<0.001$) improved both pole test and grip strength performance of the MPTP-injected animals (Fig. 44 A,B). Analysis of locomotor activity ($DFn=1$; $DFd=17$) revealed that diet

Figure 43. Palmitic Acid decreases α-syn and TH in mouse dopaminergic neurons. Representative western blot (A) and optical density (B) showing that PA treatment significantly decreases the level of α-syn protein in mouse dopaminergic neurons. Real Time-RT PCR shows that PA treatment significantly decreases SNCA mRNA (B). Representative western blot (D, G) and optical density (E, H) shows that PA treatment significantly decreases TH while it does not affect $pS^{40}TH$ protein levels, respectively, in mouse dopaminergic neurons. (F) shows that PA treatment differentially affects TH mRNA levels at varying concentrations. The one-way ANOVA was used to assess the significance of differences among the samples including more than two groups assuming the data was of parametric nature followed by Tukey's post hoc test. Western blots are expressed as fold change over β-Actin (n=3) including 3 technical replicates. Real Time-RT PCR for SNCA and TH is expressed as fold change over 18S rRNA using the $\Delta\Delta C_T$ method (n=3) including 3 technical replicates. *$p<0.05$, **$p<0.01$ versus control.

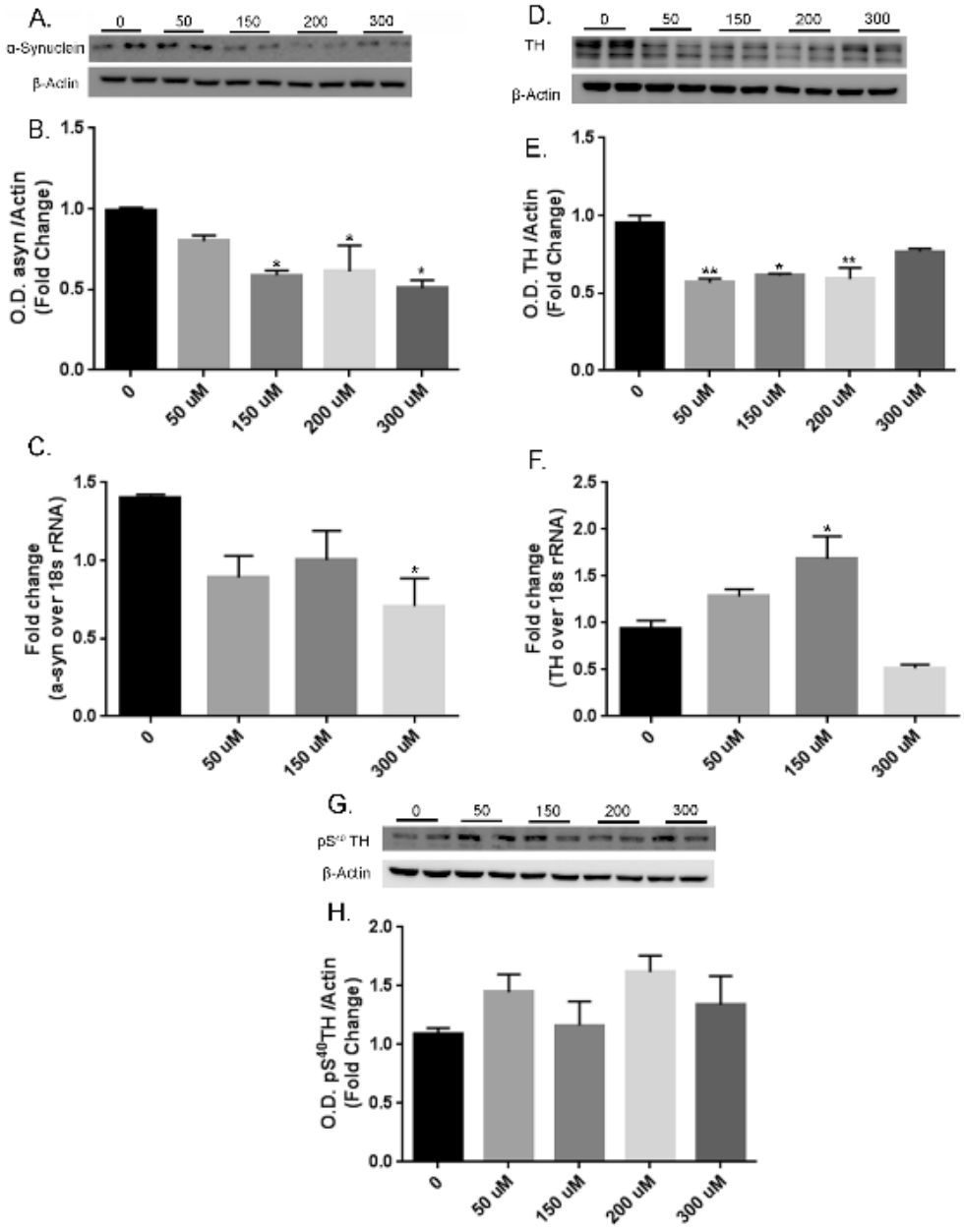
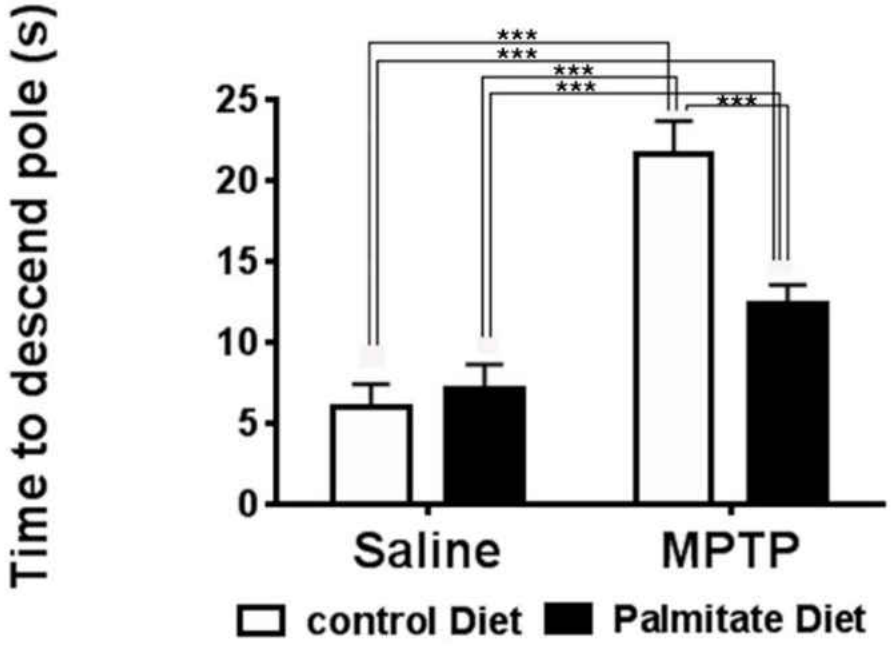
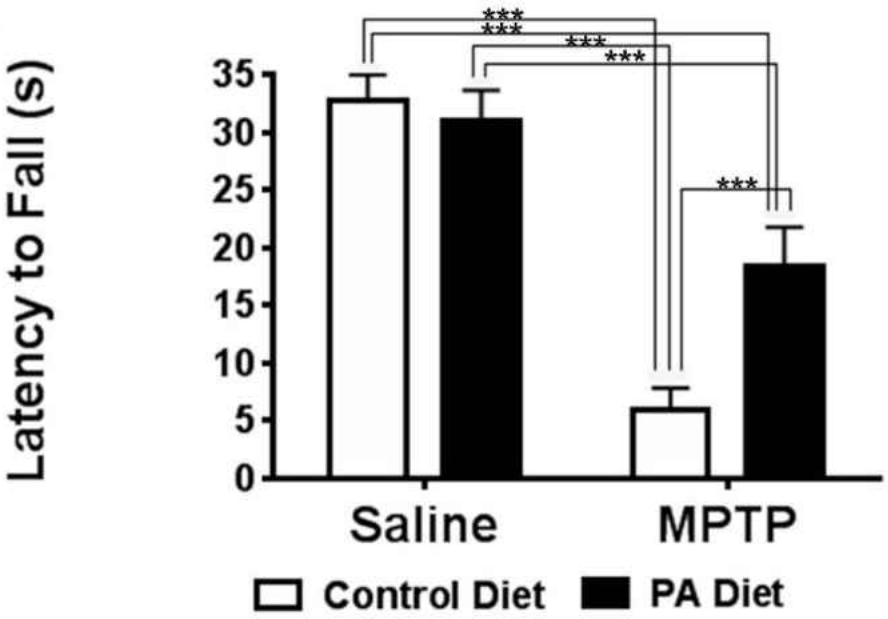

A.
0
50
150
200
300
α-Synuclein
β-Actin
B.
O.D. asyn /Actin (Fold Change)
1.5
1.0
0.5
0.0
0
50 uM
150 uM
200 uM
300 uM
C.
Fold change (a-syn over 18s rRNA)
1.5
1.0
0.5
0.0
0
50 uM
150 uM
300 uM
D.
0
50
150
200
300
TH
β-Actin
E.
O.D. TH /Actin (Fold Change)
1.5
1.0
0.5
0.0
0
50 uM
150 uM
200 uM
300 uM
F.
Fold change (TH over 18s rRNA)
2.5
2.0
1.5
1.0
0.5
0.0
0
50 uM
150 uM
300 uM
G.
0
50
150
200
300
pS40 TH
β-Actin
H.
O.D. pS40TH /Actin (Fold Change)
2.0
1.5
1.0
0.5
0.0
0
50 uM
150 uM
200 uM
300 uM

Figure 44. PA diet is protective against MPTP in motor performance tests. Locomotor activity (A) and grip strength (B) of mice on control and PA diet with and without administration of MPTP were assessed via the pole test (A) and the Kondziela's inverted screen test (B). Data were analyzed via the ordinary Two-way ANOVA with multiple comparisons comparing cell means regardless of rows and columns with a Sidak post-hoc test to correct for multiple comparisons with diet (control or palmitate) and injection paradigm (saline or MPTP) as the two variables assuming the data was of nonparametric nature due to the small size. All groups contained n=5-6 animals. ***$p<0.001$ versus saline/control diet, ***$p<0.001$ versus saline/PA diet, ***$p<0.001$ versus MPTP/control diet.

A.


Time to descend pole (s)
25
20
15
10
5
0
***
***
***
***
***
Saline
MPTP
control Diet
Palmitate Diet

B.

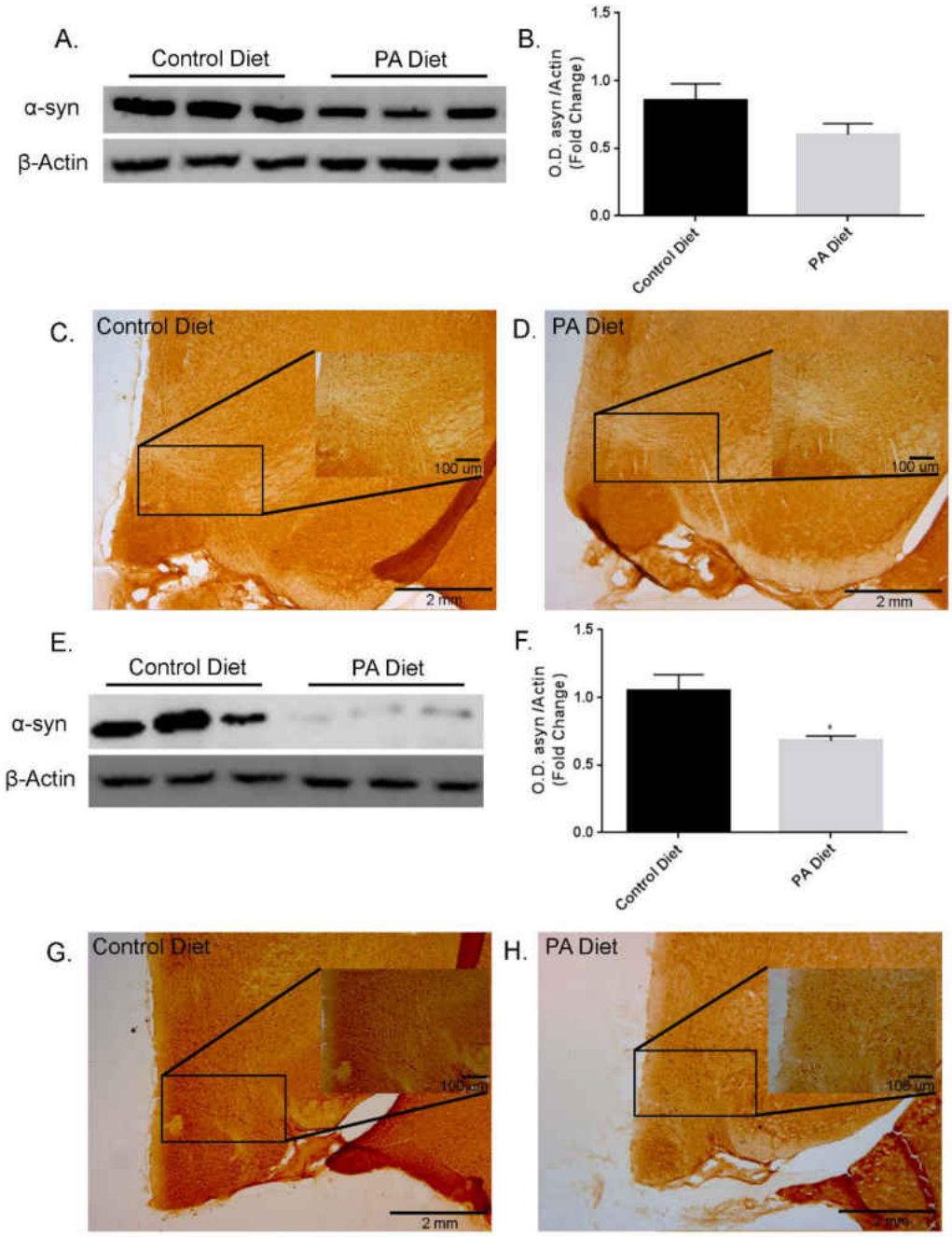

Latency to Fall (s)
35
30
25
20
15
10
5
0
***
***
***
***
***
Saline
MPTP
Control Diet
PA Diet

accounted for 10.28% of the variation (F=34.70; p<0.0001), injection accounted for 69.11% of the variation (F=233.41; p<0.0001) and an interaction between the two variables accounted for 17.11% of the variation (F=57.79; p<0.0001) observed in the system. Analysis of grip strength (DFn=1; DFd=17) revealed that diet accounted for 5.81% of the variation (F=22.25; p=0.0002), injection accounted for 80.31% of the variation (F=307.34; p<0.0001) and an interaction between the two variables accounted for 10.43% of the variation (F=39.92; p<0.0001) observed in the system. Mice fed PA diet that were injected with MPTP were able to climb down the pole at a faster rate and were able to hang on to the grip strength test apparatus for a longer period than animals fed the control diet. This data suggests that a diet enriched in PA is potentially protective against the deleterious effects of MPTP on motor performance.

PA diet decreases α-syn content in MPTP injected animals.

We determined the extent to which a PA-enriched diet affects α-syn protein content in the *substantia nigra* enriched fraction of C57BL/6 mice with or without the administration of MPTP. We found that a diet enriched in PA does not significantly affect the α-syn protein content of saline injected mice on control vs PA diet (Fig.45 A-D). We found that a PA enriched diet significantly (p<0.01) reduces α-syn protein content in relation to control fed mice treated with MPTP via western blotting analysis (Fig.45 E,F) and IHC (Fig.45 G,H). MPTP has been shown to increase α-syn protein levels in the *substantia nigra* region of C57BL/6 mice (Vila et al., 2000b) and the fact that a diet enriched in PA is capable of reducing α-syn protein content is very interesting. This data suggests a PA

Figure 45. PA diet decreases α-syn in MPTP injected mice. Representative western blot (A) and optical density (B) showing that PA diet does not significantly affect α-syn protein content in the *substantia nigra*-enriched fraction of brains from saline injected C57 mice. Immunohistochemistry of *substantia nigra* shows that saline injected C57 mice on PA diet (D) have similar α-syn protein content to control fed animals (C). Representative western blot (E) and optical density (F) showing that PA diet decreases α-syn protein content in the *substantia nigra*-enriched fraction of brains from MPTP injected C57 mice. Immunohistochemistry of *substantia nigra* shows that MPTP injected C57 mice on PA diet (H) have less α-syn protein content than control fed animals (G). Data were analyzed using the nonparametric, unpaired student's t test with the Mann-Whitney post hoc test. Western blots are expressed as fold change over β-Actin (n=5) including 2 technical replicates. **$p<0.01$ versus control diet.


A.
Control Diet
PA Diet
α-syn
β-Actin
B.
O.D. asyn /Actin (Fold Change)
1.5
1.0
0.5
0.0
Control Diet
PA Diet
C.
Control Diet
100 um
2 mm
D.
PA Diet
100 um
2 mm
E.
Control Diet
PA Diet
α-syn
β-Actin
F.
O.D. asyn /Actin (Fold Change)
1.5
1.0
0.5
0.0
Control Diet
PA Diet
G.
Control Diet
2 mm
H.
PA Diet

enriched diet is capable of modulating α-syn levels and/or may be protective against the effects of MPTP that have been shown to lead to increased α-syn accumulation.

PA diet increases TH content in saline and MPTP-injected animals.

We examined the effects of a PA-enriched diet on TH protein content in the *substantia nigra*-enriched fraction of brains from C57BL/6 mice injected acutely with saline or MPTP and found that two months of feeding with a PA-enriched diet significantly increased ($p<0.05$) TH protein levels (Fig. 46 A-B) in saline injected animals in comparison to control diet. The same was true ($p<0.01$) for the PA enriched diet among the MPTP treated animals (Fig.46 E,F). In addition, $pS^{40}TH$, the most activated form of TH (Dunkley et al., 2004b), was also shown to be significantly increased ($p<0.01$) following administration of the PA-enriched diet (Fig. 46 C,D) in relation to the control fed saline injected animals and comparing the two diets in MPTP injected animals (Fig.46 G,H). This data suggests that a PA enriched diet is capable of regulating levels of TH and that it is also potentially protective against the MPTP induced decrease in TH. This data is in accordance with findings by others (X. Yang & Cheng, 2010b) stating that a ketogenic diet is protective against MPTP induced TH loss.

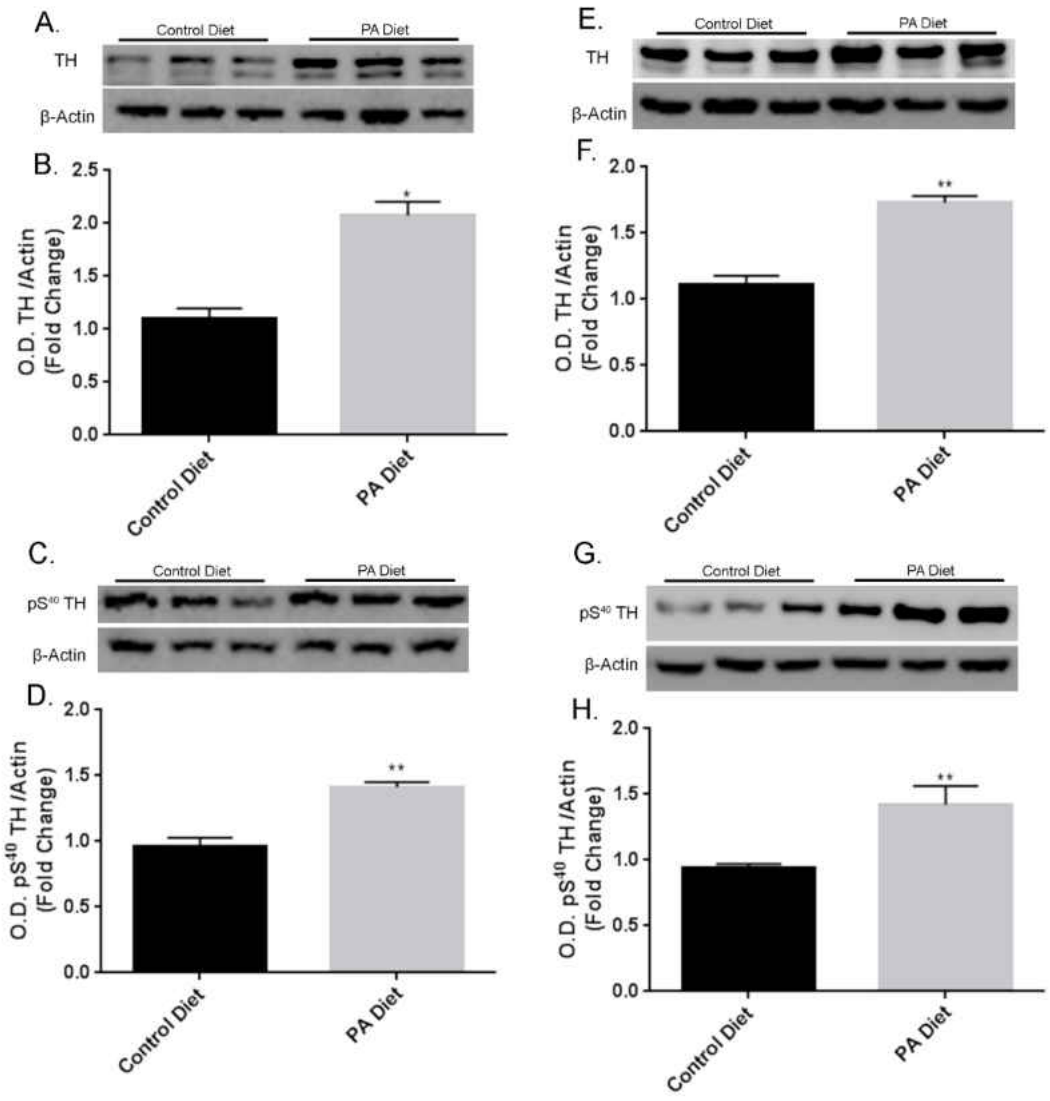
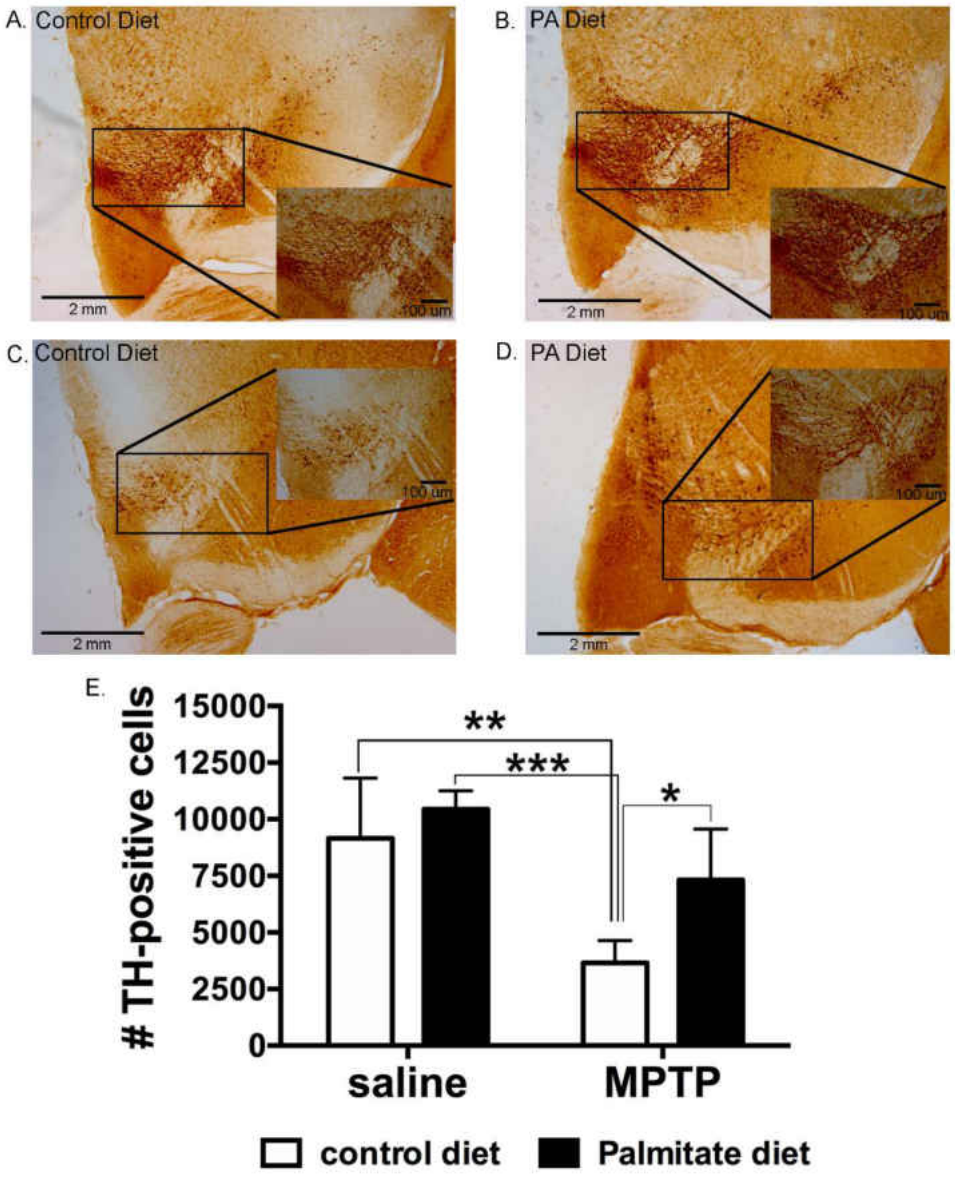


Figure 46. PA diet increases TH in saline and MPTP injected mice. Representative western blots (A, C) and optical density (B, D) showing that a PA diet increases TH and pS^{40}TH respectively in the *substantia nigra*-enriched fraction of saline injected C57 mice. Representative western blots (E, G) and optical density (F, H) showing that a PA diet increases TH and pS^{40}TH respectively in the *substantia nigra*-enriched fraction of MPTP injected C57 mice. Data were analyzed using the nonparametric, unpaired student's t test with the Mann-Whitney post hoc test. Western blots are expressed as fold change over β-Actin (n=5) including 2 technical replicates. *p<0.05, **p<0.01 versus control diet.

PA diet increases survival of TH positive neurons in MPTP-injected animals.

We performed immunohistochemistry to visualize TH-positive neurons in the *substantia nigra* of control fed saline injected (Fig.47 A), PA fed saline injected (Fig.47 B), control fed MPTP injected (Fig.47 C), and PA fed MPTP injected (Fig.47 D) animals. We then used design-based stereology to quantify the number of TH-positive neurons (Fig.47 E) and found that the MPTP-injected control fed animals had significantly fewer TH-positive neurons than saline-injected control fed ($p<0.01$) and saline-injected PA fed ($p<0.001$) animals. Interestingly, of the MPTP injected animals, the PA enriched diet group had significantly more surviving TH-positive neurons ($p<0.05$) than the control fed MPTP animals. Analysis of TH-positive neuron quantification (DFn=1; DFd=11) revealed that diet accounted for 22.54% of the variation ($F=11.24$; $p=0.0064$), injection accounted for 44.00% of the variation ($F=21.94$; $p=0.0007$) and an interaction between the two variables accounted for 6.74% of the variation ($F=3.36$; $p=0.0939$) observed in the system. This data suggests that a PA diet is protective against the MPTP-induced loss of dopaminergic neurons in the *substantia nigra*.

Figure 47. PA diet rescues TH-positive neurons from MPTP induced death. Immunohistochemistry of TH stained *substantia nigra* in saline injected control fed C57 mice (A), saline injected PA fed C57 mice (B), MPTP-injected control fed C57 mice (C), and MPTP-injected PA fed C57 mice (D). Stereological quantification of TH-positive neurons in the *substantia nigra* of the four previously mentioned groups (E). Data were analyzed via the ordinary Two-way ANOVA with multiple comparisons comparing cell means regardless of rows and columns with a Sidak post-hoc test to correct for multiple comparisons with diet (control or palmitate) and injection paradigm (saline or MPTP) as the two variables assuming the data was of nonparametric nature due to the small size. All treatment groups contained n=3-4 animals. ***$p<0.001$ versus saline/control diet, ***$p<0.001$ versus saline/PA diet, ***$p<0.001$ versus MPTP/control diet.


A. Control Diet
2 mm
100 um
B. PA Diet
2 mm
100 um
C. Control Diet
100 um
2 mm
D. PA Diet
100 um
2 mm
E.
TH-positive cells
15000
12500
10000
7500
5000
2500
0
**
***
*
saline
MPTP
control diet
Palmitate diet

Discussion

In this study, we determined the specific contribution of the fatty acid PA on expression levels of α-syn and TH, two proteins that are tightly linked to PD in primary mouse dopaminergic neurons and within a MPTP induced C57BL/6 mouse model of parkinsonism. We are the first to show that PA treatment in mouse dopaminergic neurons decreased both α-syn and TH protein levels while it differentially regulated the mRNA of both genes. Within the animal model, we are the first to show that a PA enriched diet is capable of rescuing motor function, increasing TH protein, decreasing α-syn protein, and reducing the loss of dopaminergic neurons in MPTP-injected animals.

Palmitic acid (16:0) is the most abundant saturated fatty acid in dairy products and meats. It is also synthesized *de novo* in the body and makes up 24% of total fatty acids in our blood and 28% of total fatty acids in our CSF (Guest et al., 2013). Numerous *in vitro* studies have focused on the deleterious effects of palmitic acid by itself. It has been shown to increase ER stress (Marwarha et al., 2016b), proinflammatory cytokine expression in astrocytes and microglia (Gupta et al., 2012; Tracy et al., 2013), and activation of toll-like receptors (TLRs) via NFKβ (Oberbach et al., 2012). Within our study we also showed that PA treatment alone in mouse dopaminergic neurons can decrease TH protein, the rate limiting enzyme in dopamine synthesis (Sjoerdsma et al., 1965), while it also decreases α-syn protein and mRNA levels. While these findings are very important, much of the *in vitro* work exposes cells to high levels of PA in the absence of any other vital contributing fatty acids and nutrients

present. Therefore, this research is inconclusive as to the role of PA incorporated into a wholesome diet consisting of other contributing fatty acids and nutrients. In addition, human research studies of saturated fatty acids have found positive associations (Anderson et al., 1999b; Honglei Chen, Zhang, Hernán, Willett, et al., 2003c; Johnson et al., 1999c; Giancarlo Logroscino et al., 1996b) and no significant relationship with PD risk (Honglei Chen et al., 2002; Honglei Chen, Zhang, Hernán, Willett, et al., 2003c; Hellenbrand et al., 1996b; K M Powers et al., 2003). While these studies are also very important for future research, they consist of questionnaires that involve subjects tallying what they consumed. This type of survey can be difficult to interpret because individuals may not accurately represent what, when, and how much of a given diet component they consumed. In this study, we attempted to more accurately pinpoint the contributions of the specific fatty acid PA to PD risk by feeding C57BL/6 mice a PA enriched diet that was isocaloric to control chow prior to injection with MPTP.

We found that the PA-enriched diet was protective against motor dysfunction caused by the injection of MPTP. This is in accord with other studies showing that a ketogenic diet is protective against MPTP induced motor dysfunction (Shaafi et al., 2016; X. Yang & Cheng, 2010b). It has been suggested that beta-hydroxybutyrate is protective against MPTP-induced neurodegeneration and is mediated by improved oxidative phosphorylation leading to enhanced ATP production (Tieu et al., 2003). PA can be converted into beta-hydroxybutyrate (Mashek & Grummer, 2003; Palmquist, 1972) which may be one mechanism by which a PA-enriched diet is protective against MPTP.

Abnormal accumulation of α-syn protein is a characteristic of PD and other synucleinopathies. While the cause of the accumulation remains unknown, genetic predisposition along with environmental factors such as diet are likely to contribute to the pathogenesis of PD and other synucleinopathies. MPTP has been shown to increase α-syn protein in the dopaminergic neurons of the *substantia nigra* (Fornai et al., 2005; Forno, DeLanney, Irwin, & Langston, 1993; Sonsalla & Heikkila, 1986; Vila et al., 2000a). In our study we show that a PA-enriched diet significantly reduces the MPTP-induced increase in α-syn protein. I*n vitro* studies have shown that PUFAs increase α-syn oligomerization and insoluble aggregate formation while SFAs do not (Assayag et al., 2007; Ronit Sharon et al., 2003). Studies with rodent models also reported that diets high in PUFA concentration upregulated α-syn expression (Barceló-Coblijn et al., 2003; Kitajka et al., 2004). It is possible that the increase in the saturated fatty acid PA has opposing effects to PUFA's which leads to the decrease in α-syn protein content or possibly because of the protective effects of PA against MPTP it may indirectly lead to decreased α-syn protein.

We found that in MPTP- and saline-injected mice, a PA-enriched diet increased the protein levels of TH, which is a very important enzyme in the synthesis of dopamine (Sjoerdsma et al., 1965) and the protein levels of $pS^{40}TH$, the most activated form of TH (Dunkley et al., 2004b). This is in accordance with the administration of a ketogenic diet in conjunction with MPTP (X. Yang & Cheng, 2010a). Short-chained fatty acids have been shown to upregulate TH expression through a cAMP-dependent mechanism (DeCastro et al., 2005; Mally

et al., 2004), while the roles of long-chain saturated and unsaturated fatty acids remain to be determined. To the best of our knowledge, we are the first to show that a PA-enriched diet can induce TH protein expression and that a PA-enriched diet leads to significantly higher survival of dopaminergic neurons in the *substantia nigra* of MPTP injected animals although a ketogenic diet has shown similar effects (X. Yang & Cheng, 2010a).

We have previously shown that a PA-enriched diet can lead to ER stress, increased BACE1 activity, and amyloid beta genesis in aged animals (Marwarha, Rostad, et al., 2017; Marwarha, Schommer, Lund, Schommer, & Ghribi, 2018b). Within these studies, however, animals were much more aged and were exposed to the diets for a greater amount of time. Our current study suggests a PA diet over a short span of time promotes protective effects against PD-type synucleinopathy pathology. This data is highly important to the study of PD-type synucleinopathy pathology. We have shown that PA treatment in mouse dopaminergic neurons can modulate key proteins involved in disease risk in a much different way than a diet enriched in PA affects an animal model of PD. We also show that a diet enriched in PA is protective against the motor dysfunction, α-syn accumulation, TH decrease, and loss of dopaminergic neurons caused by MPTP administration. Future studies will elucidate the mechanisms of action, and once they are understood, pharmacological means can be utilized to block the increase in α-syn and other factors to stimulate an increase in TH in order to halt or reverse the course of PD.

In summary, we demonstrate that PA treatment in mouse dopaminergic neurons decreases α-syn protein and mRNA expression as well as it decreases TH protein content. To the best of our knowledge, our study is the first to show that within MPTP-injected C57BL/6 mice a PA-enriched diet preserves motor function, decreases α-syn accumulation, increases TH protein, and increases dopaminergic neuronal survival. Altogether, our results suggest that a diet enriched in PA is protective against MPTP-induced Parkinsonism. Future studies are needed to elucidate the mechanisms by which a PA-enriched diet modulates these proteins. Establishing the effects of a smaller percentage of PA in the diet may reveal beneficial effects of this saturated free fatty acid in neurodegenerative conditions including PD and other synucleinopathies.

CHAPTER VI

DISCUSSION, CONCLUDING REMARKS, AND FUTURE DIRECTIONS

27-OHC and Parkinson's Disease

27-OHC is an active product of cholesterol metabolism made in the mitochondria of most cells by the enzyme CYP27A1 and serves many biological roles. It has been shown to promote atherosclerosis via activation of proinflammatory processes (Umetani et al., 2014), promotes breast and prostate cancer (Marwarha, Raza, et al., 2017) and functions as a ligand of the LXRs (Gabbi et al., 2014).

Ours and another lab have previously shown that the oxysterol 27-OHC evokes an increase in α-syn expression in SHSY5Y cells (Cheng et al., 2008b; Marwarha, Rhen, et al., 2011b; Rantham Prabhakara et al., 2008). The mechanism underlying the increase in α-syn was through a LXRβ dependent mechanism (Marwarha, Rhen, et al., 2011b). In my dissertation work, we aimed to recapitulate these results within normal human dopaminergic neurons that had been differentiated from normal human dopaminergic neuronal precursor cells. We found that 27-OHC increases α-syn protein levels, activates LXR as shown by an increase in ABCA1, but fails to elicit a change in α-syn mRNA. GW3965 and ECHS also failed to elicit any change in α-syn protein or mRNA content suggesting there is no link between LXR and α-syn in this model. We took our investigation further to study potential post-translational events that could

contribute to the 27-OHC induced increase in α-syn protein content. We are the first to show that 27-OHC increases α-syn protein levels through proteasomal inhibition and also a decrease in HSP70 protein content in normal dopaminergic neurons that could potentially have implications in protein folding, UPS function and autophagy-lysosomal pathways of degradation.

Proteasomes are large intracellular protein complexes whose main function is to degrade short-lived, damaged, and misfolded proteins by proteolysis. Proteasomes help control the levels of proteins necessary for normal cellular functioning. In higher organisms, proteasomes are located both in the cytoplasm and nucleus. The most common form is the 26S proteasome, which contains one 20S core catalytic particle and normally one 19S regulatory particle at each side of the 20S core particle. The 20S core, which is concealed inside the 19S particles, is the active site of the proteasome which is responsible for its caspase-like, chymotrypsin-like, and trypsin-like activities. Initially, proteins targeted for degradation are tagged with several molecules of ubiquitin. Ubiquitin is covalently attached to target proteins by three sequential enzymatic steps: ubiquitin activation by E1 enzymes, ubiquitin conjugation by E2 enzymes, and ubiquitin ligation to target proteins by E3 enzymes. Ubiquitin is normally conjugated via its carboxy-terminal glycine to an internal lysine residue (Pickart, 2001). Following many rounds of ubiquitinylation a polyubiquitin chain is formed. This chain can function as a signal for degradation by the proteasome. The proteasome unfolds substrates and threads the polypeptide chains through the inner channel, where they are cleaved into short peptides (Bhattacharyya et al.,

2014). After release from the proteasome, peptides are quickly processed into amino acids and recycled. (Reits et al., 2003)

We are aware that investigating proteasomal function is just one piece of the puzzle, *per se*, in the workings of the UPS. An approach to get a more fulfilling insight into the functionality of the UPS would be to assess the individual enzymatic activities involved in ubiquitin-dependent proteasomal degradation (Dantuma & Bott, 2014; Lindsten & Dantuma, 2003). Ubiquitinylation, is the overall result of a big family of enzymes that are involved in many processes and are therefore not straight-forward to address or to interpret. Examining proteasomal function is the final stop of all ubiquitinylated proteins to be degraded and creates a choke-point in the UPS pathway (Dantuma & Bott, 2014). Therefore, it isn't surprising that most studies focusing on the functionality of the UPS examine proteasomal function and not the individual enzymatic reactions leading up to it. However, this correlation of proteasomal activity and overall UPS impairment is complicated because it is presently unknown to what extent altered proteasomal activity affects the overall changes in degradation of ubiquitinylated proteins (Dantuma & Bott, 2014).

The decrease in HSP70 protein levels we observed could have numerous effects on cellular proteostasis. HSP70 is capable of being involved in various degradation pathways by the presence of specific chaperones and co-chaperones that aid in guiding the targeted protein to a specific degradation pathway. See (Fernández-Fernández *et al.* 2017) for a review. α-syn has been shown to bind HSP70 (Aprile et al., 2017; Dedmon et al., 2005; Luk et al., 2008)

and be degraded by the UPS and autophagy (Webb et al., 2003). The extent to which a decrease in HSP70 affects proteasomal and/or autophagy is yet to be determined in future studies.

In summary, we demonstrate that 27-OHC induces an increase in α-syn accumulation in human dopaminergic neurons, a major hallmark of PD. The mechanism by which this occurs does not involve LXRs for we did not observe any significant changes in α-syn mRNA with 27-OHC or LXR agonist and antagonistic treatments. However, we are the first to show that 27-OHC can inhibit proteasomal function. Also, 27-OHC decreases levels of HSP70 protein which is involved in protein folding, and protein degradation through the UPS and three major types of autophagy: macroautophagy, microautophagy, and chaperone-mediated autophagy (Fernández-Fernández et al., 2017). The extent to which a decrease in HSP70 protein levels leads to decreased protein folding and degradation through specific pathways needs to be further elucidated. Restoring proteasomal function and HSP70 protein levels may attenuate the 27-OHC induced increase in α-syn protein levels and needs to be further investigated.

In future studies we aim to assess the role 27-OHC has on individual enzymatic activities involved in the ubiquitin-dependent proteasomal degradation of α-syn in order to get a more fulfilling insight into the functionality of the UPS. We would also like to further investigate the mechanism by which 27-OHC is inhibiting the proteasomal degradation of α-syn. It may be through direct damage or inhibition of the proteasomal machinery and/or possibly increase the

propensity of α-syn to oligomerize which would not allow it to be properly degraded by the proteasome. Finally, we would also look to investigate the role of and mechanism by which 27-OHC effects HSP70 and identify specifically which type of degradation machinery is affected. It may be the UPS, autophagy, or both and remains to be determined.

Palmitic Acid and Parkinson's Disease

Palmitic acid (16:0), the most abundant saturated fatty acid in the body and diet, is synthesized *de novo* in the body and makes up 24% of total fatty acids in our blood and 28% of total fatty acids in our CSF (Guest et al., 2013). It has received much attention in *in vitro* and *in vivo* work. It has been shown to increase ER stress (Marwarha et al., 2016b), increase proinflammatory cytokines in astrocytes and microglia (Gupta et al., 2012; Tracy et al., 2013), activation of TLRs via NFKβ (Oberbach et al., 2012), and promote insulin resistance via phosphorylation of the insulin receptor (Reynoso, Salgado, & Calderón, 2003; Sears & Perry, 2015). While these findings are very important, much of *in vitro* work exposes cells to high levels of palmitic acid without any other fatty acids present which leaves this research inconclusive to the role of palmitic acid incorporated into a diet consisting of many other fatty acids and other nutrients. In human research studies, saturated fatty acids have shown positive associations (Anderson et al., 1999b; Honglei Chen, Zhang, Hernán, Willett, et al., 2003c; Johnson et al., 1999c; Giancarlo Logroscino et al., 1996b) and no significant relationship with PD risk (Honglei Chen et al., 2002; Honglei Chen, Zhang, Hernán, Willett, et al., 2003c; Hellenbrand et al., 1996b; K M Powers et

al., 2003). While these studies are also very important for future research, they utilize food frequency questionnaires where individuals are encouraged to eat what they will and are encouraged to fill out questionnaires describing what was eaten. This type of survey can be hard to interpret because individuals may not accurately represent what, when, and how much was consumed.

In the first of two dissertation studies, to more accurately pinpoint specific fatty acid contributions to PD risk we fed two strains of mice an isocaloric diet to control chow consisting of a slight increase in PA. We determined the extent to which a PA enriched diet affects B6D2 control and the m-Thy1 mouse model of PD biochemically.

We found that a PA enriched diet increases α-syn protein and mRNA expression in both strains of mice. It is possible that α-syn is increased following administration of PA because α-syn may function as a fatty acid binding protein that aids in the shuttling of PA around the cell (Lücke, Gantz, Klimtchuk, & Hamilton, 2006; R. Sharon et al., 2001). (Castagnet et al., 2005b) showed that when α-syn is ablated in primary astrocytes, PA incorporation into membranes is decreased. The increase in PA content in our study may therefore lead to increased α-syn expression which could function to properly traffic the excess PA to lipid membranes or to the mitochondria to be used for β-oxidation.

We found that a PA enriched diet increases TH protein and mRNA expression in both strains of mice. To the best of our knowledge, we are the first to show that a PA enriched diet can induce TH protein and mRNA expression. Short chained fatty acids have been shown to upregulate TH expression through

a cAMP dependent mechanism (DeCastro et al., 2005; Mally et al., 2004) while the role of long chain saturated and unsaturated fatty acids remains to be determined. Data not presented in this study showed that CREB protein levels are slightly upregulated in both strains of mice on the PA enriched diet. CREB has been shown to be the mediator by which cAMP upregulates TH expression in PC12 cells (Piech-Dumas & Tank, 1999). It is possible that longer chain fatty acids like palmitic acid may have the same effect on CREB activation but is yet to be determined. In addition to measuring CREB activity in this paradigm, we would also aim to pinpoint which specific receptors and intermediates are potentially involved in this mechanism.

Within the B6D2 mice on the differing diets we did not see any significant differences in levels of biogenic amines. However, the m-Thy1 mice showed much different results on the two diets. The findings of decreased dopamine content in m-Thy1 mice was very interesting as we have also shown that PA diet increases TH expression in these animals. It could be possible that m-Thy1 mice which overexpress normal human wildtype α-syn have vulnerable dopaminergic neurons to such a diet but concurrently increase the expression of TH in neurons. We also observed a significant increase in serotonin in the m-Thy1 animals on the PA diet. This increase in serotonin could be explained by the fact that saturated fatty acids have been shown to decrease serotonin binding to transporter (du Bois et al., 2006). The diet may cause less serotonin binding which could lead to an increase in serotonin production as a compensatory mechanism. The fact that these changes were only observed in the m-Thy1 mice

is perplexing. The data suggests the overexpression of α-syn must make these animals more vulnerable to changes in neurochemicals when exposed to diets of differing fat consistencies.

This data is of high importance for the study of PD. We have shown a diet enriched in PA has the ability of upregulating α-syn and TH, two very important proteins in PD, through mechanisms that are yet to be determined in these two strains of mice. Future studies will look to elucidate the aforementioned proposed mechanisms by which PA enriched diet increases α-syn and TH expression. Once the mechanisms are elucidated, pharmacological means can be utilized to block the mechanism of α-syn increase and others to enhance the mechanism of TH increase to stop or reverse the course of PD.

In our second study on PA contributions to risk in PD-type synucleinopathy, we determined the contribution of PA on expression levels of α-syn and TH in mouse dopaminergic neurons and within a MPTP induced C57BL/6 mouse model of parkinsonism.

We showed that PA treatment in mouse dopaminergic neurons decreased both α-syn and TH protein levels while it differentially regulated the mRNA of both genes. The mechanisms of such are yet to be determined. We then subjected C57Bl/6 mice to a 2 month PA enriched or control diet prior to injection with MPTP, a commonly utilized drug in *in vitro* and *in vivo* studies of PD (Meredith & Rademacher, 2011; Notter et al., 1988; Sriram et al., 1997). that has been used to model PD based on its ability to phenocopy the pathology and behavioral outcomes of PD. MPTP, first synthesized in 1947 (ZIERING & LEE,

1947), was not implicated as a potent drug that induces PD until the 1980s (Langston et al., 1983b). MPTP, itself, is not toxic but is lipid-soluble and readily crosses the blood-brain barrier (Langston et al., 1983b). Once within the brain, it is taken up by astrocytes that contain monoamine oxygenase B enzymes that convert MPTP into the toxic cation 1-methyl-4-phenylpyridinium (MPP^+) (Chiba et al., 1984; Langston et al., 1984; Ransom et al., 1987). MPP^+ is then released by astrocytes and selectively taken up by dopaminergic neurons via the dopamine transporter (Shen et al., 1985). Once inside dopaminergic neurons, it becomes concentrated in mitochondria and inhibits Complex I of the respiratory chain ultimately leading to their selective death (Ramsay et al., 1986). Mouse studies involving the administration of omega-3 polyunsaturated fatty acids (PUFAs) have shown beneficial against MPTP-induced effects (M. Bousquet et al., 2009; M Bousquet et al., 2008; Melanie Bousquet et al., 2011; Mélanie Bousquet et al., 2011; Coulombe et al., 2018), while diets high in fat either exacerbate the progression of parkinsonism by exhibiting enhanced dopamine depletion in the *substantia nigra*, striatum, and nigrostriatal pathway (M. Bousquet et al., 2012; Choi et al., 2005b; Morris et al., 2010; White et al., 2009) or have shown to to be protective against MPTP induced motor dysfunction and TH neuronal loss (X. Yang & Cheng, 2010a). However, some of these studies examined the effects of PUFAs and others utilized diets high in fat and were not isocaloric to the control diet. Within the MPTP animal model, we are the first to show that a PA enriched diet is capable of rescuing motor function, increasing TH protein, decreasing α-

syn protein, and increasing survival of dopaminergic neurons in MPTP injected animals.

We found that the PA-enriched diet was protective against motor dysfunction caused by the injection of MPTP. This is in accord with other studies showing that a ketogenic diet is protective against MPTP induced motor dysfunction (Shaafi et al., 2016; X. Yang & Cheng, 2010b). It has been suggested that beta-hydroxybutyrate is protective against MPTP induced neurodegeneration and is mediated by improved oxidative phosphorylation leading to enhanced ATP production (Tieu et al., 2003). PA can be converted into beta-hydroxybutyrate (Mashek & Grummer, 2003; Palmquist, 1972) which may be one mechanism by which a PA enriched diet is protective against MPTP.

MPTP has been shown to increase α-syn protein in the dopaminergic neurons of the *substantia nigra* (Fornai et al., 2005; Forno et al., 1993; Sonsalla & Heikkila, 1986; Vila et al., 2000a). In our study we showed that a PA enriched diet significantly reduces the MPTP induced increase in α-syn protein. I*n vitro* studies have shown that PUFAs increase α-syn oligomerization and insoluble aggregate formation while SFAs do not (Assayag et al., 2007; Ronit Sharon et al., 2003). Studies with rodent models also reported that diets high in PUFA concentration upregulated α-syn expression (Barceló-Coblijn et al., 2003; Kitajka et al., 2004). It is possible that the increase in the saturated fatty acid PA has opposing effects to PUFA's which leads to the decrease in α-syn protein content or possibly because PA remodels the mitochondrial membrane rendering it less vulnerable to the effects of MPTP.

We found that a PA diet in MPTP and saline injected mice increased the protein levels of TH, which is a very important enzyme in the synthesis of dopamine (Sjoerdsma et al., 1965) and the protein levels of $pS^{40}TH$, the most activated form of TH (Dunkley et al., 2004b). This is in accordance with the administration of a ketogenic diet in conjunction with MPTP (X. Yang & Cheng, 2010a) while the mechanisms remain elusive. Short-chained fatty acids have been shown to upregulate TH expression through a cAMP-dependent mechanism (DeCastro et al., 2005; Mally et al., 2004), while the roles of long-chain saturated and unsaturated fatty acids remain to be determined. To the best of our knowledge, we are the first to show that a PA-enriched diet can induce TH protein expression and additionally that a PA-enriched diet leads to significantly higher survival of dopaminergic neurons in the *substantia nigra* of MPTP injected animals although a ketogenic diet has shown similar effects (X. Yang & Cheng, 2010a).

This data is highly important to the study of PD-type synucleinopathy. We have shown that PA treatment alone in mouse dopaminergic neurons can modulate key proteins involved in disease risk in a much different way than a diet enriched in PA affects an animal model of PD. We also show that a diet enriched in PA is protective against the motor dysfunction, α-syn accumulation, TH decrease, and loss of dopaminergic neurons caused by MPTP administration. In future studies we would look to elucidate the mechanisms of action by which a PA diet can increase TH protein levels and examine how this diet is protective against the MPTP induced motor dysfunction, increase in α-syn, and loss of

dopaminergic neurons in the *substantia nigra*. Investigating how palmitic acid affects the mitochondria and potentially remodels the membrane to decrease its susceptibility to MPTP would be one of the first things to further explore. We would also investigate if a PA diet increases the amount of beta-hydroxybutyrate which could lead to enhanced oxidative phosphorylation and increased ATP production. Once the modes of action for a PA diets protective effects against MPTP are elucidated dietary intervention and/or pharmacological means can be utilized to slow or stop the progression of PD-type synucleinopathy.

Summary

The work presented in these studies suggest diet plays a major role in the expression of α-syn and TH, two key proteins involved in synucleinopathies (Fig. 48). 27-OHC increases α-syn protein by inhibiting its proteasomal degradation and decreasing HSP70, a key chaperone protein that is involved in many different degradation pathways. Palmitic Acid differentially effects α-syn and TH levels depending on the model system utilized and mode of administration. PA treatment alone in differentiated mouse dopaminergic neurons leads to a decrease in α-syn and TH protein content. In m-Thy1 and B6D2 mice a PA-enriched diet increases both α-syn and TH protein and mRNA expression. The diet does not affect biogenic amine content in B6D2 mice but significantly changes dopamine and serotonin levels in m-Thy1 mice relative to control fed animals. In C57Bl/6 mice treated with MPTP a PA-enriched diet decreases α-syn and increases TH and $pS^{40}TH$ protein levels. It also rescues motor function and increases survival of dopaminergic neurons.

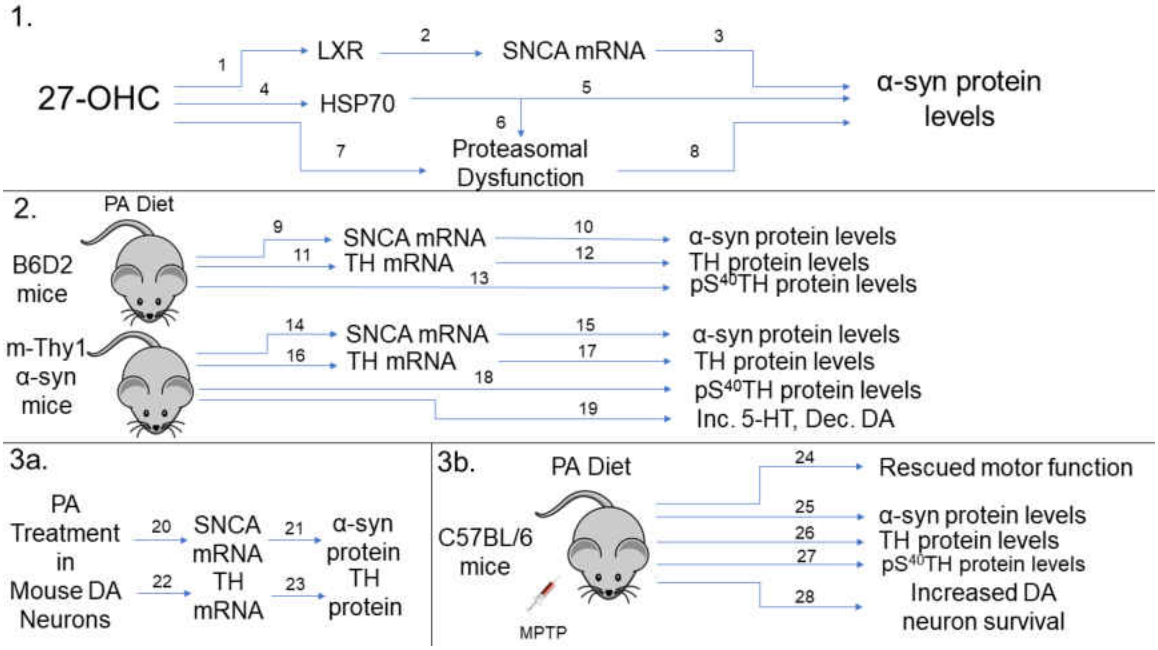


Figure 48. Summary figure. Human Dopaminergic neurons were treated with 27-OHC. (1) Treatment activated LXR receptors, (2) activated LXRs did not effect SNCA mRNA, (3) LXR agonistic and antagonistic treatments did not effect α-syn protein levels, (4) decreased HSP70 protein levels, (5) decreased HSP70 protein levels may be involved in the accumulation of α-syn protein via diminished degradatory pathway functions, (6) decreased HSP70 may be involved in proteasomal dysfunction, (7) 27-OHC decreased proteasomal function which leads to (8) increased α-syn protein levels. 2. B6D2 mice were fed a PA-enriched diet. The PA-enriched diet (9) increased SNCA mRNA, (10) increased α-syn protein levels, (11) increased TH mRNA, (12) increased TH protein levels, (13) increased $pS^{40}TH$ protein levels. m-Thy1 α-syn mice were fed a PA-enriched diet. The PA-enriched diet (14) increased SNCA mRNA, (15) increased α-syn protein levels, (16) increased TH mRNA levels, (17) increased TH protein levels, (18) increased $pS^{40}TH$ protein levels, and (19) increased 5-HT content and decreased DA content. 3a. Mouse dopaminergic neurons were treated with PA. Treatment decreased SNCA mRNA (20) and α-syn protein levels (21). Treatment increased TH mRNA levels (22) and decreased TH protein levels (23). 3b. C57BL/6 mice were fed a PA-enriched diet and subjected to MPTP injections. The PA-enriched diet rescued motor function (24), decreased α-syn protein levels (25), increased TH protein levels (26), increased $pS^{40}TH$ protein levels (27), and increased DA neuron survival (28).

REFERENCES

Aarsland, D., Andersen, K., Larsen, J. P., Lolk, A., Nielsen, H., & Kragh-Sørensen, P. (2001). Risk of dementia in Parkinson's disease: a community-based, prospective study. *Neurology*, *56*(6), 730–6. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11274306

Aarsland, D., Tandberg, E., Larsen, J. P., & Cummings, J. L. (1996). Frequency of dementia in Parkinson disease. *Archives of Neurology*, *53*(6), 538–42. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8660156

Abbott, R. D., Ross, G. W., White, L. R., Sanderson, W. T., Burchfiel, C. M., Kashon, M., … Petrovitch, H. (2003). Environmental, life-style, and physical precursors of clinical Parkinson's disease: recent findings from the Honolulu-Asia Aging Study. *Journal of Neurology*, *250 Suppl 3*(0), III30-9. http://doi.org/10.1007/s00415-003-1306-7

Abbott, R. D., Webster Ross, G., White, L. R., Sanderson, W. T., Burchfiel, C. M., Kashon, M., … Petrovitch, H. (2003a). Environmental, life-style, and physical precursors of clinical Parkinson?s disease: recent findings from the Honolulu-Asia Aging Study. *Journal of Neurology*, *250*, 1–1. http://doi.org/10.1007/s00415-003-1306-7

Abbott, R. D., Webster Ross, G., White, L. R., Sanderson, W. T., Burchfiel, C. M., Kashon, M., … Petrovitch, H. (2003b). Environmental, life-style, and physical precursors of clinical Parkinson?s disease: recent findings from the Honolulu-Asia Aging Study. *Journal of Neurology*, *250*(0), 1–1. http://doi.org/10.1007/s00415-003-1306-7

Abbott, R. D., Webster Ross, G., White, L. R., Sanderson, W. T., Burchfiel, C. M., Kashon, M., … Petrovitch, H. (2003c). Environmental, life-style, and physical precursors of clinical Parkinson?s disease: recent findings from the Honolulu-Asia Aging Study. *Journal of Neurology*, *250*(0), 1–1. http://doi.org/10.1007/s00415-003-1306-7

Acetyl Coenzyme A (Molecular Biology). (n.d.). Retrieved April 11, 2018, from http://what-when-how.com/molecular-biology/acetyl-coenzyme-a-molecular-biology/

Adamczyk, A., Kaźmierczak, A., & Strosznajder, J. B. (2006). α-Synuclein and its neurotoxic fragment inhibit dopamine uptake into rat striatal synaptosomes. *Neurochemistry International*, *49*(4), 407–412. http://doi.org/10.1016/j.neuint.2006.01.025

Adams, J. D., Chang, M. L., & Klaidman, L. (2001). Parkinson's disease--redox mechanisms. *Current Medicinal Chemistry*, *8*(7), 809–14. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11375751

Ahmed, Z., Asi, Y. T., Sailer, A., Lees, A. J., Houlden, H., Revesz, T., & Holton, J. L. (2012). The neuropathology, pathophysiology and genetics of multiple system atrophy. *Neuropathology and Applied Neurobiology*, *38*(1), 4–24. http://doi.org/10.1111/j.1365-2990.2011.01234.x

Alavanja, M. C., Sandler, D. P., McMaster, S. B., Zahm, S. H., McDonnell, C. J., Lynch, C. F., … Blair, A. (1996). The Agricultural Health Study. *Environmental Health Perspectives*, *104*(4), 362–9. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8732939

Albert, K. A., Helmer-Matyjek, E., Nairn, A. C., Müller, T. H., Haycock, J. W., Greene, L. A., … Greengard, P. (1984). Calcium/phospholipid-dependent protein kinase (protein kinase C) phosphorylates and activates tyrosine hydroxylase. *Proceedings of the National Academy of Sciences of the United States of America*, *81*(24), 7713–7. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/6151178

Alcalay, R. N., Caccappolo, E., Mejia-Santana, H., Tang, M. X., Rosado, L., Ross, B. M., … Clark, L. N. (2010). Frequency of known mutations in early-onset Parkinson disease: implication for genetic counseling: the consortium on risk for early onset Parkinson disease study. *Archives of Neurology*, *67*(9), 1116–22. http://doi.org/10.1001/archneurol.2010.194

Alim, M. A., Hossain, M. S., Arima, K., Takeda, K., Izumiyama, Y., Nakamura, M., … Ueda, K. (2002). Tubulin seeds alpha-synuclein fibril formation. *The Journal of Biological Chemistry*, *277*(3), 2112–7. http://doi.org/10.1074/jbc.M102981200

Alvarez-Castelao, B., Goethals, M., Vandekerckhove, J., & Castaño, J. G. (2014). Mechanism of cleavage of alpha-synuclein by the 20S proteasome and modulation of its degradation by the RedOx state of the N-terminal methionines. http://doi.org/10.1016/j.bbamcr.2013.11.018

Ammal Kaidery, N., Tarannum, S., & Thomas, B. (2013). Epigenetic landscape of Parkinson's disease: emerging role in disease mechanisms and therapeutic modalities. *Neurotherapeutics : The Journal of the American Society for Experimental NeuroTherapeutics*, *10*(4), 698–708. http://doi.org/10.1007/s13311-013-0211-8

Anderson, C., Checkoway, H., Franklin, G. M., Beresford, S., Smith-Weller, T., & Swanson, P. D. (1999a). Dietary factors in Parkinson's disease: the role of food groups and specific foods. *Movement Disorders : Official Journal of the Movement Disorder Society*, *14*(1), 21–7. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9918340

Anderson, C., Checkoway, H., Franklin, G. M., Beresford, S., Smith-Weller, T., & Swanson, P. D. (1999b). Dietary factors in Parkinson's disease: the role of food groups and specific foods. *Movement Disorders : Official Journal of the Movement Disorder Society*, *14*(1), 21–7. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9918340

Appel-Cresswell, S., Vilarino-Guell, C., Encarnacion, M., Sherman, H., Yu, I., Shah, B., … Farrer, M. J. (2013). Alpha-synuclein p.H50Q, a novel pathogenic mutation for Parkinson's disease. *Movement Disorders*, *28*(6), 811–813. http://doi.org/10.1002/mds.25421

Aprile, F. A., Arosio, P., Fusco, G., Chen, S. W., Kumita, J. R., Dhulesia, A., … Cremades, N. (2017). Inhibition of α-Synuclein Fibril Elongation by Hsp70 Is Governed by a Kinetic Binding Competition between α-Synuclein Species. *Biochemistry*, *56*(9), 1177–1180. http://doi.org/10.1021/acs.biochem.6b01178

Arawaka, S., Wada, M., Goto, S., Karube, H., Sakamoto, M., Ren, C.-H., … Kato, T. (2006). The role of G-protein-coupled receptor kinase 5 in pathogenesis of sporadic Parkinson's disease. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *26*(36), 9227–38. http://doi.org/10.1523/JNEUROSCI.0341-06.2006

Aridon, P., Geraci, F., Turturici, G., D'Amelio, M., Savettieri, G., & Sconzo, G. (2011). Protective Role of Heat Shock Proteins in Parkinson's Disease. *Neurodegenerative Diseases*, *8*(4), 155–168. http://doi.org/10.1159/000321548

Ascherio, A., Weisskopf, M. G., O'Reilly, E. J., McCullough, M. L., Calle, E. E., Rodriguez, C., & Thun, M. J. (2004). Coffee consumption, gender, and Parkinson's disease mortality in the cancer prevention study II cohort: the modifying effects of estrogen. *American Journal of Epidemiology*, *160*(10), 977–84. http://doi.org/10.1093/aje/kwh312

Ascherio, A., Zhang, S. M., Hernán, M. A., Kawachi, I., Colditz, G. A., Speizer, F. E., & Willett, W. C. (2001). Prospective study of caffeine consumption and risk of Parkinson's disease in men and women. *Annals of Neurology*, *50*(1), 56–63. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11456310

Asi, Y. T., Simpson, J. E., Heath, P. R., Wharton, S. B., Lees, A. J., Revesz, T., … Holton, J. L. (2014). Alpha-synuclein mRNA expression in oligodendrocytes in MSA. *Glia*, *62*(6), 964–970. http://doi.org/10.1002/glia.22653

Assayag, K., Yakunin, E., Loeb, V., Selkoe, D. J., & Sharon, R. (2007). Polyunsaturated fatty acids induce alpha-synuclein-related pathogenic changes in neuronal cells. *The American Journal of Pathology*, *171*(6), 2000–11. http://doi.org/10.2353/ajpath.2007.070373

Austin, S. A., Floden, A. M., Murphy, E. J., & Combs, C. K. (2006). -Synuclein Expression Modulates Microglial Activation Phenotype. *Journal of Neuroscience*, *26*(41), 10558–10563. http://doi.org/10.1523/JNEUROSCI.1799-06.2006

Bakshi, R., Zhang, H., Logan, R., Joshi, I., Xu, Y., Chen, X., & Schwarzschild, M. A. (2015). Neuroprotective effects of urate are mediated by augmenting astrocytic glutathione synthesis and release. *Neurobiology of Disease*, *82*, 574–579. http://doi.org/10.1016/j.nbd.2015.08.022

Baldereschi, M., Di Carlo, A., Rocca, W. A., Vanni, P., Maggi, S., Perissinotto, E., … Inzitari, D. (2000). Parkinson's disease and parkinsonism in a longitudinal study: two-fold higher incidence in men. ILSA Working Group. Italian Longitudinal Study on Aging. *Neurology*, *55*(9), 1358–63. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11087781

Barceló-Coblijn, G., Kitajka, K., Puskás, L. G., Hogyes, E., Zvara, A., Hackler, L., & Farkas, T. (2003). Gene expression and molecular composition of phospholipids in rat brain in relation to dietary n-6 to n-3 fatty acid ratio. *Biochimica et Biophysica Acta*, *1632*(1–3), 72–9. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12782153

Bartels, T., Kim, N. C., Luth, E. S., & Selkoe, D. J. (2014). N-alpha-acetylation of α-synuclein increases its helical folding propensity, GM1 binding specificity and resistance to aggregation. *PloS One*, *9*(7), e103727. http://doi.org/10.1371/journal.pone.0103727

Basso, E., Antas, P., Marijanovic, Z., Gonçalves, S., Tenreiro, S., & Outeiro, T. F. (2013). PLK2 modulates α-synuclein aggregation in yeast and mammalian cells. *Molecular Neurobiology*, *48*(3), 854–62. http://doi.org/10.1007/s12035-013-8473-z

Becker, C., Jick, S. S., & Meier, C. R. (2008a). Use of antihypertensives and the risk of Parkinson disease. *Neurology*, *70*(16 Pt 2), 1438–44. http://doi.org/10.1212/01.wnl.0000303818.38960.44

Becker, C., Jick, S. S., & Meier, C. R. (2008b). Use of statins and the risk of Parkinson's disease: a retrospective case-control study in the UK. *Drug Safety*, *31*(5), 399–407. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/18422380

Benarroch, E. E. (1993). The central autonomic network: functional organization, dysfunction, and perspective. *Mayo Clinic Proceedings*, *68*(10), 988–1001. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8412366

Bendor, J. T., Logan, T. P., & Edwards, R. H. (2013). The function of α-synuclein. *Neuron*, *79*(6), 1044–66. http://doi.org/10.1016/j.neuron.2013.09.004

Bennett, M. C., Bishop, J. F., Leng, Y., Chock, P. B., Chase, T. N., & Mouradian, M. M. (1999a). Degradation of alpha-synuclein by proteasome. *The Journal of Biological Chemistry*, *274*(48), 33855–8. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10567343

Bennett, M. C., Bishop, J. F., Leng, Y., Chock, P. B., Chase, T. N., & Mouradian, M. M. (1999b). Degradation of alpha-synuclein by proteasome. *The Journal of Biological Chemistry*, *274*(48), 33855–8. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10567343

Bensimon, G., Ludolph, A., Agid, Y., Vidailhet, M., Payan, C., Leigh, P. N., & NNIPPS Study Group. (2009). Riluzole treatment, survival and diagnostic criteria in Parkinson plus disorders: The NNIPPS Study. *Brain*, *132*(1), 156–171. http://doi.org/10.1093/brain/awn291

Berg, J. M., Tymoczko, J. L., & Stryer, L. (2002). Important Derivatives of Cholesterol Include Bile Salts and Steroid Hormones. Retrieved from https://www.ncbi.nlm.nih.gov/books/NBK22339/

Berresheim, U., & Kuhn, D. M. (1994). Dephosphorylation of tyrosine hydroxylase by brain protein phosphatases: a predominant role for type 2A. *Brain Research*, *637*(1–2), 273–6. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7910102

Bertolotti, M., Del Puppo, M., Corna, F., Anzivino, C., Gabbi, C., Baldelli, E., … Carulli, N. (2012). Increased appearance rate of 27-hydroxycholesterol in vivo in hypercholesterolemia: A possible compensatory mechanism. *Nutrition, Metabolism and Cardiovascular Diseases*, *22*(10), 823–830. http://doi.org/10.1016/j.numecd.2011.02.009

Bevilaqua, L. R. M., Cammarota, M., Dickson, P. W., Sim, A. T. R., & Dunkley, P. R. (2003). Role of protein phosphatase 2C from bovine adrenal chromaffin cells in the dephosphorylation of phospho-serine 40 tyrosine hydroxylase. *Journal of Neurochemistry*, *85*(6), 1368–73. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12787056

Beyer, K., Domingo-Sàbat, M., Humbert, J., Carrato, C., Ferrer, I., & Ariza, A. (2008). Differential expression of alpha-synuclein, parkin, and synphilin-1 isoforms in Lewy body disease. *Neurogenetics*, *9*(3), 163–72. http://doi.org/10.1007/s10048-008-0124-6

Bhattacharyya, S., Yu, H., Mim, C., & Matouschek, A. (2014). Regulated protein turnover: snapshots of the proteasome in action. *Nature Reviews Molecular Cell Biology*, *15*(2), 122–133. http://doi.org/10.1038/nrm3741

Björkhem, I. (2013). Five decades with oxysterols. *Biochimie*, *95*(3), 448–454. http://doi.org/10.1016/j.biochi.2012.02.029

Björkhem, I., Cedazo-Minguez, A., Leoni, V., & Meaney, S. (2009). Oxysterols and neurodegenerative diseases. *Molecular Aspects of Medicine*, *30*(3), 171–9. http://doi.org/10.1016/j.mam.2009.02.001

Björkhem, I., & Diczfalusy, U. (2002). Oxysterols: friends, foes, or just fellow passengers? *Arteriosclerosis, Thrombosis, and Vascular Biology*, *22*(5), 734–42. http://doi.org/10.1161/01.ATV.0000013312.32196.49

Boot, B. P., Orr, C. F., Ahlskog, J. E., Ferman, T. J., Roberts, R., Pankratz, V. S., … Boeve, B. F. (2013). Risk factors for dementia with Lewy bodies: a case-control study. *Neurology*, *81*(9), 833–40. http://doi.org/10.1212/WNL.0b013e3182a2cbd1

Bosco, D. A., Fowler, D. M., Zhang, Q., Nieva, J., Powers, E. T., Wentworth, P., … Kelly, J. W. (2006). Elevated levels of oxidized cholesterol metabolites in Lewy body disease brains accelerate alpha-synuclein fibrilization. *Nature Chemical Biology*, *2*(5), 249–53. http://doi.org/10.1038/nchembio782

Bousquet, M., Calon, F., & Cicchetti, F. (2011). Impact of omega-3 fatty acids in Parkinson's disease. *Ageing Research Reviews*, *10*(4), 453–463. http://doi.org/10.1016/j.arr.2011.03.001

Bousquet, M., Gibrat, C., Saint-Pierre, M., Julien, C., Calon, F., & Cicchetti, F. (2009). Modulation of brain-derived neurotrophic factor as a potential neuroprotective mechanism of action of omega-3 fatty acids in a parkinsonian animal model. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *33*(8), 1401–1408. http://doi.org/10.1016/j.pnpbp.2009.07.018

Bousquet, M., Gue, K., Emond, V., Julien, P., Kang, J. X., Cicchetti, F., & Calon, F. (2011). Transgenic conversion of omega-6 into omega-3 fatty acids in a mouse model of Parkinson's disease. *Journal of Lipid Research*, *52*(2), 263–71. http://doi.org/10.1194/jlr.M011692

Bousquet, M., Saint-Pierre, M., Julien, C., Salem, N., Cicchetti, F., & Calon, F. (2008). Beneficial effects of dietary omega-3 polyunsaturated fatty acid on toxin-induced neuronal degeneration in an animal model of Parkinson's disease. *FASEB Journal : Official Publication of the Federation of American Societies for Experimental Biology*, *22*(4), 1213–25. http://doi.org/10.1096/fj.07-9677com

Bousquet, M., St-Amour, I., Vandal, M., Julien, P., Cicchetti, F., & Calon, F. (2012). High-fat diet exacerbates MPTP-induced dopaminergic degeneration in mice. *Neurobiology of Disease*, *45*(1), 529–38. http://doi.org/10.1016/j.nbd.2011.09.009

Bousquet, M., St-Amour, I., Vandal, M., Julien, P., Cicchetti, F., & Calon, F. (2012). High-fat diet exacerbates MPTP-induced dopaminergic degeneration in mice. *Neurobiology of Disease*, *45*(1), 529–538. http://doi.org/10.1016/j.nbd.2011.09.009

Bower, J. H., Maraganore, D. M., McDonnell, S. K., & Rocca, W. A. (1997). Incidence of progressive supranuclear palsy and multiple system atrophy in Olmsted County, Minnesota, 1976 to 1990. *Neurology*, *49*(5), 1284–8. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9371909

Braak, H., Del Tredici, K., Rüb, U., De Vos, R. A. I., Jansen Steur, E. N. H., & Braak, E. (2003). Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiology of Aging*, *24*(2), 197–211. http://doi.org/10.1016/S0197-4580(02)00065-9

Brenner, S., Wersinger, C., & Gasser, T. (2015). Transcriptional regulation of the α-synuclein gene in human brain tissue. *Neuroscience Letters*, *599*, 140–145. http://doi.org/10.1016/j.neulet.2015.05.029

Brown, D. R. (2010). Oligomeric alpha-synuclein and its role in neuronal death. *IUBMB Life*, *62*(5), NA-NA. http://doi.org/10.1002/iub.316

Burkard, I., von Eckardstein, A., Waeber, G., Vollenweider, P., & Rentsch, K. M. (2007). Lipoprotein distribution and biological variation of 24S- and 27-hydroxycholesterol in healthy volunteers. *Atherosclerosis*, *194*(1), 71–78. http://doi.org/10.1016/j.atherosclerosis.2006.09.026

Burre, J., Sharma, M., Tsetsenis, T., Buchman, V., Etherton, M. R., & Sudhof, T. C. (2010). -Synuclein Promotes SNARE-Complex Assembly in Vivo and in Vitro. *Science*, *329*(5999), 1663–1667. http://doi.org/10.1126/science.1195227

Butler, B., Saha, K., Rana, T., Becker, J. P., Sambo, D., Davari, P., … Khoshbouei, H. (2015). Dopamine Transporter Activity Is Modulated by α-Synuclein. *Journal of Biological Chemistry*, *290*(49), 29542–29554. http://doi.org/10.1074/jbc.M115.691592

Cabeza-Arvelaiz, Y., Fleming, S. M., Richter, F., Masliah, E., Chesselet, M.-F., & Schiestl, R. H. (2011). Analysis of striatal transcriptome in mice overexpressing human wild-type alpha-synuclein supports synaptic dysfunction and suggests mechanisms of neuroprotection for striatal neurons. *Molecular Neurodegeneration*, *6*, 83. http://doi.org/10.1186/1750-1326-6-83

Calder, P. C. (2015). Functional Roles of Fatty Acids and Their Effects on Human Health. *Journal of Parenteral and Enteral Nutrition*, *39*(1_suppl), 18S–32S. http://doi.org/10.1177/0148607115595980

Callaghan, R. C., Cunningham, J. K., Sajeev, G., & Kish, S. J. (2010). Incidence of Parkinson's disease among hospital patients with methamphetamine-use disorders. *Movement Disorders : Official Journal of the Movement Disorder Society*, *25*(14), 2333–9. http://doi.org/10.1002/mds.23263

Campbell, D. G., Hardie, D. G., & Vulliet, P. R. (1986a). Identification of four phosphorylation sites in the N-terminal region of tyrosine hydroxylase. *The Journal of Biological Chemistry*, *261*(23), 10489–92. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2874140

Campbell, D. G., Hardie, D. G., & Vulliet, P. R. (1986b). Identification of four phosphorylation sites in the N-terminal region of tyrosine hydroxylase. *The Journal of Biological Chemistry*, *261*(23), 10489–92. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2874140

Carroll, J. M., Kim, K. S., Kim, K. T., Goodman, H. M., & Joh, T. H. (1991). Effects of second messenger system activation on functional expression of tyrosine hydroxylase fusion gene constructs in neuronal and nonneuronal cells. *Journal of Molecular Neuroscience : MN*, *3*(2), 65–74. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/1687657

Castagnet, P. I., Golovko, M. Y., Barceló-Coblijn, G. C., Nussbaum, R. L., & Murphy, E. J. (2005a). Fatty acid incorporation is decreased in astrocytes cultured from α-synuclein gene-ablated mice. *Journal of Neurochemistry*, *94*(3), 839–849. http://doi.org/10.1111/j.1471-4159.2005.03247.x

Castagnet, P. I., Golovko, M. Y., Barceló-Coblijn, G. C., Nussbaum, R. L., & Murphy, E. J. (2005b). Fatty acid incorporation is decreased in astrocytes cultured from α-synuclein gene-ablated mice. *Journal of Neurochemistry*, *94*(3), 839–849. http://doi.org/10.1111/j.1471-4159.2005.03247.x

Chandra, S., Gallardo, G., Fernández-Chacón, R., Schlüter, O. M., & Südhof, T. C. (2005). α-Synuclein Cooperates with CSPα in Preventing Neurodegeneration. *Cell*, *123*(3), 383–396. http://doi.org/10.1016/j.cell.2005.09.028

Chaudhuri, K. R., & Schapira, A. H. V. (2009). Non-motor symptoms of Parkinson's disease: dopaminergic pathophysiology and treatment. *The Lancet. Neurology*, *8*(5), 464–74. http://doi.org/10.1016/S1474-4422(09)70068-7

Chen, H., Huang, X., Guo, X., Mailman, R. B., Park, Y., Kamel, F., … Blair, A. (2010). Smoking duration, intensity, and risk of Parkinson disease. *Neurology*, *74*(11), 878–84. http://doi.org/10.1212/WNL.0b013e3181d55f38

Chen, H., Zhang, S. M., Hernán, M. A., Schwarzschild, M. A., Willett, W. C., Colditz, G. A., … Ascherio, A. (2003). Nonsteroidal anti-inflammatory drugs and the risk of Parkinson disease. *Archives of Neurology*, *60*(8), 1059–64. http://doi.org/10.1001/archneur.60.8.1059

Chen, H., Zhang, S. M., Hernán, M. A., Willett, W. C., & Ascherio, A. (2002). Diet and Parkinson's disease: A potential role of dairy products in men. *Annals of Neurology*, *52*(6), 793–801. http://doi.org/10.1002/ana.10381

Chen, H., Zhang, S. M., Hernán, M. A., Willett, W. C., & Ascherio, A. (2003a). Dietary intakes of fat and risk of Parkinson's disease. *American Journal of Epidemiology*, *157*(11), 1007–14. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12777364

Chen, H., Zhang, S. M., Hernán, M. A., Willett, W. C., & Ascherio, A. (2003b). Dietary intakes of fat and risk of Parkinson's disease. *American Journal of Epidemiology*, *157*(11), 1007–14. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12777364

Chen, H., Zhang, S. M., Hernán, M. A., Willett, W. C., & Ascherio, A. (2003c). Dietary intakes of fat and risk of Parkinson's disease. *American Journal of Epidemiology*, *157*(11), 1007–14. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12777364

Chen, H., Zhang, S. M., Schwarzschild, M. A., Hernán, M. A., & Ascherio, A. (2005). Physical activity and the risk of Parkinson disease. *Neurology*, *64*(4), 664–9. http://doi.org/10.1212/01.WNL.0000151960.28687.93

Chen, L., & Feany, M. B. (2005). Alpha-synuclein phosphorylation controls neurotoxicity and inclusion formation in a Drosophila model of Parkinson disease. *Nature Neuroscience*, *8*(5), 657–63. http://doi.org/10.1038/nn1443

Chen, L., Jin, J., Davis, J., Zhou, Y., Wang, Y., Liu, J., … Zhang, J. (2007). Oligomeric alpha-synuclein inhibits tubulin polymerization. *Biochemical and Biophysical Research Communications*, *356*(3), 548–53. http://doi.org/10.1016/j.bbrc.2007.02.163

Chen, L., Periquet, M., Wang, X., Negro, A., McLean, P. J., Hyman, B. T., & Feany, M. B. (2009). Tyrosine and serine phosphorylation of α-synuclein have opposing effects on neurotoxicity and soluble oligomer formation. *Journal of Clinical Investigation*, *119*(11), 3257–65. http://doi.org/10.1172/JCI39088

Chen, X., Burdett, T. C., Desjardins, C. A., Logan, R., Cipriani, S., Xu, Y., & Schwarzschild, M. A. (2013). Disrupted and transgenic urate oxidase alter urate and dopaminergic neurodegeneration. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(1), 300–5. http://doi.org/10.1073/pnas.1217296110

Cheng, D., Jenner, A. M., Shui, G., Cheong, W. F., Mitchell, T. W., Nealon, J. R., … Garner, B. (2011a). Lipid Pathway Alterations in Parkinson's Disease Primary Visual Cortex. *PLoS ONE*, *6*(2), e17299. http://doi.org/10.1371/journal.pone.0017299

Cheng, D., Jenner, A. M., Shui, G., Cheong, W. F., Mitchell, T. W., Nealon, J. R., … Garner, B. (2011b). Lipid Pathway Alterations in Parkinson's Disease Primary Visual Cortex. *PLoS ONE*, *6*(2), e17299. http://doi.org/10.1371/journal.pone.0017299

Cheng, D., Kim, W. S., & Garner, B. (2008a). Regulation of α-synuclein expression by liver X receptor ligands in vitro. *NeuroReport*, *19*(17), 1685–1689. http://doi.org/10.1097/WNR.0b013e32831578b2

Cheng, D., Kim, W. S., & Garner, B. (2008b). Regulation of α-synuclein expression by liver X receptor ligands in vitro. *NeuroReport*, *19*(17), 1685–1689. http://doi.org/10.1097/WNR.0b013e32831578b2

Chesselet, M.-F., Richter, F., Zhu, C., Magen, I., Watson, M. B., & Subramaniam, S. R. (2012a). A progressive mouse model of Parkinson's disease: the Thy1-aSyn ("Line 61") mice. *Neurotherapeutics : The Journal of the American Society for Experimental NeuroTherapeutics*, *9*(2), 297–314. http://doi.org/10.1007/s13311-012-0104-2

Chesselet, M.-F., Richter, F., Zhu, C., Magen, I., Watson, M. B., & Subramaniam, S. R. (2012b). A progressive mouse model of Parkinson's disease: the Thy1-aSyn ("Line 61") mice. *Neurotherapeutics : The Journal of the American Society for Experimental NeuroTherapeutics*, *9*(2), 297–314. http://doi.org/10.1007/s13311-012-0104-2

Chesselet, M.-F., Richter, F., Zhu, C., Magen, I., Watson, M. B., & Subramaniam, S. R. (2012c). A Progressive Mouse Model of Parkinson's Disease: The Thy1-aSyn ("Line 61") Mice. *Neurotherapeutics*, *9*(2), 297–314. http://doi.org/10.1007/s13311-012-0104-2

Chesselet, M.-F., Richter, F., Zhu, C., Magen, I., Watson, M. B., & Subramaniam, S. R. (2012d). A Progressive Mouse Model of Parkinson's Disease: The Thy1-aSyn ("Line 61") Mice. *Neurotherapeutics*, *9*(2), 297–314. http://doi.org/10.1007/s13311-012-0104-2

Chiba-Falek, O., Kowalak, J. A., Smulson, M. E., & Nussbaum, R. L. (2005). Regulation of alpha-synuclein expression by poly (ADP ribose) polymerase-1 (PARP-1) binding to the NACP-Rep1 polymorphic site upstream of the SNCA gene. *American Journal of Human Genetics*, *76*(3), 478–92. http://doi.org/10.1086/428655

Chiba, K., Trevor, A., & Castagnoli, N. (1984). Metabolism of the neurotoxic tertiary amine, MPTP, by brain monoamine oxidase. *Biochemical and Biophysical Research Communications*, *120*(2), 574–8. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/6428396

Choi, J.-Y., Jang, E.-H., Park, C.-S., & Kang, J.-H. (2005a). Enhanced susceptibility to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine neurotoxicity in high-fat diet-induced obesity. *Free Radical Biology & Medicine*, *38*(6), 806–16. http://doi.org/10.1016/j.freeradbiomed.2004.12.008

Choi, J.-Y., Jang, E.-H., Park, C.-S., & Kang, J.-H. (2005b). Enhanced susceptibility to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine neurotoxicity in high-fat diet-induced obesity. *Free Radical Biology and Medicine*, *38*(6), 806–816. http://doi.org/10.1016/j.freeradbiomed.2004.12.008

Cholesterol: Synthesis, Metabolism, Regulation. (n.d.). Retrieved April 11, 2018, from http://themedicalbiochemistrypage.org/cholesterol.php

Chrysostome, V., Tison, F., Yekhlef, F., Sourgen, C., Baldi, I., & Dartigues, J. F. (2004). Epidemiology of Multiple System Atrophy: A Prevalence and Pilot Risk Factor Study in Aquitaine, France. *Neuroepidemiology*, *23*(4), 201–208. http://doi.org/10.1159/000078506

Clough, R. L., Dermentzaki, G., Haritou, M., Petsakou, A., & Stefanis, L. (2011). Regulation of α-synuclein expression in cultured cortical neurons. *Journal of Neurochemistry*, *117*(2), 275–285. http://doi.org/10.1111/j.1471-4159.2011.07199.x

Clough, R. L., Dermentzaki, G., & Stefanis, L. (2009). Functional dissection of the α-synuclein promoter: transcriptional regulation by ZSCAN21 and ZNF219. *Journal of Neurochemistry*, *110*(5), 1479–1490. http://doi.org/10.1111/j.1471-4159.2009.06250.x

Clough, R. L., & Stefanis, L. (2007). A novel pathway for transcriptional regulation of α-synuclein. *The FASEB Journal*, *21*(2), 596–607. http://doi.org/10.1096/fj.06-7111com

Collins, J. L., Fivush, A. M., Watson, M. A., Galardi, C. M., Lewis, M. C., Moore, L. B., … Willson, T. M. (2002). Identification of a nonsteroidal liver X receptor agonist through parallel array synthesis of tertiary amines. *Journal of Medicinal Chemistry*, *45*(10), 1963–6. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11985463

Cook, C., & Petrucelli, L. (2009). A critical evaluation of the ubiquitin–proteasome system in Parkinson's disease. *BBA - Molecular Basis of Disease*, *1792*, 664–675. http://doi.org/10.1016/j.bbadis.2009.01.012

Coulombe, K., Kerdiles, O., Tremblay, C., Emond, V., Lebel, M., Boulianne, A.-S., … Calon, F. (2018). Impact of DHA intake in a mouse model of synucleinopathy. *Experimental Neurology*, *301*(Pt A), 39–49. http://doi.org/10.1016/j.expneurol.2017.12.002

Crowther, R. A., Daniel, S. E., & Goedert, M. (2000). Characterisation of isolated alpha-synuclein filaments from *substantia nigra* of Parkinson's disease brain. *Neuroscience Letters*, *292*(2), 128–30. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10998565

Cuervo, A. M., Stefanis, L., Fredenburg, R., Lansbury, P. T., & Sulzer, D. (2004). Impaired Degradation of Mutant -Synuclein by Chaperone-Mediated Autophagy. *Science*, *305*(5688), 1292–1295. http://doi.org/10.1126/science.1101738

Cummings, J. L. (1988). Intellectual impairment in Parkinson's disease: clinical, pathologic, and biochemical correlates. *Journal of Geriatric Psychiatry and Neurology*, *1*(1), 24–36. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2908099

Curtin, K., Fleckenstein, A. E., Robison, R. J., Crookston, M. J., Smith, K. R., & Hanson, G. R. (2015). Methamphetamine/amphetamine abuse and risk of Parkinson's disease in Utah: a population-based assessment. *Drug and Alcohol Dependence*, *146*, 30–8. http://doi.org/10.1016/j.drugalcdep.2014.10.027

Damier, P., Hirsch, E. C., Agid, Y., & Graybiel, A. M. (1999). The *substantia nigra* of the human brain. II. Patterns of loss of dopamine-containing neurons in Parkinson's disease. *Brain : A Journal of Neurology*, *122 (Pt 8)*, 1437–48. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10430830

Dantuma, N. P., & Bott, L. C. (2014). The ubiquitin-proteasome system in neurodegenerative diseases: precipitating factor, yet part of the solution. *Frontiers in Molecular Neuroscience*, *7*, 70. http://doi.org/10.3389/fnmol.2014.00070

de Lau, L. M. L., Bornebroek, M., Witteman, J. C. M., Hofman, A., Koudstaal, P. J., & Breteler, M. M. B. (2005a). Dietary fatty acids and the risk of Parkinson disease: the Rotterdam study. *Neurology*, *64*(12), 2040–5. http://doi.org/10.1212/01.WNL.0000166038.67153.9F

de Lau, L. M. L., Bornebroek, M., Witteman, J. C. M., Hofman, A., Koudstaal, P. J., & Breteler, M. M. B. (2005b). Dietary fatty acids and the risk of Parkinson disease: the Rotterdam study. *Neurology*, *64*(12), 2040–5. http://doi.org/10.1212/01.WNL.0000166038.67153.9F

de Lau, L. M. L., Bornebroek, M., Witteman, J. C. M., Hofman, A., Koudstaal, P. J., & Breteler, M. M. B. (2005c). Dietary fatty acids and the risk of Parkinson disease: the Rotterdam study. *Neurology*, *64*(12), 2040–5. http://doi.org/10.1212/01.WNL.0000166038.67153.9F

de Lau, L. M. L., Bornebroek, M., Witteman, J. C. M., Hofman, A., Koudstaal, P. J., & Breteler, M. M. B. (2005). Dietary fatty acids and the risk of Parkinson disease: The Rotterdam Study. *Neurology*, *64*(12), 2040–2045. http://doi.org/10.1212/01.WNL.0000166038.67153.9F

de Lau, L. M. L., Stricker, B. H. C., & Breteler, M. M. B. (2007). Serum cholesterol, use of lipid-lowering drugs, and risk of Parkinson disease. *Movement Disorders : Official Journal of the Movement Disorder Society*, *22*(13), 1985. http://doi.org/10.1002/mds.21582

Deacon, R. M. J. (2013). Measuring the Strength of Mice. *Journal of Visualized Experiments*, (76). http://doi.org/10.3791/2610

DeCastro, M., Nankova, B. B., Shah, P., Patel, P., Mally, P. V., Mishra, R., & La Gamma, E. F. (2005). Short chain fatty acids regulate tyrosine hydroxylase gene expression through a cAMP-dependent signaling pathway. *Molecular Brain Research*, *142*(1), 28–38. http://doi.org/10.1016/j.molbrainres.2005.09.002

Dedmon, M. M., Christodoulou, J., Wilson, M. R., & Dobson, C. M. (2005). Heat shock protein 70 inhibits alpha-synuclein fibril formation via preferential binding to prefibrillar species. *The Journal of Biological Chemistry*, *280*(15), 14733–40. http://doi.org/10.1074/jbc.M413024200

Dementia with Lewy bodies: diagnostic and predictive biomarkers - ppt video online download. (n.d.). Retrieved December 15, 2017, from http://slideplayer.com/slide/9263923/

Dementia with Lewy Bodies | Family Caregiver Alliance. (n.d.). Retrieved January 18, 2018, from https://www.caregiver.org/dementia-lewy-bodies

Desplats, P., Spencer, B., Coffee, E., Patel, P., Michael, S., Patrick, C., … Masliah, E. (2011). Alpha-synuclein sequesters Dnmt1 from the nucleus: a novel mechanism for epigenetic alterations in Lewy body diseases. *The Journal of Biological Chemistry*, *286*(11), 9031–7. http://doi.org/10.1074/jbc.C110.212589

Desplats, P., Spencer, B., Crews, L., Pathel, P., Morvinski-Friedmann, D., Kosberg, K., … Masliah, E. (2012). α-Synuclein induces alterations in adult neurogenesis in Parkinson disease models via p53-mediated repression of Notch1. *The Journal of Biological Chemistry*, *287*(38), 31691–702. http://doi.org/10.1074/jbc.M112.354522

Diao, J., Burré, J., Vivona, S., Cipriano, D. J., Sharma, M., Kyoung, M., … Brunger, A. T. (2013). Native α-synuclein induces clustering of synaptic-vesicle mimics via binding to phospholipids and synaptobrevin-2/VAMP2. *ELife*, *2*, e00592. http://doi.org/10.7554/eLife.00592

Dickson, D. W. (2002). Dementia with Lewy Bodies: Neuropathology. *Journal of Geriatric Psychiatry and Neurology*, *15*(4), 210–216. http://doi.org/10.1177/089198870201500406

Dietschy, J. M. (1984). Regulation of cholesterol metabolism in man and in other species. *Klinische Wochenschrift*, *62*(8), 338–45. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/6328101

Dijkstra, A. A., Voorn, P., Berendse, H. W., Groenewegen, H. J., Netherlands Brain Bank, A. J. M., Rozemuller, A. J. M., & van de Berg, W. D. J. (2014). Stage-dependent nigral neuronal loss in incidental Lewy body and Parkinson's disease. *Movement Disorders : Official Journal of the Movement Disorder Society*, *29*(10), 1244–51. http://doi.org/10.1002/mds.25952

Dikiy, I., & Eliezer, D. (2014). N-terminal acetylation stabilizes N-terminal helicity in lipid- and micelle-bound α-synuclein and increases its affinity for physiological membranes. *The Journal of Biological Chemistry*, *289*(6), 3652–65. http://doi.org/10.1074/jbc.M113.512459

Dong, J., Beard, J. D., Umbach, D. M., Park, Y., Huang, X., Blair, A., … Chen, H. (2014). Dietary fat intake and risk for Parkinson's disease. *Movement Disorders : Official Journal of the Movement Disorder Society*, *29*(13), 1623–30. http://doi.org/10.1002/mds.26032

Doxakis, E. (2010). Post-transcriptional Regulation of α-Synuclein Expression by mir-7 and mir-153. *Journal of Biological Chemistry*, *285*(17), 12726–12734. http://doi.org/10.1074/jbc.M109.086827

Dra. Carla Abdelnour Ruiz. (n.d.). Dementia with Lewy bodies: diagnostic and predictive biomarkers. Retrieved April 11, 2018, from http://slideplayer.com/slide/9263923/

Driver, J. A., Smith, A., Buring, J. E., Gaziano, J. M., Kurth, T., & Logroscino, G. (2008). Prospective cohort study of type 2 diabetes and the risk of Parkinson's disease. *Diabetes Care*, *31*(10), 2003–5. http://doi.org/10.2337/dc08-0688

du Bois, T. M., Deng, C., Bell, W., & Huang, X.-F. (2006). Fatty acids differentially affect serotonin receptor and transporter binding in the rat brain. *Neuroscience*, *139*(4), 1397–1403. http://doi.org/10.1016/j.neuroscience.2006.02.068

Duan, W., Ladenheim, B., Cutler, R. G., Kruman, I. I., Cadet, J. L., & Mattson, M. P. (2002). Dietary folate deficiency and elevated homocysteine levels endanger dopaminergic neurons in models of Parkinson's disease. *Journal of Neurochemistry*, *80*(1), 101–10. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11796748

Dunkley, P. R., Bobrovskaya, L., Graham, M. E., Von Nagy-Felsobuki, E. I., & Dickson, P. W. (2004a). Tyrosine hydroxylase phosphorylation: regulation and consequences. *Journal of Neurochemistry*, *91*(5), 1025–1043. http://doi.org/10.1111/j.1471-4159.2004.02797.x

Dunkley, P. R., Bobrovskaya, L., Graham, M. E., Von Nagy-Felsobuki, E. I., & Dickson, P. W. (2004b). Tyrosine hydroxylase phosphorylation: regulation and consequences. *Journal of Neurochemistry*, *91*(5), 1025–1043. http://doi.org/10.1111/j.1471-4159.2004.02797.x

Dyall, S. C. (2015). Long-chain omega-3 fatty acids and the brain: a review of the independent and shared effects of EPA, DPA and DHA. *Frontiers in Aging Neuroscience*, *7*, 52. http://doi.org/10.3389/fnagi.2015.00052

Ebrahimi-Fakhari, D., Saidi, L.-J., & Wahlster, L. (2013). Molecular chaperones and protein folding as therapeutic targets in Parkinson's disease and other synucleinopathies. *Acta Neuropathologica Communications*, *1*(1), 79. http://doi.org/10.1186/2051-5960-1-79

Ebrahimi-Fakhari, D., Wahlster, L., & McLean, P. J. (2011). Molecular chaperones in Parkinson's disease--present and future. *Journal of Parkinson's Disease*, *1*(4), 299–320. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/22279517

Ebrahimi-Fakhari, D., Wahlster, L., & McLean, P. J. (2012). Protein degradation pathways in Parkinson's disease: curse or blessing. *Acta Neuropathologica*, *124*(2), 153–172. http://doi.org/10.1007/s00401-012-1004-6

Edelman, A. M., Raese, J. D., Lazar, M. A., & Barchas, J. D. (1978). In vitro phosphorylation of a purified preparation of bovine corpus striatal tyrosine hydroxylase. *Communications in Psychopharmacology*, *2*(6), 461–5. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/34498

Emre, M. (2003). Dementia associated with Parkinson's disease. *The Lancet. Neurology*, *2*(4), 229–37. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12849211

Engelender, S., Kaminsky, Z., Guo, X., Sharp, A. H., Amaravi, R. K., Kleiderlein, J. J., … Ross, C. A. (1999). Synphilin-1 associates with α-synuclein and promotes the formation of cytosolic inclusions. *Nature Genetics*, *22*(1), 110–114. http://doi.org/10.1038/8820

Eriksson, A.-K., Löfving, S., Callaghan, R. C., & Allebeck, P. (2013). Alcohol use disorders and risk of Parkinson's disease: findings from a Swedish national cohort study 1972-2008. *BMC Neurology*, *13*(1), 190. http://doi.org/10.1186/1471-2377-13-190

Fader, D., & Lewis, E. J. (1990). Interaction of cyclic AMP and cell-cell contact in the control of tyrosine hydroxylase RNA. *Brain Research. Molecular Brain Research*, *8*(1), 25–9. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/1974315

Fanciulli, A., & Wenning, G. K. (2015a). Multiple-System Atrophy. *New England Journal of Medicine*, *372*(3), 249–263. http://doi.org/10.1056/NEJMra1311488

Fanciulli, A., & Wenning, G. K. (2015b). Multiple-System Atrophy. *New England Journal of Medicine*, *372*(3), 249–263. http://doi.org/10.1056/NEJMra1311488

Fatty Acids and Triacylglycerols - Lipid and Amino Acid Metabolism. (n.d.). Retrieved April 11, 2018, from http://schoolbag.info/chemistry/mcat_biochemistry/72.html

Fearnley, J. M., & Lees, A. J. (1991). Ageing and Parkinson's disease: *substantia nigra* regional selectivity. *Brain : A Journal of Neurology*, *114 (Pt 5)*, 2283–301. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/1933245

Fernagut, P. O., Hutson, C. B., Fleming, S. M., Tetreaut, N. A., Salcedo, J., Masliah, E., & Chesselet, M. F. (2007). Behavioral and histopathological consequences of paraquat intoxication in mice: effects of alpha-synuclein over-expression. *Synapse (New York, N.Y.)*, *61*(12), 991–1001. http://doi.org/10.1002/syn.20456

Fernández-Fernández, M. R., Gragera, M., Ochoa-Ibarrola, L., Quintana-Gallardo, L., & Valpuesta, J. M. (2017). Hsp70 - a master regulator in protein degradation. *FEBS Letters*, *591*(17), 2648–2660. http://doi.org/10.1002/1873-3468.12751

Fleming, S. M., Jordan, M. C., Mulligan, C. K., Masliah, E., Holden, J. G., Millard, R. W., … Roos, K. P. (2013). Impaired baroreflex function in mice overexpressing alpha-synuclein. *Frontiers in Neurology*, *4*, 103. http://doi.org/10.3389/fneur.2013.00103

Fleming, S. M., Salcedo, J., Fernagut, P.-O., Rockenstein, E., Masliah, E., Levine, M. S., & Chesselet, M.-F. (2004). Early and progressive sensorimotor anomalies in mice overexpressing wild-type human alpha-synuclein. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *24*(42), 9434–40. http://doi.org/10.1523/JNEUROSCI.3080-04.2004

Fleming, S. M., Salcedo, J., Hutson, C. B., Rockenstein, E., Masliah, E., Levine, M. S., & Chesselet, M.-F. (2006). Behavioral effects of dopaminergic agonists in transgenic mice overexpressing human wildtype alpha-synuclein. *Neuroscience*, *142*(4), 1245–53. http://doi.org/10.1016/j.neuroscience.2006.07.005

Fleming, S. M., Tetreault, N. A., Mulligan, C. K., Hutson, C. B., Masliah, E., & Chesselet, M.-F. (2008). Olfactory deficits in mice overexpressing human wildtype alpha-synuclein. *The European Journal of Neuroscience*, *28*(2), 247–56. http://doi.org/10.1111/j.1460-9568.2008.06346.x

Fornai, F., Schluter, O. M., Lenzi, P., Gesi, M., Ruffoli, R., Ferrucci, M., … Sudhof, T. C. (2005). Parkinson-like syndrome induced by continuous MPTP infusion: Convergent roles of the ubiquitin-proteasome system and -synuclein. *Proceedings of the National Academy of Sciences*, *102*(9), 3413–3418. http://doi.org/10.1073/pnas.0409713102

Forno, L. S., DeLanney, L. E., Irwin, I., & Langston, J. W. (1993). Similarities and differences between MPTP-induced parkinsonsim and Parkinson's disease. Neuropathologic considerations. *Advances in Neurology*, *60*, 600–8. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8380528

Frumkin, H. (1998). Multiple system atrophy following chronic carbon disulfide exposure. *Environmental Health Perspectives*, *106*(9), 611–3. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9721261

Fujiwara, H., Hasegawa, M., Dohmae, N., Kawashima, A., Masliah, E., Goldberg, M. S., … Iwatsubo, T. (2002). α-Synuclein is phosphorylated in synucleinopathy lesions. *Nature Cell Biology*, *4*(2), 160–164. http://doi.org/10.1038/ncb748

Gabbi, C., Warner, M., & Gustafsson, J.-Å. (2014). Action mechanisms of Liver X Receptors. *Biochemical and Biophysical Research Communications*, *446*(3), 647–50. http://doi.org/10.1016/j.bbrc.2013.11.077

Gähwiler, B. H., Capogna, M., Debanne, D., McKinney, R. A., & Thompson, S. M. (1997). Organotypic slice cultures: a technique has come of age. *Trends in Neurosciences*, *20*(10), 471–7. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9347615

Gao, X., Cassidy, A., Schwarzschild, M. A., Rimm, E. B., & Ascherio, A. (2012). Habitual intake of dietary flavonoids and risk of Parkinson disease. *Neurology*, *78*(15), 1138–45. http://doi.org/10.1212/WNL.0b013e31824f7fc4

Gao, X., Chen, H., Schwarzschild, M. A., & Ascherio, A. (2011). Use of ibuprofen and risk of Parkinson disease. *Neurology*, *76*(10), 863–9. http://doi.org/10.1212/WNL.0b013e31820f2d79

Gao, X., Simon, K. C., Schwarzschild, M. A., & Ascherio, A. (2012a). Age, statin use, and the risk for incident Parkinson disease-reply. *Archives of Neurology*, *69*(10), 1381. http://doi.org/10.1001/archneurol.2012.2032

Gao, X., Simon, K. C., Schwarzschild, M. A., & Ascherio, A. (2012b). Age, Statin Use, and the Risk for Incident Parkinson Disease—Reply. *Archives of Neurology*, *69*(10), 1381. http://doi.org/10.1001/archneurol.2012.2032

Gao, X., Simon, K. C., Schwarzschild, M. A., & Ascherio, A. (2012c). Prospective study of statin use and risk of Parkinson disease. *Archives of Neurology*, *69*(3), 380–4. http://doi.org/10.1001/archneurol.2011.1060

Genetic Variant Increases Risk for Dementia in Lewy Body Diseases | Lewy Body Dementia Association. (n.d.). Retrieved January 18, 2018, from https://www.lbda.org/content/genetic-mutation-increases-risk-dementia-lewy-body-diseases

George, J. M., & Yang, M.-L. (2013). α-Synuclein Physiology and Membrane Binding. Retrieved from https://www.ncbi.nlm.nih.gov/books/NBK6143/

Giasson, B. I., Duda, J. E., Murray, I. V, Chen, Q., Souza, J. M., Hurtig, H. I., … Lee, V. M. (2000). Oxidative damage linked to neurodegeneration by selective alpha-synuclein nitration in synucleinopathy lesions. *Science (New York, N.Y.)*, *290*(5493), 985–9. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11062131

Goc, A., & Stachowiak, M. K. (1994). Bovine tyrosine hydroxylase gene-promoter regions involved in basal and angiotensin II-stimulated expression in nontransformed adrenal medullary cells. *Journal of Neurochemistry*, *62*(3), 834–43. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7906719

Goedert, M., Jakes, R., & Spillantini, M. G. (2017). The Synucleinopathies: Twenty Years On. *Journal of Parkinson's Disease*, *7*(s1), S53–S71. http://doi.org/10.3233/JPD-179005

Gonçalves, S., & Outeiro, T. F. (2013). Assessing the subcellular dynamics of alpha-synuclein using photoactivation microscopy. *Molecular Neurobiology*, *47*(3), 1081–92. http://doi.org/10.1007/s12035-013-8406-x

Gong, L., Zhang, Q.-L., Zhang, N., Hua, W.-Y., Huang, Y.-X., Di, P.-W., … Luo, W.-F. (2012). Neuroprotection by urate on 6-OHDA-lesioned rat model of Parkinson's disease: linking to Akt/GSK3β signaling pathway. *Journal of Neurochemistry*, *123*(5), 876–85. http://doi.org/10.1111/jnc.12038

Grant, L. M., Richter, F., Miller, J. E., White, S. A., Fox, C. M., Zhu, C., … Ciucci, M. R. (2014). Vocalization deficits in mice over-expressing alpha-synuclein, a model of pre-manifest Parkinson's disease. *Behavioral Neuroscience*, *128*(2), 110–21. http://doi.org/10.1037/a0035965

Grima, B., Lamouroux, A., Boni, C., Julien, J.-F., Javoy-Agid, F., & Mallet, J. (1987). A single human gene encoding multiple tyrosine hydroxylases with different predicted functional characteristics. *Nature*, *326*(6114), 707–711. http://doi.org/10.1038/326707a0

Grima, B., Lamouroux, A., Boni, C., Julien, J. F., Javoy-Agid, F., & Mallet, J. (1987). A single human gene encoding multiple tyrosine hydroxylases with different predicted functional characteristics. *Nature*, *326*(6114), 707–11. http://doi.org/10.1038/326707a0

Guerreiro, R., Ross, O. A., Kun-Rodrigues, C., Hernandez, D. G., Orme, T., Eicher, J. D., … Bras, J. (2018). Investigating the genetic architecture of dementia with Lewy bodies: a two-stage genome-wide association study. *The Lancet Neurology*, *17*(1), 64–74. http://doi.org/10.1016/S1474-4422(17)30400-3

Guerreiro, S., Ponceau, A., Toulorge, D., Martin, E., Alvarez-Fischer, D., Hirsch, E. C., & Michel, P. P. (2009). Protection of midbrain dopaminergic neurons by the end-product of purine metabolism uric acid: potentiation by low-level depolarization. *Journal of Neurochemistry*, *109*(4), 1118–28. http://doi.org/10.1111/j.1471-4159.2009.06040.x

Guest, J., Garg, M., Bilgin, A., & Grant, R. (2013). Relationship between central and peripheral fatty acids in humans. *Lipids in Health and Disease*, *12*, 79. http://doi.org/10.1186/1476-511X-12-79

Guilarte, T. R., Nihei, M. K., McGlothan, J. L., & Howard, A. S. (2003). Methamphetamine-induced deficits of brain monoaminergic neuronal markers: distal axotomy or neuronal plasticity. *Neuroscience*, *122*(2), 499–513. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/14614914

Gupta, S., Knight, A. G., Gupta, S., Keller, J. N., & Bruce-Keller, A. J. (2012). Saturated long-chain fatty acids activate inflammatory signaling in astrocytes. *Journal of Neurochemistry*, *120*(6), no-no. http://doi.org/10.1111/j.1471-4159.2012.07660.x

Haavik, J., Schelling, D. L., Campbell, D. G., Andersson, K. K., Flatmark, T., & Cohen, P. (1989a). Identification of protein phosphatase 2A as the major tyrosine hydroxylase phosphatase in adrenal medulla and corpus striatum: evidence from the effects of okadaic acid. *FEBS Letters*, *251*(1–2), 36–42. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2568951

Haavik, J., Schelling, D. L., Campbell, D. G., Andersson, K. K., Flatmark, T., & Cohen, P. (1989b). Identification of protein phosphatase 2A as the major tyrosine hydroxylase phosphatase in adrenal medulla and corpus striatum: evidence from the effects of okadaic acid. *FEBS Letters*, *251*(1–2), 36–42. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2568951

Halbach, O. vo. B. und, Schober, A., & Krieglstein, K. (2004). Genes, proteins, and neurotoxins involved in Parkinson's disease. *Progress in Neurobiology*, *73*(3), 151–177. http://doi.org/10.1016/j.pneurobio.2004.05.002

Hanna, P. A., Jankovic, J., & Kirkpatrick, J. B. (1999). Multiple system atrophy: the putative causative role of environmental toxins. *Archives of Neurology*, *56*(1), 90–4. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9923766

Hansen, L. A., & Samuel, W. (1997). Criteria for Alzheimer's disease and the nosology of dementia with Lewy bodies. *Neurology*, *48*(1), 126–32. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9008507

Hattiangady, B., Rao, M. S., & Shetty, A. K. (2008). Plasticity of hippocampal stem/progenitor cells to enhance neurogenesis in response to kainate-induced injury is lost by middle age. *Aging Cell*, *7*(2), 207–224. http://doi.org/10.1111/j.1474-9726.2007.00363.x

Haycock, J. W. (1991). Four forms of tyrosine hydroxylase are present in human adrenal medulla. *Journal of Neurochemistry*, *56*(6), 2139–42. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/1674001

Haycock, J. W., Ahn, N. G., Cobb, M. H., & Krebs, E. G. (1992). ERK1 and ERK2, two microtubule-associated protein 2 kinases, mediate the phosphorylation of tyrosine hydroxylase at serine-31 in situ. *Proceedings of the National Academy of Sciences of the United States of America*, *89*(6), 2365–9. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/1347949

Haycock, J. W., George, R. J., & Waymire, J. C. (1985). In situ phosphorylation of tyrosine hydroxylase in chromaffin cells: Localization to soluble compartments. *Neurochemistry International*, *7*(2), 301–8. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/20492928

Hellenbrand, W., Boeing, H., Robra, B. P., Seidler, A., Vieregge, P., Nischan, P., … Ulm, G. (1996a). Diet and Parkinson's disease. II: A possible role for the past intake of specific nutrients. Results from a self-administered food-frequency questionnaire in a case-control study. *Neurology*, *47*(3), 644–50. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8797457

Hellenbrand, W., Boeing, H., Robra, B. P., Seidler, A., Vieregge, P., Nischan, P., … Ulm, G. (1996b). Diet and Parkinson's disease. II: A possible role for the past intake of specific nutrients. Results from a self-administered food-frequency questionnaire in a case-control study. *Neurology*, *47*(3), 644–50. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8797457

Hershko, A., & Ciechanover, A. (1998). THE UBIQUITIN SYSTEM. *Annual Review of Biochemistry*, *67*(1), 425–479. http://doi.org/10.1146/annurev.biochem.67.1.425

Heverin, M., Maioli, S., Pham, T., Mateos, L., Camporesi, E., Ali, Z., … Björkhem, I. (2015). 27-Hydroxycholesterol mediates negative effects of dietary cholesterol on cognition in mice. *Behavioural Brain Research*, *278*, 356–359. http://doi.org/10.1016/j.bbr.2014.10.018

Heverin, M., Meaney, S., Lütjohann, D., Diczfalusy, U., Wahren, J., & Björkhem, I. (2005a). Crossing the barrier: net flux of 27-hydroxycholesterol into the human brain. *Journal of Lipid Research*, *46*(5), 1047–52. http://doi.org/10.1194/jlr.M500024-JLR200

Heverin, M., Meaney, S., Lütjohann, D., Diczfalusy, U., Wahren, J., & Björkhem, I. (2005b). Crossing the barrier: net flux of 27-hydroxycholesterol into the human brain. *Journal of Lipid Research*, *46*(5), 1047–52. http://doi.org/10.1194/jlr.M500024-JLR200

Heverin, M., Meaney, S., Lütjohann, D., Diczfalusy, U., Wahren, J., & Björkhem, I. (2005c). Crossing the barrier: net flux of 27-hydroxycholesterol into the human brain. *Journal of Lipid Research*, *46*(5), 1047–52. http://doi.org/10.1194/jlr.M500024-JLR200

Hietanen, M., & Teräväinen, H. (1988). The effect of age of disease onset on neuropsychological performance in Parkinson's disease. *Journal of Neurology, Neurosurgery, and Psychiatry*, *51*(2), 244–9. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/3346689

Hirayama, T., Mizokami, Y., Honda, A., Homma, Y., Ikegami, T., Saito, Y., … Matsuzaki, Y. (2009). Serum concentration of 27-hydroxycholesterol predicts the effects of high-cholesterol diet on plasma LDL cholesterol level. *Hepatology Research*, *39*(2), 149–156. http://doi.org/10.1111/j.1872-034X.2008.00450.x

Hodara, R., Norris, E. H., Giasson, B. I., Mishizen-Eberz, A. J., Lynch, D. R., Lee, V. M.-Y., & Ischiropoulos, H. (2004). Functional consequences of alpha-synuclein tyrosine nitration: diminished binding to lipid vesicles and increased fibril formation. *The Journal of Biological Chemistry*, *279*(46), 47746–53. http://doi.org/10.1074/jbc.M408906200

Hu, G., Antikainen, R., Jousilahti, P., Kivipelto, M., & Tuomilehto, J. (2008). Total cholesterol and the risk of Parkinson disease. *Neurology*, *70*(21), 1972–9. http://doi.org/10.1212/01.wnl.0000312511.62699.a8

Hu, G., Antikainen, R., Jousilahti, P., Kivipelto, M., & Tuomilehto, J. (2008). Total cholesterol and the risk of Parkinson disease. *Neurology*, *70*(21), 1972–1979. http://doi.org/10.1212/01.wnl.0000312511.62699.a8

Hu, G., Bidel, S., Jousilahti, P., Antikainen, R., & Tuomilehto, J. (2007). Coffee and tea consumption and the risk of Parkinson's disease. *Movement Disorders*, *22*(15), 2242–2248. http://doi.org/10.1002/mds.21706

Hu, G., Jousilahti, P., Nissinen, A., Antikainen, R., Kivipelto, M., & Tuomilehto, J. (2006). Body mass index and the risk of Parkinson disease. *Neurology*, *67*(11), 1955–9. http://doi.org/10.1212/01.wnl.0000247052.18422.e5

Huang, X., Alonso, A., Guo, X., Umbach, D. M., Lichtenstein, M. L., Ballantyne, C. M., … Chen, H. (2015). Statins, plasma cholesterol, and risk of Parkinson's disease: a prospective study. *Movement Disorders : Official Journal of the Movement Disorder Society*, *30*(4), 552–9. http://doi.org/10.1002/mds.26152

Huang, Z., Xu, Z., Wu, Y., & Zhou, Y. (2011). Determining nuclear localization of alpha-synuclein in mouse brains. *Neuroscience*, *199*, 318–332. http://doi.org/10.1016/j.neuroscience.2011.10.016

Hughes, T. M., Rosano, C., Evans, R. W., & Kuller, L. H. (2013). Brain cholesterol metabolism, oxysterols, and dementia. *Journal of Alzheimer's Disease : JAD*, *33*(4), 891–911. http://doi.org/10.3233/JAD-2012-121585

Human, J. A., Ubbink, J. B., Jerling, J. J., Delport, R., Vermaak, W. J., Vorster, H. H., … Potgieter, H. C. (1997). The effect of Simvastatin on the plasma antioxidant concentrations in patients with hypercholesterolaemia. *Clinica Chimica Acta; International Journal of Clinical Chemistry*, *263*(1), 67–77. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9247729

Humpel, C. (2015). Organotypic brain slice cultures: A review. *Neuroscience*, *305*, 86–98. http://doi.org/10.1016/j.neuroscience.2015.07.086

Huxley, R., Lewington, S., & Clarke, R. (2002). Cholesterol, Coronary Heart Disease and Stroke: A Review of Published Evidence from Observational Studies and Randomized Controlled Trials. *Seminars in Vascular Medicine*, *02*(3), 315–324. http://doi.org/10.1055/s-2002-35402

Iacono, D., Geraci-Erck, M., Rabin, M. L., Adler, C. H., Serrano, G., Beach, T. G., & Kurlan, R. (2015). Parkinson disease and incidental Lewy body disease: Just a question of time? *Neurology*, *85*(19), 1670–9. http://doi.org/10.1212/WNL.0000000000002102

Inglis, K. J., Chereau, D., Brigham, E. F., Chiou, S.-S., Schöbel, S., Frigon, N. L., … Anderson, J. P. (2009). Polo-like kinase 2 (PLK2) phosphorylates alpha-synuclein at serine 129 in central nervous system. *The Journal of Biological Chemistry*, *284*(5), 2598–602. http://doi.org/10.1074/jbc.C800206200

Ishii, A., Nonaka, T., Taniguchi, S., Saito, T., Arai, T., Mann, D., … Hasegawa, M. (2007). Casein kinase 2 is the major enzyme in brain that phosphorylates Ser129 of human alpha-synuclein: Implication for alpha-synucleinopathies. *FEBS Letters*, *581*(24), 4711–7. http://doi.org/10.1016/j.febslet.2007.08.067

Itoh, K., Kasai, T., Tsuji, Y., Saito, K., Mizuta, I., Harada, Y., … Fushiki, S. (2014). Definite familial multiple system atrophy with unknown genetics. *Neuropathology*, *34*(3), 309–313. http://doi.org/10.1111/neup.12092

Ivanova, T., & Beyer, C. (2003). Estrogen regulates tyrosine hydroxylase expression in the neonate mouse midbrain. *Journal of Neurobiology*, *54*(4), 638–647. http://doi.org/10.1002/neu.10193

Javed, J., Latoo, L., & Jan, F. (2008). Dementia with Dementia with Dementia with Dementia with Lewy Lewy Lewy Lewy Bodies: Clinical Review Bodies: Clinical Review Bodies: Clinical Review Bodies: Clinical Review. *British Journal of Medical Practitioners BJMP.Org BJMP*, *11*(101), 10–14. Retrieved from http://www.bjmp.org/files/sept2008/bjmp0908jlatoo.pdf

Jecmenica-Lukic, M., Poewe, W., Tolosa, E., & Wenning, G. K. (2012). Premotor signs and symptoms of multiple system atrophy. *The Lancet Neurology*, *11*(4), 361–368. http://doi.org/10.1016/S1474-4422(12)70022-4

Jellinger, K. A. (2009). Formation and development of Lewy pathology: a critical update. *Journal of Neurology*, *256 Suppl 3*(S3), 270–9. http://doi.org/10.1007/s00415-009-5243-y

Jiang, W., Ju, C., Jiang, H., & Zhang, D. (2014). Dairy foods intake and risk of Parkinson's disease: a dose–response meta-analysis of prospective cohort studies. *European Journal of Epidemiology*, *29*(9), 613–619. http://doi.org/10.1007/s10654-014-9921-4

Joh, T. H., Park, D. H., & Reis, D. J. (1978). Direct phosphorylation of brain tyrosine hydroxylase by cyclic AMP-dependent protein kinase: mechanism of enzyme activation. *Proceedings of the National Academy of Sciences of the United States of America*, *75*(10), 4744–8. http://doi.org/10.1073/PNAS.75.10.4744

John Goers, ‡,§, Amy B. Manning-Bog, ⊥, Alison L. McCormack, ⊥, Ian S. Millett, #, Sebastian Doniach, #, Donato A. Di Monte, ⊥, ⋯ Anthony L. Fink*, ‡. (2003). Nuclear Localization of α-Synuclein and Its Interaction with Histones†. http://doi.org/10.1021/BI0341152

Johnsen, J. A., & Miller, V. T. (1986). Tobacco intolerance in multiple system atrophy. *Neurology*, *36*(7), 986–8. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/3714059

Johnson, C. C., Gorell, J. M., Rybicki, B. A., Sanders, K., & Peterson, E. L. (1999a). Adult nutrient intake as a risk factor for Parkinson's disease. *International Journal of Epidemiology*, *28*(6), 1102–9. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10661654

Johnson, C. C., Gorell, J. M., Rybicki, B. A., Sanders, K., & Peterson, E. L. (1999b). Adult nutrient intake as a risk factor for Parkinson's disease. *International Journal of Epidemiology*, *28*(6), 1102–9. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10661654

Johnson, C. C., Gorell, J. M., Rybicki, B. A., Sanders, K., & Peterson, E. L. (1999c). Adult nutrient intake as a risk factor for Parkinson's disease. *International Journal of Epidemiology*, *28*(6), 1102–9. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10661654

Joseph, S. B., McKilligin, E., Pei, L., Watson, M. A., Collins, A. R., Laffitte, B. A., … Tontonoz, P. (2002). Synthetic LXR ligand inhibits the development of atherosclerosis in mice. *Proceedings of the National Academy of Sciences*, *99*(11), 7604–7609. http://doi.org/10.1073/pnas.112059299

Julien, C., Berthiaume, L., Hadj-Tahar, A., Rajput, A. H., Bédard, P. J., Paolo, T. Di, … Calon, F. (2006). Postmortem brain fatty acid profile of levodopa-treated Parkinson disease patients and parkinsonian monkeys. *Neurochemistry International*, *48*(5), 404–414. http://doi.org/10.1016/J.NEUINT.2005.12.002

Kabaria, S., Choi, D. C., Chaudhuri, A. D., Mouradian, M. M., & Junn, E. (2015). Inhibition of miR-34b and miR-34c enhances α-synuclein expression in Parkinson's disease. *FEBS Letters*, *589*(3), 319–25. http://doi.org/10.1016/j.febslet.2014.12.014

Kalivendi, S. V, Yedlapudi, D., Hillard, C. J., & Kalyanaraman, B. (2010). Oxidants induce alternative splicing of alpha-synuclein: Implications for Parkinson's disease. *Free Radical Biology & Medicine*, *48*(3), 377–83. http://doi.org/10.1016/j.freeradbiomed.2009.10.045

Kamel, F., Goldman, S. M., Umbach, D. M., Chen, H., Richardson, G., Barber, M. R., … Tanner, C. M. (2014). Dietary fat intake, pesticide use, and Parkinson's disease. *Parkinsonism & Related Disorders*, *20*(1), 82–87. http://doi.org/10.1016/j.parkreldis.2013.09.023

Kaneda, N., Kobayashi, K., Ichinose, H., Kishi, F., Nakazawa, A., Kurosawa, Y., … Nagatsu, T. (1987). Isolation of a novel cDNA clone for human tyrosine hydroxylase: alternative RNA splicing produces four kinds of mRNA from a single gene. *Biochemical and Biophysical Research Communications*, *146*(3), 971–5. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2887169

Kappock, T. J., & Caradonna, J. P. (1996). Pterin-Dependent Amino Acid Hydroxylases. *Chemical Reviews,* 96(7), 2659–2756. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11848840

Karube, H., Sakamoto, M., Arawaka, S., Hara, S., Sato, H., Ren, C.-H., … Kato, T. (2008). N-terminal region of α-synuclein is essential for the fatty acid-induced oligomerization of the molecules. *FEBS Letters*, *582*(25–26), 3693–3700. http://doi.org/10.1016/j.febslet.2008.10.001

Karuna, R., Holleboom, A. G., Motazacker, M. M., Kuivenhoven, J. A., Frikke-Schmidt, R., Tybjaerg-Hansen, A., … Rentsch, K. M. (2011). Plasma levels of 27-hydroxycholesterol in humans and mice with monogenic disturbances of high density lipoprotein metabolism. *Atherosclerosis*, *214*(2), 448–455. http://doi.org/10.1016/j.atherosclerosis.2010.10.042

Katz, I. R., Yamauchi, T., & Kaufman, S. (1976). Activation of tyrosine hydroxylase by polyanions and salts. An electrostatic effect. *Biochimica et Biophysica Acta*, *429*(1), 84–95. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/4122

Kha, H. T., Basseri, B., Shouhed, D., Richardson, J., Tetradis, S., Hahn, T. J., & Parhami, F. (2004). Oxysterols regulate differentiation of mesenchymal stem cells: pro-bone and anti-fat. *Journal of Bone and Mineral Research : The Official Journal of the American Society for Bone and Mineral Research*, *19*(5), 830–40. http://doi.org/10.1359/JBMR.040115

Kim, H., Kim, E., Park, M., Lee, E., & Namkoong, K. (2013). Organotypic hippocampal slice culture from the adult mouse brain: A versatile tool for translational neuropsychopharmacology. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *41*, 36–43. http://doi.org/10.1016/j.pnpbp.2012.11.004

Kim, K. S., Lee, M. K., Carroll, J., & Joh, T. H. (1993). Both the basal and inducible transcription of the tyrosine hydroxylase gene are dependent upon a cAMP response element. *The Journal of Biological Chemistry*, *268*(21), 15689–95. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8101843

Kim, K. S., Tinti, C., Song, B., Cubells, J. F., & Joh, T. H. (1994). Cyclic AMP-dependent protein kinase regulates basal and cyclic AMP-stimulated but not phorbol ester-stimulated transcription of the tyrosine hydroxylase gene. *Journal of Neurochemistry*, *63*(3), 834–42. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7914223

Kim, M., Jung, W., Lee, I.-H., Bhak, G., Paik, S. R., & Hahn, J.-S. (2008). Impairment of microtubule system increases alpha-synuclein aggregation and toxicity. *Biochemical and Biophysical Research Communications*, *365*(4), 628–35. http://doi.org/10.1016/j.bbrc.2007.11.020

Kim, Y. M., Jang, W. H., Quezado, M. M., Oh, Y., Chung, K. C., Junn, E., & Mouradian, M. M. (2011). Proteasome inhibition induces α-synuclein SUMOylation and aggregate formation. *Journal of the Neurological Sciences*, *307*(1–2), 157–61. http://doi.org/10.1016/j.jns.2011.04.015

Kirik, D., Rosenblad, C., Burger, C., Lundberg, C., Johansen, T. E., Muzyczka, N., … Björklund, A. (2002). Parkinson-like neurodegeneration induced by targeted overexpression of alpha-synuclein in the nigrostriatal system. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *22*(7), 2780–91. http://doi.org/20026246

Kitajka, K., Sinclair, A. J., Weisinger, R. S., Weisinger, H. S., Mathai, M., Jayasooriya, A. P., … Puskas, L. G. (2004). Effects of dietary omega-3 polyunsaturated fatty acids on brain gene expression. *Proceedings of the National Academy of Sciences*, *101*(30), 10931–10936. http://doi.org/10.1073/pnas.0402342101

Kleiger, G., & Mayor, T. (2014). Perilous journey: a tour of the ubiquitin–proteasome system. *Trends in Cell Biology*, *24*(6), 352–359. http://doi.org/10.1016/j.tcb.2013.12.003

Kobayashi, K., Kaneda, N., Ichinose, H., Kishi, F., Nakazawa, A., Kurosawa, Y., … Nagatsu, T. (1988). Structure of the human tyrosine hydroxylase gene: alternative splicing from a single gene accounts for generation of four mRNA types. *Journal of Biochemistry*, *103*(6), 907–12. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2902075

Köllensperger, M., Geser, F., Ndayisaba, J.-P., Boesch, S., Seppi, K., Ostergaard, K., … EMSA-SG. (2010). Presentation, diagnosis, and management of multiple system atrophy in Europe: Final analysis of the European multiple system atrophy registry. *Movement Disorders*, *25*(15), 2604–2612. http://doi.org/10.1002/mds.23192

Kontopoulos, E., Parvin, J. D., & Feany, M. B. (2006a). Alpha-synuclein acts in the nucleus to inhibit histone acetylation and promote neurotoxicity. *Human Molecular Genetics*, *15*(20), 3012–23.

http://doi.org/10.1093/hmg/ddl243Kontopoulos, E., Parvin, J. D., & Feany, M. B. (2006b). α-synuclein acts in the nucleus to inhibit histone acetylation and promote neurotoxicity. *Human Molecular Genetics*, *15*(20), 3012–3023. http://doi.org/10.1093/hmg/ddl243

Kosmider, B., Loader, J. E., Murphy, R. C., & Mason, R. J. (2010). Apoptosis induced by ozone and oxysterols in human alveolar epithelial cells. *Free Radical Biology and Medicine*, *48*(11), 1513–1524. http://doi.org/10.1016/j.freeradbiomed.2010.02.032

Krüger, R., Kuhn, W., Müller, T., Woitalla, D., Graeber, M., Kösel, S., … Riess, O. (1998a). Ala30Pro mutation in the gene encoding alpha-synuclein in Parkinson's disease. *Nature Genetics*, *18*(2), 106–8. http://doi.org/10.1038/ng0298-106

Krüger, R., Kuhn, W., Müller, T., Woitalla, D., Graeber, M., Kösel, S., … Riess, O. (1998b). Ala30Pro mutation in the gene encoding alpha-synuclein in Parkinson's disease. *Nature Genetics*, *18*(2), 106–8. http://doi.org/10.1038/ng0298-106

Krumova, P., Meulmeester, E., Garrido, M., Tirard, M., Hsiao, H.-H., Bossis, G., … Weishaupt, J. H. (2011). Sumoylation inhibits α-synuclein aggregation and toxicity. *The Journal of Cell Biology*, *194*(1), 49–60. http://doi.org/10.1083/jcb.201010117

Kuczenski, R. T., & Mandell, A. J. (1972). ALLOSTERIC ACTIVATION OF HYPOTHALAMIC TYROSINE HYDROXYLASE BY IONS AND SULPHATED MUCOPOLYSACCHARIDES. *Journal of Neurochemistry*, *19*(1), 131–137. http://doi.org/10.1111/j.1471-4159.1972.tb01262.x

Kudo, T., Loh, D. H., Truong, D., Wu, Y., & Colwell, C. S. (2011). Circadian dysfunction in a mouse model of Parkinson's disease. *Experimental Neurology*, *232*(1), 66–75. http://doi.org/10.1016/J.EXPNEUROL.2011.08.003

Kumer, S. C., & Vrana, K. E. (1996). Intricate regulation of tyrosine hydroxylase activity and gene expression. *Journal of Neurochemistry*, *67*(2), 443–62. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8764568

Kyrozis, A., Ghika, A., Stathopoulos, P., Vassilopoulos, D., Trichopoulos, D., & Trichopoulou, A. (2013). Dietary and lifestyle variables in relation to incidence of Parkinson's disease in Greece. *European Journal of Epidemiology*, *28*(1), 67–77. http://doi.org/10.1007/s10654-012-9760-0

Lai, Y., Kim, S., Varkey, J., Lou, X., Song, J.-K., Diao, J., … Shin, Y.-K. (2014). Nonaggregated α-synuclein influences SNARE-dependent vesicle docking via membrane binding. *Biochemistry*, *53*(24), 3889–96. http://doi.org/10.1021/bi5002536

Lam, H. A., Wu, N., Cely, I., Kelly, R. L., Hean, S., Richter, F., … Maidment, N. T. (2011). Elevated tonic extracellular dopamine concentration and altered dopamine modulation of synaptic activity precede dopamine loss in the striatum of mice overexpressing human α-synuclein. *Journal of Neuroscience Research*, *89*(7), 1091–1102. http://doi.org/10.1002/jnr.22611

Langston, J. W., Ballard, P., Tetrud, J. W., & Irwin, I. (1983a). Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis. *Science (New York, N.Y.)*, *219*(4587), 979–80. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/6823561

Langston, J. W., Ballard, P., Tetrud, J. W., & Irwin, I. (1983b). Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis. *Science (New York, N.Y.)*, *219*(4587), 979–80. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/6823561

Langston, J. W., Irwin, I., Langston, E. B., & Forno, L. S. (1984). 1-Methyl-4-phenylpyridinium ion (MPP+): identification of a metabolite of MPTP, a toxin selective to the *substantia nigra*. *Neuroscience Letters*, *48*(1), 87–92. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/6332288

Lazar, M. A., Truscott, R. J., Raese, J. D., & Barchas, J. D. (1981). Thermal denaturation of native striatal tyrosine hydroxylase: increased thermolability of the phosphorylated form of the enzyme. *Journal of Neurochemistry*, *36*(2), 677–82. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/6109765

Le Bourdellès, B., Boularand, S., Boni, C., Horellou, P., Dumas, S., Grima, B., & Mallet, J. (1988). Analysis of the 5’ region of the human tyrosine hydroxylase gene: combinatorial patterns of exon splicing generate multiple regulated tyrosine hydroxylase isoforms. *Journal of Neurochemistry*, *50*(3), 988–91. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2892893

Le Bourdellès, B., Horellou, P., Le Caer, J. P., Denèfle, P., Latta, M., Haavik, J., … Mallet, J. (1991). Phosphorylation of human recombinant tyrosine hydroxylase isoforms 1 and 2: an additional phosphorylated residue in isoform 2, generated through alternative splicing. *The Journal of Biological Chemistry*, *266*(26), 17124–30. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/1680128

Leal, R. B., Sim, A. T. R., Gonçalves, C. A. S., & Dunkley, P. R. (2002). Tyrosine hydroxylase dephosphorylation by protein phosphatase 2A in bovine adrenal chromaffin cells. *Neurochemical Research*, *27*(3), 207–13. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11958518

Lee, H.-J., Khoshaghideh, F., Lee, S., & Lee, S.-J. (2006). Impairment of microtubule-dependent trafficking by overexpression of alpha-synuclein. *The European Journal of Neuroscience*, *24*(11), 3153–62. http://doi.org/10.1111/j.1460-9568.2006.05210.x

Lee, H.-J., Khoshaghideh, F., Patel, S., & Lee, S.-J. (2004). Clearance of alpha-synuclein oligomeric intermediates via the lysosomal degradation pathway. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *24*(8), 1888–96. http://doi.org/10.1523/JNEUROSCI.3809-03.2004

Lee, M. K., Marszalek, J. R., & Cleveland, D. W. (1994). A mutant neurofilament subunit causes massive, selective motor neuron death: implications for the pathogenesis of human motor neuron disease. *Neuron*, *13*(4), 975–88. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7946341

Lee, S.-J., Jeon, H., & Kandror, K. V. (2008). Alpha-synuclein is localized in a subpopulation of rat brain synaptic vesicles. *Acta Neurobiologiae Experimentalis*, *68*(4), 509–15. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/19112474

Lee, Y.-C., Lin, C.-H., Wu, R.-M., Lin, J.-W., Chang, C.-H., & Lai, M.-S. (2014). Antihypertensive agents and risk of Parkinson's disease: a nationwide cohort study. *PloS One*, *9*(6), e98961. http://doi.org/10.1371/journal.pone.0098961

Lehninger. (n.d.). the formation of palmitate. Retrieved April 11, 2018, from https://www.google.com/search?client=firefox-b-1-ab&biw=1920&bih=943&tbm=isch&sa=1&ei=HFnOWuf3GIKQjgTzirjIDw&q=the+formation+of+palmitate+lehninger&oq=the+formation+of+palmitate+lehninger&gs_l=psy-ab.3...19358.23890.0.24110.10.10.0.0.0.0.390.1214.7j2j0j1.

Leoni, V., & Caccia, C. (2011a). Oxysterols as biomarkers in neurodegenerative diseases. *Chemistry and Physics of Lipids*, *164*(6), 515–24. http://doi.org/10.1016/j.chemphyslip.2011.04.002

Leoni, V., & Caccia, C. (2011b). Oxysterols as biomarkers in neurodegenerative diseases. *Chemistry and Physics of Lipids*, *164*(6), 515–24. http://doi.org/10.1016/j.chemphyslip.2011.04.002

Leoni, V., Masterman, T., Mousavi, F. S., Wretlind, B., Wahlund, L.-O., Diczfalusy, U., … Björkhem, I. (2004a). Diagnostic use of cerebral and extracerebral oxysterols. *Clinical Chemistry and Laboratory Medicine*, *42*(2). http://doi.org/10.1515/CCLM.2004.034

Leoni, V., Masterman, T., Mousavi, F. S., Wretlind, B., Wahlund, L. O., Diczfalusy, U., … Björkhem, I. (2004b). Diagnostic use of cerebral and extracerebral oxysterols. *Clinical Chemistry and Laboratory Medicine*, *42*(2), 186–191. http://doi.org/10.1515/CCLM.2004.034

Lesage, S., Anheim, M., Letournel, F., Bousset, L., Honoré, A., Rozas, N., … French Parkinson's Disease Genetics Study Group. (2013). G51D α-synuclein mutation causes a novel parkinsonian-pyramidal syndrome. *Annals of Neurology*, *73*(4), 459–71. http://doi.org/10.1002/ana.23894

Lewy body dementia - Symptoms and causes - Mayo Clinic. (n.d.). Retrieved December 15, 2017, from https://www.mayoclinic.org/diseases-conditions/lewy-body-dementia/symptoms-causes/syc-20352025

lewy body dementia going gentle into that good night. (n.d.). Retrieved April 11, 2018, from https://goinggentleintothatgoodnight.files.wordpress.com/2016/01/lewy-body-dementia-going-gentle-into-that-good-night.jpg

Liani, E., Eyal, A., Avraham, E., Shemer, R., Szargel, R., Berg, D., … Engelender, S. (2004). Ubiquitylation of synphilin-1 and alpha-synuclein by SIAH and its presence in cellular inclusions and Lewy bodies imply a role in Parkinson's disease. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(15), 5500–5. http://doi.org/10.1073/pnas.0401081101

Lin, K.-D., Yang, C.-Y., Lee, M.-Y., Ho, S.-C., Liu, C.-K., & Shin, S.-J. (2016). Statin therapy prevents the onset of Parkinson disease in patients with diabetes. *Annals of Neurology*, *80*(4), 532–40. http://doi.org/10.1002/ana.24751

Lindsten, K., & Dantuma, N. P. (2003). Monitoring the ubiquitin/proteasome system in conformational diseases. *Ageing Research Reviews*, *2*, 433–449. http://doi.org/10.1016/S1568-1637(03)00031-X

Litvan, I., Mohr, E., Williams, J., Gomez, C., & Chase, T. N. (1991). Differential memory and executive functions in demented patients with Parkinson's and Alzheimer's disease. *Journal of Neurology, Neurosurgery, and Psychiatry*, *54*(1), 25–9. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2010755

Liu, C.-W., Corboy, M. J., DeMartino, G. N., & Thomas, P. J. (2003). Endoproteolytic Activity of the Proteasome. *Science*, *299*(5605), 408–411. http://doi.org/10.1126/science.1079293

Liu, R., Gao, X., Lu, Y., & Chen, H. (2011). Meta-analysis of the relationship between Parkinson disease and melanoma. *Neurology*, *76*(23), 2002–9. http://doi.org/10.1212/WNL.0b013e31821e554e

Liu, R., Guo, X., Park, Y., Huang, X., Sinha, R., Freedman, N. D., … Chen, H. (2012). Caffeine intake, smoking, and risk of Parkinson disease in men and women. *American Journal of Epidemiology*, *175*(11), 1200–7. http://doi.org/10.1093/aje/kwr451

Liu, X., Lee, Y. J., Liou, L.-C., Ren, Q., Zhang, Z., Wang, S., & Witt, S. N. (2011). Alpha-synuclein functions in the nucleus to protect against hydroxyurea-induced replication stress in yeast. *Human Molecular Genetics*, *20*(17), 3401–14. http://doi.org/10.1093/hmg/ddr246

Liu, Y., Qiang, M., Wei, Y., & He, R. (2011). A novel molecular mechanism for nitrated {alpha}-synuclein-induced cell death. *Journal of Molecular Cell Biology*, *3*(4), 239–49. http://doi.org/10.1093/jmcb/mjr011

Lloyd, T. (1979). The effects of phosphatidylinositol on tyrosine hydroxylase. Stimulation and inactivation. *The Journal of Biological Chemistry*, *254*(15), 7247–54. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/37250

Lloyd, T., & Kaufman, S. (1974). The stimulation of partially purified bovine caudate tyrosine hydroxylase by phosphatidyl-L-serine. *Biochemical and Biophysical Research Communications*, *59*(4), 1262–70. http://doi.org/10.1016/0006-291X(74)90450-1

Logroscino, G., Marder, K., Cote, L., Tang, M.-X., Shea, S., & Mayeux, R. (1996a). Dietary lipids and antioxidants in Parkinson's disease: A population-based, case-control study. *Annals of Neurology*, *39*(1), 89–94. http://doi.org/10.1002/ana.410390113

Logroscino, G., Marder, K., Cote, L., Tang, M.-X., Shea, S., & Mayeux, R. (1996b). Dietary lipids and antioxidants in Parkinson's disease: A population-based, case-control study. *Annals of Neurology*, *39*(1), 89–94. http://doi.org/10.1002/ana.410390113

Logroscino, G., Sesso, H. D., Paffenbarger, R. S., & Lee, I.-M. (2006). Physical activity and risk of Parkinson's disease: a prospective cohort study. *Journal of Neurology, Neurosurgery & Psychiatry*, *77*(12), 1318–1322. http://doi.org/10.1136/jnnp.2006.097170

Lopes da Fonseca, T., Villar-Piqué, A., & Outeiro, T. (2015). The Interplay between Alpha-Synuclein Clearance and Spreading. *Biomolecules*, *5*(2), 435–471. http://doi.org/10.3390/biom5020435

Lordan, S., Mackrill, J. J., & O'Brien, N. M. (2009). Oxysterols and mechanisms of apoptotic signaling: implications in the pathology of degenerative diseases☆. *The Journal of Nutritional Biochemistry*, *20*(5), 321–336. http://doi.org/10.1016/j.jnutbio.2009.01.001

Lücke, C., Gantz, D. L., Klimtchuk, E., & Hamilton, J. A. (2006). Interactions between fatty acids and α-synuclein. *Journal of Lipid Research*, *47*(8), 1714–1724. http://doi.org/10.1194/jlr.M600003-JLR200

Luk, K. C., Mills, I. P., Trojanowski, J. Q., & Lee, V. M.-Y. (2008). Interactions between Hsp70 and the Hydrophobic Core of α-Synuclein Inhibit Fibril Assembly †. *Biochemistry*, *47*(47), 12614–12625. http://doi.org/10.1021/bi801475r

Luo, X., Wu, J., Jing, S., & Yan, L.-J. (2016). Hyperglycemic Stress and Carbon Stress in Diabetic Glucotoxicity. *Aging and Disease*, *7*(1), 90–110. http://doi.org/10.14336/AD.2015.0702

Lütjohann, D., Lizard, G., & Iuliano, L. (2017). Oxysterols: Players in different metabolic leagues. *The Journal of Steroid Biochemistry and Molecular Biology*, *169*, 1–3. http://doi.org/10.1016/j.jsbmb.2017.03.011

Ma, W.-W., Li, C.-Q., Yu, H.-L., Zhang, D.-D., Xi, Y.-D., Han, J., … Xiao, R. (2015). The Oxysterol 27-Hydroxycholesterol Increases Oxidative Stress and Regulate Nrf2 Signaling Pathway in Astrocyte Cells. *Neurochemical Research*, *40*(4), 758–766. http://doi.org/10.1007/s11064-015-1524-2

Magen, I., Fleming, S. M., Zhu, C., Garcia, E. C., Cardiff, K. M., Dinh, D., … Chesselet, M.-F. (2012). Cognitive deficits in a mouse model of pre-manifest Parkinson's disease. *The European Journal of Neuroscience*, *35*(6), 870–82. http://doi.org/10.1111/j.1460-9568.2012.08012.x

Maharjan, S., Serova, L., & Sabban, E. L. (2005). Transcriptional regulation of tyrosine hydroxylase by estrogen: opposite effects with estrogen receptors α and β and interactions with cyclic AMP. *Journal of Neurochemistry*, *93*(6), 1502–1514. http://doi.org/10.1111/j.1471-4159.2005.03142.x

Mak, S. K., McCormack, A. L., Manning-Boğ, A. B., Cuervo, A. M., & Di Monte, D. A. (2010). Lysosomal Degradation of α-Synuclein *in Vivo*. *Journal of Biological Chemistry*, *285*(18), 13621–13629. http://doi.org/10.1074/jbc.M109.074617

Mally, P., Mishra, R., Gandhi, S., Decastro, M. H., Nankova, B. B., & Lagamma, E. F. (2004). Stereospecific Regulation of Tyrosine Hydroxylase and Proenkephalin Genes by Short-Chain Fatty Acids in Rat PC12 Cells. *Pediatric Research*, *55*(5), 847–854. http://doi.org/10.1203/01.PDR.0000119365.21770.45

Maltsev, A. S., Ying, J., & Bax, A. (2012). Impact of N-terminal acetylation of α-synuclein on its random coil and lipid binding properties. *Biochemistry*, *51*(25), 5004–13. http://doi.org/10.1021/bi300642h

Manocha, G. D., Floden, A. M., Puig, K. L., Nagamoto-Combs, K., Scherzer, C. R., & Combs, C. K. (2017). Defining the contribution of neuroinflammation to Parkinson's disease in humanized immune system mice. *Molecular Neurodegeneration*, *12*(1), 17. http://doi.org/10.1186/s13024-017-0158-z

Marder, K. S., Tang, M. X., Mejia-Santana, H., Rosado, L., Louis, E. D., Comella, C. L., … Clark, L. N. (2010). Predictors of parkin mutations in early-onset Parkinson disease: the consortium on risk for early-onset Parkinson disease study. *Archives of Neurology*, *67*(6), 731–8. http://doi.org/10.1001/archneurol.2010.95

Maroteaux, L., Campanelli, J. T., & Scheller, R. H. (1988). Synuclein: a neuron-specific protein localized to the nucleus and presynaptic nerve terminal. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *8*(8), 2804–15. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/3411354

Marras, C., Hincapié, C. A., Kristman, V. L., Cancelliere, C., Soklaridis, S., Li, A., … Cassidy, J. D. (2014). Systematic Review of the Risk of Parkinson's Disease After Mild Traumatic Brain Injury: Results of the International Collaboration on Mild Traumatic Brain Injury Prognosis. *Archives of Physical Medicine and Rehabilitation*, *95*(3), S238–S244. http://doi.org/10.1016/j.apmr.2013.08.298

Martin, W. E., Loewenson, R. B., Resch, J. A., & Baker, A. B. (1973). Parkinson's disease. Clinical analysis of 100 patients. *Neurology*, *23*(8), 783–90. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/4578348

Mart�, M. J., Tolosa, E., & Campdelacreu, J. (2003). Clinical overview of the synucleinopathies. *Movement Disorders*, *18*(S6), 21–27. http://doi.org/10.1002/mds.10559

Marwarha, G., Claycombe, K., Schommer, J., Collins, D., & Ghribi, O. (2016a). Palmitate-induced Endoplasmic Reticulum stress and subsequent C/EBPα Homologous Protein activation attenuates leptin and Insulin-like growth factor 1 expression in the brain. *Cellular Signalling*, *28*(11), 1789–805. http://doi.org/10.1016/j.cellsig.2016.08.012

Marwarha, G., Claycombe, K., Schommer, J., Collins, D., & Ghribi, O. (2016b). Palmitate-induced Endoplasmic Reticulum stress and subsequent C/EBPα Homologous Protein activation attenuates leptin and Insulin-like growth factor 1 expression in the brain. *Cellular Signalling*, *28*(11), 1789–1805. http://doi.org/10.1016/j.cellsig.2016.08.012

Marwarha, G., Dasari, B., Prasanthi, J. R. P., Schommer, J., & Ghribi, O. (2010a). Leptin reduces the accumulation of A?? and phosphorylated tau induced by 27-hydroxycholesterol in rabbit organotypic slices. *Journal of Alzheimer's Disease*, *19*(3), 1007–1019.

Marwarha, G., Dasari, B., Prasanthi, J. R. P., Schommer, J., & Ghribi, O. (2010b). Leptin reduces the accumulation of Abeta and phosphorylated tau induced by 27-hydroxycholesterol in rabbit organotypic slices. *Journal of Alzheimer's Disease : JAD*, *19*(3), 1007–19. http://doi.org/10.3233/JAD-2010-1298

Marwarha, G., & Ghribi, O. (2015). Does the oxysterol 27-hydroxycholesterol underlie Alzheimer's disease-Parkinson's disease overlap? *Experimental Gerontology*, *68*, 13–8. http://doi.org/10.1016/j.exger.2014.09.013

Marwarha, G., Prasanthi, J. R., Schommer, J., Dasari, B., & Ghribi, O. (2011a). Molecular interplay between leptin, insulin-like growth factor-1, and β-amyloid in organotypic slices from rabbit hippocampus. *Molecular Neurodegeneration*, *6*(1), 41.

Marwarha, G., Prasanthi, J. R., Schommer, J., Dasari, B., & Ghribi, O. (2011b). Molecular interplay between leptin, insulin-like growth factor-1, and β-amyloid in organotypic slices from rabbit hippocampus. *Molecular Neurodegeneration*, *6*(1), 41. http://doi.org/10.1186/1750-1326-6-41

Marwarha, G., Raza, S., Hammer, K., & Ghribi, O. (2017). 27-hydroxycholesterol: A novel player in molecular carcinogenesis of breast and prostate cancer. *Chemistry and Physics of Lipids*. http://doi.org/10.1016/j.chemphyslip.2017.05.012

Marwarha, G., Rhen, T., Schommer, T., & Ghribi, O. (2011a). The oxysterol 27-hydroxycholesterol regulates α-synuclein and tyrosine hydroxylase expression levels in human neuroblastoma cells through modulation of liver X receptors and estrogen receptors-relevance to Parkinson's disease. *Journal of Neurochemistry*, *119*(5), 1119–1136. http://doi.org/10.1111/j.1471-4159.2011.07497.x

Marwarha, G., Rhen, T., Schommer, T., & Ghribi, O. (2011b). The oxysterol 27-hydroxycholesterol regulates α-synuclein and tyrosine hydroxylase expression levels in human neuroblastoma cells through modulation of liver X receptors and estrogen receptors-relevance to Parkinson's disease. *Journal of Neurochemistry*, *119*(5), 1119–1136. http://doi.org/10.1111/j.1471-4159.2011.07497.x

Marwarha, G., Rostad, S., Lilek, J., Kleinjan, M., Schommer, J., & Ghribi, O. (2017). Palmitate Increases β-site AβPP-Cleavage Enzyme 1 Activity and Amyloid-β Genesis by Evoking Endoplasmic Reticulum Stress and Subsequent C/EBP Homologous Protein Activation. *Journal of Alzheimer's Disease*, *57*(3), 907–925. http://doi.org/10.3233/JAD-161130

Marwarha, G., Schommer, J., Lund, J., Schommer, T., & Ghribi, O. (2018a). Palmitate-induced C/EBP homologous protein activation leads to NF-κB-mediated increase in BACE1 activity and amyloid beta genesis. *Journal of Neurochemistry*. http://doi.org/10.1111/jnc.14292

Marwarha, G., Schommer, J., Lund, J., Schommer, T., & Ghribi, O. (2018b). Palmitate-induced C/EBP homologous protein activation leads to NF-κB-mediated increase in BACE1 activity and amyloid beta genesis. *Journal of Neurochemistry*, *144*(6), 761–779. http://doi.org/10.1111/jnc.14292

Mashek, D. G., & Grummer, R. R. (2003). Effects of Long Chain Fatty Acids on Lipid and Glucose Metabolism in Monolayer Cultures of Bovine Hepatocytes. *Journal of Dairy Science*, *86*(7), 2390–2396. http://doi.org/10.3168/jds.S0022-0302(03)73833-8

Matsumoto, L., Takuma, H., Tamaoka, A., Kurisaki, H., Date, H., Tsuji, S., & Iwata, A. (2010). CpG demethylation enhances alpha-synuclein expression and affects the pathogenesis of Parkinson's disease. *PloS One*, *5*(11), e15522. http://doi.org/10.1371/journal.pone.0015522

Matsuura, K., Kabuto, H., Makino, H., & Ogawa, N. (1997). Pole test is a useful method for evaluating the mouse movement disorder caused by striatal dopamine depletion. *Journal of Neuroscience Methods*, *73*(1), 45–8. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9130677

Mayeux, R., Denaro, J., Hemenegildo, N., Marder, K., Tang, M. X., Cote, L. J., & Stern, Y. (1992). A population-based investigation of Parkinson's disease with and without dementia. Relationship to age and gender. *Archives of Neurology*, *49*(5), 492–7. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/1580811

Mbefo, M. K., Paleologou, K. E., Boucharaba, A., Oueslati, A., Schell, H., Fournier, M., … Lashuel, H. A. (2010). Phosphorylation of Synucleins by Members of the Polo-like Kinase Family. *Journal of Biological Chemistry*, *285*(4), 2807–2822. http://doi.org/10.1074/jbc.M109.081950

McDowell, K. A., Shin, D., Roos, K. P., & Chesselet, M.-F. (2014). Sleep dysfunction and EEG alterations in mice overexpressing alpha-synuclein. *Journal of Parkinson's Disease*, *4*(3), 531–9. http://doi.org/10.3233/JPD-140374

McLean, J. R., Hallett, P. J., Cooper, O., Stanley, M., & Isacson, O. (2012). Transcript expression levels of full-length alpha-synuclein and its three alternatively spliced variants in Parkinson's disease brain regions and in a transgenic mouse model of alpha-synuclein overexpression. *Molecular and Cellular Neurosciences*, *49*(2), 230–9. http://doi.org/10.1016/j.mcn.2011.11.006

McLean, P. J., Kawamata, H., & Hyman, B. T. (2001). Alpha-synuclein-enhanced green fluorescent protein fusion proteins form proteasome sensitive inclusions in primary neurons. *Neuroscience*, *104*(3), 901–12. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11440819

McNaught, K. S. P., Björklund, L. M., Belizaire, R., Isacson, O., Jenner, P., & Olanow, C. W. (2002). Proteasome inhibition causes nigral degeneration with inclusion bodies in rats. *Neuroreport*, *13*(11), 1437–41. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12167769

McNaught, K. S. P., Jackson, T., JnoBaptiste, R., Kapustin, A., & Olanow, C. W. (2006). Proteasomal dysfunction in sporadic Parkinson's disease. *Neurology*, *66*(10 Suppl 4), S37-49. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/16717251

McNaught, K. S. P., Mytilineou, C., Jnobaptiste, R., Yabut, J., Shashidharan, P., Jennert, P., & Olanow, C. W. (2002). Impairment of the ubiquitin-proteasome system causes dopaminergic cell death and inclusion body formation in ventral mesencephalic cultures. *Journal of Neurochemistry*, *81*(2), 301–6. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12064477

McNaught, K. S. P., Olanow, C. W., Halliwell, B., Isacson, O., & Jenner, P. (2001). Failure of the ubiquitin–proteasome system in Parkinson's disease. *Nature Reviews Neuroscience*, *2*(8), 589–594. http://doi.org/10.1038/35086067

Meredith, G. E., & Rademacher, D. J. (2011). MPTP mouse models of Parkinson's disease: an update. *Journal of Parkinson's Disease*, *1*(1), 19–33. http://doi.org/10.3233/JPD-2011-11023

Mewes, A., Franke, H., Singer, D., Gähwiler, B., Stoppini, L., Buchs, P., … Pommier, Y. (2012). Organotypic Brain Slice Cultures of Adult Transgenic P301S Mice—A Model for Tauopathy Studies. *PLoS ONE*, *7*(9), e45017. http://doi.org/10.1371/journal.pone.0045017

Michael W King. (2017). Cholesterol: Synthesis, Metabolism, Regulation. Retrieved from https://themedicalbiochemistrypage.org/cholesterol.php

Miñones-Moyano, E., Porta, S., Escaramís, G., Rabionet, R., Iraola, S., Kagerbauer, B., … Martí, E. (2011). MicroRNA profiling of Parkinson's disease brains identifies early downregulation of miR-34b/c which modulate mitochondrial function. *Human Molecular Genetics*, *20*(15), 3067–78. http://doi.org/10.1093/hmg/ddr210

Miyake, Y., Sasaki, S., Tanaka, K., Fukushima, W., Kiyohara, C., Tsuboi, Y., … Fukuoka Kinki Parkinson's Disease Study Group. (2010). Dietary fat intake and risk of Parkinson's disease: a case-control study in Japan. *Journal of the Neurological Sciences*, *288*(1–2), 117–22. http://doi.org/10.1016/j.jns.2009.09.021

Miyake, Y., Tanaka, K., Fukushima, W., Sasaki, S., Kiyohara, C., Tsuboi, Y., … al., et. (2010). Case-control study of risk of Parkinson's disease in relation to hypertension, hypercholesterolemia, and diabetes in Japan. *Journal of the Neurological Sciences*, *293*(1–2), 82–6. http://doi.org/10.1016/j.jns.2010.03.002

Moog, C., Deloulme, J. C., Baudier, J., Revel, M. O., Bischoff, P., Hietter, H., & Luu, B. (1991). Membrane-related oxysterol function: preliminary results on the modification of protein kinase C activity and substrate phosphorylation by 7 beta,25-dihydroxycholesterol. *Biochimie*, *73*(10), 1321–6. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/1782225

Morens, D. M., Grandinetti, A., Reed, D., White, L. R., & Ross, G. W. (1995). Cigarette smoking and protection from Parkinson's disease: false association or etiologic clue? *Neurology*, *45*(6), 1041–51. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7783862

Morris, J. K., Bomhoff, G. L., Stanford, J. A., & Geiger, P. C. (2010). Neurodegeneration in an animal model of Parkinson's disease is exacerbated by a high-fat diet. *AJP: Regulatory, Integrative and Comparative Physiology*, *299*(4), R1082–R1090. http://doi.org/10.1152/ajpregu.00449.2010

Multhoff, G. (2007). Heat shock protein 70 (Hsp70): Membrane location, export and immunological relevance. *Methods*, *43*(3), 229–237. http://doi.org/10.1016/j.ymeth.2007.06.006

Multiple-System Atrophy Research Collaboration. (2013). Mutations in *COQ2* in Familial and Sporadic Multiple-System Atrophy. *New England Journal of Medicine*, *369*(3), 233–244. http://doi.org/10.1056/NEJMoa1212115

Murdolo, G., Piroddi, M., Tortoioli, C., Bartolini, D., Schmelz, M., Luchetti, F., … Galli, F. (2016). Free Radical-derived Oxysterols: Novel Adipokines Modulating Adipogenic Differentiation of Adipose Precursor Cells. *The Journal of Clinical Endocrinology & Metabolism*, *101*(12), 4974–4983. http://doi.org/10.1210/jc.2016-2918

Mutez, E., Duhamel, A., Defebvre, L., Bordet, R., Destée, A., & Kreisler, A. (2009). Lipid-lowering drugs are associated with delayed onset and slower course of Parkinson's disease. *Pharmacological Research*, *60*(1), 41–45. http://doi.org/10.1016/j.phrs.2009.03.010

Muthane, U., Ramsay, K. A., Jiang, H., Jackson-Lewis, V., Donaldson, D., Fernando, S., … Przedborski, S. (1994). Differences in Nigral Neuron Number and Sensitivity to 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine in C57/bl and CD-1 Mice. *Experimental Neurology*, *126*(2), 195–204. http://doi.org/10.1006/exnr.1994.1058

Nagai, Y., Kitazawa, R., Nakagawa, M., Komoda, M., Kondo, T., Haraguchi, R., & Kitazawa, S. (2012). Multiple-System Atrophy in Long-Term Professional Painter: A Case Report. *Case Reports in Pathology*, *2012*, 1–5. http://doi.org/10.1155/2012/613180

Nagatsu, T. (1995). Tyrosine hydroxylase: human isoforms, structure and regulation in physiology and pathology. *Essays in Biochemistry*, *30*, 15–35. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8822146

Naik, S. U., Wang, X., Da Silva, J. S., Jaye, M., Macphee, C. H., Reilly, M. P., … Rader, D. J. (2005). Pharmacological Activation of Liver X Receptors Promotes Reverse Cholesterol Transport In Vivo. *Circulation*, *113*(1), 90–97. http://doi.org/10.1161/CIRCULATIONAHA.105.560177

Namba, Y., Tomonaga, M., Ohtsuka, K., Oda, M., & Ikeda, K. (1991). [HSP 70 is associated with abnormal cytoplasmic inclusions characteristic of neurodegenerative diseases]. *No to Shinkei = Brain and Nerve*, *43*(1), 57–60. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2054224

Nee, L. E., Gomez, M. R., Dambrosia, J., Bale, S., Eldridge, R., & Polinsky, R. J. (1991). Environmental-occupational risk factors and familial associations in multiple system atrophy: a preliminary investigation. *Clinical Autonomic Research : Official Journal of the Clinical Autonomic Research Society*, *1*(1), 9–13. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/1821673

Norlin, M., & Wikvall, K. (2007). Enzymes in the conversion of cholesterol into bile acids. *Current Molecular Medicine*, *7*(2), 199–218. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/17346171

Notter, M. F., Irwin, I., Langston, J. W., & Gash, D. M. (1988). Neurotoxicity of MPTP and MPP+ in vitro: characterization using specific cell lines. *Brain Research*, *456*(2), 254–62. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/3264740

O'Malley, K. L., Anhalt, M. J., Martin, B. M., Kelsoe, J. R., Winfield, S. L., & Ginns, E. I. (1987). Isolation and characterization of the human tyrosine hydroxylase gene: identification of 5' alternative splice sites responsible for multiple mRNAs. *Biochemistry*, *26*(22), 6910–4. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2892528

O'Reilly, E. J., McCullough, M. L., Chao, A., Henley, S. J., Calle, E. E., Thun, M. J., & Ascherio, A. (2005). Smokeless tobacco use and the risk of Parkinson's disease mortality. *Movement Disorders : Official Journal of the Movement Disorder Society*, *20*(10), 1383–4. http://doi.org/10.1002/mds.20587

Oberbach, A., Schlichting, N., Heinrich, M., Till, H., Stolzenburg, J.-U., Neuhaus, J., … Park, S. (2012). Free Fatty Acid Palmitate Impairs the Vitality and Function of Cultured Human Bladder Smooth Muscle Cells. *PLoS ONE*, *7*(7), e41026. http://doi.org/10.1371/journal.pone.0041026

Okochi, M., Walter, J., Koyama, A., Nakajo, S., Baba, M., Iwatsubo, T., … Haass, C. (2000). Constitutive phosphorylation of the Parkinson's disease associated alpha-synuclein. *The Journal of Biological Chemistry*, *275*(1), 390–7. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10617630

Olkkonen, V. M. (2008). New Functions for Oxysterols and Their Cellular Receptors. *Lipid Insights*, *2 1*, 1–9.

Olkkonen, V. M., Béaslas, O., & Nissilä, E. (2012). Oxysterols and Their Cellular Effectors. *Biomolecules*, *2*(4), 76–103. http://doi.org/10.3390/biom2010076

Oueslati, A., Fournier, M., & Lashuel, H. A. (2010). Role of post-translational modifications in modulating the structure, function and toxicity of α-synuclein. In *Progress in brain research* (Vol. 183, pp. 115–145). http://doi.org/10.1016/S0079-6123(10)83007-9

Paleologou, K. E., Oueslati, A., Shakked, G., Rospigliosi, C. C., Kim, H.-Y., Lamberto, G. R., … Lashuel, H. A. (2010). Phosphorylation at S87 is enhanced in synucleinopathies, inhibits alpha-synuclein oligomerization, and influences synuclein-membrane interactions. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *30*(9), 3184–98. http://doi.org/10.1523/JNEUROSCI.5922-09.2010

Palmquist, D. L. (1972). Palmitic Acid as a Source of Endogenous Acetate and β-Hydroxybutyrate in Fed and Fasted Ruminants. *The Journal of Nutrition*, *102*(11), 1401–1406. http://doi.org/10.1093/jn/102.11.1401

Parkinson Study Group QE3 Investigators, M. F., Beal, M. F., Oakes, D., Shoulson, I., Henchcliffe, C., Galpern, W. R., … Boyar, K. (2014). A randomized clinical trial of high-dosage coenzyme Q10 in early Parkinson disease: no evidence of benefit. *JAMA Neurology*, *71*(5), 543–52. http://doi.org/10.1001/jamaneurol.2014.131

Pasternak, B., Svanström, H., Nielsen, N. M., Fugger, L., Melbye, M., & Hviid, A. (2012). Use of calcium channel blockers and Parkinson's disease. *American Journal of Epidemiology*, *175*(7), 627–35. http://doi.org/10.1093/aje/kwr362

Peng, X. M., Peng, X. M., Tehranian, R., Dietrich, P., Stefanis, L., & Perez, R. G. (2005). -Synuclein activation of protein phosphatase 2A reduces tyrosine hydroxylase phosphorylation in dopaminergic cells. *Journal of Cell Science*, *118*(15), 3523–3530. http://doi.org/10.1242/jcs.02481

Pickart, C. M. (2001). Ubiquitin enters the new millennium. *Molecular Cell*, *8*(3), 499–504. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11583613

Piech-Dumas, K. M., & Tank, A. W. (1999). CREB mediates the cAMP-responsiveness of the tyrosine hydroxylase gene: use of an antisense RNA strategy to produce CREB-deficient PC12 cell lines. *Brain Research. Molecular Brain Research*, *70*(2), 219–30. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10407170

Pillon, B., Deweer, B., Agid, Y., & Dubois, B. (1993). Explicit memory in Alzheimer's, Huntington's, and Parkinson's diseases. *Archives of Neurology*, *50*(4), 374–9. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8460958

Pillon, B., Dubois, B., Lhermitte, F., & Agid, Y. (1986). Heterogeneity of cognitive impairment in progressive supranuclear palsy, Parkinson's disease, and Alzheimer's disease. *Neurology*, *36*(9), 1179–85. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/3748383

Pillon, B., Dubois, B., Ploska, A., & Agid, Y. (1991). Severity and specificity of cognitive impairment in Alzheimer's, Huntington's, and Parkinson's diseases and progressive supranuclear palsy. *Neurology*, *41*(5), 634–43. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/1827513

Pinter, B., Diem-Zangerl, A., Wenning, G. K., Scherfler, C., Oberaigner, W., Seppi, K., & Poewe, W. (2015). Mortality in Parkinson's disease: a 38-year follow-up study. *Movement Disorders : Official Journal of the Movement Disorder Society*, *30*(2), 266–9. http://doi.org/10.1002/mds.26060

Pirillo, A., Zhu, W., Roma, P., Galli, G., Caruso, D., Pellegatta, F., & Catapano, A. L. (1999). Oxysterols from oxidized LDL are cytotoxic but fail to induce hsp70 expression in endothelial cells. *FEBS Letters*, *462*(1–2), 113–6. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10580102

Poewe, W., Seppi, K., Tanner, C. M., Halliday, G. M., Brundin, P., Volkmann, J., … Lang, A. E. (2017). Parkinson disease. *Nature Reviews Disease Primers*, *3*, 17013. http://doi.org/10.1038/nrdp.2017.13

Polymeropoulos, M. H., Lavedan, C., Leroy, E., Ide, S. E., Dehejia, A., Dutra, A., … Nussbaum, R. L. (1997a). Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. *Science (New York, N.Y.)*, *276*(5321), 2045–7. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9197268

Polymeropoulos, M. H., Lavedan, C., Leroy, E., Ide, S. E., Dehejia, A., Dutra, A., … Nussbaum, R. L. (1997b). Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. *Science (New York, N.Y.)*, *276*(5321), 2045–7. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9197268

Powers, K. M., Smith-Weller, T., Franklin, G. M., Longstreth, W. T., Swanson, P. D., & Checkoway, H. (2003). Parkinson's disease risks associated with dietary iron, manganese, and other nutrient intakes. *Neurology*, *60*(11), 1761–6. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12796527

Powers, K. M., Smith-Weller, T., Franklin, G. M., Longstreth, W. T., Swanson, P. D., & Checkoway, H. (2009a). Dietary fats, cholesterol and iron as risk factors for Parkinson's disease. *Parkinsonism & Related Disorders*, *15*(1), 47–52. http://doi.org/10.1016/j.parkreldis.2008.03.002

Powers, K. M., Smith-Weller, T., Franklin, G. M., Longstreth, W. T., Swanson, P. D., & Checkoway, H. (2009b). Dietary fats, cholesterol and iron as risk factors for Parkinson's disease. *Parkinsonism & Related Disorders*, *15*(1), 47–52. http://doi.org/10.1016/j.parkreldis.2008.03.002

Pradhan, S., & Andreasson, K. (2013). Commentary: Progressive inflammation as a contributing factor to early development of Parkinson's disease. *Experimental Neurology*, *241*, 148–55. http://doi.org/10.1016/j.expneurol.2012.12.008

Prasanthi, J. R. P., Larson, T., Schommer, J., & Ghribi, O. (2011). Silencing GADD153/CHOP gene expression protects against Alzheimer's disease-like pathology induced by 27-hydroxycholesterol in rabbit hippocampus. *PloS One*, *6*(10), e26420. http://doi.org/10.1371/journal.pone.0026420

Pringsheim, T., Jette, N., Frolkis, A., & Steeves, T. D. L. (2014). The prevalence of Parkinson's disease: A systematic review and meta-analysis. *Movement Disorders*, *29*(13), 1583–1590. http://doi.org/10.1002/mds.25945

Pronin, A. N., Morris, A. J., Surguchov, A., & Benovic, J. L. (2000). Synucleins are a novel class of substrates for G protein-coupled receptor kinases. *The Journal of Biological Chemistry*, *275*(34), 26515–22. http://doi.org/10.1074/jbc.M003542200

Proukakis, C., Dudzik, C. G., Brier, T., MacKay, D. S., Cooper, J. M., Millhauser, G. L., … Schapira, A. H. (2013). A novel -synuclein missense mutation in Parkinson disease. *Neurology*, *80*(11), 1062–1064. http://doi.org/10.1212/WNL.0b013e31828727ba

Psychopharmacology - 4th Generation of Progress - ACNP. (n.d.). Retrieved May 7, 2018, from https://acnp.org/digital-library/psychopharmacology-4th-generation-progress/

Raab, H., Pilgrim, C., & Reisert, I. (1995). Effects of sex and estrogen on tyrosine hydroxylase mRNA in cultured embryonic rat mesencephalon. *Molecular Brain Research*, *33*(1), 157–164. http://doi.org/10.1016/0169-328X(95)00125-C

Rabl, R., Breitschaedel, C., Flunkert, S., Duller, S., Amschl, D., Neddens, J., … Hutter-Paier, B. (2017). Early start of progressive motor deficits in Line 61 α-synuclein transgenic mice. *BMC Neuroscience*, *18*(1), 22. http://doi.org/10.1186/s12868-017-0341-8

Raese, J., Patrick, R. L., & Barchas, J. D. (1976). Phospholipid-induced activation of tyrosine hydroxylase from rat brain striatal synaptosomes. *Biochemical Pharmacology*, *25*(20), 2245–2250. http://doi.org/10.1016/0006-2952(76)90005-8

Rajput, A. H., Offord, K. P., Beard, C. M., & Kurland, L. T. (1987). A case-control study of smoking habits, dementia, and other illnesses in idiopathic Parkinson's disease. *Neurology*, *37*(2), 226–32. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/3808303

Ramsay, R. R., Salach, J. I., & Singer, T. P. (1986). Uptake of the neurotoxin 1-methyl-4-phenylpyridine (MPP+) by mitochondria and its relation to the inhibition of the mitochondrial oxidation of NAD+-linked substrates by MPP+. *Biochemical and Biophysical Research Communications*, *134*(2), 743–8. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2868716

Ransom, B. R., Kunis, D. M., Irwin, I., & Langston, J. W. (1987). Astrocytes convert the parkinsonism inducing neurotoxin, MPTP, to its active metabolite, MPP+. *Neuroscience Letters*, *75*(3), 323–8. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/3495754

Rantham Prabhakara, J. P., Feist, G., Thomasson, S., Thompson, A., Schommer, E., & Ghribi, O. (2008). Differential effects of 24-hydroxycholesterol and 27-hydroxycholesterol on tyrosine hydroxylase and alpha-synuclein in human neuroblastoma SH-SY5Y cells. *Journal of Neurochemistry*, *107*(6), 1722–9. http://doi.org/10.1111/j.1471-4159.2008.05736.x

Rao, M. S., Hattiangady, B., & Shetty, A. K. (2006). The window and mechanisms of major age-related decline in the production of new neurons within the dentate gyrus of the hippocampus. *Aging Cell*, *5*(6), 545–558. http://doi.org/10.1111/j.1474-9726.2006.00243.x

Reits, E., Griekspoor, A., Neijssen, J., Groothuis, T., Jalink, K., van Veelen, P., … Neefjes, J. (2003). Peptide diffusion, protection, and degradation in nuclear and cytoplasmic compartments before antigen presentation by MHC class I. *Immunity*, *18*(1), 97–108. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12530979

Reyes, J. F., Rey, N. L., Bousset, L., Melki, R., Brundin, P., & Angot, E. (2014). Alpha-synuclein transfers from neurons to oligodendrocytes. *Glia*, *62*(3), 387–398. http://doi.org/10.1002/glia.22611

Reynoso, R., Salgado, L. M., & Calderón, V. (2003). High levels of palmitic acid lead to insulin resistance due to changes in the level of phosphorylation of the insulin receptor and insulin receptor substrate-1. *Molecular and Cellular Biochemistry*, *246*(1–2), 155–62. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12841357

Ribeiro, C. S., Carneiro, K., Ross, C. A., Menezes, J. R. L., & Engelender, S. (2002). Synphilin-1 is developmentally localized to synaptic terminals, and its association with synaptic vesicles is modulated by alpha-synuclein. *The Journal of Biological Chemistry*, *277*(26), 23927–33. http://doi.org/10.1074/jbc.M201115200

Rideout, H. J., Lang-Rollin, I., & Stefanis, L. (2004). Involvement of macroautophagy in the dissolution of neuronal inclusions. *The International Journal of Biochemistry & Cell Biology*, *36*(12), 2551–62. http://doi.org/10.1016/j.biocel.2004.05.008

Rideout, H. J., Larsen, K. E., Sulzer, D., & Stefanis, L. (2001). Proteasomal inhibition leads to formation of ubiquitin/alpha-synuclein-immunoreactive inclusions in PC12 cells. *Journal of Neurochemistry*, *78*(4), 899–908. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11520910

Rideout, H. J., & Stefanis, L. (2002). Proteasomal inhibition-induced inclusion formation and death in cortical neurons require transcription and ubiquitination. *Molecular and Cellular Neurosciences*, *21*(2), 223–38. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12401444

Risé, P., Eligini, S., Ghezzi, S., Colli, S., & Galli, C. (2007). Fatty acid composition of plasma, blood cells and whole blood: relevance for the assessment of the fatty acid status in humans. *Prostaglandins, Leukotrienes, and Essential Fatty Acids*, *76*(6), 363–9. http://doi.org/10.1016/j.plefa.2007.05.003

Ritz, B., Manthripragada, A. D., Qian, L., Schernhammer, E., Wermuth, L., Olsen, J., & Friis, S. (2010). Statin use and Parkinson's disease in Denmark. *Movement Disorders : Official Journal of the Movement Disorder Society*, *25*(9), 1210–6. http://doi.org/10.1002/mds.23102

Ritz, B., Rhodes, S. L., Qian, L., Schernhammer, E., Olsen, J. H., & Friis, S. (2010). L-type calcium channel blockers and Parkinson disease in Denmark. *Annals of Neurology*, *67*(5), 600–6. http://doi.org/10.1002/ana.21937

Rockenstein, E., Mallory, M., Hashimoto, M., Song, D., Shults, C. W., Lang, I., & Masliah, E. (2002a). Differential neuropathological alterations in transgenic mice expressing ?-synuclein from the platelet-derived growth factor and Thy-1 promoters. *Journal of Neuroscience Research*, *68*(5), 568–578. http://doi.org/10.1002/jnr.10231

Rockenstein, E., Mallory, M., Hashimoto, M., Song, D., Shults, C. W., Lang, I., & Masliah, E. (2002b). Differential neuropathological alterations in transgenic mice expressing alpha-synuclein from the platelet-derived growth factor and Thy-1 promoters. *Journal of Neuroscience Research*, *68*(5), 568–78. http://doi.org/10.1002/jnr.10231

Roskoski, R., Vulliet, P. R., & Glass, D. B. (1987). Phosphorylation of tyrosine hydroxylase by cyclic GMP-dependent protein kinase. *Journal of Neurochemistry*, *48*(3), 840–5. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2879892

Ross, G. W., Abbott, R. D., Petrovitch, H., Morens, D. M., Grandinetti, A., Tung, K. H., … White, L. R. (n.d.). Association of coffee and caffeine intake with the risk of Parkinson disease. *JAMA*, *283*(20), 2674–9. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10819950

Rott, R., Szargel, R., Haskin, J., Bandopadhyay, R., Lees, A. J., Shani, V., & Engelender, S. (2011). α-Synuclein fate is determined by USP9X-regulated monoubiquitination. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(46), 18666–71. http://doi.org/10.1073/pnas.1105725108

Rott, R., Szargel, R., Haskin, J., Shani, V., Shainskaya, A., Manov, I., … Engelender, S. (2008). Monoubiquitylation of alpha-synuclein by seven in absentia homolog (SIAH) promotes its aggregation in dopaminergic cells. *The Journal of Biological Chemistry*, *283*(6), 3316–28. http://doi.org/10.1074/jbc.M704809200

Royo, M., Daubner, S. C., & Fitzpatrick, P. F. (2004). Specificity of the MAP kinase ERK2 for phosphorylation of tyrosine hydroxylase. *Archives of Biochemistry and Biophysics*, *423*(2), 247–252. http://doi.org/10.1016/j.abb.2003.12.027

Ryan, L., O'Callaghan, Y. C., & O'Brien, N. M. (2004). Comparison of the apoptotic processes induced by the oxysterols 7beta-hydroxycholesterol and cholesterol-5beta,6beta-epoxide. *Cell Biology and Toxicology*, *20*(5), 313–23. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/15685934

Samii, A., Carleton, B. C., & Etminan, M. (2008). Statin use and the risk of Parkinson disease: a nested case control study. *Journal of Clinical Neuroscience : Official Journal of the Neurosurgical Society of Australasia*, *15*(11), 1272–3. http://doi.org/10.1016/j.jocn.2008.01.016

Sampson, T. R., Debelius, J. W., Thron, T., Janssen, S., Shastri, G. G., Ilhan, Z. E., … Mazmanian, S. K. (2016). Gut Microbiota Regulate Motor Deficits and Neuroinflammation in a Model of Parkinson's Disease. *Cell*, *167*(6), 1469–1480.e12. http://doi.org/10.1016/j.cell.2016.11.018

Sato, H., Kato, T., & Arawaka, S. (2013a). The role of Ser129 phosphorylation of α-synuclein in neurodegeneration of Parkinson's disease: a review of in vivo models. *Reviews in the Neurosciences*, *24*(2), 115–23. http://doi.org/10.1515/revneuro-2012-0071

Sato, H., Kato, T., & Arawaka, S. (2013b). The role of Ser129 phosphorylation of α-synuclein in neurodegeneration of Parkinson's disease: a review of in vivo models. *Reviews in the Neurosciences*, *24*(2), 115–23. http://doi.org/10.1515/revneuro-2012-0071

Savica, R., Grossardt, B. R., Bower, J. H., Boeve, B. F., Ahlskog, J. E., & Rocca, W. A. (2013). Incidence of Dementia With Lewy Bodies and Parkinson Disease Dementia. *JAMA Neurology*, *70*(11), 1396. http://doi.org/10.1001/jamaneurol.2013.3579

Savolainen, M. H., Albert, K., Airavaara, M., & Myöhänen, T. T. (2017). Nigral injection of a proteasomal inhibitor, lactacystin, induces widespread glial cell activation and shows various phenotypes of Parkinson's disease in young and adult mouse. *Experimental Brain Research*, *235*(7), 2189–2202. http://doi.org/10.1007/s00221-017-4962-z

Schapira, A. H. (1997). Pathogenesis of Parkinson's disease. *Bailliere's Clinical Neurology*, *6*(1), 15–36. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9426866

Schell, H., Hasegawa, T., Neumann, M., & Kahle, P. J. (2009). Nuclear and neuritic distribution of serine-129 phosphorylated alpha-synuclein in transgenic mice. *Neuroscience*, *160*(4), 796–804. http://doi.org/10.1016/j.neuroscience.2009.03.002

Scherzer, C. R., Grass, J. A., Liao, Z., Pepivani, I., Zheng, B., Eklund, A. C., … Schlossmacher, M. G. (2008). GATA transcription factors directly regulate the Parkinson's disease-linked gene alpha-synuclein. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(31), 10907–12. http://doi.org/10.1073/pnas.0802437105

Schoch, S., Deák, F., Königstorfer, A., Mozhayeva, M., Sara, Y., Südhof, T. C., & Kavalali, E. T. (2001). SNARE Function Analyzed in Synaptobrevin/VAMP Knockout Mice. *Science*, *294*(5544), 1117–1122. http://doi.org/10.1126/science.1064335

Scholz, S. W., Houlden, H., Schulte, C., Sharma, M., Li, A., Berg, D., … Gasser, T. (2009). *SNCA* variants are associated with increased risk for multiple system atrophy. *Annals of Neurology*, *65*(5), 610–614. http://doi.org/10.1002/ana.21685

Schommer, J., Marwarha, G., Schommer, T., Flick, T., Lund, J., & Ghribi, O. (2018). 27-Hydroxycholesterol increases α-synuclein protein levels through proteasomal inhibition in human dopaminergic neurons. *BMC Neuroscience*, *19*(1), 17. http://doi.org/10.1186/s12868-018-0420-5

Schommer, J., Schrag, M., Nackenoff, A., Marwarha, G., & Ghribi, O. (2017). Method for organotypic tissue culture in the aged animal. *MethodsX*, *4*, 166–171. http://doi.org/10.1016/j.mex.2017.03.003

Schrag, A., Wenning, G. K., Quinn, N., & Ben-Shlomo, Y. (2008). Survival in multiple system atrophy. *Movement Disorders*, *23*(2), 294–296. http://doi.org/10.1002/mds.21839

Schrag, M., Sharma, S., Brown-Borg, H., & Ghribi, O. (2008). Hippocampus of Ames dwarf mice is resistant to β-amyloid-induced tau hyperphosphorylation and changes in apoptosis-regulatory protein levels. *Hippocampus*, *18*(3), 239–244. http://doi.org/10.1002/hipo.20387

Sears, B., & Perry, M. (2015). The role of fatty acids in insulin resistance. *Lipids in Health and Disease*, *14*, 121. http://doi.org/10.1186/s12944-015-0123-1

Seidl, S. E., Santiago, J. A., Bilyk, H., & Potashkin, J. A. (2014). The emerging role of nutrition in Parkinson's disease. *Frontiers in Aging Neuroscience*, *6*, 36. http://doi.org/10.3389/fnagi.2014.00036

Serrano-Pozo, A., Vega, G. L., Lütjohann, D., Locascio, J. J., Tennis, M. K., Deng, A., … Growdon, J. H. (2010). Effects of simvastatin on cholesterol metabolism and Alzheimer disease biomarkers. *Alzheimer Disease and Associated Disorders*, *24*(3), 220–6. http://doi.org/10.1097/WAD.0b013e3181d61fea

Shaafi, S., Najmi, S., Aliasgharpour, H., Mahmoudi, J., Sadigh-Etemad, S., Farhoudi, M., & Baniasadi, N. (2016). The efficacy of the ketogenic diet on motor functions in Parkinson's disease: A rat model. *Iranian Journal of Neurology*, *15*(2), 63–9. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/27326359

Shafaati, M., Marutle, A., Pettersson, H., Lovgren-Sandblom, A., Olin, M., Pikuleva, I., … Bjorkhem, I. (2011). Marked accumulation of 27-hydroxycholesterol in the brains of Alzheimer's patients with the Swedish APP 670/671 mutation. *The Journal of Lipid Research*, *52*(5), 1004–1010. http://doi.org/10.1194/jlr.M014548

Shafaati, M., Marutle, A., Pettersson, H., Lövgren-Sandblom, A., Olin, M., Pikuleva, I., … Björkhem, I. (2011). Marked accumulation of 27-hydroxycholesterol in the brains of Alzheimer's patients with the Swedish APP 670/671 mutation. *Journal of Lipid Research*, *52*(5), 1004–10. http://doi.org/10.1194/jlr.M014548

Shahpasandzadeh, H., Popova, B., Kleinknecht, A., Fraser, P. E., Outeiro, T. F., & Braus, G. H. (2014). Interplay between sumoylation and phosphorylation for protection against α-synuclein inclusions. *The Journal of Biological Chemistry*, *289*(45), 31224–40. http://doi.org/10.1074/jbc.M114.559237

Shang, F., & Taylor, A. (2011). Ubiquitin–proteasome pathway and cellular responses to oxidative stress. *Free Radical Biology and Medicine*, *51*(1), 5–16. http://doi.org/10.1016/j.freeradbiomed.2011.03.031

Sharon, R., Bar-Joseph, I., Mirick, G. E., Serhan, C. N., & Selkoe, D. J. (2003). Altered fatty acid composition of dopaminergic neurons expressing alpha-synuclein and human brains with alpha-synucleinopathies. *The Journal of Biological Chemistry*, *278*(50), 49874–81. http://doi.org/10.1074/jbc.M309127200

Sharon, R., Goldberg, M. S., Bar-Josef, I., Betensky, R. A., Shen, J., & Selkoe, D. J. (2001). -Synuclein occurs in lipid-rich high molecular weight complexes, binds fatty acids, and shows homology to the fatty acid-binding proteins. *Proceedings of the National Academy of Sciences*, *98*(16), 9110–9115. http://doi.org/10.1073/pnas.171300598

Shen, R. S., Abell, C. W., Gessner, W., & Brossi, A. (1985). Serotonergic conversion of MPTP and dopaminergic accumulation of MPP+. *FEBS Letters*, *189*(2), 225–30. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/3876242

Shibasaki, Y., Baillie, D. A. M., St. Clair, D., & Brookes, A. J. (1995). High-resolution mapping of SNCA encoding α-synuclein, the non-Aβ component of Alzheimer’s disease amyloid precursor, to human chromosome 4q21.3→q22 by fluorescence in situ hybridization. *Cytogenetic and Genome Research*, *71*(1), 54–55. http://doi.org/10.1159/000134061

Shin, Y., Klucken, J., Patterson, C., Hyman, B. T., & McLean, P. J. (2005). The co-chaperone carboxyl terminus of Hsp70-interacting protein (CHIP) mediates alpha-synuclein degradation decisions between proteasomal and lysosomal pathways. *The Journal of Biological Chemistry*, *280*(25), 23727–34. http://doi.org/10.1074/jbc.M503326200

Shults, C. W., Oakes, D., Kieburtz, K., Beal, M. F., Haas, R., Plumb, S., … Parkinson Study Group. (2002). Effects of coenzyme Q10 in early Parkinson disease: evidence of slowing of the functional decline. *Archives of Neurology*, *59*(10), 1541–50. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12374491

Siddiqui, A., Chinta, S. J., Mallajosyula, J. K., Rajagopolan, S., Hanson, I., Rane, A., … Andersen, J. K. (2012). Selective binding of nuclear alpha-synuclein to the PGC1alpha promoter under conditions of oxidative stress may contribute to losses in mitochondrial function: implications for Parkinson's disease. *Free Radical Biology & Medicine*, *53*(4), 993–1003. http://doi.org/10.1016/j.freeradbiomed.2012.05.024

Simon, K. C., Chen, H., Schwarzschild, M., & Ascherio, A. (2007). Hypertension, hypercholesterolemia, diabetes, and risk of Parkinson disease. *Neurology*, *69*(17), 1688–95. http://doi.org/10.1212/01.wnl.0000271883.45010.8a

Sjoerdsma, A., Engelman, K., Spector, S., & Udenfriend, S. (1965). Inhibition of catecholamine synthesis in man with alpha-methyl-tyrosine, an inhibitor of tyrosine hydroxylase. *Lancet (London, England)*, *2*(7422), 1092–4. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/4158803

Snyder, H., & Wolozin, B. (2004). Pathological Proteins in Parkinson's Disease: Focus on the Proteasome. *Journal of Molecular Neuroscience*, *24*(3), 425–442. http://doi.org/10.1385/JMN:24:3:425

Song, C., Hiipakka, R. A., & Liao, S. (2001). Auto-oxidized cholesterol sulfates are antagonistic ligands of liver X receptors: implications for the development and treatment of atherosclerosis. *Steroids*, *66*(6), 473–9. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11182136

Song, D. D., Shults, C. W., Sisk, A., Rockenstein, E., & Masliah, E. (2004). Enhanced *substantia nigra* mitochondrial pathology in human α-synuclein transgenic mice after treatment with MPTP111-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *Experimental Neurology*, *186*(2), 158–172. http://doi.org/10.1016/S0014-4886(03)00342-X

Song, Y. J. C., Lundvig, D. M. S., Huang, Y., Gai, W. P., Blumbergs, P. C., Højrup, P., … Jensen, P. H. (2007). p25α Relocalizes in Oligodendroglia from Myelin to Cytoplasmic Inclusions in Multiple System Atrophy. *The American Journal of Pathology*, *171*(4), 1291–1303. http://doi.org/10.2353/ajpath.2007.070201

Sonsalla, P. K., & Heikkila, R. E. (1986). The influence of dose and dosing interval on MPTP-induced dopaminergic neurotoxicity in mice. *European Journal of Pharmacology*, *129*(3), 339–45. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/3490988

Sottero, B., Gamba, P., Gargiulo, S., Leonarduzzi, G., & Poli, G. (2009). Cholesterol oxidation products and disease: an emerging topic of interest in medicinal chemistry. *Current Medicinal Chemistry*, *16*(6), 685–705. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/19199932

Sottero, B., Rossin, D., Poli, G., & Biasi, F. (2017). Lipid oxidation products in the pathogenesis of inflammation-related gut diseases. *Current Medicinal Chemistry*, *24*. http://doi.org/10.2174/0929867324666170619104105

Spann, N. J., & Glass, C. K. (2013). Sterols and oxysterols in immune cell function. *Nature Immunology*, *14*(9), 893–900. http://doi.org/10.1038/ni.2681

Spillantini, M. G., Schmidt, M. L., Lee, V. M.-Y., Trojanowski, J. Q., Jakes, R., & Goedert, M. (1997). |[alpha]|-Synuclein in Lewy bodies. *Nature*, *388*(6645), 839–840. http://doi.org/10.1038/42166

Sriram, K., Pai, K. S., Boyd, M. R., & Ravindranath, V. (1997). Evidence for generation of oxidative stress in brain by MPTP: in vitro and in vivo studies in mice. *Brain Research*, *749*(1), 44–52. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9070626

Stemberger, S., Scholz, S. W., Singleton, A. B., & Wenning, G. K. (2011). Genetic players in multiple system atrophy: unfolding the nature of the beast. *Neurobiology of Aging*, *32*(10), 1924.e5-14. http://doi.org/10.1016/j.neurobiolaging.2011.04.001

Sterling, L., Walter, M., Ting, D., & Schüle, B. (2014). Discovery of functional non-coding conserved regions in the α-synuclein gene locus. *F1000Research*, *3*, 259. http://doi.org/10.12688/f1000research.3281.2

Stern, Y., Marder, K., Tang, M. X., & Mayeux, R. (1993). Antecedent clinical features associated with dementia in Parkinson's disease. *Neurology*, *43*(9), 1690–2. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8414013

Stoppini, L., Buchs, P. A., & Muller, D. (1991). A simple method for organotypic cultures of nervous tissue. *Journal of Neuroscience Methods*, *37*(2), 173–82. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/1715499

Sutherland, C., Alterio, J., Campbell, D. G., Le Bourdellès, B., Mallet, J., Haavik, J., & Cohen, P. (1993a). Phosphorylation and activation of human tyrosine hydroxylase in vitro by mitogen-activated protein (MAP) kinase and MAP-kinase-activated kinases 1 and 2. *European Journal of Biochemistry*, *217*(2), 715–22. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7901013

Sutherland, C., Alterio, J., Campbell, D. G., Le Bourdellès, B., Mallet, J., Haavik, J., & Cohen, P. (1993b). Phosphorylation and activation of human tyrosine hydroxylase in vitro by mitogen-activated protein (MAP) kinase and MAP-kinase-activated kinases 1 and 2. *European Journal of Biochemistry*, *217*(2), 715–22. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7901013

Tabrez, S., Jabir, N. R., Shakil, S., Greig, N. H., Alam, Q., Abuzenadah, A. M., … Kamal, M. A. (2012). A synopsis on the role of tyrosine hydroxylase in Parkinson's disease. *CNS & Neurological Disorders Drug Targets*, *11*(4), 395–409. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/22483313

Tagliafierro, L., & Chiba-Falek, O. (2016). Up-regulation of SNCA gene expression: implications to synucleinopathies. *Neurogenetics*, *17*(3), 145–157. http://doi.org/10.1007/s10048-016-0478-0

Tan, L. C., Koh, W.-P., Yuan, J.-M., Wang, R., Au, W.-L., Tan, J. H., … Yu, M. C. (2007). Differential Effects of Black versus Green Tea on Risk of Parkinson's Disease in the Singapore Chinese Health Study. *American Journal of Epidemiology*, *167*(5), 553–560. http://doi.org/10.1093/aje/kwm338

Tan, L. C., Koh, W.-P., Yuan, J.-M., Wang, R., Au, W.-L., Tan, J. H., … Yu, M. C. (2008a). Differential effects of black versus green tea on risk of Parkinson's disease in the Singapore Chinese Health Study. *American Journal of Epidemiology*, *167*(5), 553–60. http://doi.org/10.1093/aje/kwm338

Tan, L. C., Koh, W.-P., Yuan, J.-M., Wang, R., Au, W.-L., Tan, J. H., … Yu, M. C. (2008b). Differential effects of black versus green tea on risk of Parkinson's disease in the Singapore Chinese Health Study. *American Journal of Epidemiology*, *167*(5), 553–60. http://doi.org/10.1093/aje/kwm338

Tanner, C. M., Kamel, F., Ross, G. W., Hoppin, J. A., Goldman, S. M., Korell, M., … Langston, J. W. (2011). Rotenone, paraquat, and Parkinson's disease. *Environmental Health Perspectives*, *119*(6), 866–72. http://doi.org/10.1289/ehp.1002839

Tenreiro, S., Eckermann, K., & Outeiro, T. F. (2014). Protein phosphorylation in neurodegeneration: friend or foe? *Frontiers in Molecular Neuroscience*, *7*, 42. http://doi.org/10.3389/fnmol.2014.00042

Tenreiro, S., Reimão-Pinto, M. M., Antas, P., Rino, J., Wawrzycka, D., Macedo, D., … Outeiro, T. F. (2014). Phosphorylation modulates clearance of alpha-synuclein inclusions in a yeast model of Parkinson's disease. *PLoS Genetics*, *10*(5), e1004302. http://doi.org/10.1371/journal.pgen.1004302

Thacker, E. L., Chen, H., Patel, A. V, McCullough, M. L., Calle, E. E., Thun, M. J., … Ascherio, A. (2008). Recreational physical activity and risk of Parkinson's disease. *Movement Disorders : Official Journal of the Movement Disorder Society*, *23*(1), 69–74. http://doi.org/10.1002/mds.21772

Thacker, E. L., O'Reilly, E. J., Weisskopf, M. G., Chen, H., Schwarzschild, M. A., McCullough, M. L., … Ascherio, A. (2007). Temporal relationship between cigarette smoking and risk of Parkinson disease. *Neurology*, *68*(10), 764–8. http://doi.org/10.1212/01.wnl.0000256374.50227.4b

Thanan, R., Oikawa, S., Hiraku, Y., Ohnishi, S., Ma, N., Pinlaor, S., … Murata, M. (2015). Oxidative stress and its significant roles in neurodegenerative diseases and cancer. *International Journal of Molecular Sciences*, *16*(1), 193–217. http://doi.org/10.3390/ijms16010193

Tieu, K., Perier, C., Caspersen, C., Teismann, P., Wu, D.-C., Yan, S.-D., … Przedborski, S. (2003). D-β-Hydroxybutyrate rescues mitochondrial respiration and mitigates features of Parkinson disease. *Journal of Clinical Investigation*, *112*(6), 892–901. http://doi.org/10.1172/JCI18797

Tofaris, G. K., Kim, H. T., Hourez, R., Jung, J.-W., Kim, K. P., & Goldberg, A. L. (2011). Ubiquitin ligase Nedd4 promotes alpha-synuclein degradation by the endosomal-lysosomal pathway. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(41), 17004–9. http://doi.org/10.1073/pnas.1109356108

Tofaris, G. K., Layfield, R., & Spillantini, M. G. (2001). alpha-synuclein metabolism and aggregation is linked to ubiquitin-independent degradation by the proteasome. *FEBS Letters*, *509*(1), 22–6. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11734199

Tofaris, G. K., Razzaq, A., Ghetti, B., Lilley, K. S., & Spillantini, M. G. (2003). Ubiquitination of alpha-synuclein in Lewy bodies is a pathological event not associated with impairment of proteasome function. *The Journal of Biological Chemistry*, *278*(45), 44405–11. http://doi.org/10.1074/jbc.M308041200

Toska, K., Kleppe, R., Armstrong, C. G., Morrice, N. A., Cohen, P., & Haavik, J. (2002a). Regulation of tyrosine hydroxylase by stress-activated protein kinases. *Journal of Neurochemistry*, *83*(4), 775–83. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12421349

Toska, K., Kleppe, R., Armstrong, C. G., Morrice, N. A., Cohen, P., & Haavik, J. (2002b). Regulation of tyrosine hydroxylase by stress-activated protein kinases. *Journal of Neurochemistry*, *83*(4), 775–83. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12421349

Tracy, L. M., Bergqvist, F., Ivanova, E. V., Jacobsen, K. T., & Iverfeldt, K. (2013). Exposure to the Saturated Free Fatty Acid Palmitate Alters BV-2 Microglia Inflammatory Response. *Journal of Molecular Neuroscience*, *51*(3), 805–812. http://doi.org/10.1007/s12031-013-0068-7

Twelves, D., Perkins, K. S. M., & Counsell, C. (2003). Systematic review of incidence studies of Parkinson's disease. *Movement Disorders*, *18*(1), 19–31. http://doi.org/10.1002/mds.10305

Tysnes, O.-B., & Storstein, A. (2017). Epidemiology of Parkinson's disease. *Journal of Neural Transmission*, *124*(8), 901–905. http://doi.org/10.1007/s00702-017-1686-y

Umetani, M., Ghosh, P., Ishikawa, T., Umetani, J., Ahmed, M., Mineo, C., & Shaul, P. W. (2014). The Cholesterol Metabolite 27-Hydroxycholesterol Promotes Atherosclerosis via Proinflammatory Processes Mediated by Estrogen Receptor Alpha. *Cell Metabolism*, *20*(1), 172–182. http://doi.org/10.1016/j.cmet.2014.05.013

Undela, K., Gudala, K., Malla, S., & Bansal, D. (2013). Statin use and risk of Parkinson's disease: a meta-analysis of observational studies. *Journal of Neurology*, *260*(1), 158–65. http://doi.org/10.1007/s00415-012-6606-3

Valdinocci, D., Radford, R. A. W., Siow, S. M., Chung, R. S., & Pountney, D. L. (2017). Potential Modes of Intercellular α-Synuclein Transmission. *International Journal of Molecular Sciences*, *18*(2). http://doi.org/10.3390/ijms18020469

Van Den Eeden, S. K., Tanner, C. M., Bernstein, A. L., Fross, R. D., Leimpeter, A., Bloch, D. A., & Nelson, L. M. (2003). Incidence of Parkinson's disease: variation by age, gender, and race/ethnicity. *American Journal of Epidemiology*, *157*(11), 1015–22. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12777365

van Doormaal, J. J., Smit, N., Koopman, B. J., van der Molen, J. C., Wolthers, B. G., & Doorenbos, H. (1989). Hydroxycholesterols in serum from hypercholesterolaemic patients with and without bile acid sequestrant therapy. *Clinica Chimica Acta; International Journal of Clinical Chemistry*, *181*(3), 273–9. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2758681

Vanacore, N., Bonifati, V., Fabbrini, G., Colosimo, C., De Michele, G., Marconi, R., … ESGAP Consortium. (2005). Case-control study of multiple system atrophy. *Movement Disorders*, *20*(2), 158–163. http://doi.org/10.1002/mds.20303

Vanacore, N., Bonifati, V., Fabbrini, G., Colosimo, C., Marconi, R., Nicholl, D., … Meco, G. (2000). Smoking habits in multiple system atrophy and progressive supranuclear palsy. European Study Group on Atypical Parkinsonisms. *Neurology*, *54*(1), 114–9. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10636135

Vargas, K. J., Makani, S., Davis, T., Westphal, C. H., Castillo, P. E., & Chandra, S. S. (2014). Synucleins regulate the kinetics of synaptic vesicle endocytosis. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *34*(28), 9364–76. http://doi.org/10.1523/JNEUROSCI.4787-13.2014

Vidal, J.-S., Vidailhet, M., Elbaz, A., Derkinderen, P., Tzourio, C., & Alpérovitch, A. (2008). Risk factors of multiple system atrophy: A case-control study in French patients. *Movement Disorders*, *23*(6), 797–803. http://doi.org/10.1002/mds.21857

Vila, M., Vukosavic, S., Jackson-Lewis, V., Neystat, M., Jakowec, M., & Przedborski, S. (2000a). Alpha-synuclein up-regulation in *substantia nigra* dopaminergic neurons following administration of the parkinsonian toxin MPTP. *Journal of Neurochemistry*, *74*(2), 721–9. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10646524

Vila, M., Vukosavic, S., Jackson-Lewis, V., Neystat, M., Jakowec, M., & Przedborski, S. (2000b). Alpha-synuclein up-regulation in *substantia nigra* dopaminergic neurons following administration of the parkinsonian toxin MPTP. *Journal of Neurochemistry*, *74*(2), 721–9. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10646524

Villar-Menéndez, I., Porta, S., Buira, S. P., Pereira-Veiga, T., Díaz-Sánchez, S., Albasanz, J. L., … Barrachina, M. (2014). Increased striatal adenosine A2A receptor levels is an early event in Parkinson's disease-related pathology and it is potentially regulated by miR-34b. *Neurobiology of Disease*, *69*, 206–14. http://doi.org/10.1016/j.nbd.2014.05.030

Vogiatzi, T., Xilouri, M., Vekrellis, K., & Stefanis, L. (2008). Wild Type α-Synuclein Is Degraded by Chaperone-mediated Autophagy and Macroautophagy in Neuronal Cells. *Journal of Biological Chemistry*, *283*(35), 23542–23556. http://doi.org/10.1074/jbc.M801992200

Vrana, K. E., Allhiser, C. L., & Roskoski, R. (1981). Tyrosine hydroxylase activation and inactivation by protein phosphorylation conditions. *Journal of Neurochemistry*, *36*(1), 92–100. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/6109760

Vrana, K. E., & Roskoski, R. (1983). Tyrosine hydroxylase inactivation following cAMP-dependent phosphorylation activation. *Journal of Neurochemistry*, *40*(6), 1692–700. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/6133915

Vulliet, P. R., Woodgett, J. R., & Cohen, P. (1984). Phosphorylation of tyrosine hydroxylase by calmodulin-dependent multiprotein kinase. *The Journal of Biological Chemistry*, *259*(22), 13680–3. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/6150037

Vurusaner, B., Leonarduzzi, G., Gamba, P., Poli, G., & Basaga, H. (2016). Oxysterols and mechanisms of survival signaling. *Molecular Aspects of Medicine*, *49*, 8–22. http://doi.org/10.1016/j.mam.2016.02.004

Wang, H., Tang, C., Jiang, Z., Zhou, X., Chen, J., Na, M., … Lin, Z. (2017). Glutamine promotes Hsp70 and inhibits α-Synuclein accumulation in pheochromocytoma PC12 cells. *Experimental and Therapeutic Medicine*, *14*(2), 1253–1259. http://doi.org/10.3892/etm.2017.4580

Wang, L., Fleming, S. M., Chesselet, M.-F., & Taché, Y. (2008). Abnormal colonic motility in mice overexpressing human wild-type alpha-synuclein. *Neuroreport*, *19*(8), 873–6. http://doi.org/10.1097/WNR.0b013e3282ffda5e

Wang, L., Magen, I., Yuan, P.-Q., Subramaniam, S. R., Richter, F., Chesselet, M.-F., & Taché, Y. (2012). Mice overexpressing wild-type human alpha-synuclein display alterations in colonic myenteric ganglia and defecation. *Neurogastroenterology and Motility : The Official Journal of the European Gastrointestinal Motility Society*, *24*(9), e425-36. http://doi.org/10.1111/j.1365-2982.2012.01974.x

Watson, M. B., Richter, F., Lee, S. K., Gabby, L., Wu, J., Masliah, E., … Chesselet, M.-F. (2012). Regionally-specific microglial activation in young mice over-expressing human wildtype alpha-synuclein. *Experimental Neurology*, *237*(2), 318–34. http://doi.org/10.1016/j.expneurol.2012.06.025

Watts, J. C., Giles, K., Oehler, A., Middleton, L., Dexter, D. T., Gentleman, S. M., … Prusiner, S. B. (2013). Transmission of multiple system atrophy prions to transgenic mice. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(48), 19555–60. http://doi.org/10.1073/pnas.1318268110

Webb, J. L., Ravikumar, B., Atkins, J., Skepper, J. N., & Rubinsztein, D. C. (2003). α-Synuclein Is Degraded by Both Autophagy and the Proteasome. *Journal of Biological Chemistry*, *278*(27), 25009–25013. http://doi.org/10.1074/jbc.M300227200

Wenning, G. K., Colosimo, C., Geser, F., & Poewe, W. (2004). Multiple system atrophy. *The Lancet. Neurology*, *3*(2), 93–103. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/14747001

Wenning, G. K., Tison, F., ben Shlomo, Y., Daniel, S. E., & Quinn, N. P. (1997). Multiple system atrophy: A review of 203 pathologically proven cases. *Movement Disorders*, *12*(2), 133–147. http://doi.org/10.1002/mds.870120203

West, M. J., & Gundersen, H. J. G. (1990). Unbiased stereological estimation of the number of neurons in the human hippocampus. *The Journal of Comparative Neurology*, *296*(1), 1–22. http://doi.org/10.1002/cne.902960102

White, C. L., Pistell, P. J., Purpera, M. N., Gupta, S., Fernandez-Kim, S.-O., Hise, T. L., … Bruce-Keller, A. J. (2009). Effects of high fat diet on Morris maze performance, oxidative stress, and inflammation in rats: Contributions of maternal diet. http://doi.org/10.1016/j.nbd.2009.04.002

Withers, G. S., George, J. M., Banker, G. A., & Clayton, D. F. (1997). Delayed localization of synelfin (synuclein, NACP) to presynaptic terminals in cultured rat hippocampal neurons. *Brain Research. Developmental Brain Research*, *99*(1), 87–94. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9088569

Wolozin, B., Wang, S. W., Li, N.-C., Lee, A., Lee, T. A., & Kazis, L. E. (2007). Simvastatin is associated with a reduced incidence of dementia and Parkinson's disease. *BMC Medicine*, *5*(1), 20. http://doi.org/10.1186/1741-7015-5-20

Wu, N., Joshi, P. R., Cepeda, C., Masliah, E., & Levine, M. S. (2010). Alpha-synuclein overexpression in mice alters synaptic communication in the corticostriatal pathway. *Journal of Neuroscience Research*, *88*(8), 1764–76. http://doi.org/10.1002/jnr.22327

Wüllner, U., Schmitz-Hübsch, T., Abele, M., Antony, G., Bauer, P., & Eggert, K. (2007). Features of probable multiple system atrophy patients identified among 4770 patients with parkinsonism enrolled in the multicentre registry of the German Competence Network on Parkinson's disease. *Journal of Neural Transmission*, *114*(9), 1161–1165. http://doi.org/10.1007/s00702-007-0746-0

Xu, Q., Park, Y., Huang, X., Hollenbeck, A., Blair, A., Schatzkin, A., & Chen, H. (2010). Physical activities and future risk of Parkinson disease. *Neurology*, *75*(4), 341–8. http://doi.org/10.1212/WNL.0b013e3181ea1597

Xu, Q., Park, Y., Huang, X., Hollenbeck, A., Blair, A., Schatzkin, A., & Chen, H. (2011). Diabetes and risk of Parkinson's disease. *Diabetes Care*, *34*(4), 910–5. http://doi.org/10.2337/dc10-1922

Xu, S., Zhou, M., Yu, S., Cai, Y., Zhang, A., Uéda, K., & Chan, P. (2006). Oxidative stress induces nuclear translocation of C-terminus of alpha-synuclein in dopaminergic cells. *Biochemical and Biophysical Research Communications*, *342*(1), 330–5. http://doi.org/10.1016/j.bbrc.2006.01.148

Xu, W., Tan, L., & Yu, J.-T. (2015). The link between the SNCA gene and parkinsonism. *Neurobiology of Aging*, *36*(3), 1505–1518. http://doi.org/10.1016/j.neurobiolaging.2014.10.042

Xu, Y., Deng, Y., & Qing, H. (2015). The phosphorylation of α-synuclein: development and implication for the mechanism and therapy of the Parkinson's disease. *Journal of Neurochemistry*, *135*(1), 4–18. http://doi.org/10.1111/jnc.13234

Yang, F., Trolle Lagerros, Y., Bellocco, R., Adami, H.-O., Fang, F., Pedersen, N. L., & Wirdefeldt, K. (2015). Physical activity and risk of Parkinson's disease in the Swedish National March Cohort. *Brain : A Journal of Neurology*, *138*(Pt 2), 269–75. http://doi.org/10.1093/brain/awu323

Yang, F., Yang, Y., Mao, C., Liu, L., Zheng, H., Hu, L., & Liu, C. (2013). Crosstalk between the proteasome system and autophagy in the clearance of α-synuclein. *Acta Pharmacologica Sinica*, *34*(5), 674–680. http://doi.org/10.1038/aps.2013.29

Yang, X., & Cheng, B. (2010a). Neuroprotective and anti-inflammatory activities of ketogenic diet on MPTP-induced neurotoxicity. *Journal of Molecular Neuroscience : MN*, *42*(2), 145–53. http://doi.org/10.1007/s12031-010-9336-y

Yang, X., & Cheng, B. (2010b). Neuroprotective and anti-inflammatory activities of ketogenic diet on MPTP-induced neurotoxicity. *Journal of Molecular Neuroscience : MN*, *42*(2), 145–53. http://doi.org/10.1007/s12031-010-9336-y

Zarranz, J. J., Alegre, J., Gómez-Esteban, J. C., Lezcano, E., Ros, R., Ampuero, I., … de Yebenes, J. G. (2004). The new mutation, E46K, of α-synuclein causes parkinson and Lewy body dementia. *Annals of Neurology*, *55*(2), 164–173. http://doi.org/10.1002/ana.10795

Zhang, L., Zhang, C., Zhu, Y., Cai, Q., Chan, P., Uéda, K., … Yang, H. (2008). Semi-quantitative analysis of alpha-synuclein in subcellular pools of rat brain neurons: an immunogold electron microscopic study using a C-terminal specific monoclonal antibody. *Brain Research*, *1244*, 40–52. http://doi.org/10.1016/j.brainres.2008.08.067

Zhu, Y., Zhang, J., & Zeng, Y. (2012). Overview of tyrosine hydroxylase in Parkinson's disease. *CNS & Neurological Disorders Drug Targets*, *11*(4), 350–8. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/22483316

ZIERING, A., & LEE, J. (1947). PIPERIDINE DERIVATIVES. V. 1,3-DIALKYL-4-ARYL-4-ACYLOXYPIPERIDINES. *The Journal of Organic Chemistry*, *12*(6), 911–914. http://doi.org/10.1021/jo01170a024

Zigmond, R. E., Schwarzschild, M. A., & Rittenhouse, A. R. (1989). Acute Regulation of Tyrosine Hydroxylase by Nerve Activity and by Neurotransmitters Via Phosphorylation. *Annual Review of Neuroscience*, *12*(1), 415–461. http://doi.org/10.1146/annurev.ne.12.030189.002215

Zmysłowski, A., & Szterk, A. (2017). Current knowledge on the mechanism of atherosclerosis and pro-atherosclerotic properties of oxysterols. *Lipids in Health and Disease*, *16*(1), 188. http://doi.org/10.1186/s12944-017-0579-2