The role of RNA in synapse physiology and neurodegeneration in models of Parkinson's disease

Dissertation

for the award of the degree

"Doctor rerum naturalium"

of the Georg-August-Universität Göttingen

within the doctoral program

"Cellular and Molecular Physiology of the Brain"

of the Georg-August University School of Science (GAUSS)

submitted by

Maria Xylaki

from Chania, Greece

Göttingen May 2021

Thesis Committee

Prof. Dr. Tiago Fleming Outeiro

Department of Experimental Neurodegeneration, University Medical Center Göttingen

Prof. Dr. André Fischer

German Center for Neurodegenerative Diseases (DZNE) Göttingen

Prof. Dr. Paul Lingor

Department of Neurology, University Medical Center Göttingen

Members of the Examination Board

Reviewer: Prof. Dr. Tiago Outeiro

Department of Experimental Neurodegeneration, University Medical Center Göttingen

Second Reviewer: Prof. Dr. André Fischer

German Center for Neurodegenerative Diseases (DZNE) Göttingen Additional Reviewer

Further members of the Examination Board

Prof. Dr. Paul Lingor

Department of Neurology, University Medical Center Göttingen

Prof. Dr. Dr. Hannelore Ehrenreich

Clinical Neuroscience, Max Planck Institute of Experimental Medicine, Göttingen

Prof. Dr. Silvio Rizzoli

Dept. of Neuro- and Sensory Physiology, University Medical Center Göttingen

Prof. Dr. med. Christine Stadelmann-Nessler

Department of Neurology, University Medical Center Göttingen

Date of oral examination: 24/06/2021

Affirmation

I hereby declare that I have written this thesis entitled “The role of RNA in synapse physiology and neurodegeneration in models of Parkinson’s disease” independently and with no other sources and aids other than quoted. This thesis has not been submitted elsewhere for any academic degree.

Göttingen, May 2021 Maria Xylaki

Acknowledgements

Research for this thesis was conducted in the lab of Prof. Dr. Tiago Outeiro in the Department of Experimental Neurodegeneration in University Medical Center Göttingen from September 2017 to May 2021. Completing the thesis, I want to thank the people that contributed to this work or supported me in their way.

First of all, my supervisor Prof. Dr. Tiago Outeiro for the opportunity to join his lab for my PhD thesis. I am grateful for his guidance, for supporting my ideas and for always helping move forwards. I appreciate his attention to small but important details and his encouragement to have an active presence in the scientific community.

Prof. Dr. Andre Fischer and Prof. Dr. Paul Lingor for joining my thesis committee and providing valuable input to this work. Special thanks to Prof. Andre Fischer for offering his expertise on epigenetics and next generation sequencing as well as his lab equipment and constant contact with his lab members.

The members of my extended committee Prof. Dr. Dr. Hannelore Ehrenreich, Prof. Dr. Silvio Rizzoli, and Prof. Dr. med. Christine Stadelmann-Nessler for accepting the invitation to attend my thesis examination and evaluate this work.

The GGNB office for their constant support over the years and the excellent organisation of our doctoral program “Cellular and Molecular Physiology of the Brain”.

Mrs. Sonja Reisenauer, the unsung hero of our lab, for her constant help and support on lab-related matters and daily life issues. Thank you for always being there for answering our questions, for a chat, and for taking special care of us outlanders, always making sure we get our groceries before German national holidays!

Mrs. Christiane Fahlbusch, our lovely lab-technician, for organising everything and trying to accommodate everyone's needs. I loved the hidden treats popping out of your drawers, always in the right moment!

Dr. Ellen Gerhardt, our senior post-doc, for teaching and advising everyone on new techniques or troubleshooting with her unique style! Special thanks for teaching me molecular cloning and helping design the plasmids in this work. But most of all, thank you for the emotional support and making me laugh in tough moments!

All former and current post-docs of the lab: Dr Antonio Dominguez-Meijide, Dr Anka König, Dr Diana Lazaro, Dr Éva Szegö, Dr. Zeinah Wassouf, and Dr Evi Vasili for their support and contribution and for composing such a colourful lab! Special thanks to Evi for helping with recombinant α-synuclein experiments and providing valuable criticism. Having you in the lab made everything so much easier for me!

My fellow doctoral students: Mohammed Al-Azzani, Ines Bras, Renato Dominques, Dr. Isabel Paiva, Patricia Santos, Liana Shvachiy for the lab moments, worries and laughs we shared. Many thanks to Isabel for her crucial guidance in the beginning of this project, to Patricia for sharing the management of laboratory animals and to Mohammed for preparing recombinant α-synuclein.

Benedict Atzler, my first master student, for everything I learned by teaching him and for his contribution to Immunoblotting and qPCR. You did a great job and you will become a great scientist!

All the Fischer lab members for always being ready to offer help and technical assistance. Special thanks to Dr Gaurav Jain and Dr Rezaul Islam for the bioinformatic analysis and Robert Epple for the inspiring conversations and his incredible help with the production of microfluidic devices.

Anke Gregus for sharing her expertise and technical support on the Luciferase assay.

Dr Miso Mitkovski and Heiko Röhse from Light Microscopy Facility of the Max-Planck-Institute of Experimental Medicine for their assistance.

The vets and animal caretakers from the Central Animal Facility for their hard work.

My friends, office mates, and fellow doctoral students: Magda Redondo, Lujane Slitin and Carmina Warth for sharing so much of our daily life. You are such an empowering and inspiring group of women!

Closing, the biggest thanks goes to my family, my dear friends and my partner for always being there for me!

Table of Contents

List of Figures

List of Tables

List of Abbreviations

6-OHDA: 6-hydroxydopamine
AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
APP: Amyloid-Precursor-Protein
BSA: bovine serum albumin
cDNA: complementary DNA
circRNAs: circular RNAs
CpG: cytosine-phosphate-guanine
CSPα: cysteine-string protein-α
C-terminus: carboxyl-terminus
DIV: day in vitro
DLB: dementia with Lewy bodies
DMEM: Dulbecco's Modified Eagle Medium
DNMT: DNA methyltransferase
EDTA: Ethylendiamine tetraacetic acid
ER: endoplasmic reticulum
FBS: fetal bovine serum
FC: fold change,
FGFR3: fibroblast growth factor receptor 3
GABA: Gamma-Aminobutyric Acid (
GCIs: glial cytoplasmic inclusions
GFP: green fluorescent protein
GluR1: glutamate subunit receptor 1
GSEA: gene set enrichment analysis
GTP: Guanosine-5'-triphosphate
GWAS: genome wide association studies
HATs: histone acetyltransferases

HBSS: Hank's Balanced Salt Solution
HDACs: histone deacetylases
HEK 293: Human embryonic kidney 293
HEPES: 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HOTAIR: Hox transcript antisense intergenic RNA)
IL-6: interleukin-6
LBs: Lewy bodies
lncRNAs: long ncRNAs
LNs: Lewy neurites
LRRK2: Leucine-rich repeat kinase 2
LTP: long term potentiation
LTP: long-term potentiation
MAMs: mitochondria-associated ER membranes
MAP2: microtubule-associated protein 2
MAPK: mitogen-activated protein kinase
MAPT: microtubule-associated protein tau
MEM: Minimal Essential Medium
miRNA: microRNA
miRNAs: microRNAs
mRNA: messenger RNA
MSA: multiple system atrophy
NAC: non-amyloid-β component
ncRNAs: non-coding RNAs
NORAD: LncRNA activated by DNA damage
NSF: N-ethylmaleimide-sensitive factor
N-terminus: amino-terminus
PAF: pure autonomic failure
PBS: phosphate buffered saline
PCA: principal component analysis
PD: Parkindons disease
PD: Parkinson's Disease
PDMS: polymethylsiloxane
PFFs: pre-formed fibrils
PLO: Poly-L-ornithine hydrobromide,
pS129: phosphorylation at S129
PSD95: postsynaptic density protein 95
PTEN: phosphatase and tensin homolog on chromosome 10
PTMs: post-translational modifications
PVDF: polyvinylidene fluoride
qPCR: quantitative PCR

RBD: (REM) sleep behaviour disorder

RBPs: mRNA binding proteins

REM: rapid eye movement

Rhes: Ras homolog enriched in striatum

RNA ribonuclein acid

rRNA: ribosomal RNA

RT: Reverse transcription for

SAP90: synapse-associated protein 90

SAPAP3: synapse-associated protein 90 (SAP90)/postsynaptic density protein 95 (PSD95)-associated protein 3

SDS: Sodium-Dodecyl-Sulphate

SLC7A11: solute carrier family 7-member 11

SN: substantia nigra

SNARE: soluble NSF attachment protein receptor

SNARE: Soluble NSF-attachment proteins Receptor

SNCAIP: synuclein alpha interacting protein

snRNA: small nuclear RNA

TAE: Tris-acetate-EDTA

TEMED: tetramethylethylenediamine

Tg: transgenic

TH: Tyrosine Hydroxylase

TNFα: tumor necrosis factor

UPR: unfolded protein response

UTRs: untranslated regions

Wt: wild-type

αsyn: α-synuclein

Abstract

Synaptic dysfunction is an early alteration in multiple neurodegenerative disorders. Parkinson's disease (PD) is characterised by the accumulation of α-synuclein (αsyn) in pathological inclusions known as Lewy bodies and Lewy neurites. αsyn is involved in synaptic vesicle trafficking, and SNARE complex formation at the nerve terminals. In pathological conditions, it is associated with alterations of synaptic function. Interestingly, αsyn also occurs in the nucleus where it induces epigenetic changes. RNA-mediated processes contribute to synaptic remodelling by RNA translocation to the synaptic compartment. This is particularly relevant for microRNAs (miRNAs) that can regulate mRNA expression by complementary binding. Here, we sought to identify miRNAs associated with synaptic processes that may contribute to synapse degeneration.

We performed small RNA-Sequencing of the midbrain of 6-month-old transgenic mice expressing A30P mutant αsyn, present in familial forms of PD. Gene ontology (GO) functional annotation and pathway analysis of differentially expressed genes and miRNAs revealed several deregulated biological processes linked with the synaptic compartment. A negative correlation between deregulated miRNAs and gene targets highlighted the top interacting miRNAs and identified miR-101a-3p as a prominent regulator of synaptic plasticity. MiR-101a-3p was validated by qPCR in the transgenic mouse midbrain and in the cortex of Dementia with Lewy Bodies (DLB) patients. Confocal imaging of primary cortical neurons overexpressing miR-101a-3p showed reduced dendritic length and altered spine morphology. Further correlation with synaptic plasticity was provided by wild-type mice exposed to enriched environment which showed reduced levels of miR-101a-3p. Finally, exposure of primary cortical neurons to recombinant αsyn species showed a direct effect of αsyn on miR-101a-3p levels.

Our data support the emerging role of specific microRNAs as key regulators of gene expression alterations associated with αsyn. Identification of RNA based processes leading to synaptic compromise may reveal novel targets for therapeutic intervention in synucleinopathies, and may also result in the development of novel biomarkers.

1. Introduction

1.1. Synucleinopathies

Synucleinopathies is an umbrella term for a group of neurodegenerative diseases with accumulation of misfolded αsyn as a shared pathological characteristic (Maria Grazia Spillantini et al. 1997; M G Spillantini 1999). αsyn is a small protein of unknown biological function, mainly localised in presynaptic terminals, cell bodies, and axons of neuronal cells, and consists the major component of abnormal deposits in synucleinopathies known as Lewy bodies (LBs), Lewy neurites (LNs), and glial cytoplasmic inclusions (GCIs) (Iwai et al. 1995; M G Spillantini et al. 1998; M G Spillantini 1999). Synucleinopathies comprise Parkinson's disease (PD), dementia with Lewy bodies (DLB), multiple system atrophy (MSA) and pure autonomic failure (PAF).

1.1.1. Parkinson's disease

PD is a progressive motor disorder clinically characterised by resting tremor, rigidity and bradykinesia in primary stages, while in later stages non-motor symptoms like cognitive and psychiatric impairments, constipation, hyposmia, and rapid eye movement (REM) sleep behavior disorder (RBD) can develop (Jenner et al. 2013; Gibb and Lees 1988; Poewe et al. 2017). The pathological hallmark of PD is the progressive loss of dopaminergic neurons in the substantia nigra (SN) which project to the putamen and caudate nucleus, thus leading to impairments in motor control (William Dauer and Przedborski 2003). The progressive nature of the disease was highlighted by Braak and colleagues who reported stepwise spreading of αsyn pathology in brain starting from the olfactory bulb, anterior olfactory nucleus, and the dorsal motor nucleus of the vagus nerve and finally reaching the neocortex affecting primary sensory functions (H Braak et al. 2003).

The 90% of PD cases are idiopathic and are attributed to aging and environmental factors while the smaller percentage of familial cases are linked with autosomal dominant genetic variations (Billingsley et al. 2018; Pan-Montojo et al. 2012; Saito et al. 2003). In line with this, large cohort genome wide association studies (GWAS) have identified genetic loci as risk factors for idiopathic PD in addition to the highly penetrant genetic variations (Mullin and Schapira 2015).

Primarily *SNCA*, the gene encoding for αsyn, exhibits the missense mutations: A53T (Polymeropoulos et al. 1997), A30P (Krüger et al. 1998), E46K (Zarranz et al. 2004), H50Q (Appel-Cresswell et al. 2013), G51D (Lesage et al. 2013), A53E (Pasanen et al. 2014), A53V (Mohite et al. 2018) and A30G (H. Liu et al. 2021) (Figure 1). In addition to mutations, *SNCA* duplications and

triplications are included to familial disease causality (Ibanez et al. 2004; Singleton et al. 2003). Other PD associated genes termed as PARK and count 1-23, including *SNCA* as *PARK1*, have been associated with autosomal forms of parkinsonism with different degrees of inheritance certainty (Bonifati 2014; Aasly 2020). Variability in genes additional to the *PARK* group include glucocerebrosidase (*GBA*) and microtubule-associated protein tau (*MAPT*) genes causing Gaucher's disease and Frontotemporal dementia respectively, both manifesting parkinsonism symptoms (Neudorfer et al. 1996; Skipper et al. 2004). Some of these genetic variants are also associated with other synucleinopathies (Al-Chalabi et al. 2009; Chiba-Falek 2017; Blandini et al. 2019).

1.1.2. Other synucleinopathies

Other more rare forms of synucleinopathy share common characteristics with PD but differ on the succession and severity of symptoms as the Lewy pathology is observed in distinct brain regions in different disease stages (Kahle 2007). DLB is characterised by cognitive decline and dementia prior motor symptom onset as Lewy pathology prevails in the neocortex (Mayo and Bordelon 2014). Often well-established PD pathology after the development of cognitive impairments is referred to as PD Dementia (PDD) (Vasconcellos and Pereira 2015). GCI prevalence is evident in MSA where aggregates are formed in oligodendrocytes leading striatonigral and olivopontocerebellar degeneration while clinical symptoms exhibited are motor weakness, cognitive decline and autonomic failure (Fanciulli and Wenning 2015; M G Spillantini et al. 1998). PAF often precedes PD, DLB and MSA and is characterised by sympathetic and parasympathetic lesions caused by LBs and LNs in sympathetic ganglia and peripheral autonomic nerves (Hague et al. 1997; Arai et al. 2000). Finally, as RBD can develop in PD, patients with RBD often develop synucleinopathies, sometimes with evident Lewy pathology thus considered as synucleinopathy (Vilas et al. 2016; McKenna and Peever 2017).

1.2. Alpha-synuclein in physiology and pathology

1.2.1. Structural properties

αsyn is the central player of synucleinopathies and attempting to understand the diverse roles in physiology and disease the sequence and the structure of the protein have been extensively studied. αsyn is 140 amino acids long and consists of a folded amphipathic helix in the amino-terminus (N-terminus), a highly acidic dynamic carboxyl-terminus (C-terminus) and the non-amyloid-

β component (NAC) in the middle (Davidson et al. 1998; Eliezer et al. 2001; Giasson et al. 2001).The NAC domain was first isolated from amyloid plaques from AD patients' brain and is suggested responsible for αsyn oligomerisation (Ueda et al. 1993).

Upon oligomerisation, αsyn structure changes from α-helix to a β-sheet, often polymerised to form protein aggregates resembling amyloids (Vilar et al. 2008). Native αsyn is soluble in aqueous solutions and also binds on membrane, small vesicles and micelles that can act as condensation spots leading oligomerisation (Ouberai et al. 2013; Narayanan and Scarlata 2001; Eliezer et al. 2001). On the contrary to native αsyn, β-sheets are highly hydrophobic and interact with each other. Native αsyn and β-sheets exist in equilibrium, aggregation starts when this equilibrium is lost and amyloid fibrils are formed (Uversky, Li, and Fink 2001; H. T. Li et al. 2002; Vilar et al. 2008). Recent data point

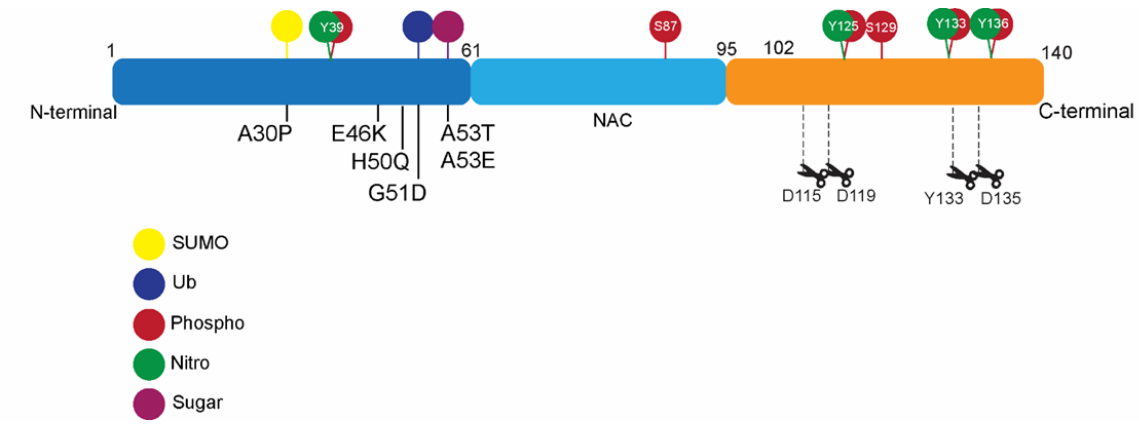

Figure 1. **Schematic representation of the polypeptide sequence of αsyn**. N-terminus, amino acids 1–61 (blue), functions as apolipoprotein and holds all known mutations A30P, E46K, H50Q, G51D, A53E, and A53T leading familial synucleinopathies. The non-amyloid-β component (NAC), amino acids 61–95 (light blue), are linked with αsyn oligomerisation. The C-terminus, amino acids 95–140 (orange), is highly plastic and polar, interacting with other proteins and metals. The C-terminus includes the majority of tyrosines (Y) where phosphorylation (P) and nitration (N) are installed. αsyn truncations, known to mediate aggregation are evident in the C-terminus as well. Acetylation (A) is installed at N-terminus, while SUMOylation (S), ubiquitination (U) and glycation (G) are usually installed at the N-terminus but can be found in other regions as well. (Brás, Xylaki and Outeiro, 2020)

out that the process of aggregation is leading toxicity instead of aggregates themselves (Mahul-Mellier et al. 2020). The oligomers formed in this process can display different structure, aggregation capacity and toxicity possibly correlating with the different disease phenotypes (Bousset et al. 2013).

Several αsyn post-translational modifications (PTMs) have been described and can affect the aggregation process and toxicity of produced species (Figure 1). Phosphorylation, the most studied PTM, and particularly phosphorylation at S129 (pS129) is identified in almost 90% of aggregated αsyn (Fujiwara et al. 2003). PS129 is shown to aggravate toxicity but the effect on αsyn fibrillation remains highly debatable (Tenreiro, Eckermann, and Outeiro 2014; Karampetsou et al. 2017). αsyn monoubiquitination seems to drive oligomer formation while polyubiquitination and SUMOylation seem to promote αsyn degradation (G. K. Tofaris et al. 2011; Rott et al. 2017). αsyn glycation, a spontaneous reaction, promotes oligomer but not fibril formation, contributing to toxicity (Paik et al. 2004; Vicente Miranda and Outeiro 2010). Additional modifications include nitration, interaction with metal cations and truncations of the C-terminus and lead to accelerated fibril formation (Hodara et al. 2004; Uversky et al. 2002; Hoyer et al. 2004).

1.2.2. Cellular localisation

Soma

αsyn was first described as a nuclear and synaptic protein, the synaptic presence of αsyn has been extensively studied due to its implication in neurodegenerative disorders while the nuclear role is still poorly understood (Maroteaux, Campanelli, and Scheller 1988; Gonçalves and Outeiro 2013). Apart from synapse and nucleus αsyn is highly abundant in the cytosol due to its high solubility and interacts with several organelles due to the lipophilic α-helix (Snead and Eliezer 2014).

αsyn interacts with mitochondrial membranes via a specialised domain with KAKEGVVAAAE repeats, and is able to further diffuse towards the inner mitochondrial membranes (Zigoneanu et al. 2012; Devi and Anandatheerthavarada 2010). Accumulation of αsyn inside the mitochondrion can disrupt the membrane architecture and physiological function, an event that is evident in synaptic mitochondria prior the somatic mitochondria (Chinta et al. 2010; Nakamura et al. 2011; Szegő et al. 2019). αsyn also interacts with mitochondria-associated membranes (MAMs) in the endoplasmic reticulum (ER) and disturbs both mitochondria and ER related processes (Guardia-Laguarta et al. 2015; Gómez-Suaga et al. 2018). Apart from ER membranes, αsyn interacts with several chaperones and ER proteins and can accumulate inside the ER inhibiting its proper function (Bellucci et al. 2011; Rodrigues et al. 2014). Downstream effects on the Golgi apparatus are also observed (Paiva et al. 2018).

αsyn peptide sequence contains a VKKDQ motif that is recognized by the lysosomes and internalized for degradation, thus abnormal accumulation in the lysosomes leads αsyn-induced autophagy impairments. Similar events apply for the proteasome complex which physiologically degrades αsyn but upon αsyn mutation or oligomerisation the proteasome degradation system displays dysfunctions or completely shuts down (George K. Tofaris, Layfield, and Spillantini 2001; Emmanouilidou, Stefanis, and Vekrellis 2010).

Finally, a couple of physiological cytoskeletal interacting partners of αsyn have been identified and although the role of interaction is not yet clear in physiology it clearly contributes to pathology. αsyn interacts with both actin and tubulin and can inhibit their proper polymerization process thus leading cytoskeletal impairments (V. L. Sousa et al. 2009; Leo Chen et al. 2007). A detrimental interaction is that of αsyn with tau which leads misfolding of both proteins (Jensen et al. 1999).

Nucleus

Considering the size of nuclear pore and that of αsyn, it is easy to assume that αsyn can passively diffuse in the nuclear space (Specht et al. 2005). Mutant forms A30P, G51D, and A53T lead increased nuclear localisation and nuclear topology seems to increase pS129 αsyn (Kontopoulos, Parvin, and Feany 2006; Pinho et al. 2019). PS129 αsyn was shown to hold an important role on DNA damage response as it is recruited to the damage cites to modulate DNA repair (Pinho et al. 2019; Schaser et al. 2019). αsyn interacts physiologically with histones and inhibits histone acetylation thus directly affecting gene expression (Goers et al. 2003; Kontopoulos, Parvin, and Feany 2006). In line with this, several epigenetic processes have been proposed in synucleinopathies (Sturm and Stefanova 2014; Pavlou et al. 2016).

Synapse

αsyn is immanent in synaptic pathogenesis of synucleinopathies and the physiological role of αsyn in synapse has been extensively studied and is implicated in synaptic vesicle cycle and neurotransmission release (Maria Grazia Spillantini and Goedert 2006; Abeliovich et al. 2000; Nemani et al. 2010). Most studies use models overexpressing different forms of αsyn or ablating αsyn to observe the synaptic phenotypes. Collectively, αsyn does not seem to affect synaptic biogenesis but a fine balance of the protein levels is required for proper synaptic function.

αsyn is critical for soluble N-ethylmaleimide-sensitive factor (NSF) attachment protein receptor (SNARE) complex formation through its interaction with synaptobrevin-2 (Burré et al. 2010). Ablation of αsyn leads to accelerated neurotransmission and inhibits the SNARE complex assembly thus disturbing exocytosis (Abeliovich et al. 2000; Burré et al. 2010). On the contrary, upon overexpression of αsyn, SNARE disturbances introduced by ablating other chaperons like cysteine-string protein-α (CSPα) can be rescued (Chandra et al. 2005). Of course overexpression is not always beneficial as it is shown to reduce reuptake of synaptic vesicles and may lead to mislocalisation of SNARE proteins SNAP-25, syntaxin-1 and synaptobrevin-2 (Nemani et al. 2010; Garcia-Reitböck et al. 2010). Similarly, αsyn aggregates can bind synaptobrevin-2 in a manner similar to the monomer and obstruct vesicle docking (B. Choi et al. 2013).

Undoubtedly, αsyn plays an important role on the different steps of a synaptic vesicle's life cycle. Overexpression of αsyn in cell models seems to reduce the reserve pool of vesicles and inhibit the vesicle priming resulting in inhibition of neurotransmitter release (Larsen et al. 2006; Nemani et al. 2010). Studies in transgenic animals expressing αsyn showed redistribution of synaptic vesicles in sites further from the active zone, which is observed to be longer but with reduced post synaptic density (Janezic et al. 2013). In another study, overexpression of αsyn in hippocampal neurons seems to result in dispersed synaptic effects mediated by loss of synaptic proteins and vacant enlarged vesicles (Scott et al. 2010). The mechanism leading the abnormalities in synaptic vesicle priming, docking, and fusion remains unknown.

Increased αsyn levels seem to directly disrupt dopaminergic neurotransmission by modulating the activity of tyrosine hydroxylase (TH), the rate limiting enzyme in dopamine production (Kirik et al. 2002; Perez et al. 2002). In addition to this, αsyn can bind the dopamine active transporter (DAT) and inhibit dopamine reuptake (Paxinou et al. 2001; Wersinger and Sidhu 2003). Based on recent findings showing that dopamine can induce αsyn oligomerisation, the event of αsyn and dopamine accumulation in the synaptic cleft can be detrimental (Mor et al. 2017). Lastly, oligomeric αsyn was found to interact with vesicles in a manner that introduces pores in the surface of the vesicles resulting in neurotransmitter leakage inside the synapse. In the case of dopamine leakage, further αsyn oligomerisation and oxidative stress in the site of synapse is expected (Volles et al. 2001; Mosharov 2006).

Upon the initial identification of NAC domain as a substance of the amyloid aggregates in AD patients' brains, αsyn was proposed to lead a synaptic pathology towards neurodegeneration (Iwai

et al. 1995). Indeed, small aggregates instead of LBs are evident in synapses prior to LBs formation, indicating that the pathology progresses from the synapse to the cell body (Marui et al. 2002; Kramer and Schulz-Schaeffer 2007). Important mechanistic evidence showed that exposure of primary cortical neurons to αsyn fibrils exhibited a pathology similar to LNs that advanced to LBs-like aggregates in the soma (Volpicelli-Daley et al. 2011). Similar data are obtained from genetic and pathogenic mouse models of synucleinopathies corroborating that synaptic dysfunction precedes neuronal death (Schirinzi et al. 2016). Upon fibril formation neuronal cells seem to completely "shut down" as synapses are lost, dendritic spines are withdrawn and synaptic activity is impaired (Q. Wu et al. 2019). Lastly, it was recently shown that the synaptic impairment is gradually developed parallel to the LB formation (Mahul-Mellier et al. 2020).

1.3. Epigenetic mechanisms in synucleinopathies

Despite extensive research on αsyn physiology and pathology, we still lack detailed understanding of the mechanisms and sequence of events initiating and progressing pathology. Moreover, genetics do not fully explain the majority of idiopathic PD cases implicating an environmental interplay. At this point epigenetic research may contribute to further understand the disease. Epigenetic mechanism is considered any process modulating gene expression without affecting the genome sequence (Dupont, Armant, and A. Brenner 2016). Epigenetics comprise of chemical modifications of DNA and histones and a wide variety of RNA mediated processes, resulting in altered genomic readout and site-specific cellular phenotypes (Figure 2).

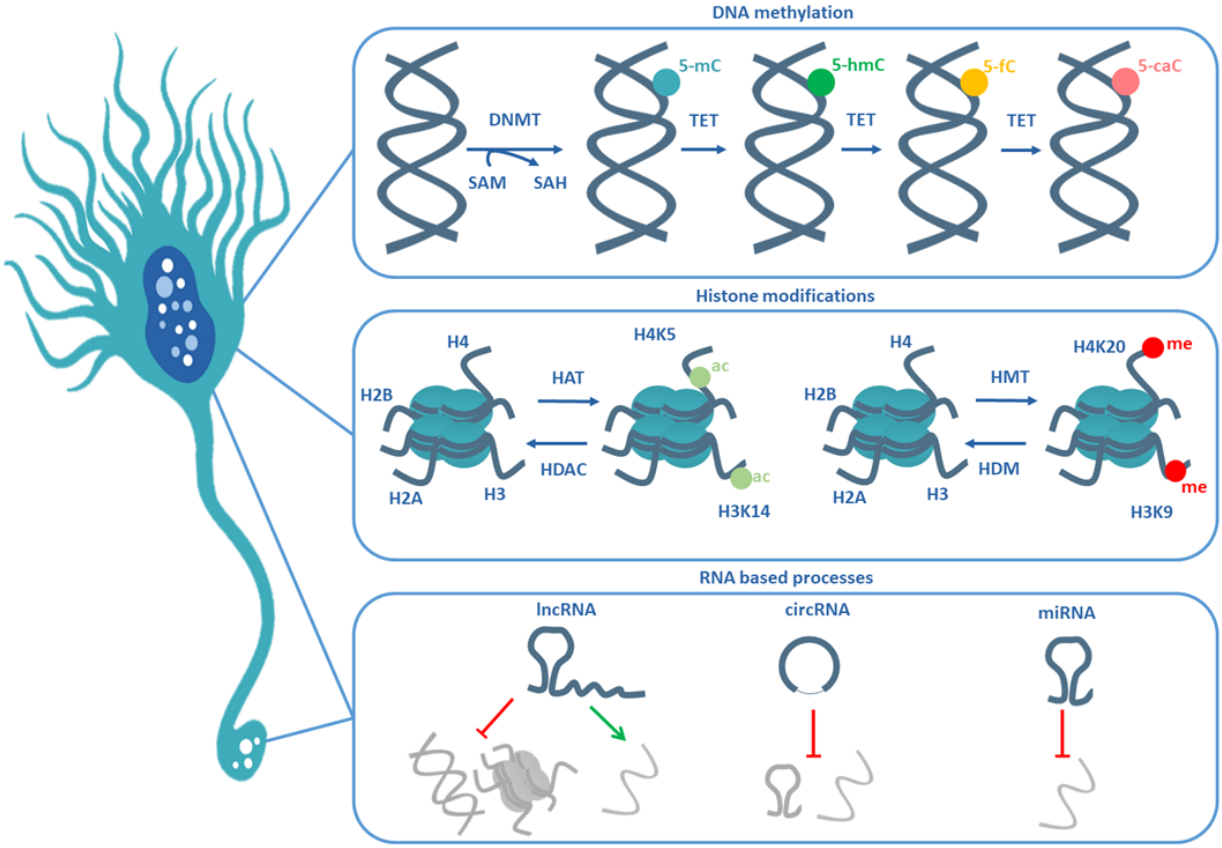

Figure 2 Epigenetic mechanisms. Epigenetic gene expression modulation is established by DNA methylation, histone modifications, or ncRNAs. DNMT enzymes regulate 5-Methylcytosine (5mC) modification on DNA. This modification is mediated by S-adenosyl (SAM) converting to S-adenosyl-homocysteine (SAH). 5mC can be oxidised by ten-eleven translocation (TET) enzymes to 5- hydroxymethyl cytosine (5hmC), 5-formylcytosine (5fC), and 5- carboxyl cytosine (5caC), to restore cytosine, conversing the methylation code. The histone code includes methylation and acetylation, usually installed on lysine residues (K) of histones (H2, H3, H4). Histone modifications are mediated by covalent bonds and can be reversed. Acetyl groups (ac) installed by histone acetyltransferases (HATs) can be removed by histone deacetylases (HDACs). Methyl groups (me) installed by histone methyltransferases (HMTs) can be removed by histone demethylases (HDMs). Histone acetylation leads an increase in transcription, whereas methylation effect depends on the modification site. LncRNAs, circRNAs, and miRNAs, well-studied RNA-based processes in the brain mediate their functions by regulating gene expression or other RNA molecules. LncRNAs can supress whole chromosomes or genes or activate small RNAs. CircRNAs are considered molecular sponges, binding and suppressing mRNAs and miRNAs. MiRNAs usually bind complementary mRNAs and block their translation or promote their decay. (Xylaki, Atzler and Outeiro, 2019)

1.3.1. DNA modifications

DNA methylation is the most studied DNA modification and conventionally represses gene expression. It is installed during aging, disease and exposure to environmental factors and can be inherited to offspring (Leonhardt et al. 1992). Methylation occurs primarily on cytosine-phosphate-guanine (CpG) islands, it is replicated by enzyme DNA methyltransferase 1 (DNMT1) and newly installed by DNMT3 (Bestor et al. 1988; Okano et al. 1999; Woodcock, Crowther, and Diver 1987).

DNMT1 was found to interact with αsyn, resulting in restriction of the enzyme to the cytosol and reduced methylation on *SNCA* intron 1 leading higher gene expression. Interestingly, reduced intron 1 methylation was identified in idiopathic PD and DLB patients shedding light to a novel disease etiology (Desplats et al. 2011; Jowaed et al. 2010; Funahashi et al. 2017). SNCA is not consistently found hypomethylated in idiopathic PD highlighting the diversity of epigenetic mechanisms accountable to pathology (De Boni et al. 2011). Alterations in DNA methylation pattern have not been reported in MSA (Sturm and Stefanova 2014).

Genome wide DNA methylation profiling in large PD cohorts has identified several hypomethylated genes including the synuclein alpha interacting protein (SNCAIP), fibroblast growth factor receptor 3 (FGFR3), and solute carrier family 7-member 11 (SLC7A11). Such methylation changes alter the expression of key proteins in neuronal susceptibility and response to αsyn toxicity (Dashtipour et al. 2017; Vallerga et al. 2020; Tsuchida et al. 2018).

1.3.2. Histone modifications

In addition to direct DNA modifications, the histones (H1/H5, H2A, H2B, H3, and H4) around which DNA is packed are subject to PTMs that can alter their structure, function and DNA storage and availability. These PTMs are both dynamic and diverse thus affecting gene expression in so many ways that are often termed as the histone code (Jenuwein 2001). PTMs include acetylation, phosphorylation, methylation, ubiquitination and sumoylation.

Histone acetylation is the most widely studied due to its dynamic nature. Histone acetylation, which results in chromatin relaxation and increased transcription, is installed by histone acetyltransferases (HATs) and deacetylation is mediated by histone deacetylases (HDACs) (Strahl and Allis 2000). Histone methylation is also irreversible and modulated by the enzyme multifamily of histone methylases and demethylases. In contrast to acetylation, methylation effect on gene

expression is unambiguous and depends on the methylated residue and the degree of methylation (Sims, Nishioka, and Reinberg 2003).

As previously mentioned αsyn can bind histones and interfere with the enzymes modulating the histone PTMs. αsyn was first shown to reduce H3 acetylation, inducing toxicity that was alleviated by HDAC inhibitors (Kontopoulos, Parvin, and Feany 2006). In line with this, treatment of neuronal cells with HDAC inhibitors like valproic acid, sodium 4-phenylbutyrate and trichostatin A was found to induce αsyn and offer protection against excitotoxicity (Leng and Chuang 2006). On the other hand, the HAT p300, that co-aggregates with αsyn was found downregulated in αsyn expressing models suggesting a role of αsyn in histone modification regulation (Kirilyuk et al. 2012; Huajun Jin et al. 2011). In another study, paraquat-induced synucleinopathy was correlated with epigenetic changes mediated by increased histone acetylation (Song et al. 2011). αsyn overexpression is also correlated with increased histone methylation suppressing gene expression and modulating important synaptic partners (Sugeno et al. 2016). These studies highlight the multiplicity and complexity of αsyn-histone interactions and the effects on toxicity.

Apart from αsyn–histone interactions, other proteins identified by GWAS in synucleinopathies have been implemented in histone remodeling. LRRK2 and Pink1 have been implemented in promoting histone deacetylation by regulating the HDAC3 localisation and phosphorylation, respectively. In both cases aberrant histone modifications are observed affecting neuronal survival (Han et al. 2017; H. K. Choi et al. 2015). The opposite regulation is evident in case of MAPT where the expression of disease associated variants are linked with histone methylation (Prendergast et al. 2012). This provides proof that events apparently irrelevant to PD pathology, can lead processes affecting PD-related genes. Finally, PD progression has been linked with an increase of hypermethylated *HDAC4* gene thus suppressing the expression of the protein and consequently increasing overall histone acetylation (Henderson-Smith et al. 2019). This is confirmed by several studies identifying progressive increase on histone acetylation levels in PD brains, accompanied by decreased HDAC levels following disease progression (Gebremedhin and Rademacher 2016; Park et al. 2016).

1.3.3. RNA-mediated processes

Considering only 2% of RNA is transcribed to mRNA and results in protein production it is of great importance to highlight the function of the rest of the genome transcribed to non-coding RNAs

(ncRNAs). NcRNAs consist of a diverse group and have proven to be extremely important modulators in gene expression and genomic programming (Mattick 2003; Yan et al. 2019; Cech and Steitz 2014). The list of ncRNAs is continuously growing and new functions are attributed to the different classes. The most studied RNAs are long ncRNAs (lncRNAs), circular RNAs (circRNAs), and microRNAs (miRNAs) that are highly enriched in the nervous system (Derrien et al. 2012; Smalheiser et al. 2008; Rybak-Wolf et al. 2014). RNA based epigenetic processes lay on mechanisms such as RNA transport, local storage, and translation, all of which are dependent on RNA-binding proteins (RBPs) (J. A. Briggs et al. 2015; Salta and Strooper 2012; Liu-Yesucevitz et al. 2011; Glanzer et al. 2005).

LncRNA are known for regulating transcription via chromatin and RNA interactions. Although they were listed as non-coding RNAs, the amplitude of their function is still under investigation as they were shown to encode small peptides with significant biological function (Andrews and Rothnagel 2014). Most lncRNAs identified in synucleinopathies are linked with apoptosis induction. LncRNAs NEAT1 and RNA-p21 were found to directly induce apoptosis and αsyn expression in cellular models (Y. Liu and Lu 2018; Xu et al. 2018). LncRNA activated by DNA damage (NORAD) upregulated in MPP+ treated cells and lncRNA Hox transcript antisense intergenic RNA (HOTAIR) upregulated along with Leucine-rich repeat kinase 2 (LRRK2) in primary neurons are both promoting caspase activation (S. Wang et al. 2017; Sang et al. 2018). Our understanding of lncRNAs is still poor but studying them can be insightful for understanding pathways leading sporadic PD and other synucleinopathies.

MiRNAs regulate gene expression by complementary binding to mRNA and result in translation inhibition or mRNA degradation. These ncRNAs are extensively studied due to their unique size of 19-22 nucleotides and specialised protein machinery supporting their function (Jonas and Izaurralde 2015). MiR-7, miR-153 and miR-34b/c downregulate SNCA by binding the 3'UTR, therefore suggesting a strong regulatory role in synuclein induced pathologies (Doxakis 2010; Miñones-Moyano et al. 2011). Of those, miR-7 has been extensively studied and is proposed for miRNA replacement therapy in synucleinopathies to reduce synuclein production and the consequent accumulation and oligomerisation of the protein (Titze-de-Almeida and Titze-de-Almeida 2018).

Apart from miRNAs targeting SNCA directly, several key miRNAs in synucleinopathy development have been identified by next generation sequencing in patient material and animal or cellular models (Heman-Ackah et al. 2013). In addition, miRNAs are identified in CSF as well as

circulating in the blood and are considered potent biomarkers (Fyfe 2020; Marques et al. 2017). Highlighting miRNA signatures in PD and other synucleinopathies and putting efforts to identify the miRNA functions and their correlation with disease initiation and progression is of great importance for diagnosis and novel therapeutic approaches. The first miRNA discovered, let-7, and the cluster miRNAs let-7a to let-7-k, miR-98 and miR-202, consist of one family upregulated in PD brain and CSF of PD patients (C. E. Briggs et al. 2015; Gui et al. 2015). This miRNA family is linked with apoptosis, negative regulation of axon guidance and regeneration, and immune response leading neurodegeneration (Li Li et al. 2017; X. Wang et al. 2019; Lehmann et al. 2012). The miR-30 family: miR-30a, miR-30b, miR-30c-1, miR-30c-2, miR-30d and miR-30e, is upregulated in substantia nigra and cingulate gyri of PD brain and is associated with neuroinflammation and disease progression serving as potent biomarker (C. E. Briggs et al. 2015; Tatura et al. 2016; D. Li et al. 2018). The miR-29 family: miR-29a, miR-29b-1, miR-29b-2 and miR-29c, is also upregulated in cingulate gyri of PD brain and is modulated by L-Dopa treatment providing insights to medical disease manipulation. In addition, miR-29 family regulates processes related to apoptosis, neuronal survival and epigenetic processes (Roshan et al. 2014; Schwienbacher et al. 2017; Tatura et al. 2016). Finally, another important miRNA family, miR-26a-1, miR-26a-2 and miR-26b, is found upregulated in PD brain substantia nigra and rodent PD model striatum (C. E. Briggs et al. 2015; Horst et al. 2018). Of note, miR-26 seems to be indirectly linked with GWAS classified *PINK1*, thus correlated with familial PD (Huse et al. 2009; Pickrell and Youle 2015). Moreover, miR-26 is correlated with long term potentiation (LTP) induced gene expression upon neuronal activity thus linking this miRNA with neuronal function impairments prior to neuronal loss (Gu et al. 2015).

Most circRNAs are produced from protein coding sequences and are implicated in transcription regulation and protein coding (Pamudurti et al. 2017). A circRNA emerging from SNCA has been identified as a miR-7 sponge thus promoting αsyn expression (Sang et al. 2018). This indicates a feedback loop of SNCA-circSNCA-miR7 positively regulating αsyn expression, in addition to the previously mentioned αsyn-DNMT1-SNCA, highlighting the complexity of interactions in molecular level. Similar to miRNAs, circRNAs have been proposed as potent biomarkers for diagnosis and disease progression due to their high stability (D'Ambra, Capauto, and Morlando 2019). Research towards this direction has identified circRNAs deregulation signatures by transcriptomic profiling in MSA brain and PD patients and mouse models (Ravanidis et al. 2021; E. Jia et al. 2020; B. J. Chen et al. 2016). Identification of such transcriptomic profiles may provide novel pathogenesis mechanisms in addition to biomarker development.

1.3.4. RNA at the synapse in physiology and neurodegeneration

Coding and non-coding RNAs are localised at synapses and modulate synaptic physiology by local transcript processing and protein translation (Steward and Schuman 2001; Tiedge and Brosius 1996). Partially, synaptic gene expression regulation is mediated by mRNA binding proteins (RBPs) that can bind different mRNA regions and control stability and translation or transcript localisation (Martin and Ephrussi 2009). In addition to mRNA transport at synapses, primary miRNAs are actively transported to pre- and post-synaptic compartment and then locally processed by miRNA machinery components that are expressed on site (Lugli et al. 2008, 2005). Increasing research highlights the importance of miRNAs on synaptic development, function and plasticity (Cohen et al. 2011; Lugli et al. 2008).

In the case of neurodegenerative disorders, dysfunction of synaptic homeostasis and plasticity lead to progressive loss of structural and functional properties of neurons eventually resulting in neuronal death. Extensive post mortem studies and animal research propose synaptic dyshomeostasis is an early event in the pathogenesis of synucleinopathies (Bellucci et al. 2016). At the very early stages of αsyn aggregation and seeding phenomena in neurons, transcriptome dysregulation of synapse-related RNA and protein levels is evident at synapses suggesting synaptopathy is a primary event in PD and other synucleinopathies (Mahul-Mellier et al. 2020). Transcriptome dysregulation can be partially attributed to miRNAs that are highly enriched at synapses and hold the potential of manipulating mRNAs. In addition, certain miRNA signatures at synapses have been linked with PD (Fiore et al. 2011; Boese et al. 2016).

Accumulating evidence link miRNAs with synucleinopathy pathogenesis and progression. The catalogue of synaptic miRNAs is rapidly increasing and the study of individual miRNAs allows for understanding of unique mechanisms linked to pathology. This mode of research is slow yet valuable as the synaptic presence of miRNAs provides leads to a targeted view of events compared to whole-cell epigenetics that often include complicated interactions. The interface between epigenetics and synaptic function is undoubtedly valuable for understanding the establishment of well-studied biochemical phenomena in PD and neurodegeneration in general. Ongoing and future studies will undoubtedly shed light into these processes, and may open novel avenues for diagnostics and even therapeutic intervention in synucleinopathies.

1.4 Objectives

In this study, we focused on PD which is characterised by gradual loss of dopaminergic neurons in the substantia nigra leading primarily to motor deficits and secondarily to cognitive dysfunctions (Heiko Braak et al. 2003). One of the hallmarks of PD is the aberrant accumulation of the presynaptic protein αsyn. αsyn is physiologically associated with synaptic vesicles, control of synaptic vesicle trafficking, and SNARE complex formation at the nerve terminal while in pathological conditions, it is implicated in the alteration of synaptic functions (Vekrellis, Rideout, and Stefanis 2004; Vekrellis et al. 2011). One of the most important characteristics of the synapse is its dynamic nature, termed synaptic plasticity. RNAs are known to reside at synapses and to actively participate in synaptic remodelling by regulating local protein synthesis. This is particularly true for mRNA (Sutton and Schuman 2006; Lugli et al. 2012) and ncRNAs such as miRNAs that can regulate mRNA expression by complementary binding (Fiore et al. 2011; Lugli et al. 2008). Synaptic compromise is observed in PD parallel to αsyn aggregation and accompanied by transcript deregulation (Mahul-Mellier et al. 2020). In this study we aimed to identify and quantify RNAs affecting synapses, and then investigate the role of selected RNAs in mechanistic studies that will contribute to the definition of the role of RNAs in synapse function and dysfunction. We used the mouse as our model organism, and specifically addressed the question of whether RNA-based processes contribute to synaptic dysfunction in the context of PD.

Aim 1. Assess the effect of αsyn on miRNA signatures

For the first aim, small RNA sequencing and differential expression analysis was performed on the midbrain of mice expressing human mutant [A30P] αsyn at an early time point prior synapse loss to investigate changes parallel to αsyn aggregation and disease progression.

Aim 2. Investigate the effect of miRNAs on transcript regulation and synaptic pathways

To investigate the effects of deregulated miRNAs we correlated the predicted targets with known deregulated mRNAs from our previous study (Paiva et al. 2018). Negative miRNA-mRNA interactions indicated the truly affected target and following pathway analysis highlighted the affected synaptic pathways.

Aim 3. Understand the role of selected RNAs in synaptic plasticity and neurodegeneration

In order to understand the effect of selected RNAs, proposed pathways were investigated in the mouse model and the RNAs were manipulated *in vitro* to characterise their action on neuronal cells and investigate their role in PD pathogenesis.

2. Materials and Methods

2.1. Human samples

RNA samples extracted from human cortex were provided by the Newcastle Brain Tissue Resource, Institute of Neuroscience, Newcastle University, Campus for Ageing and Vitality, Newcastle upon Tyne, UK. Detailed information for the samples is presented in Table 1.

Case Number	NBTR Number	Diagnosis	age	gender	PMD	pH
26383	19830263	Control	76	F	24	6,11
30783	19830307	Control	93	F	10	6,27
98089	19890980	Control	85	M	23	#
87887	19870878	Control	81	F	32	6,23
96288	19880962	Control	34	F	103	#
103688	19881036	Control	79	F	26	6,4
103689	19891036	Control	79	F	26	6,4
5690	19900056	Control	51	M	25	#
1991	19910019	Control	54	M	12	#
22991	19910229	Control	53	M	12	#
29391	19910293	Control	65	F	17	#
31991	19910319	Control	67	M	36	6,56
2793	19930027	Control	84	F	7	6,55
5095	19950050	DLB	76	F	23	6,03
20292	19920202	DLB	66	M	31	6,54
10498	19980104	DLB	86	M	41	6,08
13798	19980137	XCBD	85	M	39	6,23
131 96	19960131	DLB	77	F	23	6,24
703	20030007	DLB	88	F	16	5,92
10504	20040105	PDD/DLB	68	M	11	6,15

Table 2. Human Samples

2.2. Animal samples and procedures

2.2.1. Animals

This study employed wild-type C57BL6/J mice provided by Charles River laboratories, Wilmington, MA or Central Animal Facility University Medical Center Gottingen and transgenic animals carrying the human mutant [A30P] αsyn (B6;Tg(Thy1-SNCA*A30P)18Pjk) (Kahle et al. 2000)provided by the Central Animal Facility University Medical Center Gottingen. For tissue sampling wild-type and transgenic animals were sacrificed at ages of 4,6,8, and 12 months and for preparation of primary cortical cultures female wild-type and transgenic animals were sacrificed for donation of mouse embryos on embryonic day 15.5. All animals were housed in groups of 5 animals

in individually ventilated cages under a 12hour dark-light cycle and *ad libitum* access to food and water. All experiments were performed according to the national German animal protection law and employment of mouse embryos was performed under the project 19/3213.

2.2.2. Animal sacrifice and tissue collection

Adult animals were sacrificed by deep anesthesia with CO2 administration followed by cervical dislocation. Confirmation was by checking spine break and paw pain reflex. Dissection was performed to harvest embryos or heart, spleen, lungs, and brain. Following dissection, brain tissue was rinsed with ice-cold phosphate buffered saline (PBS) and used directly for histology protocols or placed on ice for microdissection to harvest midbrain, hippocampus, striatum, and cortex. Tissue was collected in vials, snap frozen in liquid N_2 bath and stored in -80˚C for future use.

2.2.3. Preparation of tissue for immunohistochemistry

For the preparation of brain tissue for sectioning and immunohistochemistry, mice were perfused quickly after sacrifice. In short, heart was exposed by a rib cage cut and a 20G perfusion cannula was inserted in the left heart ventricle, animals were perfused with 50 ml ice-cold PBS followed by 50 ml ice-cold freshly prepared 4% paraformaldehyde (PFA) with the help of a peristaltic pump (Miniplus 3 Peristaltic Pump, F155001, Gilson). After perfusion, brain was dissected and incubated in falcon tubes in 4% PFA for 24 hrs at 4˚C followed by sequential incubations for 24 hrs at 4 ˚C in 15% and 30% sucrose (59378, Sigma-Aldrich, MO, USA) solutions in PBS. Finally, tissue was snap frozen in -30˚C isopentane bath cooled with dry ice and stored at -80˚C until sectioning.

2.2.4. Preparation of embryonal cortical cultures

For the preparation of primary cortical cultures, embryos were harvested on embryonic day 15.5 and placed in ice-cold Hank's Balanced Salt Solution (HBSS, HBSS (10X), no calcium, no magnesium, phenol red, 14180046, Gibco, Austria) with pH 7.4 adjusted with sodium bicarbonate (S8761, Sigma-Aldrich, MO, USA). Brain tissue was collected and cortex was micro dissected with the help of a stereoscope (Leica S6 E Stereomicroscope, Leica, Germany). Tissue was digested for 20 mins at 37˚C in HBSS containing 1 ml0.25% trypsin (15090046, Invitrogen, CA, USA). Following addition of 100 µl DNAse (5 mg/mL, 11284932001, Roche, Switzerland) and 100 µl fetal bovine serum (FBS)(P30-

1502, PAN-Biotech, Germany), tissue was transferred to 1 ml FBS and dissociated by 20 ups and downs with a 1 ml pipette tip, leftover debri were allowed to precipitate for 1 minute, supernatant cell suspension was transferred to a new tube and centrifuged at 300 x g for 5 mins. The resulting pellet was resuspended in neuronal culture medium (Neurobasal,21103049, Gibco, Austria; Glutamax 125 µL 200 mM,35050 061, Gibco, Austria;1ml B27 Supplement 50x, 17504 044, Gibco, Austria; 500µl Penicillin/ Streptomycin, P06 07 100, PAN-Biotech, Germany), cells were counted with a Neubauer chamber and seeded on poly-L-ornithine (PLO, Poly-L-ornithine hydrobromide, P3655-1G, Sigma-Aldrich, MO, USA) coated plates.

2.3. Cell culture

2.3.1. Immortalised cell lines – growth conditions and treatments

Human embryonic kidney 293 (HEK 293) cells were grown at standard conditions at 37 ˚C and 5.5% CO_2 in DMEM containing 10% FBS and 1% Penicillin/Streptomycin solution (10,000 U/ml Penicillin, 10 mg/ml Streptomycin, P06-07050, PAN-Biotech, Germany). For transfection with nucleic acids, cells were seeded in 48 well plates at a density of 20.000 cells/well. Transfection was with plasmid DNA (1 µg/ml culture medium) and microRNA mimic (4 nM final concentration) in a 1:3 ratio with FuGENE® HD Transfection Reagent (E2311, Promega, WIS, USA). For this, nucleic acids and transfection reagent were diluted in two different tubes containing 25 µl Minimal Essential Medium (MEM) (Opti-MEM™ Reduced Serum Medium, 31985-047, Invitrogen, CA, USA) at room temperature. The nucleic acid solution was added to the transfection reagent solution and incubated for 30 mins at room temperature prior dropwise application on the cell culture medium. Following transfection, cells were incubated for 24 hrs prior collection for downstream analysis.

2.3.2. Primary neuronal cultures – growth conditions and treatments

Primary cultures were grown at standard conditions of 37 ˚C and 5.5% CO_2 in neuronal culture medium. In mass primary cortical cultures, cells were seeded in 24 well plates onto PLO coated coverslips (VD100, Y1A.01, Knittel Glass, Germany) for imaging purposes or PLO coated wells for RNA extraction at a density of 150.000 cells/well. For protein isolation purposes cells were seeded in PLO coated 6 well-plates at an 800.000/well density and for recombinant αsyn treatment at a 400.000/well density. In mass primary cortical cultures, cells were allowed to attach to substrate for 24 hrs prior infection. For mass cultures infection was with 1 µl viral particles per 500 µl culture

medium. For recombinant αsyn treatment cells were allowed to grow for 5 days *in vitro* before treatment with 100 nM monomeric recombinant αsyn, oligomeric recombinant αsyn species or pre-formed fibrils (PFFs). Cells were allowed to mature without any media change for 14 days in infected mass cultures, and for 25 days in recombinant αsyn treated mass cultures.

2.3.3. Seeding in microfluidic devices

For optimal separation of neuronal cell bodies and dendrites, cells were cultured in custom made microfluidic devices (MFDs) (detailed description of MFDs and production protocol in section 7). After isolation, neuronal cells were diluted to a concentration of 200.000 cells per 60 µl that were added to the top well on one side of the MFDs. After a 10 mins incubation cells have moved to the lower well through the channel and stabilized enough for addition of 300µl neuronal culture medium per well. Infection was done after one day by removing the media from the bottom well, addition of 1µl viral particles per 500 µl medium and reapplication on the top well so that viral particles are enriched in the culture channel. MFDs are incubated in petri dishes with a smaller dish with sterile double distilled H_2O (ddH_2O) to prevent media evaporation from the device. In case evaporation is observed media is added from a sister mass neuronal culture in a standard plate treated in the same manner. Neurons grow in the MFDs for 10 days before further processing for imaging purposes.

2.4. Molecular biology and biochemistry techniques

2.4.1. Cloning and virus preparation

Plasmid isolation and bacterial transformation

For this project, a vector was generated to express the miRNA miR-101a-3p or a scrambled miRNA sequence. pLKO.3G (Plasmid #14748, Addgene, MA, USA) was used as a backbone vector. First the plasmid was eluted from the commercial filter paper by soaking the paper for 30 mins in 30µl Ultra-Pure Water (400000, Cayman Chemical Company, MI, USA) and 2 µl of the extract were used for the transformation of 500µl chemically competent bacteria (One Shot™ Stbl3™ Chemically Competent E. coli, C737303, Invitrogen, CA, USA) by heat-shock on a heating block (Thermomixer comfort, Eppendorf, Germany). Bacteria-plasmid mixture was first incubated for 30 mins on ice, followed by heating at 42 ˚C for 45 sec, again on ice for 2 mins followed by addition of 500 µl SOC medium (15544034, Invitrogen, CA, USA) and incubation at 37˚C for 1 hr. Finally, mixture was applied

on LB-agar (X9653, Carl Roth, Germany) plate supplemented with 100 µg/µl ampicillin (Ampicillin Sodium Salt CellPure, HP62, Carl Roth, Germany) and grown at 37 ˚C overnight. The following day, single colonies were picked and grown in 5 ml LB medium supplemented with 100 µg/µl ampicillin (A9518, Sigma-Aldrich, MO, USA) at 37 ˚C overnight. Plasmid was isolated with NucleoBond™ Xtra Midi kit for plasmid DNA (740410, Macherey-Nagel, Germany) according to manufacturer's instructions. Diagnostic digestions were performed to confirm correct plasmid isolation prior cloning of the desired sequences.

Clonings

For the production of the plasmids expressing miRNAs the vector pLKO.3G was selected. PLKO.3G was digested at 37˚C for 30 mins by incubating 5µg of the plasmid with 2 µl FastDigest Green Buffer (B72, Thermo Fisher Scientific, MA, USA), 1 µl restriction enzymes Pac1 (ER2202, Thermo Fisher Scientific, MA, USA) and EcoR1 (ER0271, Thermo Fisher Scientific, MA, USA) in a final volume of 20µl. After digestion, the linearised plasmid was loaded on a 1.5% agarose gel (35-1020, PEQLAB Biotechnologie, Germany) in TAE buffer (Tris-acetate-EDTA, 1 mM EDTA, 20 mM acetic acid (6759,1, Carl Roth, Germany), 40 mM Tris (Trizma base, 93362, Fluka Analytical, Germany), pH 8.5) supplemented with 0.5 µg/µl ethidium bromide (OC476872, Merck, Germany). Electrophoresis was done with a PowerPac Universal Power Supply (BioRad) at 120 V, the DNA band was visualised in a FUSION Xpress chamber (PEQLAB Biotechnologie, Germany) supplemented with a UV transilluminator (VWR) and the band required for cloning was excised from the gel with a scalpel. Plasmid was extracted from the agarose gel using the QIAquick® Gel Extraction kit (28706, Qiagen, Germany) according to the manufacturer's instructions.

The desired DNA-inserts (Table2), obtained from Metabion, Germany, were phosphorylated and annealed prior cloning. The reaction mixture consisted of 100 µMsense and antisense oligosmixed with 1µl T4 ligation buffer (B0202, NEB) and 0.5µl T4 polynucleotide kinase (M0201, New England Biolabs, MA, USA) at a final volume of 10µl. Reaction was performed on MasterCycler Gradient Thermal Cycler (5331, Eppendorf, Germany) at 37˚C for 30mins, 95˚C for 5 mins and then ramp down to 25˚C at 5˚C/min. The products of the reaction were diluted with 200 times volume in sterile water prior ligation reaction.

For the ligation reaction,50ng of the linearised vector were mixed with 1 µl annealed oligos, 2 µl DNALigase buffer, 1 µl Ligase (EL0013, T4 DNA Ligase, HC, Thermo Fisher Scientific, MA, USA) at

a final volume of 20 µl and incubated at 16 ˚C overnight. Ligation was performed for miR-101a-3p and scrambled insert as well as without insert as control reaction. Following ligation transformation was performed as described previously with the use of 2 µl ligation reaction. Isolated plasmids were digested to confirm insert existence and sent for sequencing at Microsynth Seqlab GmbH, Göttingen, Germany.

For the production of the plasmids expressing the 3'-untranslated regions (UTRs) of the miRNA targets mRNAs, vector pCMV-Gluc-KDEL was selected. The 3'-UTR sequences (Table2), obtained from BioCat by custom gene synthesis, were delivered in pUC57 vector including KpnI and BamHI restriction sites. pCMV-Gluc-KDEL and the desired sequences were digested at 37˚C for 30 mins by incubating 5µg of the plasmid with 2 µl FastDigest Green Buffer (B72, Thermo Fisher Scientific, MA, USA), 1 µl restriction enzymes KpnI (ER0521, Thermo Fisher Scientific, MA, USA) and BamHI (ER0051, Thermo Fisher Scientific, MA, USA) in a final volume of 20 µl. After digestion, electrophoresis and gel extraction of the DNA fragments was performed as described above.

For the ligation reaction, 500 ng of the vector was mixed with 2µl DNA fragments, 2 µl DNA Ligase buffer, 1µl Ligase (EL0013, T4 DNA Ligase, HC, Thermo Fisher Scientific, MA, USA) at a final volume of 20 µl and incubated at 16 ˚C overnight. Ligation was performed for all 3'-UTRs fragments and without a DNA insert as control reaction. Following ligation transformation was performed as described previously with the use of 2 µl ligation reaction. Isolated plasmids were digested to confirm proper insert ligation.

Viral production

Lentivirus production was performed according to Follenzi and Naldini (Follenzi and Naldini 2002). In short, 293FT HEK cells were transfected with $CaCl_2$, HBS buffer and plasmid mix consisting of pMD2-VSV-G, pCMV-delta 8.9 (Trono lab, EPFL, Switzerland) and the respective pLKO.3G vector in a 1:2.7:2.5 ratio. Media was collected after 48 hrs, mixed with PEG-it Virus Precipitation Solution (LV825A-1-SBl, BioCat, Germany) and viral particles were isolated according the manufacturer's instructions. The viruses were tested on primary cortical neurons for transduction efficacy and toxicity and viral titers were determined using qPCR.

Name	Sequence (5'-3')
Mature mir101a-3p Anti-Sense Oligo	CAAAAATACGTACTGATAACGAACTGCAGTTCAGTTATCACAGTACTGTA
Scramble mir Anti-Sense Oligo	CAAAAAATAGTATGCAATTCGAAGATCCTGCAGGATCTTCGAATTGCATACTA
DAG1 3'-UTR fragment	TTCATAGAAAAGTCTTCGCTGCGTTTTTTGATGGCTCTGAAGCACTGTTTGAGTAGAGGTAG AAGGAGGGAGCGAGGAACCGTGAATGAACTCGCAGGCAGTGCTGGGCGGCCCCAGCTCTC TGCATTTTGCCTTTAACACTAACTGTACTGTTTTTTCTATTCACGTGTGTCTAGCTGCAGGATG TAACATGGAAAACAGTAGCTAAAGATTACATTCAAAGGACTTTCAGAAATTAAGGTTAAGTT TTTACATTTAATCTGCTGTTTACCTAAACTTGTACGTATAATTTTTGGGTGG
DLGAP3 3'-UTR fragment	CCCGCCCGGGCCGCCCAGTCCGACCCGGGCCCGCGGTTTTCTACCCGTACTGTACACCCAGC GTCGAGGTCACTGTGAACGCGGGCAGCTCCGTGCGCCCGCCCTGCCGGCACCGCACGCCCC GGCTTCTGCCCGCCGCGCTTTCGTGGGTTTTTTACCTTCCTGATCCCACGCAAAGGCGCCCGG GCTAGGCTGGGGGTCGTGCCTCTCCGCCCTGCGCCCCTCACTTGGAACTCCCATCTTCCTGGT CCGACGCTTTGACCCCCTCACCTTTTTCCCCCCATGGGCACCATCTCTGCC
GABRB2 3'-UTR fragment	ATTTCCTTCATATGACTTTTTTTTTTTTAAATTTGCCAGCCAACAGTCACCATTCTGAAATAAAT AATACAGGGTACTGAACATCTCTCTCAGCAAATCAATGCCTACAGTTCCTAAGAACATTGCAC AGTTTGACTCTACAATAGTACTGTACCTCAGAATATGAGATGTTAACTAGTGTCTGCATTGCT TGTCAAATATCCATTTGTTCTTTTAAAGGACACAGATAACACTATCCATTGGTGATTTAAAAT TGAAAAAAAAATTAGGAAAATGGGGAAAAATCCTCTTGAGAGGAGG
SHISA6 3'-UTR fragment	ATTTTGTAGTAAGGAATTCTTGAAATTCTCTAATAAAAGGCAATTCTTACTGTAACATTTTTA GTTTGGGGACACAATTTCCTAATGGGGGTTAAGGCACATTTTTACTCATTAGCTGGATTTATG GATTCTATGTTTATTATATGGTAGTATTATGAAAAGTACCTTTCTATCTGTACCTCTGCAGTTT CTCCATCACTCACAGGCCTCCATTACTGCTTCTGTACAAAGTCACCATTCTCCTTTCCTGTCAA AGCACACCCTATGTGTACTGTAAACAGAGATTGCTTCAGTAAGATT

Table 3. Cloning Sequences

2.4.2. Isolation of total RNA from solutions, cell cultures and tissue

RNA isolation was performed with TRIzol reagent (15596026, Invitrogen, CA, USA) according to the manufacturer's instructions. For solutions equal volume of liquid sample and TRIzol were mixed. For cell culture, media was aspirated and 0.5 ml TRIzol was added directly to the culture plate for cell lysis. For tissue, 1 ml TRIzol and ceramic beads (1.4 mm diameter ceramic beads, 91-PCS-CK14B, PEQLAB Biotechnologie, Germany) were added in the vials with frozen tissue. Tissue homogenisation was performed with the Precellys 24 tissue homogenizer (P000669-PR240-A, Bertin Instruments) at 65.000 x g for 2x30 sec runs with a 30 sec break in between. TRIzol containing cell lysates or tissue homogenate was transferred in Eppendorf tubes and mixed with $CHCl_3$to separate into a lower organic phase and an upper aqueous phase containing the RNA. The aqueous phase was then collected and transferred to a new tube where it was mixed with isopropanol to precipitate RNA. RNA pellet was then washed 2 times with 70% EtOH to remove solvent residues and left to air-

dry for a few minutes. Finally, RNAse-free water was added to the RNA pellet, samples were incubated on a heating block at 55 ˚C for 10 mins to promote RNA solubilisation, and RNA concentration and quality was estimated with NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, MA, USA) by measuring absorption at 260 nm and the ratios 260/280 nm and 230/280 respectively.

2.4.3. Reverse transcription PCR and Real time quantitative PCR

Reverse transcription (RT) for complementary DNA (cDNA) synthesis from total RNA was performed for both mRNAs and miRNAs using the QuantiTect RT Kit (205311, Qiagen, Germany) and miScript RT II Kit (218160, Qiagen, Germany) respectively according to the kit manuals. For both miRNA and mRNA 300 ng of isolated RNA were used per reaction. For the miRNA RT the miScript HiSpec Buffer was selected for specific transcription of mature miRNAs. The reactions were performed on MasterCycler. Produced cDNAs were diluted with 3 times volume RNAse free water prior real-time quantitative PCR (qPCR).

Quantification of relative mRNA and miRNA levels was performed with QuantStudio3 Real-Time PCR system (Applied Biosystems, CA, USA). For the miRNA qPCR the miScript SYBR Green PCR Kit (218073, Qiagen, Germany) was used with miScript primer assay (Table 3) and the miRNA expression was normalised to endogenous control RNU6. For the mRNA qPCR the Mesa blue qPCR Mastermix plus for SYBR assay (SY2X-03+WOUB, Eurogentec, Belgium) was used with custom primers (Table 3) and the mRNA expression was normalised to *ACTB*. A dissociation (melting) curve analysis was included in every run and relative expression levels (fold change, FC)were calculated using the Livak method (Livak and Schmittgen 2001).

Primers	Sequence/ID	Company
ACTB forward	GCG AGA AGA TGA CCC AGA TC	Metabion
ACTB reverse	CCA GTG GTA CGG CCA GAG G	Metabion
DAG1 forward	TTG ACA GGG TAG ATG CCT GG	Metabion
DAG1 reverse	ATA CAT GAG CTG GCT GTT GG	Metabion
GABRB2 forward	GCC TGC ATG ATG GAC CTA AG	Metabion
GABRB2 reverse	CCT GTG GAG AAA ACA ACT TTC TTG	Metabion
DLGAP3 forward	GCT CCT CCT TCA ACT TCA GA	Metabion
DLGAP3 reverse	GGA CTG GCT CGG GGT GG	Metabion
SHISA6 forward	AGT TCG AGT GCA ACA ACA GC	Metabion

SHISA6 reverse	AGT TGG TCT TGT CCT TCT CC	Metabion
Hs_RNU6-2_11 miScript Primer Assay	MS00033740	Qiagen
Mm_miR-101a_3 miScript Primer Assay	MS00011011	Qiagen

Table 4. Primer List

2.4.4. Small RNA sequencing and analysis of sequencing data

Small RNA sequencing was performed in the lab of Prof. Dr. André Fischer (DZNE, Göttingen) on an Illumina HiSeq 2000 system. Small RNA libraries were prepared from 1 µg total RNA using the Illumina TruSeq Small RNA Sample Preparation kit. Analysis of sequencing data was performed by the group of Prof. Dr. André Fischer (DZNE, Göttingen) using custom pipelines. For quality check and demultiplexing the CASAVA 1.8.2 software (Illumina) was used. For alignment the basic local alignment search tool (BLAST) was used. Reads were aligned to MiRBase version 19 with a cut off score of $p < 5 \times 10\text{-}7$ and a single mismatch allowed. In order to compare the miRNA expression levels between samples, a differential expression analysis was performed using R and the DESeq package. Heatmaps were created using the ggplot2 package. Computational miRNA target prediction analysis was performed using TargetScanMouse 6.2 (Lewis et al. 2005). For functional annotation of predicted miRNA targets the Panther database (version 15.0) was used (Thomas et al. 2006).

2.4.5. Synaptosome Isolation

Synaptosomes were isolated from pooled midbrain tissue of two 6 month old Wt mice according to Carlin and colleagues (Carlin et al. 1980) with some modifications. Freshly dissected tissue was homogenised in 3 ml homogenisation solution (0.32 M sucrose, 1 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES, 9105.4, Carl Roth, Germany) pH 7.4, 1 mM $MgCl_2$ (63033, Fluka Analytical, Germany), 0.5 mM $CaCl_2$ (C3306, Sigma-Aldrich, MO, USA) in a 5ml glass-Teflon homogenizer with 12 strokes at 900rpm on a motorised stirrer (D-91126, Heidolph Instruments). Homogenate was transferred to a falcon tube and centrifuged at 1.400 x g for 10mins at 4 ˚C. Supernatant was transferred to a fresh falcon tube and pellet was resuspended with 3 ml homogenisation solution and re-homogenised (5 strokes, 900rpm) to ensure maximum synaptosome extraction. The new homogenate was centrifuged at 710 x g for 10mins at 4 ˚C, supernatants were pooled and centrifuged at 13.800 x g for 10mins at 4 ˚C. Pellet was collected, resuspended in 2ml resuspension solution (0.32 M sucrose, 1 mM HEPES pH 7.4) with the use of the homogeniser (5 strokes, 900 rpm) and loaded on a sucrose gradient. Sucrose gradient was prepared in centrifuge

tubes (Ultra-Clear, 344059, Beckman Coulter) with 4 ml 1.2 M sucrose, 3 ml 1.0 M sucrose and 3ml 0.85M sucrose. Ultracentrifugation was performed at 82.000 x g for 2hr at 4 ˚C in a TH641 swinging bucket rotor in an ultracentrifuge (Sorvall Discovery 90SE Thermo Fisher Scientific, MA, USA) and the synaptosome fraction, concentrated at the 1.0 / 1.2 M sucrose interface, was collected with a syringe to an Eppendorf tube. All solutions were supplemented with RNase inhibitor (ProtectRNA™ RNase Inhibitor 500× Concentrate, R7397, Thermo Fisher Scientific, MA, USA). Purified synaptosomes were immediately processed for RNA extraction.

2.4.6. Preparation of protein lysates from cell culture and tissue

Cell culture protein extracts were prepared by washing the culture wells with ice cold PBS and addition of ice-cold RIPA buffer (50 mM Tris pH 8.0, 150 mM NaCl (3957,2, Carl Roth, Germany), 0.1% Sodium-Dodecyl-Sulphate (SDS, CN30.2, Carl Roth, Germany), 1% Nonidet NP40 (74385, Fluka Analytical, Germany), 0.5% Sodium-Deoxycholate (3484,3, Carl Roth, Germany), and protease inhibitor (cOmplete™ Protease Inhibitor Cocktail, 11836145001, Roche, Switzerland)) to harvest the cells. Cell lysate in RIPA buffer was then transferred to a tube and incubated on ice for 20 mins to allow complete protein extraction from cell membranes. Cell lysates where then centrifuged at 13.000 x g for 15mins at 4 ˚C to pellet debri and supernatant was transferred to a new tube. For tissue protein extracts, 100µl RIPA buffer / 10 mg tissue was added to vials along with ceramic beads and tissue was homogenized with the Precellys 24 tissue homogenizer at 65.000 x g for 2 x 30 sec runs with a 30 sec break in between. Following homogenization, the sample was allowed to rest for 20 mins on ice to allow protein extraction and transferred to ultracentrifuge tubes. Ultracentrifugation was performed to remove debri at 100.000 x g for 1hr at 4 ˚C in a Micro-Ultracentrifuge (Sorvall™ MTX 150, 46962, Thermo Fisher Scientific, MA, USA) and supernatant was collected to a new tube.

Protein concentration was estimated with the Bradford assay(Bradford 1976). The assay was performed by mixing 1 µl of protein extract with 49 µl ddH_2O, and 150 µl of dye (Bio-Rad Protein Assay Dye Reagent Concentrate, 5000006, Bio-Rad Laboratories, Inc., Hercules, CA, USA). A standard curve was prepared with standard concentrations of bovine serum albumin (BSA, Albumin Bovine Fraction V, MB04603, Nzytech, Portugal). Triplicates of all samples and standards were measured at 595 nm using the plate reader Infinite M200 PRO (Tecan Ltd., Maennedorf, Switzerland).

2.4.7. Protein electrophoresis and Immunoblotting

Electrophoresis was performed with equal amount of total protein for each sample in denaturing conditions according to Laemmli (Laemmli 1970). Protein extracts were mixed with Laemmli buffer (250 mM Tris pH 6.8 (9090,3, Carl Roth, Germany), 10% SDS, 1.25% Bromophenol Blue (B0126, Sigma-Aldrich, MO, USA), 5% β-mercaptoethanol (63690, Fluka Analytical, Germany), 50% Glycerol (G5516, Sigma-Aldrich, MO, USA)) and incubated at 95 °C for 5 mins. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed with the use of 12 or 15% separating gels with 7.5% stacking gels (ROTIPHORESE®Gel 30 (37,5:1) (3029,1, Carl Roth, Germany), 0.01 % SDS in ddH2O, 0.1 % tetramethylethylenediamine (TEMED, A1148,0100, Applichem, Germany), and 1% ammoniumperoxodisulfate (APS, 9592,2, Carl Roth, Germany). Samples and a marker for protein molecular weights (PageRuler™ Prestained Protein Ladder, 10 to 180 kDa, 26617, Thermo Fisher Scientific, MA, USA) were electrophoresed in Tris-Glycin-SDS buffer (ROTIPHORESE®10x SDS-PAGE, 3060.2, Carl Roth, Germany) in vertical apparatus (Mini PROTEAN Tetra-Cell, 1658000EDU, Bio-Rad Laboratories, Inc., Hercules, CA, USA) with constant voltage provided by the PowerPac Universal Power Supply until the dye front reached the bottom of the gel.

Following electrophoresis proteins were transferred onto nitrocellulose (IB23001X3, Thermo Fisher Scientific, MA, USA) or polyvinylidene fluoride (PVDF) membranes (IB24002X3, Thermo Fisher Scientific, MA, USA) using the iBlot 2 Gel Transfer device (IB21001, Thermo Fisher Scientific, MA, USA) by applying 20 V constantly for 10 mins. After transfer, the membranes were incubated at ambient temperature for 1 hr in blocking buffer (5 % skim milk (70166, Fluka Analytical, Germany) or 5% BSA in tris-buffered saline-tween (TBS-T, 10 mM Tris-HCl, 150 mM NaCl (3957,2, Carl Roth, Germany), 0.1% Tween-20 (P2287, Sigma-Aldrich, MO, USA)) followed by incubation with the primary antibodies (Table 4) in blocking buffer at 4°C overnight. The next day, membranes were washed in TBS-T 3 times for 10 mins each and incubated with the secondary antibodies at ambient temperature for 1 hr. After performing washes again, membranes were shortly incubated with Immobilon Western Chemiluminiscent HRP Substrate (WBKLS0500, Millipore, MA, USA) and the protein bands were visualized with a chemiluminescence imaging system (Fusion FX, Vilber Lourmat, Marne-la-Vallée, France). Semi-quantitative analysis of protein levels was performed with densitometric quantification using the Fiji software (Schindelin et al. 2012).

2.4.8. Luciferase and β-galactosidase activity assay

HEK cells were transfected with the vectors expressing Gaussia luciferase including the KDEL sequence, an endoplasmic reticulum retention signal which causes retention of the Gaussia luciferase in the endoplasmic reticulum and results in high levels of intracellular luciferase expression. Cells growing in 48 well plates were rinsed with PBS and 100µl Glycylglycin lysis buffer (25 mM Glycylglycin pH 7.8 (G1002,Sigma-Aldrich, MO, USA), 1% Triton X-100, 15 mM $MgSO_4$ (M7506, Sigma-Aldrich, MO, USA), 4 mM EGTA (03780, Sigma-Aldrich, MO, USA), 0.3% DTT (A1101, AppliChem, Germany), 1x protease inhibitors (Pefabloc® SC,11429868001, Roche, Switzerland and cOmplete™, 11697498001, Roche, Switzerland) was added to the wells and left for 15 mins at ambient temperature to ensure cell lysis. Lysates were transferred to Eppendorf tubes, centrifuged for 15 mins at 15.000 x g at 4 °C and the supernatant was collected.

For the luciferase assay, 20 µl of cell lysate was added in a 96 well plate and luciferase activity was estimated by measuring luminescence on the plate reader Infinite M200 PRO after injecting the Luciferase Assay Reagent (E1483, Promega, WIS, USA) with the Te-Inject™ reagent injector.

For the β-galactosidase assay, 20 µl of cell lysate was added in a new Eppendorf tube and 280 µl of β-galactosidase reagent (1 mg/ml o-nitrophenyl-β-D-galactopyranoside (ONPG, 34055, Thermo Fisher Scientific, MA, USA), 25 mM Sodium Phosphate pH 7.1 (S9638, Sigma-Aldrich, MO, USA), 2 µM $MgSO_4$) was added to the tube and incubated at ambient temperature until colour development was evident. Reaction was stopped with the addition of 500µl 0.5 M Sodium Carbonate (S5761, Sigma-Aldrich, MO, USA), 150 µl of the reaction mixture was transferred to a 96 well plate and absorbance at 420 nm was measured on the plate reader Infinite M200 PRO.

2.4.9. Immunocytochemistry

Cells grown on coverslips were fixed with 2% PFA at ambient temperature for 20 mins. Following incubation with blocking buffer (3% BSA, 0.1% Triton-X100 (T9284, Sigma-Aldrich, MO, USA), PBS) at ambient temperature for 1 hr, primary antibody (Table 4) diluted in blocking buffer was added and incubated at 4 °C overnight. The next day cells were washed 3 times with PBS and secondary antibody (Table 4) diluted in blocking buffer was incubated at ambient temperature for 2hr or at 4 °C overnight for the MFDs. For visualisation of the nuclei cells were then incubated with at ambient temperature for 10 mins with Hoechst (1:5000 Hoechst (Hoechst 33258, pentahydrate (bis-benzimide), H-1398, Thermo Fisher Scientific, MA, USA), PBS). Finally, cells were washed 3 times

with PBS, coverslips were mounted on glass slides (VA113001FKB.01, Knittel Glass, Germany) with Mowiol mounting medium (1.18 M 1,4 Diazabicyclo (2.2.2) octane (D2522, Sigma-Aldrich, MO, USA), 3.6 M glycerol, 0.13 M Tris pH 7.2) and MFDs were stored with PBS at 4°C until imaging.

Antibody	Species	Company	Cat- Nr
Primary detection			
β-actin	mouse	Sigma	A5441
Dystroglycan-α	mouse	Millipore	05-298
GABA A receptor β2	rabbit	Abcam	ab186875
MAP2	rabbit	Proteintech	17490-1-AP
PSD-95	rabbit	Cell Signalling	3450X
SAPAP3	rabbit	Abcam	ab67224
SHISA6	rabbit	Novus Bio	NBP1-93747
SNAP-25	rabbit	Synaptic System	111002
Synapsin 2	mouse	Synaptic System	106-002
Synaptophysin	mouse	Sigma	S5768
Syntaxin	goat	Santa Cruz	SC-7562
α-Synuclein	mouse	BD	610787
α-Synuclein (211)	mouse	Santa Cruz	SC-12767
α-synuclein phospho S129	rabbit	Abcam	ab51253
Tyrosine Hydroxylase	mouse	Millipore	AB152
α-tubulin	rabbit	Cell signalling	5335S
Secondary detection Western blot			
HRP conjugated anti-mouse IgG	sheep	Amersham	NXA931
HRP conjugated anti-rabbit IgG	donkey	Amersham	NA934V
AP anti-goat IgG	donkey	Santa Cruz	sc 2022
Secondary detection Immunofluorescence			
Alexa Fluor 488 anti-rabbit IgG	donkey	Invitrogen	A21206
Alexa Fluor 555 anti-rabbit IgG	goat	Invitrogen	A21428
Alexa Fluor 488 anti-mouse IgG	goat	Invitrogen	A11029
Alexa Fluor 555 anti-mouse IgG	goat	Invitrogen	A21422

Table 5. Antibody List

2.4.10. Immunohistochemistry

Serial coronal 30 µm thick brain sections were produced from fixed frozen tissue with a CM3050 S cryostat (Leica, Germany) and stored free floating in PBS containing 0.03% NaN_3 (K305.1, Carl Roth, Germany). Sections were incubated at ambient temperature for 1 hr in blocking buffer (2% natural goat serum (B15-035, PAA Cell Culture Company, Germany), 0.2% Triton-X100, PBS) prior

incubation with the primary antibody (Table 4) diluted in blocking buffer at 4°C for 48 hrs. After that, sections were washed 3 times 10 mins each with PBS and incubated with the secondary antibody (Table 4) diluted in blocking buffer at ambient temperature for 2hrs. Finally, sections were incubated with Hoechst at ambient temperature for 30 mins to stain the nuclei and washed again with PBS 3 times for 10 mins each prior mounting on glass slides. Mounting was on SuperFrost Plus™ Adhesion slides (10149870, Thermo Fisher Scientific, MA, USA) and sections were allowed to dry for 24 hrs prior addition of Mowiol mounting medium and 1mm thick coverslips (VD12560Y1A.01, Knittel Glass, Germany).

2.5. Histology – Golgi Cox staining

Visualisation of the dendritic spines in mouse midbrain was performed with the Golgi-Cox staining (Golgi 1873) according to the step by step protocol published by Zaqout and Kaindl (Zaqout and Kaindl 2016). Freshly dissected brain tissue was washed once with H_2O, cut in sagittal halves and each half was incubated in a glass vial with Golgi Cox solution (5% w/v $K_2Cr_2O_7$ (1.04862, Merck, Germany), 5% w/v $HgCl_2$ (KK04.2, Carl Roth, Germany), 5%K_2CrO_4 (HN33.2, Carl Roth, Germany)) overnight at room temperature in the dark. The next day tissue was transferred in fresh Golgi Cox solution in a new vial and incubated for 10 days at room temperature in the dark. After that tissue was transferred in a new vial and incubated with tissue protection solution (0.87 M sucrose, 1% w/v polyvinylpyrrolidone (PVP40, Sigma-Aldrich, MO, USA), 30% v/v ethylene glycol (E-9129, Sigma-Aldrich, MO, USA), 0.05 M phosphate buffer pH 7.2 (8461.0005, Geyer, Germany)) at 4°Covernight in the dark. The next day, tissue was transferred to fresh tissue protection solution in a new vial and incubated at 4°Cfor 5 days. Tissue sectioning was performed with a vibratome (Leica VT1000 S Vibrating blade microtome, Leica, Germany). Tissue was embedded in 4% agarose (35-2010, PEQLAB Biotechnologie, Germany) blocks, glued (Super glue 35, 7341, World Precision Instruments, Germany) on the vibratome plate and the vibratome chamber was filled with tissue protection solution. Sections were produced sagittal, 100 µm thick, on vibration frequency 60 Hz, at a speed of 15 mm/s, collected with a brush and placed onto gelatin coated glass slides. Sections were blotted by pressing absorbent paper moistened with tissue protection solution and left to dry overnight. For developing the staining, glass slides were placed on racks in histological staining boxes and incubated according to the following procedure. Slides were first washed in distilled water twice for 5 mins, then 50% EtOH for 5 mins and developed by 8 mins in 3:1 ammonia solution, washed with distilled water twice

for 5mins and 5% sodium thiosulfate for 10 mins in the dark following by a quick 1 min wash with distilled water. Slides were then dehydrated in sequential 6 min incubations with 70, 95, 100% EtOH and xylol. Lastly, when the slides dried, a few drops of Eukitt (quick-hardening mounting medium; 03989, Fluka Analytical, Germany) were added and the cover glass was applied with light pressure. Slides were left for 48 hrs in the dark to dry and brightfield images were acquired with confocal microscope.

2.6. Microscopy

2.6.1. Epifluorescence

Imaging of MFDs was performed with a Leica DMI6000 B microscope equipped with a Leica DFC320 colour camera and LAS Application Suite software (Leica, Germany). All images in one experiment were acquired with the same exposure time. Images were analysed with the Fiji software.

2.6.2. Confocal microscopy

Imaging of primary cell culture and brain sections was performed with a TCS SP5 confocal microscope (Leica, Germany) at the Light Microscope facility of the Max Planck Institute for Experimental Medicine. Images were analysed with Fiji software and dendritic spine analysis was done with Imaris V 5.1.1, Imaris XT, Bitplane AG, software available at http://bitplane.com.

2.7. Preparation of microfluidic chambers

2.7.1. Photolithography

MFDs were manufactured from polymethylsiloxane (PDMS, DOWSIL™ 5-7222 LF Emulsion, Dow, Germany) with soft-lithography(Duffy et al. 1998; Quake and Scherer 2000) in a clean room at the IV Physics Institute, Georg-August-University Göttingen. MDFs consist of 2 chambers connected with microgrooves (500 x 7500 µm). To construct the microgrooves a SU-8 3025 resist (MicroChem, MA, USA) was spin-coated on a silicon wafer (one side polished, CZ Test grade, diameter 50,8+/-0,5mm, Silchem, Germany) by spinning at 500 rpm for 15 sec at 100 ramp followed by 4000 rpm for 45 sec at 200 ramp to achieve a 10 µm height for the microgrooves. The microgrooves pattern was baked on the resist layer by photolithography using a 20.000 dpi printed transparency mask (provided by the Fischer lab, DZNE, Göttingen) and a mask aligner (Karl SUSS MJB4, Suss MicroTec, Germany)

with exposure to 200-Watt UV light for 7 sec. To construct the chambers and perfusion channels a SU-8 3050 resist (MicroChem, MA, USA) was spin-coated on top of the microgroove layer by spinning at 500 rpm for 15 sec at 100 ramp followed by 1500 rpm for 45 sec at 200 ramp to achieve a 80 µm height for these compartments. Similar to the microgrooves pattern the chambers and perfusions pattern was baked using an appropriate mask and the mask aligner with exposure to 200-Watt UV light for 17 sec. Design was developed by incubating the wafer for 10mins in developer (mr-DEV 600, Micro Resist Technology, Germany).

2.7.2. Moulding, mounting and coating

Wafers were placed in 10 cm dishes and coated with trichlorosilane (175552, Sigma-Aldrich, MO, USA) to prevent polymer from sticking on the surface. To prepare the polymer one volume of catalyst was mixed with 9 volumes of PDMS and after degassing it was poured on the molds. Following backing at 60 °C for 2 hrs polymer was hardened, the PDMS design was cut out of the moulds, washed with soap water overnight, rinsed excessively with water, washed with EtOH for 2 hrs and mounted on glass slides by plasma application with a tesla coil. After mounting MFDs were washed with water, EtOH, incubated under UV light to sterilise and coated with PLO by incubating at 37 °C overnight. Finally, coating medium was aspirated, MFDs were washed with water and culture medium and left with culture medium to equilibrate at 37 °C at least for 2 hrs prior cell seeding.

2.8. Statistical analysis

For statistical analysis was performed using the Graphpad software version 5.03 for Windows (GraphPad Software, La Jolla California USA, www.graphpad.com). For group comparisons, one-way ANOVA with Dunnet´s post-hoc test was used, while comparisons of two groups of means were done with Wilcoxon-Mann-Whitney test. All data are expressed as mean ± SEM. Differences are considered significant with $p < 0.05$(* p-value < 0.05, ** p-value < 0.01, ***p-value < 0.001). Statistical tests, number of values, and degree of significance are indicated in each figure legends.

3. Results

3.1. Small RNA profiling and pathway analysis in [A30P] αsyn mice

In order to identify RNA based processes affecting synapses in PD, we employed a mouse PD model, namely the [A30P] αsyn mice that will be referred as Tg mice onwards for simplicity. Previous studies demonstrated that these mice display motor and cognitive impairments at 12 months while abnormal αsyn accumulation at cell bodies and neurites is already evident at 6 months (Freichel et al. 2007; Kahle et al. 2000). We have previously performed transcriptional analysis through RNA-seq of the midbrain of this mouse at 6 months and identified downregulation of biological pathways related to neuronal development and synaptic signaling (Paiva et al. 2018). Thus, we decided to pursue further analysis through small RNA-seq using the same RNA samples from which RNA-seq was performed. Small RNA-seq and bioinformatical analyses were performed in collaboration with the Lab of Prof. Dr. André Fischer.

3.1.1. Small RNA profiling

The majority of small RNA mapped reads in all samples consisted of miRNAs accounting for 90.27 % of reads, 5.62 % other small RNAs and 3.98 % was ribosomal RNA (rRNA) left over (Figure 3A). Clustering of samples was evaluated by principal component analysis (PCA) which showed a clear separation between Wt and Tg animals (Figure 3B). The first step to differential expression analysis was the visualisation of microRNAs in a scatter plot, known as MA plot, depicting data as M (log ratio) on y axis and A (mean average) on x axis (Figure 3C). MA plot showed the proportion of differentially expressed miRNAs as well as the tendency of their majority being downregulated. The differential expression analysis was based on statistical significance ($p_{adj} \leq 0.05$) and levels of expression defined by a strict $\log_2$FC cut-off (±1.00). In total 124 miRNAs were found differentially expressed (Figure 3D), 99 downregulated and 25 upregulated. The top 10 upregulated are: miR-690, miR-582-3p, miR-183-5p, miR-101a-3p, miR-182-5p, miR-184-3p, miR-143-3p, miR-467a-5p, miR-7a-5p, miR-10a-5p and the top 10 downregulated are: miR-702-3p, miR-1188-5p, miR-491-5p, miR-7080-5p, miR-29b-2-5p, miR-339-5p, miR-503-3p, miR-107-3p, miR-1982-3p, and miR-23b-5p.

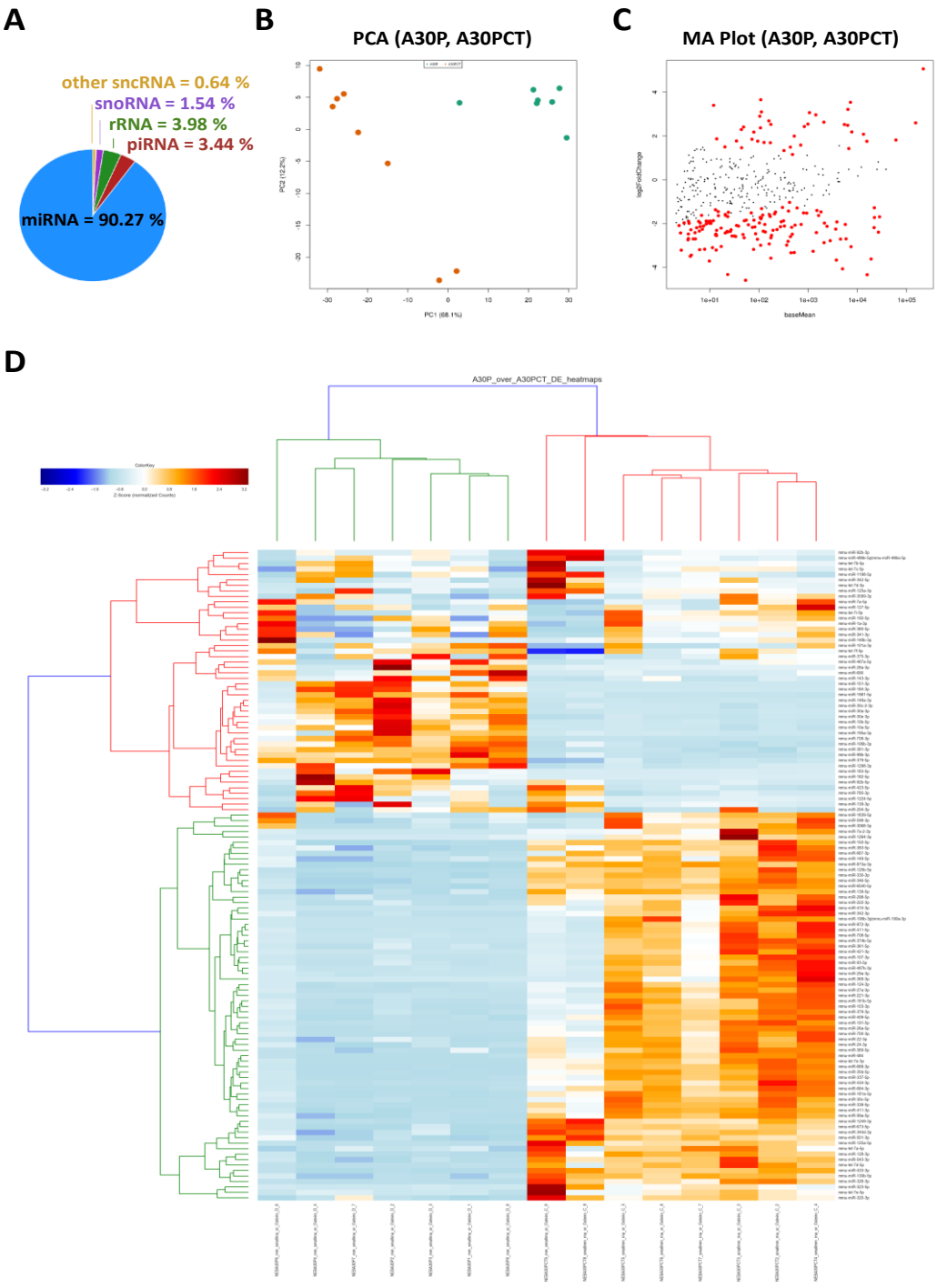

Figure 3. miRNAome changes revealed in midbrain of [A30P] αsyn mice by small-RNA-seq. A. Pie chart depicting average percentages of the different RNA classes detected in the small RNA libraries as a readout for quality of the sequencing technique; B. Principal component analysis (PCA) plots showing the clustering of Wt and Tg; C. MA plots visualise the differential expression analysis of microRNAs by plotting data data as M (log ratio) on y axis and A (mean average) on x axis; D. Heatmap of deregulated miRNAs for Wt and Tg mice. The color key indicates expression levels ranging from lower (blue) to higher (red) expression. The dendrograms indicate hierarchical clustering based on expression levels among miRNAs and individual mice.

3.1.2. Functional pathway analysis

After profiling the miRNA signatures in Tg mice, the target genes of these miRNAs were identified to obtain data on the affected pathways. For this, the predicted target genes of the top 20 upregulated and downregulated miRNAs were identified and gene set enrichment analysis (GSEA) was performed using ToppGene Suite (J. Chen et al. 2009). We selected the top 20 significant pathways from each category, namely biological process, cellular component, and molecular function and ranked them based on the enrichment ratio. The enrichment ratio indicates the degree of representation of a gene set to the entire gene set (Subramanian et al. 2005).It is important to note that as upregulated miRNAs target their complementary genes to prevent their function the predicted pathways of upregulated miRNAs are expected to be prevented as well and contrariwise for downregulated miRNAs.

Analysis of the biological processes linked to the top 20 upregulated miRNAs (Figure 4.1.A) revealed processes related to development and morphogenesis. This finding is striking as αsyn has not been linked to any gross morphological deficits as demonstrated by mouse models lacking αsyn (Abeliovich et al. 2000) or expressing different αsyn variants (Fernagut and Chesselet 2004). Nevertheless, αsyn has been implicated in impairments of adult neurogenesis (Winner et al. 2012) and Notch-1 signaling (Crews et al. 2008)thus pointing out biological processes that may be worth investigating. On the contrary, the top 20 downregulated miRNAs (Figure 4.1.B) seem to target genes that in the vast majority are regulating secretion and in particular protein exocytosis.

The cellular component ontology indicated neuronal spines and post synapses for both upregulated and downregulated miRNA target genes (Figure 4.2). This consistency pointed the post synapse as the compartment mostly affected by miRNA mediated processes in the Tg mice.

Finally, the molecular component of the top 20 upregulated miRNAs (Figure 4.3.A) revealed processes related to kinase activity/binding and transmembrane transporters. The top 20 downregulated miRNAs (Figure 4.3.B) indicated protein and DNA related processes. The protein part regards components like Ras guanosine-5'-triphosphatase (GTPase) binding and kinase activity indicating the deregulation of one of the Ras signaling pathways affecting the cytoskeleton integrity or cell proliferation, adhesion, migration and apoptosis. The DNA related processes regard DNA binding and RNA polymerase II activity which transcribes DNA to messenger RNA (mRNA), small nuclear RNA (snRNA) and miRNA. Collectively, these data indicate miRNA driven changes in the

midbrain of 6-month-old Tg animals. These changes confirm an early post synaptic phenotype and exocytosis impairments.

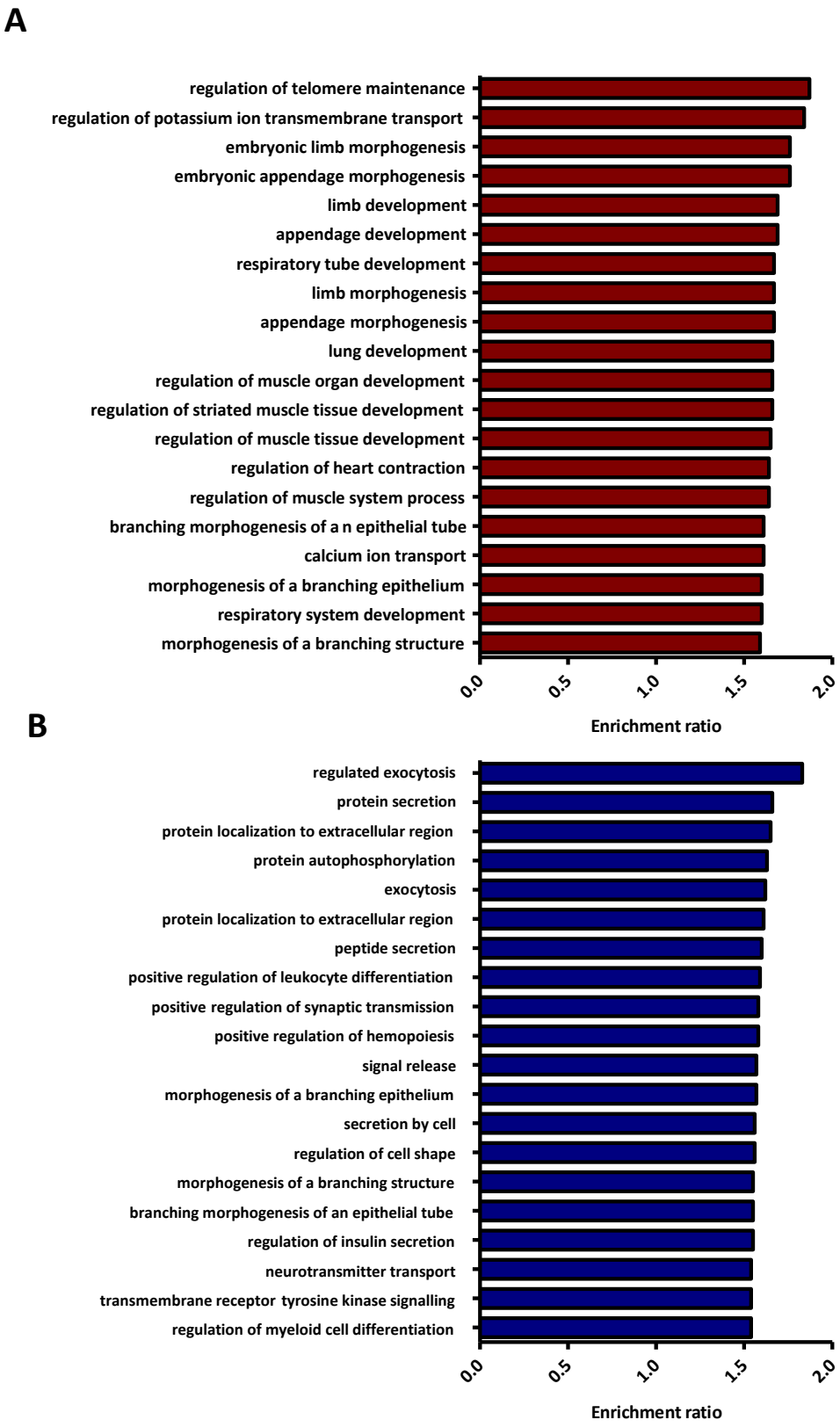

Figure 4.1. miRNAome changes affect biological function. Top 20 biological processes of top 20 A. upregulated (red) and B. downregulated (blue) miRNA target genes in Tg mouse midbrain with the respective enrichment ratio.

Why use 4.1, 4.2, 4.3, etc, and not simply Fig. 4, Fig 5, Fig 6… it would seem more logical…

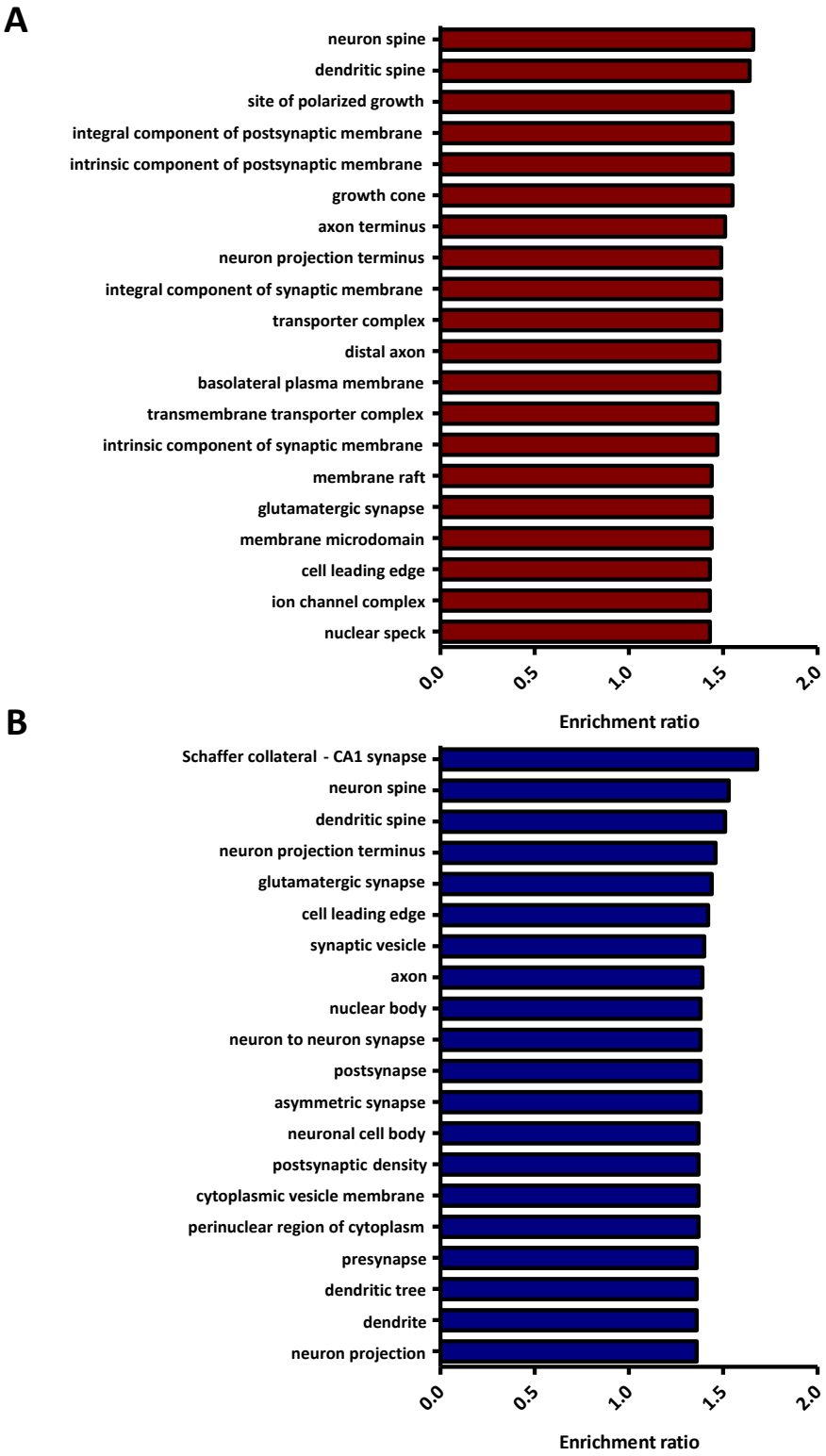

Figure 4.2. miRNAome changes affect cellular components. Top 20 biological cellular components of top 20 A. upregulated (red) and B. downregulated (blue) miRNA target genes in Tg mouse midbrain with the respective enrichment ratio.

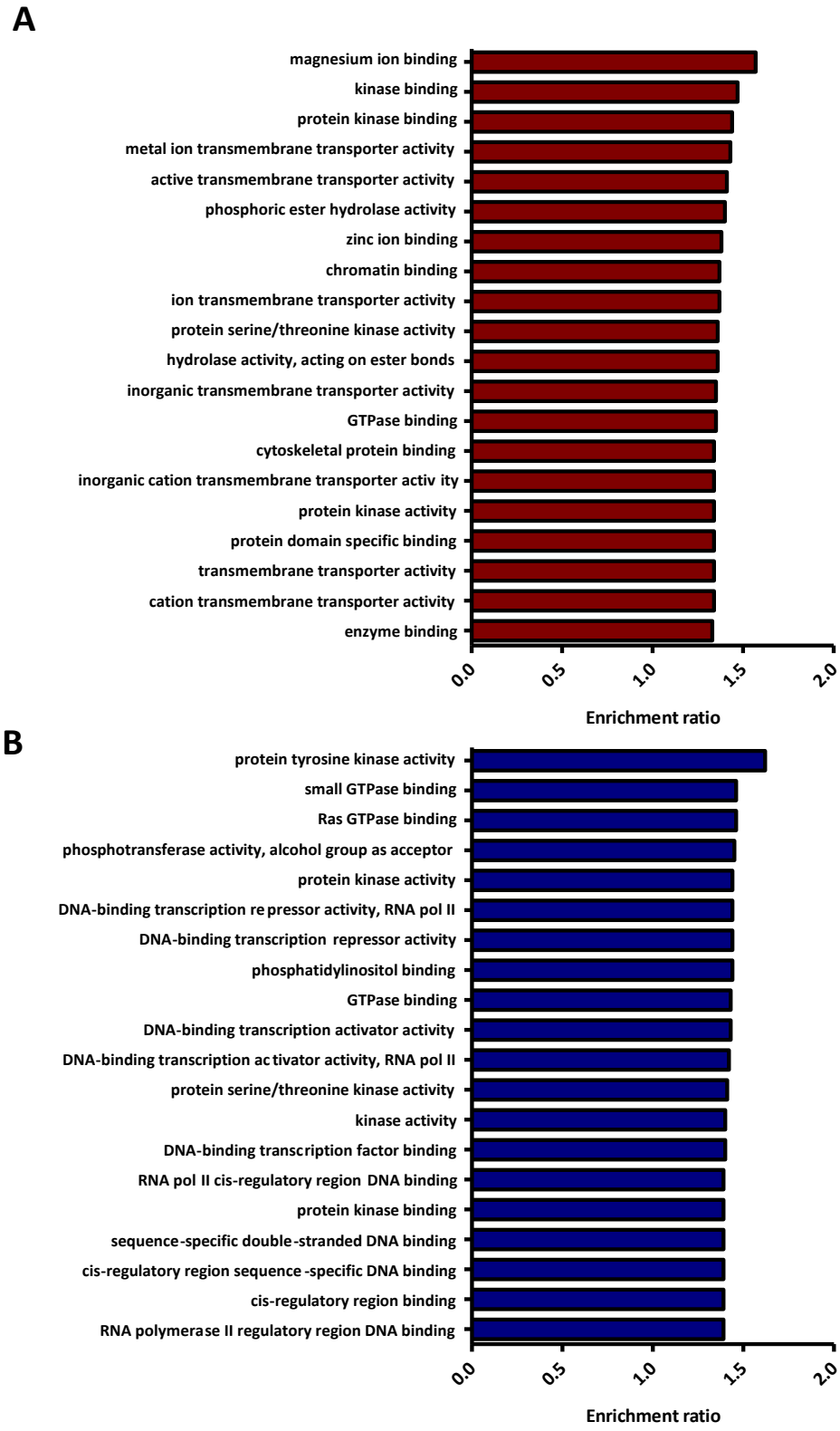

Figure 4.3. miRNAome changes affect molecular function. Top 20 molecular functions of top 20 A. upregulated (red) and B. downregulated (blue) miRNA target genes in Tg mouse midbrain

3.2. MiRNA effects on transcript regulation and synaptic pathways

3.2.1. Confirmed miRNA - mRNA interactions in [A30P] αsyn mice

For the next step of analysis the miRNA data were correlated with the mRNA data we have previously acquired from the exact RNA samples (Paiva et al. 2018). Correlation of the predicted target mRNAs of significant deregulated miRNAs with the significant deregulated mRNAs identified the significant confirmed deregulated target mRNAs. From this data set, the negative interactions were filtered i.e., the downregulated target mRNAs of each upregulated miRNA and the upregulated target mRNAs of each downregulated miRNA (Figure 5.1.A). Next the miRNAs were filtered according to the number of target mRNAs (Figure 5.1.B) and the levels of expression (Figure 5.1.C). A highly expressed miRNA with small number of target mRNAs holds the potential of producing a strong phenotype. Based on this, miR-543-3p and miR-101a-3p were selected as the most effective miRNAs showing high expression and few target mRNAs. In particular, miR-543-3p has 2133 reads on average in all samples and 53 target mRNAs and miR-101a-3p has 1424 reads on average in all samples and 60 targets.

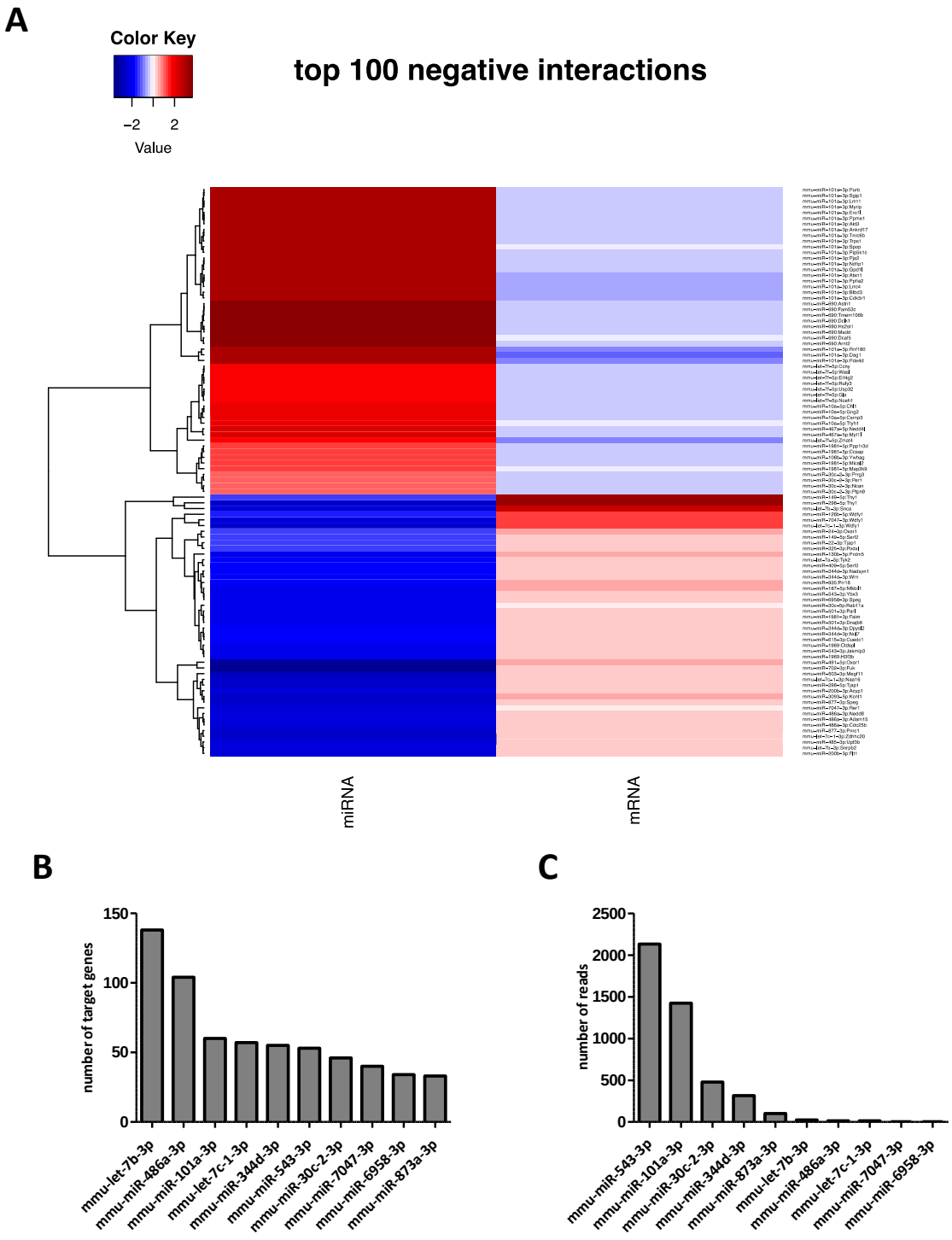

Figure 5.1. Identification of confirmed significant negative correlations in miRNA-mRNA expression. A. Heatmap of negative interactions among deregulated miRNAs and mRNAs. The color key indicates expression levels ranging from lower (blue) to higher (red). The dendrograms indicate hierarchical clustering based on expression levels. B. Histogram plotting top interacting miRNAs according to number of confirmed target mRNAs. C. Histogram plotting top interacting miRNAs according to number of reads indicating expression levels.

3.2.2. Functional pathway analysis of most effective miRNA

Next, GSEA was performed as previously described for the confirmed target genes of miR-543-3p and miR-101a-3p. The top 10 significant pathways were selected for each category based on the enrichment ratio (Figure 5.2 A).

The cellular component for both miRNAs indicated the dendritic spines and post synapses as the affected compartments in agreement with the cellular component from the bulk data set.

Analysis of the biological process linked miR-101a-3p to cytoskeletal and synaptic structural changes and amyloid-beta response. Interestingly, miR-101a-3p targeting of amyloid beta and amyloid beta effect on synaptic plasticity have been confirmed (Long and Lahiri 2011; Parihar and Brewer 2010). On the other hand, miR-543-3p was linked to biological processes regulating rectifying potassium channels and morphogenesis. Rectifying potassium channels are highly expressed in cardiac and skeletal muscle, blood vessels, brain, and kidney supporting cell depolarisation (Hibino et al. 2010). Dopaminergic neurons are particularly vulnerable to ion dyshomeostasis and even small changes in the potassium equilibrium can trigger PD (Duda, Pötschke, and Liss 2016).

Finally, the molecular component analysis identified protein kinase A catalytic subunit binding as the affected pathway for miR-101a-3p, indicating that the affected biological processes are potentially mediated by the deregulation of a kinase. Molecular component analysis for miR-543-3p did not identify any molecular pathways.

Collectively, functional pathway analysis of the most effective miRNAs included several of the components identified in the bulk analysis highlighting these miRNAs are driving strong phenotypes. As both miRNAs affect the postsynapse and their deregulation sings are opposite they can possibly drive opposite effects. For this, the validated target genes of both miRNAs were compared and no overlap was found, excluding this possibility.

A

miR-101a-3p

GO cellular component	Fold Enrichment
dendritic spine	11.16
neuron spine	10.83
growth cone	8.76
site of polarized growth	8.43
postsynaptic specialization	8.26
postsynapse	7.90
postsynaptic density	7.25
asymmetric synapse	7.18
neuron to neuron synapse	6.73
glutamatergic synapse	6.36

GO biological process	Fold Enrichment
cytoskeletal anchoring at plasma membrane	> 100
cardiac muscle cell membrane depolarization	> 100
cellular response to amyloid-beta	31.69
response to amyloid-beta	26.93
synapse assembly	21.38
regulation of neuronal synaptic plasticity	21.12
regulation of synaptic vesicle cycle	14.76
regulation of postsynapse organization	13.71
regulation of protein localization to nucleus	13.40
regulation of heart contraction	12.10

GO molecular function	Fold Enrichment
protein kinase A catalytic subunit binding	71.82
protein kinase A binding	27.62
protein domain specific binding	5.03
protein binding	1.85
binding	1.51

miR-543-3p

GO cellular component	Fold Enrichment
dendritic spine	10.68
neuron spine	10.36
neuron to neuron synapse	8.69
asymmetric synapse	8.25
postsynaptic membrane	7.50
postsynaptic density	7.29
postsynaptic specialization	6.63
postsynapse	6.24
synaptic membrane	6.17
cell-cell junction	5.81

GO biological process	Fold Enrichment
positive regulation of delayed rectifier potassium channel activity	> 100
regulation of delayed rectifier potassium channel activity	65.10
positive regulation of organ growth	28.93
regulation of potassium ion transmembrane transporter activity	27.95
dendrite morphogenesis	22.29
regulation of cardiac muscle tissue growth	22.29
regulation of heart growth	20.62
regulation of potassium ion transmembrane transport	19.63
regulation of organ growth	18.08
regulation of cardiac muscle tissue development	16.83

GO molecular function	Fold Enrichment
-	-

B

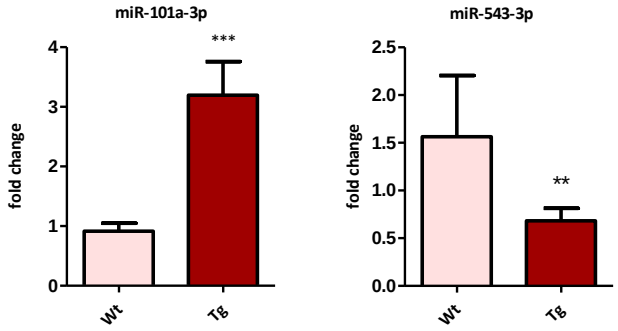

Figure 5.2 Mir-543-3p and mir-101a-3p are the most effective miRNAs. A. Top 10 significant pathways of cellular components, biological processes, and molecular functions with the respective enrichment ratio for mir-543-3p and mir-101a-3p. B. Validation by real time qPCR of mir-543-3p and mir-101a-3p levels in Wt (n = 8) and Tg (n = 7) mouse midbrain. All data are expressed as mean ± SEM; Student's t-test; * p-value < 0.05, ** p-value < 0.01, ***p-value < 0.001);

3.2.3. Real-Time qPCR validation of the most effective miRNA

The levels of miR-543-3p and miR-101a-3pwere validated by real time qPCR to confirm the small RNA-seq data (Figure 3.2 B). Confirmation on the direction of deregulation was sought with this validation, as the two quantification methods are based on different analysis. Fold change of RNA-seq data is calculated based on absolute expression levels that are correlated between groups while fold change of real-time qPCR data is calculated based on expression levels relevant to an endogenous control and then correlated between groups. The relative levels of miR-101a-3pin Tg mice compared to Wt mice were found significantly increased (3.19 ± 0.56 FC, p = 0.0002), in agreement with the small RNA-seq data (7.78± 1.71FC, p = 0.0006). Similarly, the relative levels of miR-543-3p in Tg mice compared to Wt mice were found significantly decreased (0.68 ± 0.13FC, p = 0.0064), in agreement with the small RNA-seq data (0.26± 0.085FC, p = 0.00005). The relevant levels of miR-543-3p in Wt mice showed high variation resulting in high average (1.563 ± 0.6420 FC) thus compromising the validity of qPCR. Based on this, miR-101a-3p was selected for investigating downstream pathways and phenotypes.

3.2.4. Validation of miR-101a-3p predicted target genes by luciferase reporter assay

The focus of this study was set on the post synaptic compartment as the data analysis indicated. Four downregulated miR-101a-3p target mRNAs identified from RNA-seq: *DAG1* (0.70 ± 0.06 FC, p = 0.00017), *DLGAP3* (0.66 ± 0.07 FC, p = 0.0012), *SHISA6* (0.53 ± 0.05 FC, p = 0.000001), and *GABRB2* (0.48± 0.07 FC, p = 0.000001) were associated with the post-synapse (Figure 5.2.B).

In order to verify the miRNA-mRNA interactions we tested the interaction of miR-101a-3p and its targeting sequence in the 3'-UTR of the target mRNAs by the Gaussia luciferase reporter assay. The luciferase assay was performed for transfected HEK cells expressing the luciferase alone or under the control of the 3'-UTR of the miRNA target genes. In parallel, β-gal expression was used as an internal standard to normalize expression between replicates. MiR-101a-3psuccessfully targeted all four genes' 3'-UTRs as shown by the reduced expression of Gaussia luciferase (Figure 6.1).

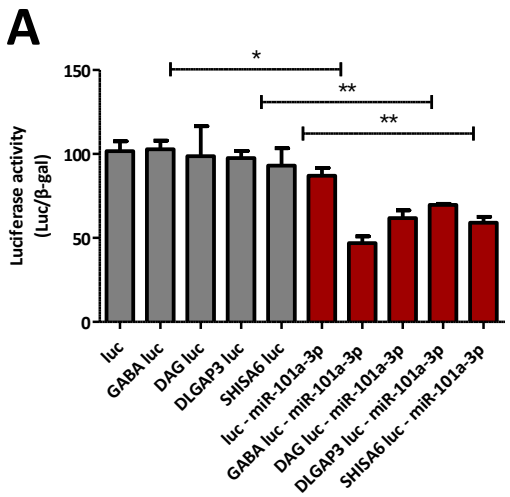

Figure 6.1. MiR-101a-3p targets 3'-UTRs of target mRNAs. A. Validation by Gaussia luciferase assay of mir-101a-3p targeting the 3'UTRs of genes DAG1, GABRB2, DLGAP3, and SHISA6 (n = 6). Luciferase activity is normalised to β-galactosidase expression. All data are expressed as mean ± SEM; Student's t-test; * p-value < 0.05, ** p-value < 0.01, ***p-value < 0.001);

3.2.5. Levels of miR-101a-3p target genes do not follow RNA-seq pattern

These mRNAs were validated with real-time qPCR (Figure 6.2.A). mRNA levels were calculated relative to the expression of *ACTB* that encodes for β-actin and was not found deregulated in the RNA-seq dataset. mRNA levels were found significantly downregulated only for GABRB2 and DLGAP3 (*DAG1* (1.19 ± 0.06 FC, p=0.29), *DLGAP3* (0.60 ± 0.06 FC, p< 0,0001), *SHISA6* (1.28± 0.11FC, p=0.045), and *GABRB2* (0.64 ± 0.14 FC, p=0.01)).

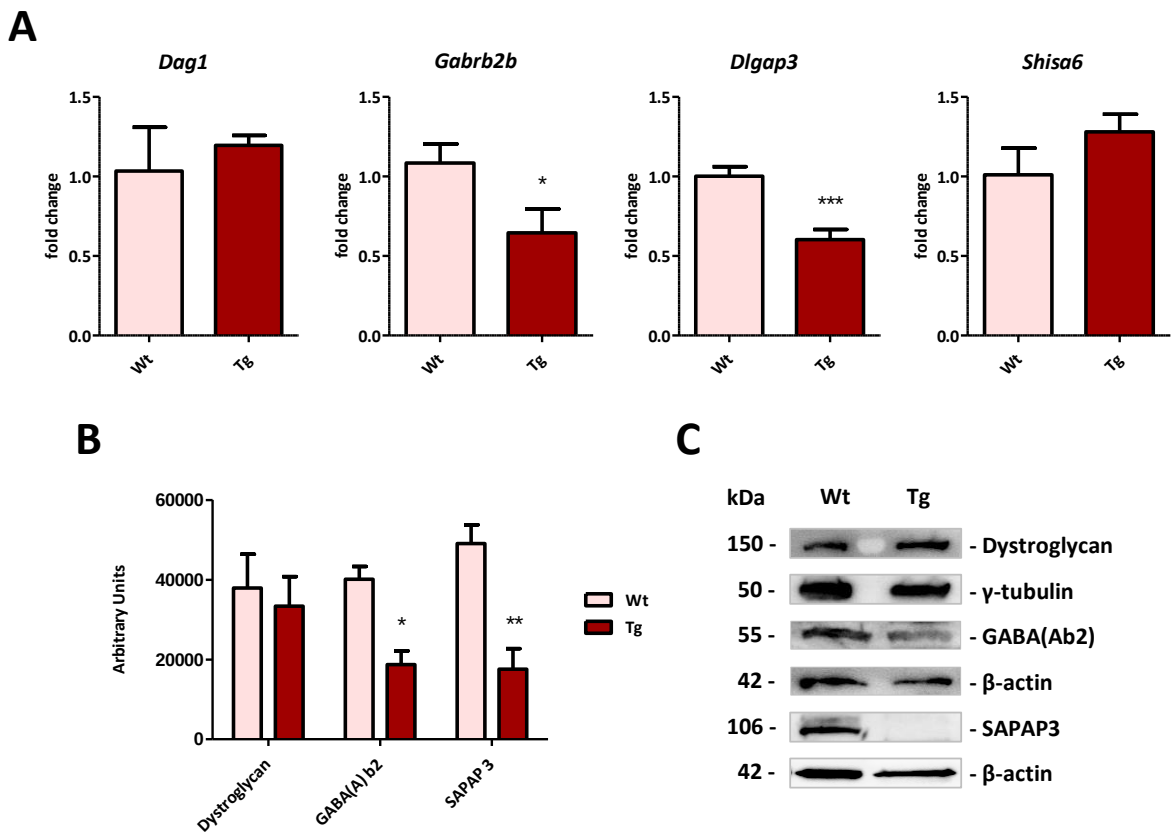

Figure 6.2. MiR-101a-3p regulates target mRNAs and proteins. A. Validation by real time qPCR of mir-101a-3p target genes DAG1, GABRB2, DLGAP3, and SHISA6 levels in Wt (n = 8) and Tg (n = 7) mouse midbrain. B. Quantification of target protein levels in Wt (n = 4) and Tg (n = 4) mouse midbrain by immunoblotting analysis and C. representative immunoblots. All data are expressed as mean ± SEM; Student's t-test; * p-value < 0.05, ** p-value < 0.01, ***p-value < 0.001);

3.2.6. MiR-101a-3p target gene encoded proteins are downregulated

Translation of target genes was evaluated by immunoblotting analysis (Figure 6.2.B, C). Shisa 6 was not detected in both Wt and Tg animals probably due to low enrichment in the brain tissue lysate. Dystroglycan showed only a slight decrease in Tg animals accounting to 12% of the levels detected in Wt mice. GABA(A)b2 and SAPAP3 showed significant decrease of 53% and 64% respectively. In order to verify this decrease is due to the miRNA effect and not due to cell or synapse loss, further analysis was performed.

To quantify dopaminergic neurons, TH+ cells were counted at four different bregma levels (Figure 6.3.). Quantification did not show any cell death in Tg animals at 6 months. Immunoblotting analysis (Figure 6.4.) did not show any changes of TH levels supporting no evidence of dopaminergic

neuron loss or neurotransmitter imbalance. Moreover, the post synaptic marker PSD95 validated no loss of post synaptic compartments in Tg animals indicating the downregulation of protein levels is due to miRNA mediated process affecting only the targeted proteins.

Accordingly, pre-synaptic proteins, markers of synaptic vesicles (synaptophysin and synapsin2) and Soluble NSF-attachment proteins Receptor (SNARE) complex (syntaxin and SNAP25), were quantified. Quantification showed no significant changes in Tg mice compared to Wt animals (Figure 4.4) indicating no loss in presynaptic structures.

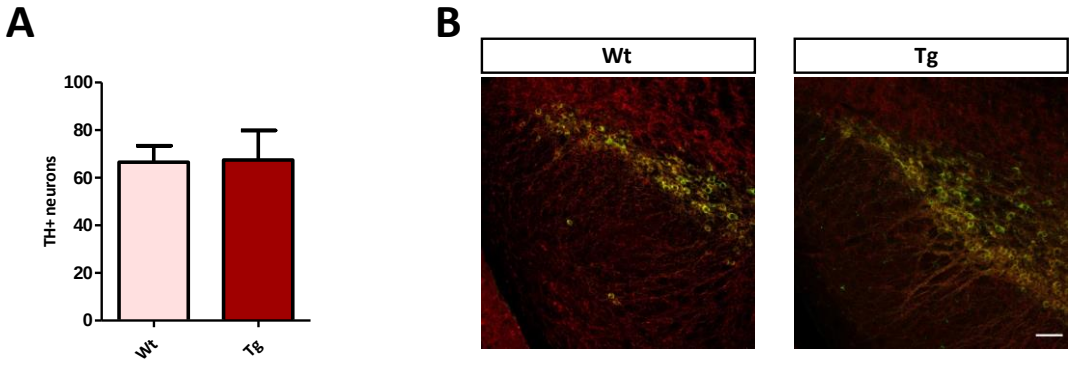

Figure 6.3. No evidence of dopaminergic cell death in substantia nigra at 6 months. A. Quantification of TH+ positive cells at levels -2.26, -2.8, -3.34, and -3.88 from bregma (n = 8 x 4 bregma levels) and B. representative images of level -3.34 from bregma for Wt and Tg animals; TH - green; MAP2 - red; scale bar =100 µm. All data are expressed as mean ± SEM; Student's t-test; * p-value < 0.05, ** p-value < 0.01, ***p-value < 0.001);

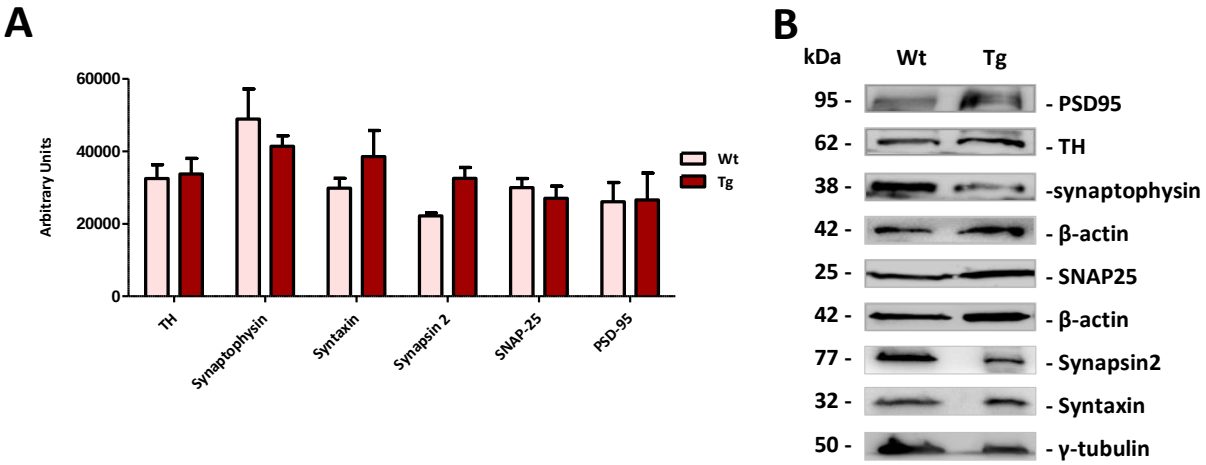

Figure 6.4. MiR-101a-3p has no effect on synaptic proteins. A. Quantification of synaptic protein levels in Wt (n = 4) and Tg (n = 4) mouse midbrain by immunoblotting analysis and B. representative immunoblots. All data are expressed as mean ± SEM; Student's t-test; * p-value < 0.05, ** p-value < 0.01, ***p-value < 0.001);

3.2.7. MiR-101a-3p expression peaks at 6 months and is specific for midbrain

In order to further characterise miR-101a-3p, the expression pattern in different time points and tissues was analysed by real time qPCR. In particular, in order to associate the miRNA with a specific timeframe of the phenotype development miR-101a-3p levels were assessed at 4, 8, and 12 months. miRNA was found elevated at 4 months (1.58 ± 0.24 FC), it peaks at 6 months and at 8 and 12 months it drops to basal levels (Figure 6.5.A). At 6 months when the miRNA levels peak, neuronal plasticity deficits are already evident (Heinrich Schell et al. 2012) and at 8 months the first dopaminergic deficits appear (Ekmark-Lewén et al. 2018).

Relevant expression levels of miR-101a-3p in different time points were assessed by comparing the difference in cycle time (ΔCt) for miR-101a-3p and the endogenous control RNU6 (Figure 6.5.D). The higher the ΔCt the longer it takes for miR-101a-3p to be detectable compared to the endogenous control so the lesser the levels. In addition, based on the calculations of the Livak method each ΔCt corresponds to 2 folds change in expression. In Wt mice we observe reduced ΔCt values with ageing (ΔCt_4 = 4.39±0.26, ΔCt_6 = 3.57±0.28, ΔCt_8= 3.01±0.22) interpreted as a stable increase of the miRNA with time. This pattern is followed in Tg mice as well only the increase rate is higher from 4 to 6 months (ΔCt_4 = 3.93±0.23, ΔCt_6 = 2.82±0.06, ΔCt_8 = 3.01±0.20). These data present miR-101a-3p increase as a normal phenomenon in the ageing midbrain while the abrupt increase in Tg animals provides indications of early senescence or degeneration.

miR-101a-3p levels assessment in other brain regions at 6 months showed no differences in cortex or hippocampus but an insignificant increase is observed in striatum (1.62 ± 0.28 FC) (Figure 6.5.B). This indicates either the miRNA is overexpressed in striatum as well or it is transported via the nigrostriatal pathway by axonal transport.

Finally, in order to evaluate the specificity of the observation thus far, the levels of miR-101a-3p were evaluated in other tissues, namely the heart, lungs, and spleen (Figure 6.5.C). MiRNA levels were not altered in the organs indicating brain specificity and particularly midbrain specificity of miR-101a-3p increase.

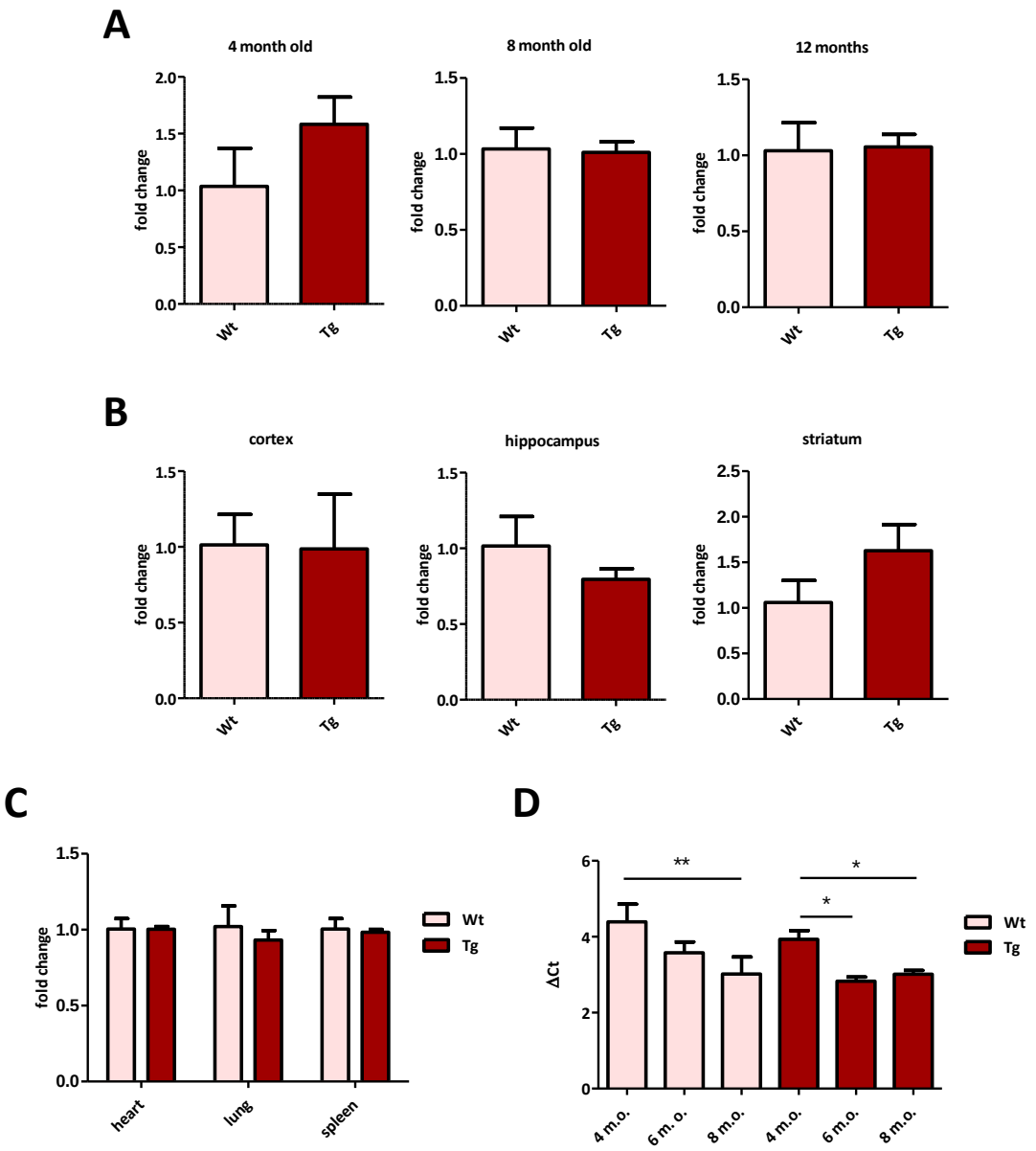

Figure 6.5. MiR-101a-3p expression pattern. Real time qPCR quantification of mir-101a-3p in A. midbrain of 4 (n = 4), 8 (n = 4) and 12 (n = 4) month old animals; B. different brain regions at 6 month old animals (n = 4), and C. different tissues at 6 month old animals (n = 4). D. Comparative expression of miR-101a-3p ΔCt; difference in cycle time of detection to endogenous control in midbrain at different time points; All data are expressed as mean ± SEM; Student's t-test; * p-value < 0.05, ** p-value < 0.01, ***p-value < 0.001);

3.3. The role of miR-101a-3p in synaptic plasticity and neurodegeneration

3.3.1. MiR-101a-3p is enriched in synapses

Considering miR-101a-3p affects primarily post synapses we next asked whether it is enriched in the specific cellular compartments. For this we isolated midbrain cytosol and synaptosome fractions of 6-month-old Wt mice. RNA was extracted form samples and miR-101a-3p was quantified by real time qPCR. To assess the miRNA levels in synaptosomes vs cytosolic fractions the enrichment ratio was calculatedas the fraction of the synaptosomal or cytosolic ΔCt to the total homogenate ΔCt as in Lugli et al (Lugli et al. 2012). Cytosolic miRNA levels were found similar to those in total homogenate while miRNA levels in synaptosomes were found enriched by 2-fold (2.42 ±0.26) (Figure 6.6). This highlights that miR-101a-3p has a regulatory role near synapses even in physiological conditions.

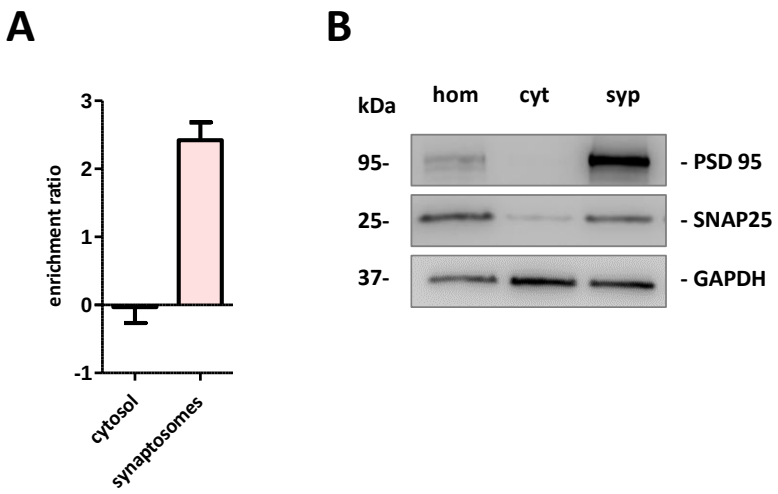

Figure 6.6. MiR-101a-3p is enriched in midbrain synaptosomes of 6 month old Wt mice. A. Bar graph of miR-101a-3p enrichment ratio in cytosol and synaptosomes compared to total homogenate and B. representative immunoblot of synaptic markers in the crude midbrain homogenate (hom), cytosolic fraction (cyt) and purified synaptosomes (syp).

3.3.2. Midbrain neurons display altered dendritic morphology

The cellular component of functional pathway analysis indicated the dendritic spines and post synapses as the affected compartments of miR-101a-3p, thus we sought to visualise the dendritic spines with the Golgi-Cox staining (Figure 6.7.C). Quantification was performed manually by inspection of at least 10 dendritic spine segments of 10 µm per mouse. Absolute number of dendritic spines was not different among Wt (6,471 ± 0,1583 / 10 µm) and Tg (6,485 ± 0,3969 / 10 µm) animals

(Figure 6.7.A). Further classification of dendritic spines to mushroom, thin and stubby revealed significant differences in the Tg mouse midbrain (Figure 6.7.B). Mushroom and thin spines are reduced by 24% (p = 0,0429) and 35% (p = 0.0007) respectively, while stubby spines are increased by 69% (p = 0.0002). Mushroom spines are considered mature structures forming strong synaptic connections with bigger head relative to the thin spines that are considered more plastic structures (Bourne and Harris 2007). Stubby spines are considered immature structures, sparse in adult brain, while their increase is correlated with loss of mushroom spines (Hering and Sheng 2001). Decrease of mushroom and thin spines indicates an early phenotype of synaptic failure and impairments of synaptic plasticity.

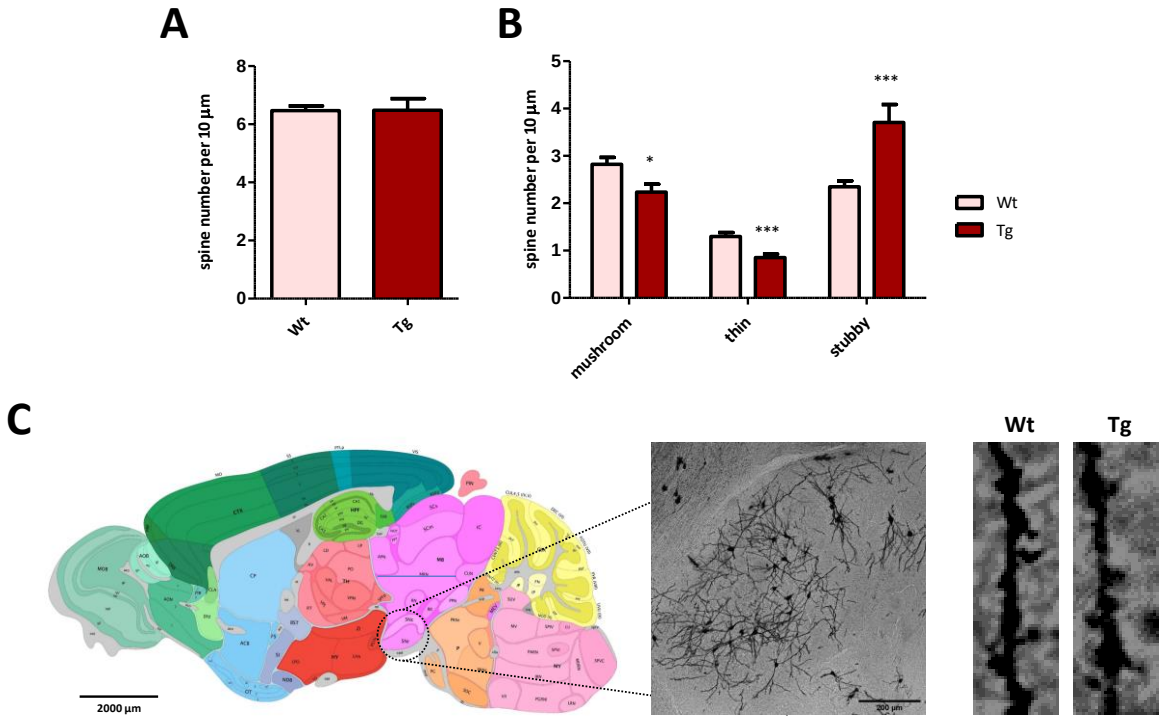

Figure 6.7. Dendritic spine morphology is altered in Tg mouse midbrain. A. Quantification of total dendritic spine number and B. classification of mushroom, thin and stubby dendritic spines per 10 µm dendrite in Wt (n = 3) and Tg (n = 3) mice. (n ≥ 45 x 10 µm segments per condition manually counted with Fiji software). C. Schematic of the analysed brain region; sagittal level 14; lateral level 1.35 mm; scale bar = 2000 µm; Image credit: Allen Institute; and representative images of the analysed brain region in Wt mouse; brightfield; scale bar = 200 µm; and dendritic spine segments of Wt and Tg animals; brightfield; total length = 20 µm. All data are expressed as mean ± SEM; Student's t-test; * p-value < 0.05, ** p-value < 0.01, ***p-value < 0.001);

3.3.3 MiR-101a-3p effects *in vitro*

In order to assess whether the observed effect on dendritic spines is attributed to miR-101a-3p increase we tested the effect of the miRNA overexpression in primary cortical mouse neurons. Neurons were infected with viral particles that induced the expression of miR-101a-3p or a scrambled miRNA as control. The scrambled miRNA was designed so that it showed no homology to the mouse genome excluding the possibility of RNA binding and downstream effects. Both viruses expressed green fluorescent protein (GFP) as a reporter for gene expression and for this, a virus expressing only GFP was used as an additional control. The analysis was focused on the miRNA target genes levels and neuronal morphological effects.

3.3.4. MiR-101a-3p induced expression in primary cortical neurons

MiR-101a-3p expression was induced on day in vitro (DIV) 1 and cells were allowed to grow until DIV 14. The relative levels of miR-101a-3p were quantified by real time qPCR to confirm miRNA induction. MiR-101a-3p was found significantly increased in miR-101a-3p induced neurons (1.33 ± 0.11 FC) compared to scrambled induced neurons (0.92 ± 0.13 FC) (Figure 7.1.A). In order to verify the validity of these data the infection efficiency was estimated by calculating the percentage of infected neurons in the mass cultures and estimating the expressed levels of GFP per condition. Fluorescent images of the infected cultures (Figure 7.1.C) were used to calculate the percentage of infected cells counted as GFP positive cells to the total number of neurons stained with the neuronal marker microtubule-associated protein 2 (MAP2) positive cells. The percentages of infected cells showed consistency among the three conditions (control 65%, scrambled 72% and miR-101a-3p 67%) indicating that similar numbers of cells are infected in each condition (Figure 7.1.B). In addition, the percentage of infected cells is high enough to exclude the possibility of diluted observed effects. The levels of expressed GFP were estimated by immunoblotting analysis to verify all viruses are expressed in similar levels. GFP showed no significant changes among the three conditions (Figure 7.1.D, E). Collectively these data verify the scrambled miRNA and the miR-101a-3p are expressed in similar levels with the GFP control, in a comparative number of neurons.

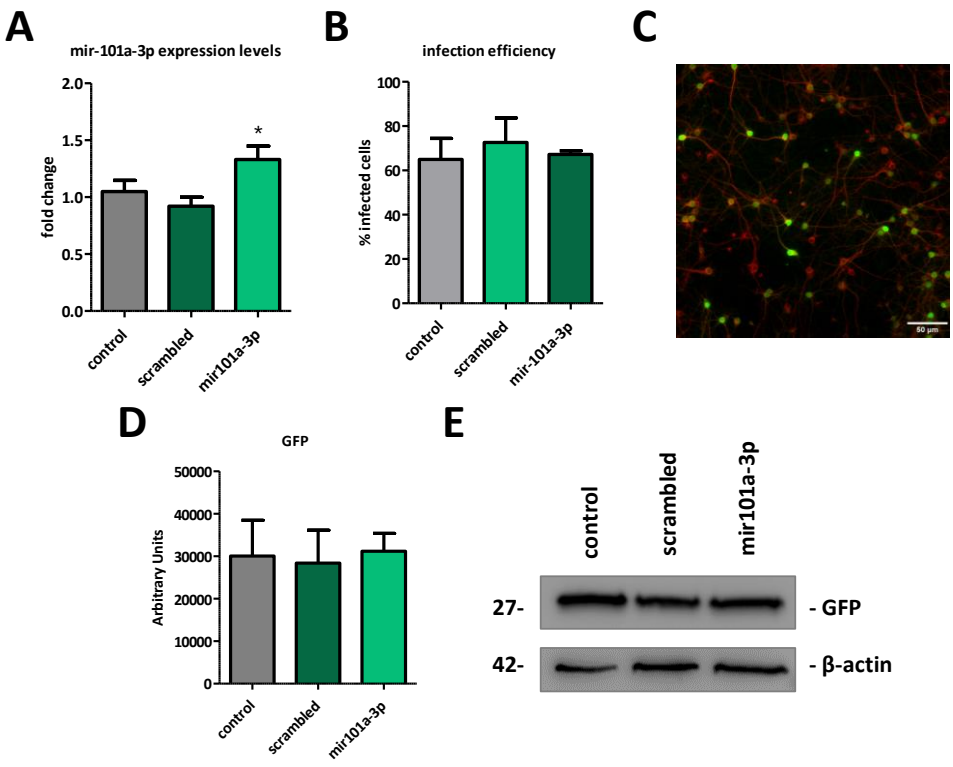

Figure 7.1. Viral induction of mir101a-3p in vitro. A. Mir-101a-3p levels quantified by real-time qPCR in neurons infected with control vector expressing only GFP (control) (n = 4), vector expressing GFP and scrambled miRNA sequence (scrambled) (n = 4) and vector expressing GFP and miR-101a-3p (mir101a-3p) (n = 4). B. Quantification of infected neurons in mass primary cultures expressed as the percentage of GFP positive cells to total neuron number. C. Representative image of infected mass cultures (infection with control vector is depicted); GFP - green; MAP2 - red; scale bar = 50 µm. D. Quantification of GFP levels in infected neurons by immunoblotting analysis and E. representative immunoblots. All data are expressed as mean ± SEM; Student's t-test; * p-value < 0.05, ** p-value < 0.01, ***p-value < 0.001);

3.3.5. MiR-101a-3p targets are downregulated

The levels of target genes were validated again in this system to test the miRNA-mRNA interaction (Figure 7.2.A.). *DAG1* was not altered upon miR-101a-3p expression (0.92 ± 0.11 FC) and *SHISA6* showed similar increase with scrambled miRNA expression (miR-101a-3p (1.33 ± 0.28 FC) and scrambled (1.41 ± 0.29 FC)) compromising the validity of this interaction. Similar to the data from the mouse model, *DLGAP3* and *GABRB2* mRNAs were found significantly downregulated as expected (*DLGAP3* (0.61 ± 0.20 FC, p=0.049) and *GABRB2* (0.51 ± 0.24 FC, p=0.02)).

Translation of target genes was evaluated by immunoblotting analysis (Figure 7.2.B, C). Dystroglycan was not detected in primary cultures. Shisa6 showed only a slight decrease upon miR-101a-3p expression accounting to 15% of the levels detected in neurons expressing only GFP. Despite

the observed effect in mRNA levels of neurons expressing scrambled miRNA, there was no evident change in the protein levels minimising the significance of the result. GABA (A)b2 and SAPAP3 showed significant decrease of 54% and 30% respectively confirming the downregulation of miR-101a-3p targets *in vitro* in a similar manner as the *in vivo* data. This confirms miR-101a-3p targets *GABRB2* and *DLGAP3*. In order to validate miR-101a-3p has no gross effect on the synaptic compartment *in vitro*, the post synaptic marker PSD95 and pre-synaptic marker synaptophysin were quantified by immunoblotting and no significant changes were observed (Figure 7.2.D, E) in agreement with the *in vivo* data. For further investigation of the miRNA correlation with αsyn pathology we asked whether αsyn levels are increased in vitro upon miR-101a-3p induction. It was previously reported that miR-101a-3p regulates autophagy (Frankel et al. 2011), a major culprit in synucleinopathies, leading αsyn accumulation (Ebrahimi-Fakhari et al. 2011). In addition, miR-101a-3p was shown to block autophagy and increase αsyn levels in oligodendroglial cells indicating its role in multiple system atrophy pathology (Valera et al. 2017). For this, levels of αsyn were assessed by immunoblotting but showed no alterations in neuronal cells expressing miR-101a-3p (Figure 7.2.D, E). This finding confirms a specific role of miR-101a-3p towards the postsynapses of neuronal cells and highlights the distinct effects of single miRNA in different cellular backgrounds.

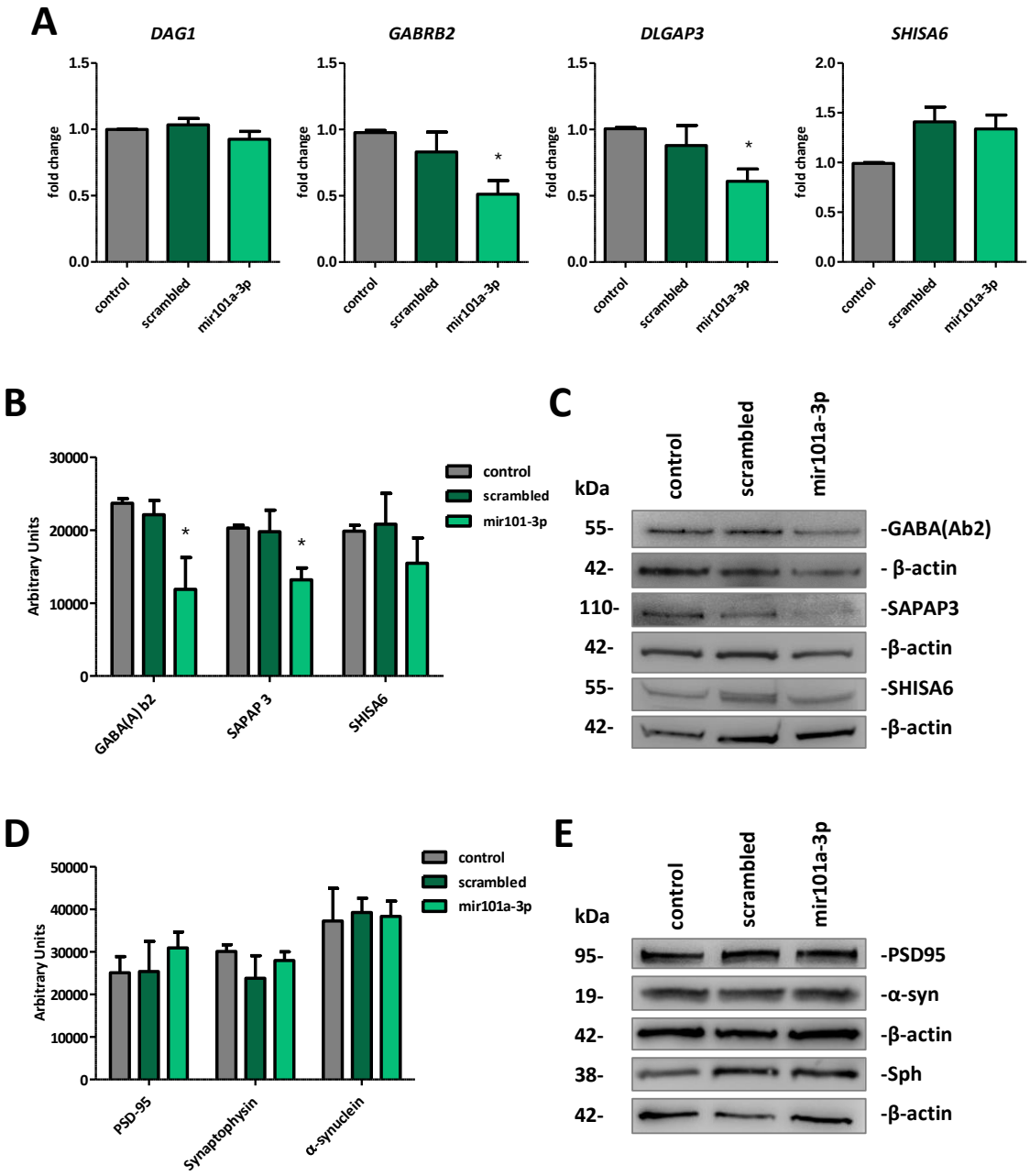

Figure 7.2. MiR-101a-3p regulates target mRNAs and proteins but not synaptic proteins in vitro. A. Validation by real time qPCR of mir-101a-3p target genes DAG1, GABRB2, DLGAP3, and SHISA6 levels in neurons infected with control vector expressing only GFP (control) (n = 4), vector expressing GFP and scrambled miRNA sequence (scrambled) (n = 4) and vector expressing GFP and miR-101a-3p (mir101a-3p) (n = 4). B. Quantification of target protein levels in infected neurons by immunoblotting analysis and C. representative immunoblots. D. Quantification of synaptic protein levels in infected neurons (n = 4) by immunoblotting analysis and B. representative immunoblots. All data are expressed as mean ± SEM; Student's t-test; * p-value < 0.05, ** p-value < 0.01, ***p-value < 0.001);

3.3.6. MiR-101a-3p reduces dendritic length and alters dendritic spine morphology

To assess the dendritic effects upon miR-101a-3p induction in vitro neurons were cultured in the custom made MFDs. This device allows the separation of neuronal bodies from dendrites which are growing through microgrooves towards a different compartment and can be easily visualised with fluorescent microscopy with the MAP2 marker (Figure 7.3.A). Using this approach, the dendritic spine length was assessed by measuring the dendritic length starting from the point of exist to the empty compartment, counting in the length of the microgrooves. miR-101a-3p induction upon DIV1 resulted in significantly shorter dendrites (537.2 ± 17.4 μm) compared to GFP control and scrambled miRNA (749.1 ± 21.77 μm and 683.8 ± 22.11 μm respectively) (Figure 7.3.B-C). Considering those account only for the distal dendrites that can grow past the microgroove compartment, neurons were sparsely grown in order to assess the length of apical dendrites as well. Similarly, dendrite length was found significantly shorter upon miR-101a-3p expression (54.6 ± 2.8 μm) compared to GFP control and scrambled miRNA (160.0 ± 10.61 μm and 136.6 ± 6.7 μm respectively) (Figure 7.3.D-E).

For the dendritic spine assessment, the spines were visualised with the MAP2 marker with confocal microscopy at an x 120 magnification. Quantification was performed manually by inspection of at least 30 dendritic spine segments of 10 μm per condition. Interestingly, in this isolated *in vitro* system absolute spine number was significantly decreased upon miR-101a-3p expression (5.8 ± 0.4/ 10 μm) compared to GFP control and scrambled miRNA (7.2 ± 0.4/ 10 μm and 7.3 ± 0.3/ 10 μm respectively) (Figure 7.3.F). Further classification of dendritic spines to mushroom, thin and stubby revealed significant differences in the miR-101a-3p group (Figure 7.3.G). Mushroom spines were significantly reduced by 30% (Figure 7.3.D) while thin and stubby spines did not show any alterations. Reduced mushroom spines implicate an early phenotype of synaptic failure similar to the *in vivo* data while the increase of stubby spines *in vivo*, which is not observed here, is probably due to a compensatory mechanism.

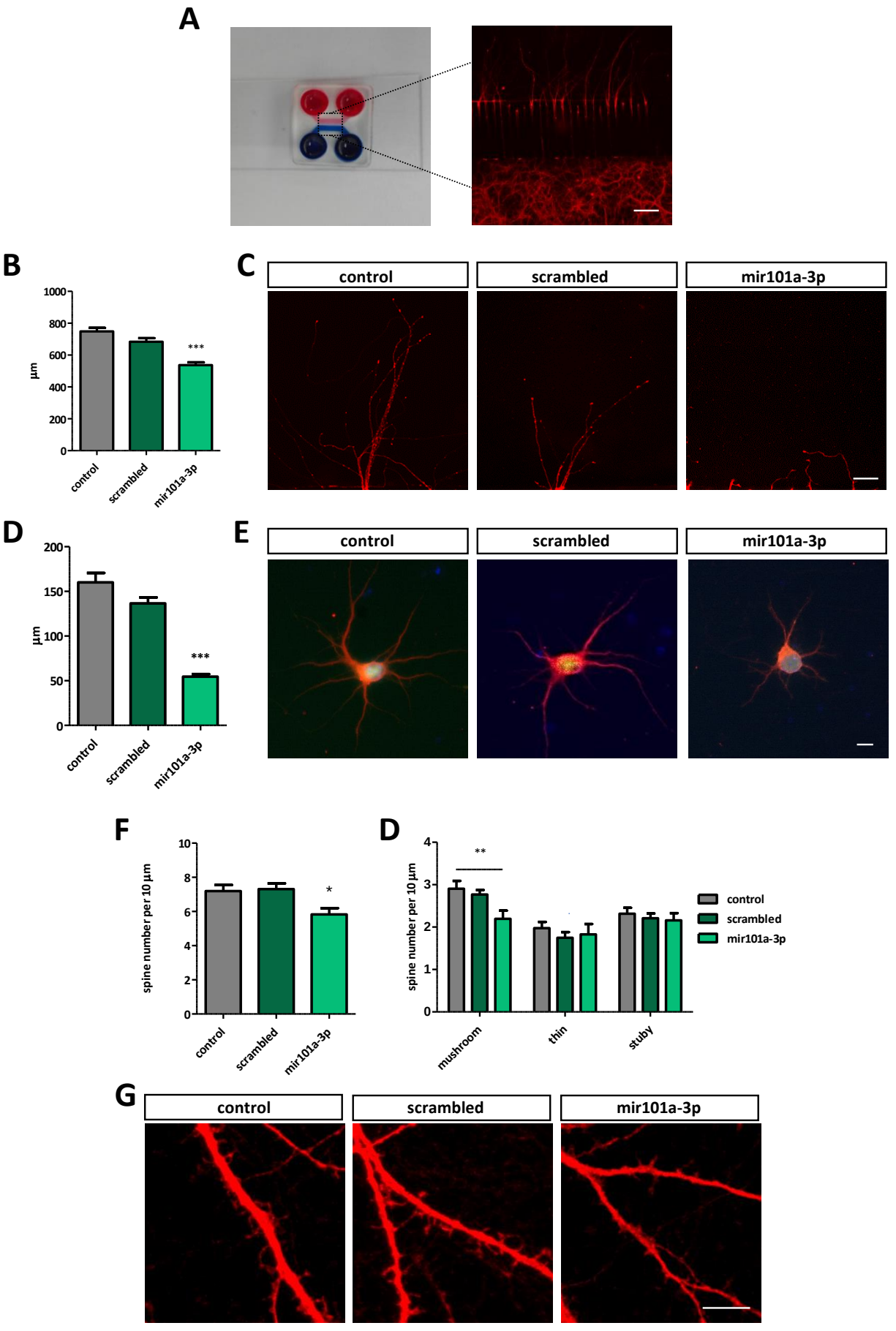
A
B
µm
1000
800
600
400
200
0
***
control
scrambled
mir101a-3p
C
control
scrambled
mir101a-3p
D
µm
200
150
100
50
0
***
control
scrambled
mir101a-3p
E
control
scrambled
mir101a-3p
F
spine number per 10 µm
10
8
6
4
2
0
*
control
scrambled
mir101a-3p
D
spine number per 10 µm
4
3
2
1
0
**
mushroom
thin
stuby
control
scrambled
mir101a-3p
G
control
scrambled
mir101a-3p

Figure 7.3. MiR-101a-3p reduces dendritic length and alters dendritic spine morphology in vitro. A. Photo of the custom made MFD; upper wells and chamber are filled with red dye and the bottom with blue dye. Fluorescent image of neurons cultured in the bottom chamber and the dendrites growing to the upper chamber through the microgrooves; MAP2 - red; scale bar =200 µm. B Bar graph of average length (µm) of distal dendrites growing through the microgrooves of MFDs of neurons infected with control vector expressing only GFP (control), vector expressing GFP and scrambled miRNA sequence (scrambled) and vector expressing GFP and miR-101a-3p (mir101a-3p) (n = 3 individual experiments x 3 MFDs per condition). C. Representative images of dendrites at the exit point to the upper chamber of the MFDs; MAP2 - red; scale bar = 50 µm. D. Bar graph of average length (µm) of apical dendrites in sparsely cultured infected neurons (n ≥ 25 cells from 4 individual experiments per condition). E. Representative images of single infected neurons; GFP – green; MAP2 - red; Hoechst – blue; scale bar = 10 µm. F. Quantification of total dendritic spine number and D. classification of mushroom, thin and stuby dendritic spines per 10 µm dendrite of infected neurons (n ≥ 32 x 10 µm segments per condition manually counted with Fiji software). G. Representative images of the dendritic spine segments; MAP2 – red; scale bar = 50 µm. All data are expressed as mean ± SEM; Student's t-test; * p-value < 0.05, ** p-value < 0.01, ***p-value < 0.001);

3.3.7. MiR-101a-3p is induced by αsyn species

A direct correlation between αsyn levels and miR-101a-3p levels was sought. For this, primary neurons on DIV 5 were exposed to recombinant monomeric and oligomeric αsyn species as well as pre-formed fibrils (PFF) and incubated for 20 days according to recently published protocol (Mahul-Mellier et al. 2020). MiR-101a-3p levels were quantified by qPCR (Figure 7.4.A) following the incubation period and a significant increase was identified in neuronal cells exposed to monomeric and oligomeric αsyn species (1.75 ± 0.38 FC and 1.75 ± 0.09 FC respectively). On the contrary, miR-101a-3p increase was not observed in the PFF exposed group at DIV25 (1.02 ± 0.06 FC). This coincides with synaptic loss, as shown by reduced PSD95 and synaptophysin estimated by on immunoblots (Figure 7.4.B). This observation suggests either PFFs fail to induce miRNA increase or the miRNA is lost along with the synapses. Interestingly, the PFF exposure seems to increase αsyn phosphorylation at Ser129 (Figure 7.4.C).

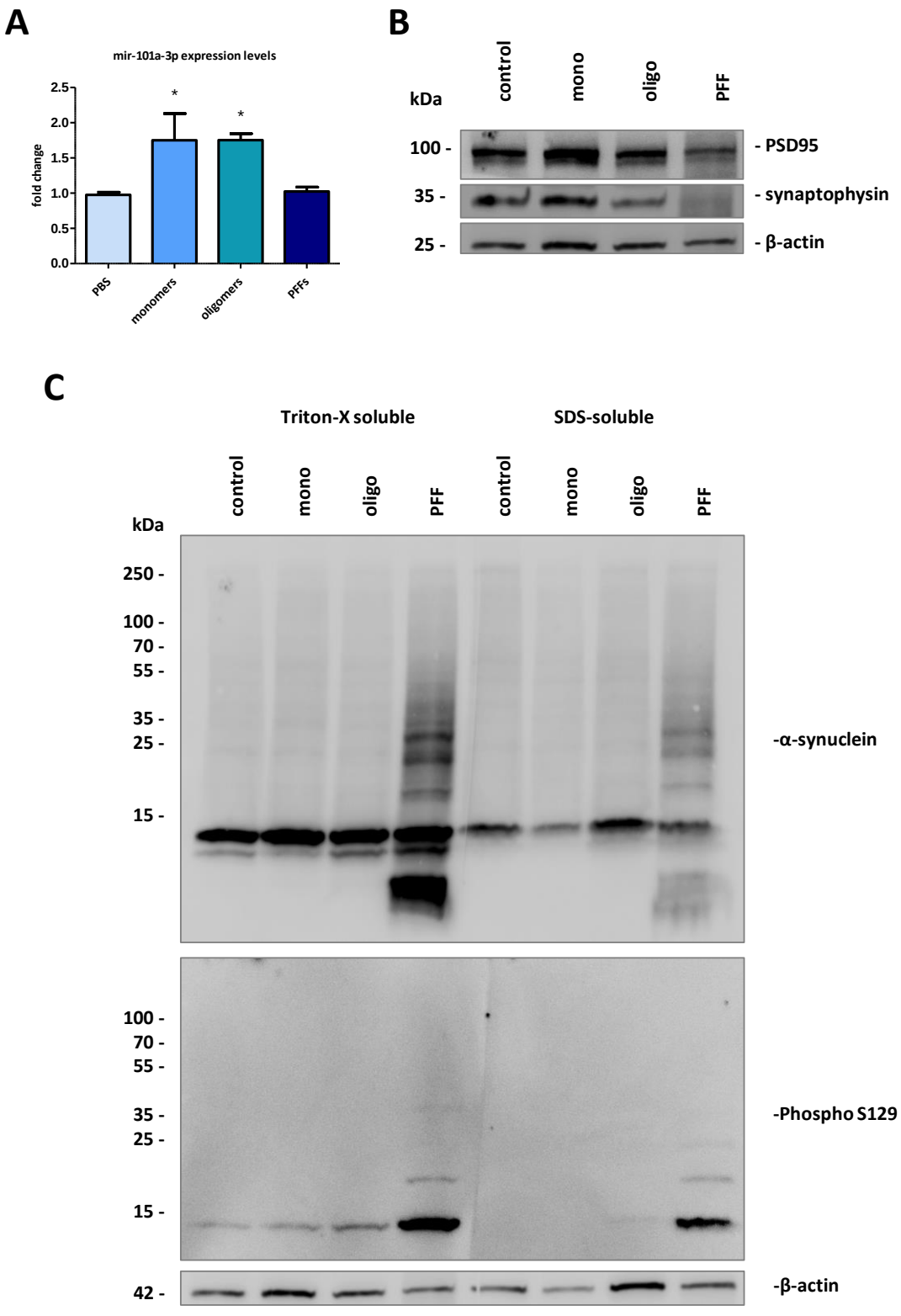

Figure 7.4. Primary neurons exposed to exogenous αsyn species show increased mir101a-3p. A. Mir-101a-3p levels quantified by real-time qPCR in neurons exposed to monomeric and oligomeric αsyn species and PFFs. B. Immunoblot of synaptic markers in neurons exposed to the different αsyn species. C. Immunoblot of αsyn and phospho S129 αsyn sequentially extracted with detergents Triton-X100 and SDS from neurons exposed to the different αsyn species.

3.3.8. MiR-101a-3p is a negative modulator of synaptic plasticity

Evidence so far indicates a direct negative role of miR-101a-3p in dendrite development and synaptic plasticity, to further validate this, we tested the levels of miR-101a-3p upon environmental enrichment which is known to exert the opposite effects in neurons. We quantified miR-101a3p in midbrain of 12-month-old mice grown in enriched environment (EE) and identified a significant reduction (0.6235 ± 0.02611 FC) (Figure 8) confirming the dynamic role of miR-101a-3p on synaptic phenotype.

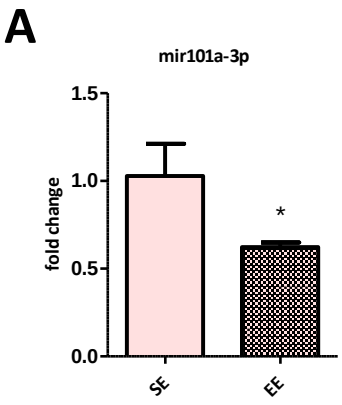

Figure 8. Midbrain of mice grown in EE shows decreased mir-101a-3p levels. A. Mir-101a-3p levels quantified by real-time qPCR in midbrain of mice grown in standard environment (n = 4) or enriched environment (n = 4). All data are expressed as mean ± SEM; Student's t-test; * p-value < 0.05, ** p-value < 0.01, ***p-value < 0.001);

3.3.9. MiR-101a-3p is increased in the cortex of patients with Dementia with Lewy Bodies

Finally, to validate the importance of our findings we assessed the miRNA levels in RNA samples isolated from cortex of 8 Dementia with Lewy Bodies (DLB) patients: 2 females and 6 males, aged 77.5 ± 8.2 years and 23±11.8 hrs post-mortem delay and 14 healthy individuals: 8 females and 6 males aged 73 ± 16.3 years and 26±23.8 hrs post-mortem delay (Tissue from Newcastle University NBTR) (Table 1). Quantification of miR-101a-3p was with qPCR and showed significant increase in DLB samples (4.994 ± 0.93 FC) compared to the healthy individuals (1.728 ± 0.5134 FC) (Figure 9). This increase in cortex of DLB patients highlighted the potential miR-101a-3p as biomarker or therapeutic target.

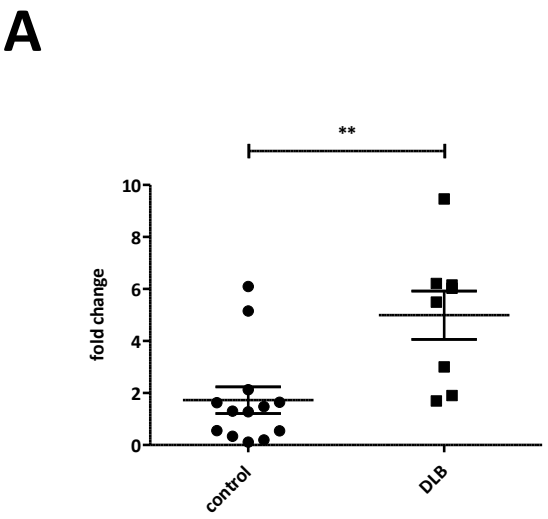

Figure 9. MiR-101a-3p is increased in the cortex of patients with Dementia with Lewy Bodies. A. Mir-101a-3p levels quantified by real-time qPCR in cortex of 8 DLB patients and 14 control samples of healthy individuals; p = 0.0027; All data are expressed as mean ± SEM; Student's t-test; * p-value < 0.05, ** p-value < 0.01, ***p-value < 0.001);

4. Discussion

4.1. Small RNA profiling and pathway analysis in [A30P] αsyn mice

PD is a chronic neurodegenerative disease only second to AD in incidence rate (de Lau and Breteler 2006). Research over the past decades has pointed out that PD is a multifactorial disorder (Rocha, De Miranda, and Sanders 2018; Navarro-Sánchez et al. 2018). Since the 70's the only treatment available is the pharmacological supplementation of dopamine with L-DOPA, that only slows down the motor symptoms arising from the pathology, without rescuing disease progression (Lees, Tolosa, and Olanow 2015; You et al. 2018). PD treatment inefficiency is mostly due to the late diagnosis which comes when motor symptoms appear and brain degeneration is already at an advanced stage (Postuma et al. 2015). After extensive studies on PD and other synucleinopathies' pathogenesis and mechanisms of disease progression, investigation is focusing on biochemical and molecular changes in early disease stages. (Schirinzi et al. 2016; Ghiglieri, Calabrese, and Calabresi 2018). As the disease is multifaceted, so are the studies evaluating early pathology with part of them focusing on gene expression analysis (Urbizu and Beyer 2020).

Genetic factors have been extensively studied in synucleinopathies, and GWAS have associated several genes with the different disease forms. Mutations in one or more of the *PARK* genes induce familial forms of PD and other synucleinopathies while other polymorphisms identified by GWAS lead to higher chances of developing familial or sporadic PD (Y. I. Li et al. 2019). These extensive genetic studies provide hints on the susceptibility of certain population to develop PD and other synucleinopathies. In order to fully understand the genetic impact on PD development, underlying epigenetic changes in early stages need to be studied (Jakubowski and Labrie 2017; Pavlou et al. 2016). In this study, we focused on epigenetic regulation by RNAs and presented small RNA deregulation in a mouse model of familial PD at an early stage, prior neuron loss and phenotype onset.

PD is characterised by progressive loss of dopaminergic neurons in the SN. The majority of PD mouse models aiming to replicate a robust cell loss employ the use of toxins like 6-hydroxydopamine (6-OHDA), MPTP, paraquat, and rotenone (Betarbet et al. 2000; Meredith and Rademacher 2011; Manning-Bog et al. 2002; Ungerstedt 1968) or injection of αsyn species in different brain regions (Luk et al. 2012; Mason et al. 2016; Paumier et al. 2015). These models offer acute neurodegeneration replicating many phenotypic characteristics of PD but their major disadvantage is they do not replicate the chronic progressive development of disease. On the contrary, transgenic mouse models

expressing high levels of human Wt αsyn or mutant αsyn forms show a middle age onset with gradual development of neuropathology and phenotype (Masliah et al. 2000; Kahle et al. 2000; Giasson et al. 2002).

The mouse model expressing human mutant [A30P] αsyn has been well charaterised in terms of neuropathology and phenotype. This model expresses [A30P] αsyn under the Thy-1 promoter and shows two-fold αsyn expression relative to endogenous levels and accumulation of the protein in both soma and neurites of neurons in SN, cerebellum, neocortex and brainstem (Kahle et al. 2000). Misfolded phosphorylated αsyn formations resistant to proteinase K digestion with amyloid characteristics start appearing at 8 month-old mice, accompanied by locomotor impairments and reduced TH levels (Neumann et al. 2002). Fine motor impairments and hyperactivity are already evident at 2 month-old mice and progressively deteriorate till later age (Ekmark-Lewén et al. 2018; Freichel et al. 2007). Phenotype fully features at 12 months when mice show severe locomotor impairments and cognitive decline (Freichel et al. 2007). Collectively, this model resembles age-dependent neuropathology of synucleinopathies and cognitive impairments simulating DLB.

The [A30P] mouse biochemistry is obviously extensively studied. However, epigenetic studies in this model are still minimum. Phosphorylated αsyn species were shown to accumulate in nucleus and differential αsyn DNA binding was observed accompanied by transcriptional deregulation (Paiva et al. 2018; H. Schell et al. 2009). One study has looked into RNA based processes in this model by identifying miRNA signatures by chip analysis in the brainstem of 12-month-old mice (Gillardon et al. 2008). The brainstem is abundant of accumulated αsyn but not affected by neuronal cell loss thus the effect of αsyn burden and cell stress is identified in this study. Here we analysed the midbrain miRNAome by small RNA-seq at 6-month-old Tg mice and littermate controls. Based on the characterisation of this model at this time point αsyn has already started to aggregate to amyloid-like species and neuronal function is disturbed while neurons and synapses are intact. Assessing the neurons known to degenerate from αsyn burden before this happens provides the possibility to identify processes that lead to cell loss. To our best knowledge, this study is the first attempt to assess miRNAs at an early age in [A30P] mouse midbrain. We identified significant deregulation of microRNAs (Figure 1) highlighting the epigenetic potential on phenotype development. The deregulated miRNAs from our dataset showed some consistency with data sets from PD patients and other PD models (Singh and Sen 2017; Mouradian 2012; Gillardon et al. 2008), while many novel miRNAs were identified.

The majority of upregulated miRNAs target genes related to development and morphogenesis while the downregulated miRNAs target genes related to secretion and exocytosis (Figure 2.1.). As previously mentioned, αsyn is not traditionally producing gross developmental deficits (Abeliovich et al. 2000; Fernagut and Chesselet 2004) although the protein has been implicated in some developmental processes. The direction of the effect is still not clear as αsyn was shown to negatively regulate development of enteric neurons and primary midbrain neurons (Swaminathan et al. 2019; Koch et al. 2015) and enhance development of corticostriatal glutamatergic projections (Schechter et al. 2020). Interestingly, the role of αsyn in these neuronal subtypes correlates with their susceptibility to the protein as enteric and dopaminergic neurons are known to degenerate in synucleinopathies while the corticostriatal pathway is only indirectly affected (Baumuratov et al. 2016; W Dauer and Przedborski 2003). These pathways are probably attributed to the top upregulated miRNAs miR-690, miR-582-3p and the cluster miR-183-5p, miR-182-5p, all of which are increased upon neuronal injury and correlated with dendritic and/or neuronal development (Hunsberger et al. 2013; Roser et al. 2018; Fang et al. 2015).

Lastly, we should note that the process showing the highest enrichment is the “regulation of telomere maintenance”. One study in this mouse model has identified shorter telomere size as an important factor accelerating αsyn pathology (Scheffold et al. 2016). Telomere size assessment in patients with PD and DLB indicated shorter telomeres in comparison with healthy population which is indicative for both accelerated senescence and accelerated disease progression (Kume et al. 2012; Jing et al. 2008).

The implication of downregulated miRNAs target genes in secretory pathways indicate a responsive role of ncRNAs in processes like protein secretion and synaptic transmission. Asyn is clearly implicated in SNARE exocytosis, synaptic vesicle cycle and neurotransmission release (Maria Grazia Spillantini and Goedert 2006; Abeliovich et al. 2000; Nemani et al. 2010). The protein itself is secreted to the extracellular space as it is found incorporated in exosomes and synaptic vesicles (Emmanouilidou et al. 2010, 2016; El-Agnaf 2003). Both monomers and oligomers are incorporated into vesicles due to the high affinity of the protein to membranes of high curvature leading to disturbances in vesicle-related pathways like the ER-Golgi secretory pathway (Ouberai et al. 2013; Credle et al. 2015; H. J. Lee, Patel, and Lee 2005). In addition to that, ER-Golgi pathway is impaired by αsyn accumulation that triggers the activation of a defense mechanism known as the unfolded protein response (UPR) leading to neuronal death (Bellucci et al. 2011; Colla 2019). MiR-702-3p, miR-

339-5p, and miR-29-5p identified in the top downregulated miRNAs are downregulated by ER stress and are implicated in UPR and apoptosis (Mcmahon, Samali, and Chevet 2017; Berry, Lal, and Binukumar 2018; Long, Ray, and Lahiri 2014). Of note, miR-29-5p has been previously linked with neurodegeneration as it is found downregulated in blood serum of PD patients and brain of AD patients (Bai et al. 2017; Shioya et al. 2010; Roshan et al. 2014). In addition, the miR-491-5p and miR-7080-5p are both defined as negative modulators of neurotransmission (Sun et al. 2018; X. Jia et al. 2016).

In terms of cellular localisation of the miRNA target genes, the synapse was strongly projected either as pre- or post-synaptic compartment (Figure 2.2.). This correlation validated our initial strategy for identifying pathways implicated in synaptic impairment prior neuronal loss. Neurodegenerative disorders are often classified as synaptopathies, meaning synaptic impairments are evident prior neuronal degeneration (Schirinzi et al. 2016; Spires-Jones and Hyman 2015; Fogarty 2019; J. Y. Li, Plomann, and Brundin 2003). Synaptic dyshomeostasis is seen as an early event in the pathogenesis of synucleinopathies in both human studies and animal models (Bellucci et al. 2016).

The unique characteristic of synapse is its dynamic nature. The fine-tuned homeostatic mechanisms leading neuronal plasticity which can maintain or alter neuronal networks are vital for synapses. Minor synaptic imbalances can render this characteristic the major cause of susceptibility to synaptic damage. Biological pathways identified from the target genes of miRNAs indicated changes in neuronal development and transmission, both disturbing synaptic plasticity (Picconi, Piccoli, and Calabresi 2012; Ghiglieri, Calabrese, and Calabresi 2018). Synaptic plasticity requires local dendritic protein synthesis which is partially controlled by local post-transcriptional regulation (Sutton and Schuman 2006). MiRNAs and the machinery for their biogenesis is present in dendritic spines providing a new dimension on the identified cellular components related to terms such as “spine” and “postsynapse” which may be indicative of local events (Lugli et al. 2008, 2012).

Moreover, two of the cellular components identified drew our attention. The first is related to upregulated miRNA target genes and is about glutamatergic synapse. Midbrain is rich in glutamatergic neurons but they have not been reported to degenerate in midbrain upon αsyn mediated stress (Morales and Root 2014). On the contrary, studies on animal models and PD patients indicate significant glutamatergic synapse loss, dendritic spine pathology and microcircuit impairments in the striatum, which is considered an indirect effect mediated by dopamine loss (Villalba, Mathai, and Smith 2015). It would be interesting to study whether glutamate neurons suffer

prior the dopamine neurons leading the imbalance that eventually causes dopaminergic neuron loss. The second component is listed in the downregulated miRNA target genes where the highest enrichment ratio is for Schaffer collateral. The Schaffer collateral is an integral part of memory formation and requires midbrain dopamine neurons for the long-term potentiation (LTP) (Rosen, Cheung, and Siegelbaum 2015). This is particularly interesting considering the memory deficits displayed by our mouse model.

Finally, the majority of the enriched molecular processes linked with both upregulated and downregulated target genes correlated with Ras pathway, kinases and GTPases. Leading proteins of the Ras signalling pathway have been correlated with PD and synucleinopathies (E. K. Kim and Choi 2010; Bohush, Niewiadomska, and Filipek 2018; Obergasteiger et al. 2018). Ras pathway includes many kinases and GTPases but we cannot exclude the possibility of independent kinases regulated by miRNA processes. Many kinases have been implemented in PD pathogenesis and are even considered promising therapeutic targets (Tönges et al. 2012; Valente et al. 2004; Price et al. 2018; Tatenhorst et al. 2016). In addition to these components a great percentage of the genes targeted by downregulated miRNAs were DNA-related processes. Although αsyn physiology has been implicated in DNA damage response, the link with neurodegeneration and PD development is still not clear but holds great potential (Schaser et al. 2019; Pinho et al. 2019; Merlo et al. 2016).

4.2. MiRNA effects on transcript regulation and synaptic pathways

MiRNAs target mRNAs and mediate gene silencing by complementary binding of a 6-8 nucleotide region (Brennecke et al. 2005). Although silencing mechanisms are well studied accurate prediction of target genes is still a challenge. Factors like stereochemistry and thermodynamics affect the stability of mRNA and accessibility of the complementary site defining the actual binding and outcome (J. T. Roberts and Borchert 2017). Despite the different computational methods employed and the development of different tools for miRNA target gene prediction, about 30% of the predicted targets are false positives (Enright et al. 2003; Hsu et al. 2011; Lewis et al. 2003). To bypass this issue and increase the confidence of predicted miRNA effects we integrated the transcriptomic data set previously produced from RNA seq of the same RNA samples (Paiva et al. 2018). Correlating the miRNAome with the transcriptomic profile we identified the predicted miRNA target genes in the transcriptomic data set and selected the negative miRNA-mRNA interactions (Figure 3.1.A). This means, only downregulated mRNAs were appointed to upregulated miRNAs and contrariwise. Only

those valid miRNA-mRNA interacting pairs were considered for downstream analysis. Finally, in order to select the most relevant pairs the miRNAs were filtered according to number of target genes (Figure 3.1.B) and expression levels (Figure 3.1.C). This approach obviously restricted the candidate miRNAs but significantly increased the confidence of validity, reducing the possibility of identifying mRNAs targeted via non-canonical interactions or low proficiency matches with low biological impact (Agarwal et al. 2015; Garcia et al. 2010).

The downregulated let-7b-3p and miR-486a-3p were identified as the top interacting miRNAs with 138 and 104 upregulated target mRNAs respectively (Figure 3.1.B). Those miRNAs showed a low number of reads (Figure 3.1.C) indicative of lower expression in the mouse midbrain reducing the chances of producing a strong phenotype alone. Considering the large number of targets, we cannot exclude the possibility of synergistic effects from other miRNAs targeting the same genes to produce the phenotype.

Let-7 is very well conserved across species and shows a high homology in mouse and human (Pasquinelli et al. 2000). Let-7a-g, let-7i, and let-7k share the same sequence for target recognition but are produced from different genomic locations (Roush and Slack 2008). As previously mentioned, let-7 is an important regulator of immune response in PD related to apoptosis and axon guidance (X. Wang et al. 2019; Lehmann et al. 2012). Let-7 was found downregulated in Caenorhabditis elegans PD models and in a manganese induced synucleinopathy cellular model (Asikainen et al. 2010; He et al. 2017). On the contrary, different let-7 homologues, including let-7b, have been found upregulated in human CSF and brain tissue from synucleinopathy patients (C. E. Briggs et al. 2015; Gui et al. 2015; Tatura et al. 2016; Dorval et al. 2014; Burgos et al. 2014). Interestingly, let-7 is found downregulated in blood of PD patients only before they start treatment (Lei Chen et al. 2018). This could be the reason behind the disagreement on let-7 deregulation results coming from synucleinopathy models and studies from PD patients. Similar discrepancy is observed in AD studies and it would be interesting to investigate whether drug related effects apply in this case as well (Gámez-Valero et al. 2019).

miR-486a-3p has not been studied in the context of αsyn pathology but has been previously linked with Amyotrophic Lateral Sclerosis and Huntington´s disease which indicates it might be an interesting player in the context of neurodegeneration (Hoss et al. 2016; Waller et al. 2018).

Following the top 2 interacting miRNAs, a group of 4 miRNAs showed comparable number of target genes. The upregulated miR-101a-3p with 60 target genes and the downregulated let-7c-1-3p,

miR-344d-3p, and miR-543-3p with 57, 55, and 53 target genes respectively (Figure 3.1.B). Of those, miR-543-3p and miR-101a-3p are the miRNAs with highest expression levels providing confidence of producing a strong phenotype. This is partially confirmed by cellular component analysis for both miRNA target genes (Figure 3.2.A) indicating dendritic spines as the affected compartments which is reflected to the bulk data set (Figure 2.2).

MiR-543 has not been extensively studied in the context of neurobiology, regardless it was found highly expressed in mouse midbrain (Figure 3.1.C). One study in MPTP treated mice identified miR-543 inhibition has neuroprotective effects implicated in glutamate excitotoxicity (X. Wu et al. 2019). Downregulation of miR-543 has been previously linked with increased NF-κB expression and mediated pro-inflammatory response which decreased the level of apoptosis and promoted the release of nerve repair factors. (Zhao, Cui, and Zhang 2021). In addition, phosphatase and tensin homolog on chromosome 10 (PTEN) is a direct target of miR-543. MiR-543 downregulation can elevate PTEN which is a crucial regulator of neuronal development, neuronal survival, axonal regeneration and synaptic plasticity. (Ismail et al. 2012; G. Liu, Zhou, and Dong 2019). Collectively, these data indicate downregulation of miR-543 as a compensatory mechanism in a cellular attempt to escape early injury and enhance neuronal survival.

MiR-543 target genes, all of which were upregulated due to the negative correlation analysis, indicated a positive regulation of potassium channel activity and dendrite morphogenesis (Figure 3.2.A). These findings are not surprising as potassium channels are highly expressed in neuronal dendrites and key players in synaptic plasticity (Yuan and Chen 2006; Johnston et al. 2003). In agreement with our finding, potassium channels are upregulated in surviving neurons in PD brain indicating electrical activity is enhanced to compensate for neurotransmitter loss (Schiemann et al. 2012; Dragicevic, Schiemann, and Liss 2015). This compensatory mechanism may offer harmful effects as enhanced activity of potassium channels and the subsequent depolarisation may activate calcium channels leading calcium overload which in turn results in excitotoxicity and further αsyn secretion creating a deleterious feedback loop (Chan et al. 2007; Chan, Gertler, and Surmeier 2009; Emmanouilidou et al. 2010). MiR-543 seems to modulate a network of great interest in synapse physiology and synucleinopathy.

In contrast to miR-543, miR-101 has been studied in the context of physiology and pathology of nervous system. Expression of miR-101 in the postnatal hippocampus was shown to regulate GABAergic signaling maturation to limit spontaneous activity and block uncontrollable dendritic

growth. In addition to that, it regulates glutamatergic synapses by preventing pre-synaptic components from increasing synaptic density (Lippi et al. 2016). High levels of miR-101 are identified in the postnatal cortex as well, suggesting the observed effects may apply to other regions of postnatal brain (Chi et al. 2009). Studies focusing on miR-101 in the adult brain indicated modulation of different pathways highlighting the miRNA has distinct roles in postnatal and adult brain (Vilardo et al. 2010; Y. Lee et al. 2008).

While miR-101 shows a physiological role in postnatal brain, it is linked with neurodegenerative disorders in the adult brain. miR-101 participates in the posttranscriptional regulation of mutant ataxin1 gene and effectively results in reduction of the abnormal accumulation of polyglutamine-expanded ataxin1 which is implicated in spinocerebellar ataxia (Y. Lee et al. 2008). In addition, miR-101 targets Ras homolog enriched in striatum (Rhes), a small GTP-binding protein implicated in Huntington's disease (Mizuno and Taketomi 2018). Considering there are only a few known polyglutamine disorders including Huntington´s and spinocerebellar ataxia, it would be interesting to further investigate the effect of miR-101 in this context (Dong and Cong 2019).

Amyloid-Precursor-Protein (APP) is also a target of miR-101, rendering the miRNA a potent modulator of amyloid beta (Aβ) accumulation and fibril formation (Vilardo et al. 2010; Long and Lahiri 2011). MiR-101 is further identified as a modulator of neuroinflammation by targeting cyclooxygenase-2 which is an important player in neurodegeneration modulating both protein aggregation and inflammation (Tanaka et al. 2009; Gresa-Arribas et al. 2012; Figueiredo-Pereira et al. 2014). In microglia, miR-101 seems to induce their development by blocking mitogen-activated protein kinase (MAPK) and promote the expression of pro-inflammatory cytokines like interleukin-6 (IL-6) and tumour necrosis factor (TNFα) (Gao et al. 2014; Saika et al. 2017). Similar effects are observed in macrophages indicating miR-101 is a positive regulator of inflammatory processes (Q.-Y. Zhu et al. 2010).

MiR-101 was also studied in the context of synucleinopathy, with increased expression identified in the striatum of MSA patients. MiR-101 was further found to inhibit autophagy in oligodendrocytes and promote αsyn accumulation (Valera et al. 2017). In addition, αsyn was found to downregulate lncRNA-T199678, which acts as a miR-101 sponge. In this study, αsyn overexpression was correlated with increased miR-101 and neuronal injury but downstream gene targets were not studied (Bu et al. 2020).

The downregulated gene set targeted by miR-101 includes Aβ related processes confirming APP targeting as in previous studies. In addition, downregulation of genes related to biological processes like cytoskeletal anchoring, synapse assembly and synaptic plasticity resemble events observed in miR-101 studies in postnatal stages. Interestingly, both APP and Aβ regulate synaptic plasticity while Aβ pathology is associated with cognitive decline in PD, and a phenotype in our mouse model (Lim et al. 2019; Parihar and Brewer 2010; Jendroska et al. 1996). MiR-101 is tightly correlated with early neuronal physiology while in later age it seems to enhance neurodegeneration. Moreover, the distinct effects observed in different cells highlight the dynamic nature of miRNAs and the diversity of posttranscriptional regulation programmes.

Collectively miR-543 and miR-101 are excellent candidates for mechanistic studies in the context of synucleinopathy. In order to validate these findings with a second method, the levels of both miRNAs were assessed qPCR on independent biological replicate samples (Figure 3.2.B). Deregulation was confirmed for both miRNAs but miR-543 expression showed high variability in midbrain of Wt mice so this study was focused on miR-101a-3p. The target genes of miR-101a-3p were filtered for dendritic localisation in order to identify processes taking place locally at synapses at this early disease stage (Figure 3.2.C). The dendrite-relevant differentially expressed genes targeted by miR-101a-3p were *DAG1, DLGAP3, SHISA6,* and *GABRB2.*

DAG1 encodes for α- and β-dystroglycan, two major structural proteins and cell adhesion receptors expressed in the nervous system and found within the PSD of dendritic spines (Durbeej et al. 1998; Dansie and Ethell 2011). Dystroglycan is essential for embryonic development and participates in synaptic plasticity by facilitating LTP (Satz et al. 2010; Williamson et al. 1997). In addition, it is implicated in axon guidance and synapse formation (Sato et al. 2008; Wright et al. 2012). There is a discrepancy between studies on the importance of dystroglycan in dendritic spines, attributed mainly on the different cell types used. Data so far indicate dystroglycan is a key player in dendritic formation of inhibitory neurons (Bijata, Wlodarczyk, and Figiel 2015; Lévi et al. 2002; Satz et al. 2010; Levy, Omar, and Koleske 2014). There are several disorders related to *DAG1* mutations which can completely eliminate dystroglycan expression resulting disrupted nervous system development and progressive muscular dystrophy (Cormand et al. 2001; Barresi and Campbell 2006). One study has linked dystroglycan with PD and AD, identifying higher levels of the protein in CSF of patients compared to healthy controls (Yin et al. 2009). While this does not correlate with our

observation in the mouse model, several parameters including the species, disease stage and cohort characteristics need to be considered in addition to further studies to confirm such findings.

DLGAP3 encodes for synapse-associated protein 90 (SAP90)/postsynaptic density protein 95 (PSD95)-associated protein 3 (SAPAP3), a guanylate kinase associated protein that participates in purine metabolism (E. Kim et al. 1997). SAPAP3 mRNA is targeted to dendritic spines and is mainly identified in excitatory synapses (Welch, Wang, and Feng 2004a). SAPAP3 interacts with many proteins at the PSD serving both as an anchoring protein maintaining the structure of PSD by concentrating its components to the membrane but also as a signaling complex via interactions with signaling molecules thus exerting many functions (Takeuchi et al. 1997; E. Kim and Sheng 2004; Welch, Wang, and Feng 2004b). Blocking the synaptic interaction of SAPAP3 with PSD95 was shown to have a negative effect on dendritic spine development in cultured neurons (J. Zhu et al. 2017). Due to the multiple functions of SAPAP3 in synaptic morphology and plasticity, mutations and deletion of the protein lead to psychiatric disorders and neurodevelopmental diseases (Grant 2012; Marín 2012). In particular, it is linked with neuropathology of Autism Spectrum Disorders, Obsessive-Compulsive Disorders, and Tourette's syndrome in humans (Bienvenu et al. 2009; Crane et al. 2011; Züchner et al. 2009). *DLGAP3* has been identified as a promising candidate gene for PD by microarray-based gene expression profiling of human blood samples (S. Liu et al. 2016). Interestingly, PD patients often develop Obsessive-Compulsive Disorder due to impairments of the basal ganglia circuitry (Alegret et al. 2001; Mallet et al. 2002).

SHISA6 encodes for another protein interacting with PSD95, the homonymous Shisa6. Little is known about Shisa6 physiology. It is enriched in dendritic spines and is identified as an auxiliary subunit of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors and regulates the stability of the receptor at the active site preventing desensitization during excessive neuronal activity (Klaassen et al. 2016). Shisa6 modulates synaptic function while in the absence of the protein significant impairments develop in memory formation (Sa sa Peter et al. 2020). One study in stem cells reported Shisa6 is a Wnt inhibitor, indicating an important role in development (Tokue et al. 2017). This is further validated by several studies associating Shisa6 with eye and neuronal development in persons with myopia (J. Li et al. 2015; Oishi et al. 2013; Kiefer et al. 2013). Finally, RNA seq of blood samples from PD patients identified Shisa6 downregulation as an aggravating gene in PD pathology (Xue et al. 2020).

Lastly, *GABRB2* encodes for Gamma-Aminobutyric Acid (GABA) Type A Receptor Subunit Beta2 (GABA(A)b2) that is necessary but not sufficient for the formation of the multi-subunit chloride channel that mediates the fast inhibitory neurotransmission (Enna 2007). GABA is an important player in maintaining a healthy neurotransmission pace (Taketo and Yoshioka 2000; Barbin et al. 1993; Behar et al. 1996). Different GABA receptor compositions have the dynamic to induce changes in neuronal migration, differentiation, proliferation and outgrowth (Hong Jin et al. 2003; Y. Wu et al. 2007). *GABRB2* knock out in mice results in loss of more than 50% GABA A receptors and results in hyperactivity, memory deficits, and anxiety (Yeung et al. 2018; Sur et al. 2001). GABA release in early postnatal stages mediates dendritic spine formation via GABA A receptor activation while in adult it regulates competitive interactions between enlargement and shrinkage of spines (Oh et al. 2016; Hayama et al. 2013). PD is characterised by lower GABAergic neurotransmission as shown by both PD models and patient CSF (Feng et al. 2014; Teychenné et al. 1982; Abbott, Pye, and Nahorski 1982; Manyam 1982; B. M. Roberts et al. 2020; Mograbi et al. 2017).

The identified miR-101a-3p target genes are all potent modulators of synapse function and formation and dendritic spine development thus modulating synaptic plasticity. Interestingly, they have all been previously correlated with PD or other neurodegenerative and neurodevelopmental disorders indicating their importance in neuronal physiology. Since all four genes display interest in regards with PD pathology and synaptic function we decided to proceed with further validation. A targeting sequence for miR-101a-3p was identified in the 3'-UTR of the mRNAs and the interaction was tested with an adapted luciferase reporter gene assay for the effect of microRNAs (Y. Jin et al. 2013). With this, we validated all 4 mRNAs are effectively targeted by the miRNA (Figure 4.1). We identified significant downregulation of *DLGAP3, SHISA6,* and *GABRB2. DAG1,* was also decreased but did not display statistical significance. Of note, the 3'-UTR of *SHISA6* includes 2 target sequences for miR-101a-3p but this did not correlate with increased mRNA targeting and/or reduction. This validation certified our initial filtering criteria for selecting only true miRNA-mRNA interactions.

Proceeding to the quantification of the target gene levels by qPCR for second degree validation we found downregulation of *GABRB2* and *DLGAP3* as expected based on the DESeq analysis (Figure 3.2.C) and Luciferase assay (Figure 4.1). In particular, *GABRB2* and *DLGAP3* showed a significant decrease in Tg mouse midbrain compared to Wt mice and *DAG1 and SHISA6* showed a slight change with no statistical significance (Figure 4.2.A). To validate the effect of miR-101a-3p in mRNAs translation, protein levels were estimated by immunoblotting analysis. We found that GABA

Ab2 and SAPAP3 are highly reduced, dystroglycan is not affected, while Shisa6 was not detected at all in mouse midbrain (Figure 4.2.B, C). Interestingly, both luciferase assay and qPCR verified miR-101a-3p effect on *DAG1* is milder compared to the other genes. Next, we verified there is no evident neuronal loss (Figure 4.3) or any robust changes in presynaptic or postsynaptic components (Figure 4.4) at this early time point. This is in agreement with previous characterisation of the mouse model (Neumann et al. 2002) and indicates the synaptic compartment is still intact although possibly already affected by the downregulation of GABA A receptor and SAPAP3.

Finally, in order to further characterise miR-101a-3p we sought to identify the expression pattern in different time points and tissues. Assessment of miR-101a-3p in different brain regions (Figure 4.5.B) and tissues (Figure 4.5.C) at 6-month-old mice showed that the miRNA increase is specific for midbrain. Then we looked at the midbrain in different time points and found in Tg mice the expression of miR-101a-3p is already increased at 4 months, it peaks at 6 months and is equalised with Wt mice at 8 and 12 months (Figure 4.5.A). Correlating the different ages in Wt and Tg mice we found out that the miRNA levels equalised at 8 months because miR-101a-3p is physiologically increased with age (Figure 4.5.D). In our PD mouse model, a faster increase is observed starting already at 4 months indicating miR-101a-3p mediates faster senescence or affects common pathways in neurodegeneration and ageing. PD is known for developing neuronal senescence of early onset (Martínez-Cué and Rueda 2020) and here we show for the first time miR-101a-3p is not only correlated with disease in adult brain (Vilardo et al. 2010; Y. Lee et al. 2008) but is also related with the ageing brain. Further studies will be needed to identify the physiology of miR-101a-3p and the transcripts modulated from postnatal stages to the ageing brain.

4.3. The role of miR-101a-3p in synaptic plasticity and neurodegeneration

The list of miRNAs involved in synaptic development, function and plasticity is continuously increasing and so are the miRNAs identified in pre- and post-synaptic compartment (Cohen et al. 2011; Lugli et al. 2008, 2005). Identification of miRNA processing machinery at the synapse highlighted that the local action of miRNAs in remote cellular compartments is one of their core features (Steward and Schuman 2001; Tiedge and Brosius 1996). We speculated miR-101a-3p localises at synapses due to the direct link with synaptic processes. Previous studies identified miR-101a-3p enrichment in synaptosomes prepared from hippocampus of 3-month-old mice and forebrain of 11-month-old mice (Zongaro et al. 2013; Epple et al. 2021). Here we confirmed miR-

101a-3p is enriched in synaptosomes prepared from midbrain of 6-month-old mice (Figure 4.6). These findings confirm miR-101a-3p localises at mouse brain synapses in different ages and regions highlighting its importance in synaptic physiology. Despite the findings in mouse, one study identified miR-101a-3p is less abundant in synaptosomes from human frontal cortex. In this study, the contradicting result could be due to the different species but synaptosomes were prepared from frozen tissue and the quality of synaptosomes as well as the miRNA stability are questionable (Yoshino, Roy, and Dwivedi 2021).

Several miRNAs residing at synapses have been correlated with synaptic plasticity and dendritic spine morphology in AD and prion diseases (Boese et al. 2016; Reza-Zaldivar et al. 2020). Accumulating evidence implicate dendritic spine dysfunction in the pathogenesis of neurodegenerative disorders but this is a common observation in normal ageing (Mostany et al. 2013). Dendritic loss is mediated by alterations of the presynaptic input or changes in neuron-autonomous functions. Apart from evident spine loss which alters dendritic spine numbers, dendritic spines can change in terms of shape or cytosolic and membranous content (Herms and Dorostkar 2016)

In PD, dopaminergic neurotransmission blockage by αsyn accumulation and impairments in synaptic vesicle cycle are potent events for altering dendritic spine number and morphology (Nemani et al. 2010). Although in PD the most common hypotheses regarding synaptic plasticity concentrate in presynaptic mechanisms mediated by abnormal αsyn species, other mechanisms are gradually unravelled. For example, αsyn oligomerisation increases glutamate subunit receptor 1 (GluR1) suggesting imbalances in GluR1 and GluR2 subunit ratio which is critical for LTP induction and maintenance, consequently affecting dendritic spine morphology (Diogenes et al. 2012; Pozo and Goda 2010). Mutant A53T αsyn was correlated with reduced dendritic plasticity upon ageing. In Wt mice reduction of dendritic spines during ageing was compensated by an increase in spine head size while this was not evident in Tg mice expressing mutant A53T αsyn (Parajuli et al. 2020). An established impairment in synaptic plasticity was also observed in mice expressing mutant A30P αsyn, where newly formed neurons in olfactory bulb failed to develop normal dendritic branches and spines (Neuner et al. 2014). Studies in both animal models and postmortem human samples showed reduction in spine density along with changes in spine morphology in striatum either due to loss of neurotransmitter input or due to αsyn burden (Villalba and Smith 2010; McNeill et al. 1988; Zaja-Milatovic et al. 2005).

The cellular component analysis and biological relevance of miR-101a-3p target genes indicated the dendritic spines as the affected site. Interestingly, both proteins downregulated by miR-101a-3p, namely GABA Ab2 and SAPAP3 are linked with alterations in dendritic spine morphology. Reduction of GABA A receptor has been correlated with increased elimination of existing dendritic spines and reduction of SAPAP3 results in reduction of mature spines (J. Zhu et al. 2017; Y. Chen 2014). Here we show the absolute number of dendritic spines in mouse midbrain was not affected (Figure 4.7.A) while the shape of spines differed significantly among Wt and Tg mouse midbrain (Figure 4.7.B). Mature mushroom spines and plastic thin spines were substituted by immature stubby spines. Normally, stubby spines are increased as a compensatory mechanism upon mushroom spine loss (Hering and Sheng 2001). These observations can be attributed to the downregulation of the proteins based on existing literature and are indicative of synaptic failure and synaptic plasticity impairments prior to synapse loss.

Bioinformatic analysis indicated miR-101a-3p is the most potent modulator of transcripts in the midbrain affecting the dendritic spines although the deregulation of several miRNAs and mRNAs cannot exclude the possibility of the observed phenotype being a synergistic effect of other miRNAs or biochemical processes. In order to identify whether the observed effects in dendritic morphology are mediated by miR-101a-3p we delivered the mature miRNA sequence via a viral vector in primary cortical neurons to analyse the produced phenotype. The mode of expression and virus load we selected resulted in moderate upregulation of the miRNA (Figure 5.1.A) close to the endogenous levels so that we exclude the possibility of off-target effects. Excessive upregulation of the miRNA could favour non-specific binding and ectopic expression at concentrations greatly exceeding physiology (Witwer and Halushka 2016).

Similar to the *in vivo* analysis, miR-101a-3p upregulation in neuronal culture resulted in downregulation of GABA Ab2 and SAPAP3 without producing any gross changes in synapses (Figure 5.2). A novel observation in this system was the significant reduction of dendrite length. Both apical and distal dendrites appeared much shorter in primary neurons expressing miR-101a-3p (Figure 5.3.B, C). This finding resembles a well-established phenotype in synucleinopathies. Overexpression of Wt αsyn and mutant A53T αsyn was associated with negative impact on dendrite development leading reduced dendrite length and complexity (Winner et al. 2012; Czaniecki et al. 2019). Decrease of dendritic length is evident in dopaminergic neurons of the SN and medium spiny neurons of striatum in PD and in cortical neurons in DLB (Patt S et al. 1991; Zaja-Milatovic et al. 2005; Kramer

and Schulz-Schaeffer 2007). Dendritic morphology displays significant changes in many neurodegenerative, neurodevelopmental and psychiatric disorders (Yamada et al. 1988; Moolman et al. 2004; Dierssen and Ramakers 2006; Teskey et al. 2006; N. Sousa et al. 2000; Radley et al. 2004; Cook and Wellman 2004). Although changes in dendritic spine morphology occur in response to learning and enriched environment, dendrites display an incredible stability to sustain experience-dependent changes of brain connectome and are only disturbed upon serious insults (Black et al. 1989; Trachtenberg et al. 2002; Grutzendler, Kasthuri, and Gan 2002).

Upregulation of miR-101a-3p in primary neurons, resulted in a significant reduction in the total number of spines attributed exclusively to the reduction of mushroom spines (Figure 5.3.F, D). Neurons *in vivo* show a reduction in mushroom and thin spines (Figure 4.7.B) while *in vitro* only mushroom spines are reduced (Figure 5.3.D). In addition, dendritic spine reduction *in vivo* is rescued by a compensatory increase of young stubby spines while this is not evident *in vitro*. Neurons in mouse brain are exposed to A30P αsyn and many other deregulated miRNAs possibly mediating a phenotype as well. The common finding of mushroom spine reduction can be attributed to miR-101a-3p increase and GABA Ab2 and SAPAP3 decrease which are implemented in reduction of mature spines (J. Zhu et al. 2017; Y. Chen 2014). In addition to that, the lack of a compensatory mechanisms *in vitro* is expected as the neuronal network does not present the same complexity and glial-mediated processes favouring positive neuronal plasticity are missing (F. Wang et al. 2016). Lastly, neurons *in vitro* are growing upon the influence of miR-101a-3p upregulation while neurons *in vivo* are already mature when exposed to higher concentrations of miR-101a-3p (Figure 4.5.A) illustrating observed differences in dendritic plasticity.

So far, we observed and discussed the effect of αsyn and miR-101a-3p at the synapse but it is still unknown whether a causal link among αsyn and miR-101a-3p exists. For this, we employed a neuronal culture model of synucleinopathy replicating αsyn pathology progression. There are many different ways to model synucleinopathies *in vitro* offering advantages and disadvantages, primary neurons have been used for studies on post-transcriptional regulation and αsyn pathology progression (Lázaro, Pavlou, and Outeiro 2017). These models used mainly recombinant αsyn species to study cell-to-cell transmission and produced very robust results unraveling many pathways involved in the pathogenesis of synucleinopathies (Volpicelli-Daley et al. 2011). A recent study presented a modified protocol using lower levels of recombinant αsyn species for longer incubation time replicated key events of αsyn pathology featuring disruption of cellular functions including

synaptic dysfunctions. They also present transcriptomic data of the PFF-treated neurons indicating differential expression of genes related to neurotransmission and synapse organization (Mahul-Mellier et al. 2020).

Based on this model, we chose to assess miR-101a-3p levels in primary neurons exposed to PFFs as well as recombinant monomeric and oligomeric αsyn for control in the same conditions. Interestingly, monomeric and oligomeric recombinant αsyn induced the upregulation of miR-101a-3p verifying a direct link among the miRNA and the protein (Figure 5.4.A). In the PFF treated neurons we did not observe the upregulation of miR-101a-3p most probably due to the evident synapse loss mediated by fibril toxicity (Figure 5.4.A, B). Knowing the miR-101a-3p is enriched in synapses we can assume a portion of the miRNA is lost along with the lost synapses and this is reflected to the miRNA levels. Although PS129 αsyn is correlated with pathology and implicated in epigenetic processes (Pinho et al. 2019; Schaser et al. 2019; Kontopoulos, Parvin, and Feany 2006; Fujiwara et al. 2003; Tenreiro, Eckermann, and Outeiro 2014), we failed to observe a link with miR-101a-3p possibly indicating RNA processes at the synapse in this case are irrelevant or the phosphorylation state of the protein (Figure 5.4.C).

Evidence so far indicate miR-101a-3p is upregulated in response to αsyn burden and acts as negative modulator of synaptic plasticity. MiR-101a-3p is linked with plasticity events in mouse postnatal brain physiology and adult brain disorders (Lippi et al. 2016; Y. Lee et al. 2008; Vilardo et al. 2010). We then asked if miR-101a-3p is linked with synaptic plasticity in adult brain physiology. Synaptic plasticity is mediated by enhanced motor and cognitive stimulation, which is modelled by EE caging i.e. bigger space, alternating objects, and running wheels (C.-J. Wang et al. 2019; Bayat et al. 2015). We assessed the midbrain of 12-month-old Wt mice grown in EE and found that miR-101a-3p was downregulated compared to WT littermates grown in standard environment (Figure 6). This finding indicated miR-101a-3p is reduced to allow plastic events to occur. EE is reported to enhance production of synaptic proteins, brain neurotrophins and promote dendritic branching and synaptogenesis (Nithianantharajah and Hannan 2006; Frick and Fernandez 2003; van Praag, Kempermann, and Gage 2000). Accumulating research highlights the benefits of EE in neurodegenerative disorders mediated by both transcriptional and translational neuroprotective events (Lingzhi Li and Bor 2005; Laviola et al. 2008). In particular, many differentially expressed miRNAs have been identified following EE in both healthy animals and disease models (Kuznetsova et al. 2020).

Here we showed miR-101a-3p is decreased upon EE and increased in αsyn pathology indicating a dynamic role in neuronal plasticity in physiology and disease. While these findings are relevant in mouse, the translational potential to humans is always questionable (Burkhardt and Zlotnik 2013). For this we assessed miR-101a-3p levels in the cortex of DLB patients and healthy individuals and confirmed a significant increase in the DLB group (Figure 7). DLB cortex shows the highest pathology appearing in some regions in early stages and progressively developing in most cortical sub regions (Marui et al. 2002). Synapse loss and cognitive dysfunction of DLB patients is mediated by reduced dendritic spines due to presynaptic αsyn aggregates accumulation in the cortex (Kramer and Schulz-Schaeffer, 2007). Cortical pathology and cognitive impairments in the A30P mouse model resemble the DLB phenotype (Freichel et al. 2007; Kahle et al. 2000). In line with this, the reduction of mature dendritic spines we observed is now added to the list of DLB resembling pathological events. This phenotype seems to be mediated by miR-101a-3p possibly both in mouse model and human brain. MiR-101a-3p was upregulated in the striatum of MSA patients which also displays alteration in dendritic spine morphology (Zaja-Milatovic et al. 2005; Valera et al. 2017).

These data indicate miR-101a-3p as a potent candidate for biomarker and therapeutic target. MiR-101a-3p holds the potential of developing an excellent biomarker since we show here it is increased in early stages of pathology prior symptom onset. Finally, targeting such miRNAs that lead synaptic deficits through the structural alteration of dendritic spines could form part of therapeutic strategies to improve synaptic plasticity and to ameliorate impairments in many neurodegenerative diseases.

5. Conclusions

One of the major obstacles in PD treatment is the late diagnosis. Symptomatology develops when most of the dopaminergic neurons in the SN are irreversibly lost. Despite advancements in understanding mechanisms involved in PD initiation and progression we still lack the identification of critical events prior symptom onset that would lead the development of biomarkers for in-time diagnosis and prevention. Research so far points at the synapse as the compartment first affected in synucleinopathies.

The present study provides further evidence synapse physiology is disrupted in early PD stages. We observed miRNA deregulation in response to αsyn expression in the midbrain of a mouse model of familial synucleinopathy resembling DLB. The miRNA signatures we identified prior phenotype onset regulated processes involved in neuronal development and plasticity. Bioinformatic analysis and correlation with mRNA signatures indicated miR-101a-3p as the most effective miRNA. MiR-101a-3p upregulation was confirmed in the mouse midbrain and in the cortex of DLB patients highlighting its importance in pathology establishment and progression.

Analysis of miR-101a-3p levels in isolated synaptosomes indicated the miRNA is enriched in synapses. Study of the miR-101a-3p mediated phenotype *in vivo* and *in vitro* suggested it effectively targets GABA Ab2 subunit and SAPAP3 reducing significantly the protein levels. Both GABA Ab2 and SAPAP3 reduction are linked with alterations in dendritic spine morphology. Indeed miR-101a-3p expression resulted in reduced dendritic length and number of mushroom spines indicative of early synaptic damage and synaptic plasticity impairments. In addition, miR-101a-3p levels increased with age in the Wt mouse midbrain. Senescence is correlated with physiological reduction in synaptic plasticity. Further analysis of miR-101a-3p in midbrain of mice grown in EE, modelling enhanced synaptic plasticity, indicated a negative correlation. Our results provide strong evidence miR-101a-3p is a negative modulator of synaptic plasticity.

In addition, miR-101a-3p was directly modulated by αsyn levels *in vitro* as shown by the application of different species of recombinant αsyn. This indicates a direct effect of αsyn burden on synapses parallel to the miRNA increase. With this experiment we verified miR-101a-3p is increased upon αsyn mediated synaptic toxicity prior synapse loss as shown by neurons exposed to PFFs where synapses are lost and miR-101a-3p increase is not observed.

Collectively, our data indicate miR-101a-3p is a synaptic miRNA induced by αsyn accumulation that acts as a negative modulator of synaptic plasticity. These findings highlight the emerging role of

miRNAs as key regulators of gene expression related to αsyn pathology. Unravelling RNA based processes implicated in synaptic compromise may point out novel targets for the development of biomarkers in synucleinopathies, and may also result in the design of novel therapeutic interventions.

References

Aasly, Jan O. 2020. “Long-Term Outcomes of Genetic Parkinson’s Disease.” *Journal of Movement Disorders* 13 (2): 81–96. https://doi.org/10.14802/jmd.19080.

Abbott, R. J., I. F. Pye, and S. R. Nahorski. 1982. “CSF and Plasma GABA Levels in Parkinson’s Disease.” *Journal of Neurology Neurosurgery and Psychiatry* 45 (3): 253–56. https://doi.org/10.1136/jnnp.45.3.253.

Abeliovich, Asa, Yvonne Schmitz, Isabel Fariñas, Derek Choi-Lundberg, Wei-Hsien Ho, Pablo E Castillo, Natasha Shinsky, et al. 2000. “Mice Lacking α-Synuclein Display Functional Deficits in the Nigrostriatal Dopamine System.” *Neuron* 25 (1): 239–52. https://doi.org/10.1016/S0896-6273(00)80886-7.

Agarwal, Vikram, George W. Bell, Jin Wu Nam, and David P. Bartel. 2015. “Predicting Effective MicroRNA Target Sites in Mammalian MRNAs.” *ELife* 4 (AUGUST2015). https://doi.org/10.7554/eLife.05005.

Al-Chalabi, A, A Durr, N W Wood, M H Parkinson, A Camuzat, J S Hulot, K E Morrison, et al. 2009. “Genetic Variants of the Alpha-Synuclein Gene SNCA Are Associated with Multiple System Atrophy.” *PLoS One* 4 (9): e7114. https://doi.org/10.1371/journal.pone.0007114.

Alegret, M., C. Junqué, F. Valldeoriola, P. Vendrell, M. J. Martí, and E. Tolosa. 2001. “Obsessive-Compulsive Symptoms in Parkinson’s Disease.” *Journal of Neurology Neurosurgery and Psychiatry* 70 (3): 394–96. https://doi.org/10.1136/jnnp.70.3.394.

Andrews, Shea J., and Joseph A. Rothnagel. 2014. “Emerging Evidence for Functional Peptides Encoded by Short Open Reading Frames.” *Nature Reviews Genetics*. Nat Rev Genet. https://doi.org/10.1038/nrg3520.

Appel-Cresswell, S, C Vilarino-Guell, M Encarnacion, H Sherman, I Yu, B Shah, D Weir, et al. 2013. “Alpha-Synuclein p.H50Q, a Novel Pathogenic Mutation for Parkinson’s Disease.” *Mov Disord* 28 (6): 811–13. https://doi.org/10.1002/mds.25421.

Arai, K, N Kato, K Kashiwado, and T Hattori. 2000. “Pure Autonomic Failure in Association with Human Alpha-Synucleinopathy.” *Neurosci Lett* 296 (2–3): 171–73. https://doi.org/10.1016/s0304-3940(00)01623-2.

Asikainen, Suvi, Martina Rudgalvyte, Liisa Heikkinen, Kristiina Louhiranta, Merja Lakso, Garry Wong, and Richard Nass. 2010. “Global MicroRNA Expression Profiling of Caenorhabditis Elegans Parkinson’s Disease Models.” *Journal of Molecular Neuroscience* 41 (1): 210–18. https://doi.org/10.1007/s12031-009-9325-1.

Bai, Xiaochen, Yilin Tang, Mei Yu, Lei Wu, Fengtao Liu, Jianliang Ni, Zishan Wang, et al. 2017. “Downregulation of Blood Serum MicroRNA 29 Family in Patients with Parkinson’s Disease.” *Scientific Reports* 7 (1): 1–7. https://doi.org/10.1038/s41598-017-03887-3.

Barbin, G., H. Pollard, J. L. Gaïarsa, and Y. Ben-Ari. 1993. “Involvement of GABAA Receptors in the Outgrowth of Cultured Hippocampal Neurons.” *Neuroscience Letters* 152 (1–2): 150–54. https://doi.org/10.1016/0304-3940(93)90505-F.

Barresi, Rita, and Kevin P. Campbell. 2006. “Dystroglycan: From Biosynthesis to Pathogenesis of Human Disease.” *Journal of Cell Science* 119 (2): 199–207. https://doi.org/10.1242/jcs.02814.

Baumuratov, A. S., P. M.A. Antony, M. Ostaszewski, F. He, L. Salamanca, L. Antunes, J. Weber, et al. 2016. “Enteric Neurons from Parkinson’s Disease Patients Display Ex Vivo Aberrations in Mitochondrial Structure.” *Scientific Reports* 6 (1): 1–12. https://doi.org/10.1038/srep33117.

Bayat, Mahnaz, Mohammad Davood Sharifi, Masoud Haghani, and Mohammad Shabani. 2015. “Enriched Environment Improves Synaptic Plasticity and Cognitive Deficiency in Chronic Cerebral Hypoperfused Rats.” *Brain Research Bulletin* 119 (October): 34–40. https://doi.org/10.1016/j.brainresbull.2015.10.001.

Behar, Toby N., Yong Xin Li, Hung T. Tran, Wu Ma, Veronica Dunlap, Catherine Scott, and Jeffery L. Barker. 1996. “GABA Stimulates Chemotaxis and Chemokinesis of Embryonic Cortical Neurons via Calcium-Dependent Mechanisms.” *Journal of Neuroscience* 16 (5): 1808–18. https://doi.org/10.1523/jneurosci.16-05-01808.1996.

Bellucci, Arianna, Nicola Biagio Mercuri, Annalena Venneri, Gaia Faustini, Francesca Longhena, Marina Pizzi, Cristina Missale, and Pierfranco Spano. 2016. “Parkinson’s Disease: From Synaptic Loss to Connectome Dysfunction.” *Neuropathology and Applied Neurobiology*. Blackwell Publishing Ltd.

https://doi.org/10.1111/nan.12297.

Bellucci, Arianna, Laura Navarria, Michela Zaltieri, Elisa Falarti, Serena Bodei, Sandra Sigala, Leontino Battistin, Mariagrazia Spillantini, Cristina Missale, and Pierfranco Spano. 2011. "Induction of the Unfolded Protein Response by α-Synuclein in Experimental Models of Parkinson's Disease." *Journal of Neurochemistry* 116 (4): 588–605. https://doi.org/10.1111/j.1471-4159.2010.07143.x.

Berry, Chinar, Megha Lal, and B. K. Binukumar. 2018. "Crosstalk between the Unfolded Protein Response, MicroRNAs, and Insulin Signaling Pathways: In Search of Biomarkers for the Diagnosis and Treatment of Type 2 Diabetes." *Frontiers in Endocrinology*. Frontiers Media S.A. https://doi.org/10.3389/fendo.2018.00210.

Bestor, Timothy, Andrew Laudano, Robert Mattaliano, and Vernon Ingram. 1988. "Cloning and Sequencing of a CDNA Encoding DNA Methyltransferase of Mouse Cells. The Carboxyl-Terminal Domain of the Mammalian Enzymes Is Related to Bacterial Restriction Methyltransferases." *Journal of Molecular Biology* 203 (4): 971–83. https://doi.org/10.1016/0022-2836(88)90122-2.

Betarbet, R., T. B. Sherer, G. MacKenzie, M. Garcia-Osuna, A. V. Panov, and J. T. Greenamyre. 2000. "Chronic Systemic Pesticide Exposure Reproduces Features of Parkinson's Disease." *Nature Neuroscience* 3 (12): 1301–6. https://doi.org/10.1038/81834.

Bienvenu, O.J., Y. Wang, Y.Y. Shugart, J.M. Welch, M.A. Grados, A.J. Fyer, S.L. Rauch, et al. 2009. "*Sapap3* and Pathological Grooming in Humans: Results from the OCD Collaborative Genetics Study." *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics* 150B (5): 710–20. https://doi.org/10.1002/ajmg.b.30897.

Bijata, Monika, Jakub Wlodarczyk, and Izabela Figiel. 2015. "Dystroglycan Controls Dendritic Morphogenesis of Hippocampal Neurons in Vitro." *Frontiers in Cellular Neuroscience* 9 (MAY). https://doi.org/10.3389/fncel.2015.00199.

Billingsley, K J, S Bandres-Ciga, S Saez-Atienzar, and A B Singleton. 2018. "Genetic Risk Factors in Parkinson's Disease." *Cell Tissue Res* 373 (1): 9–20. https://doi.org/10.1007/s00441-018-2817-y.

Black, James E., Anita M. Sirevaag, Christopher S. Wallace, Michael H. Savin, and William T. Greenough. 1989. "Effects of Complex Experience on Somatic Growth and Organ Development in Rats." *Developmental Psychobiology* 22 (7): 727–52. https://doi.org/10.1002/dev.420220707.

Blandini, F, R Cilia, S Cerri, G Pezzoli, A H V Schapira, S Mullin, and J L Lanciego. 2019. "Glucocerebrosidase Mutations and Synucleinopathies: Toward a Model of Precision Medicine." *Mov Disord* 34 (1): 9–21. https://doi.org/10.1002/mds.27583.

Boese, Amrit S., Reuben Saba, Kristyn Campbell, Anna Majer, Sarah Medina, Lynn Burton, Timothy F. Booth, et al. 2016. "MicroRNA Abundance Is Altered in Synaptoneurosomes during Prion Disease." *Molecular and Cellular Neuroscience* 71: 13–24. https://doi.org/10.1016/j.mcn.2015.12.001.

Bohush, Anastasiia, Grazyna Niewiadomska, and Anna Filipek. 2018. "Role of Mitogen Activated Protein Kinase Signaling in Parkinson's Disease." *International Journal of Molecular Sciences* 19 (10): 2973. https://doi.org/10.3390/ijms19102973.

Boni, L. De, S. Tierling, S. Roeber, J. Walter, A. Giese, and Hans A. Kretzschmar. 2011. "Next-Generation Sequencing Reveals Regional Differences of the α-Synuclein Methylation State Independent of Lewy Body Disease." *NeuroMolecular Medicine* 13 (4): 310–20. https://doi.org/10.1007/s12017-011-8163-9.

Bonifati, Vincenzo. 2014. "Genetics of Parkinson's Disease--State of the Art, 2013." *Parkinsonism & Related Disorders* 20 Suppl 1 (January): S23-8. https://doi.org/10.1016/S1353-8020(13)70009-9.

Bourne, Jennifer, and Kristen M. Harris. 2007. "Do Thin Spines Learn to Be Mushroom Spines That Remember?" *Current Opinion in Neurobiology*. Elsevier Current Trends. https://doi.org/10.1016/j.conb.2007.04.009.

Bousset, Luc, Laura Pieri, Gemma Ruiz-Arlandis, Julia Gath, Poul Henning Jensen, Birgit Habenstein, Karine Madiona, et al. 2013. "Structural and Functional Characterization of Two Alpha-Synuclein Strains." *Nat Commun* 4: 2575. https://doi.org/10.1038/ncomms3575.

Braak, H, U. Rob, W P Gai, and K Del Tredici. 2003. "Idiopathic Parkinson's Disease: Possible Routes by Which

Vulnerable Neuronal Types May Be Subject to Neuroinvasion by an Unknown Pathogen." *Journal of Neural Transmission* 110 (5): 517–36. https://doi.org/10.1007/s00702-002-0808-2.

Braak, Heiko, Kelly Del Tredici, Udo Rüb, Rob A I De Vos, Ernst N H Jansen Steur, and Eva Braak. 2003. "Staging of Brain Pathology Related to Sporadic Parkinson's Disease." *Neurobiology of Aging* 24 (2): 197–211. https://doi.org/10.1016/S0197-4580(02)00065-9.

Bradford, Marion M. 1976. "A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding." *Analytical Biochemistry* 72 (1–2): 248–54. https://doi.org/10.1016/0003-2697(76)90527-3.

Brennecke, Julius, Alexander Stark, Robert B. Russell, and Stephen M. Cohen. 2005. "Principles of MicroRNA-Target Recognition." In *PLoS Biology*, 3:0404–18. Public Library of Science. https://doi.org/10.1371/journal.pbio.0030085.

Briggs, Christine E., Yulei Wang, Benjamin Kong, Tsung Ung W. Woo, Lakshmanan K. Iyer, and Kai C. Sonntag. 2015. "Midbrain Dopamine Neurons in Parkinson's Disease Exhibit a Dysregulated MiRNA and Target-Gene Network." *Brain Research* 1618 (August): 111–21. https://doi.org/10.1016/j.brainres.2015.05.021.

Briggs, James A, Ernst J Wolvetang, John S Mattick, John L Rinn, and Guy Barry. 2015. "Review Mechanisms of Long Non-Coding RNAs in Mammalian Nervous System Development , Plasticity , Disease , and Evolution." *Neuron* 88 (5): 861–77. https://doi.org/10.1016/j.neuron.2015.09.045.

Bu, Lu-Lu, Ying-Yu Xie, Dan-Yu Lin, Ying Chen, Xiu-Na Jing, Yan-Ran Liang, Su-Dan Peng, Kai-Xun Huang, and En-Xiang Tao. 2020. "LncRNA-T199678 Mitigates α-Synuclein-Induced Dopaminergic Neuron Injury via MiR-101-3p." *Frontiers in Aging Neuroscience* 12 (November): 599246. https://doi.org/10.3389/fnagi.2020.599246.

Burgalossi, Andrea, Sang Yong Jung, Kwun nok Mimi Man, Ramya Nair, Wolf J. Jockusch, Sonja M. Wojcik, Nils Brose, and Jeong Seop Rhee. 2012. "Analysis of Neurotransmitter Release Mechanisms by Photolysis of Caged Ca2+ in an Autaptic Neuron Culture System." *Nature Protocols* 7 (7): 1351–65. https://doi.org/10.1038/nprot.2012.074.

Burgos, Kasandra, Ivana Malenica, Raghu Metpally, Amanda Courtright, Benjamin Rakela, Thomas Beach, Holly Shill, et al. 2014. "Profiles of Extracellular MiRNA in Cerebrospinal Fluid and Serum from Patients with Alzheimer's and Parkinson's Diseases Correlate with Disease Status and Features of Pathology." Edited by Andrew Francis Hill. *PLoS ONE* 9 (5): e94839. https://doi.org/10.1371/journal.pone.0094839.

Burkhardt, Amanda M., and Albert Zlotnik. 2013. "Translating Translational Research: Mouse Models of Human Disease." *Cellular and Molecular Immunology*. Nature Publishing Group. https://doi.org/10.1038/cmi.2013.19.

Burré, Jacqueline, Manu Sharma, Theodoros Tsetsenis, Vladimir Buchman, Mark R Etherton, and Thomas C Südhof. 2010. "Alpha-Synuclein Promotes SNARE-Complex Assembly in Vivo and in Vitro." *Science (New York, N.Y.)* 329 (5999): 1663–67. https://doi.org/10.1126/science.1195227.

Carlin, Richard K., Dennis J. Grab, Rochelle S. Cohen, and Philip Siekevitz. 1980. "Isolation and Characterization of Postsynaptic Densities from Various Brain Regions: Enrichment of Different Types of Postsynaptic Densities." *Journal of Cell Biology* 86 (3): 831–43. https://doi.org/10.1083/jcb.86.3.831.

Cech, Thomas R, and Joan A Steitz. 2014. "Review The Noncoding RNA Revolution — Trashing Old Rules to Forge New Ones." *Cell* 157 (1): 77–94. https://doi.org/10.1016/j.cell.2014.03.008.

Chan, C. Savio, Tracy S. Gertler, and D. James Surmeier. 2009. "Calcium Homeostasis, Selective Vulnerability and Parkinson's Disease." *Trends in Neurosciences* 32 (5): 249–56. https://doi.org/10.1016/j.tins.2009.01.006.

Chan, C. Savio, Jaime N. Guzman, Ema Ilijic, Jeff N. Mercer, Caroline Rick, Tatiana Tkatch, Gloria E. Meredith, and D. James Surmeier. 2007. "'Rejuvenation' Protects Neurons in Mouse Models of Parkinson's Disease." *Nature* 447 (7148): 1081–86. https://doi.org/10.1038/nature05865.

Chandra, Sreeganga, Gilbert Gallardo, Rafael Fernández-Chacón, Oliver M. Schlüter, and Thomas C. Südhof. 2005. "α-Synuclein Cooperates with CSPα in Preventing Neurodegeneration." *Cell* 123 (3): 383–96. https://doi.org/10.1016/j.cell.2005.09.028.

Chen, Bei Jun, James D. Mills, Konii Takenaka, Nicola Bliim, Glenda M. Halliday, and Michael Janitz. 2016. "Characterization of Circular RNAs Landscape in Multiple System Atrophy Brain." *Journal of Neurochemistry* 139 (3): 485–96. https://doi.org/10.1111/jnc.13752.

Chen, Jing, Eric E. Bardes, Bruce J. Aronow, and Anil G. Jegga. 2009. "ToppGene Suite for Gene List Enrichment Analysis and Candidate Gene Prioritization." *Nucleic Acids Research* 37 (SUPPL. 2): 305–11. https://doi.org/10.1093/nar/gkp427.

Chen, Lei, Junxiu Yang, Jinhui Lü, Shanshan Cao, Qian Zhao, and Zuoren Yu. 2018. "Identification of Aberrant Circulating MiRNAs in Parkinson's Disease Plasma Samples." *Brain and Behavior* 8 (4). https://doi.org/10.1002/brb3.941.

Chen, Leo, Jinghua Jin, Jeanne Davis, Yong Zhou, Yan Wang, Jun Liu, Paul J. Lockhart, and Jing Zhang. 2007. "Oligomeric α-Synuclein Inhibits Tubulin Polymerization." *Biochemical and Biophysical Research Communications* 356 (3): 548–53. https://doi.org/10.1016/j.bbrc.2007.02.163.

Chen, Yachi. 2014. "GABA-A Receptor-Dependent Mechanisms Prevent Excessive Spine Elimination during Postnatal Maturation of the Mouse Cortex in Vivo." *FEBS Letters* 588 (24): 4551–60. https://doi.org/10.1016/j.febslet.2014.10.023.

Chi, Sung Wook, Julie B. Zang, Aldo Mele, and Robert B. Darnell. 2009. "Argonaute HITS-CLIP Decodes MicroRNA-MRNA Interaction Maps." *Nature* 460 (7254): 479–86. https://doi.org/10.1038/nature08170.

Chiba-Falek, O. 2017. "Structural Variants in SNCA Gene and the Implication to Synucleinopathies." *Curr Opin Genet Dev* 44: 110–16. https://doi.org/10.1016/j.gde.2017.01.014.

Chinta, Shankar J., Jyothi K. Mallajosyula, Anand Rane, and Julie K. Andersen. 2010. "Mitochondrial Alpha-Synuclein Accumulation Impairs Complex I Function in Dopaminergic Neurons and Results in Increased Mitophagy in Vivo." *Neuroscience Letters* 486 (3): 235–39. https://doi.org/10.1016/j.neulet.2010.09.061.

Choi, Bong-kyu, Mal-gi Choi, Jae-yeol Kim, Yoosoo Yang, Ying Lai, Dae-hyuk Kweon, and Nam Ki. 2013. "Large α -Synuclein Oligomers Inhibit Neuronal SNARE-Mediated Vesicle Docking" 110 (10). https://doi.org/10.1073/pnas.1218424110.

Choi, Hyo Kyoung, Youngsok Choi, Hee Bum Kang, Eun jin Lim, Soo Yeon Park, Hyun Seob Lee, Ji Min Park, et al. 2015. "Pink1 Positively Regulates HDAC3 to Suppress Dopaminergic Neuronal Cell Death." *Human Molecular Genetics* 24 (4): 1127–41. https://doi.org/10.1093/hmg/ddu526.

Cohen, J. E., P. R. Lee, S. Chen, W. Li, and R. D. Fields. 2011. "MicroRNA Regulation of Homeostatic Synaptic Plasticity." *Proceedings of the National Academy of Sciences* 108 (28): 11650–55. https://doi.org/10.1073/pnas.1017576108.

Colla, Emanuela. 2019. "Linking the Endoplasmic Reticulum to Parkinson's Disease and Alpha-Synucleinopathy." *Frontiers in Neuroscience* 13 (May): 1–10. https://doi.org/10.3389/fnins.2019.00560.

Cook, Susan C, and Cara L Wellman. 2004. "Chronic Stress Alters Dendritic Morphology in Rat Medial Prefrontal Cortex." *Wiley Online Library* 60 (2): 236–48. https://doi.org/10.1002/neu.20025.

Cormand, B., H. Pihko, M. Bayés, L. Valanne, P. Santavuori, B. Talim, R. Gershoni-Baruch, et al. 2001. "Clinical and Genetic Distinction between Walker-Warburg Syndrome and Muscle-Eye-Brain Disease." *Neurology* 56 (8): 1059–69. https://doi.org/10.1212/WNL.56.8.1059.

Crane, Jacquelyn, Jesen Fagerness, Lisa Osiecki, Boyd Gunnell, S. Evelyn Stewart, David L. Pauls, and Jeremiah M. Scharf. 2011. "Family-Based Genetic Association Study of DLGAP3 in Tourette Syndrome." *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics* 156 (1): 108–14. https://doi.org/10.1002/ajmg.b.31134.

Credle, Joel J., Patrick A. Forcelli, Michael Delannoy, Adam W. Oaks, Eva Permaul, Deborah L. Berry, Valeriy Duka, Jonathan Wills, and Anita Sidhu. 2015. "α-Synuclein-Mediated Inhibition of ATF6 Processing into COPII Vesicles Disrupts UPR Signaling in Parkinson's Disease." *Neurobiology of Disease* 76: 112–25. https://doi.org/10.1016/j.nbd.2015.02.005.

Crews, Leslie, Hideya Mizuno, Paula Desplats, Edward Rockenstein, Anthony Adame, Christina Patrick, Beate Winner, Juergen Winkler, and Eliezer Masliah. 2008. "α-Synuclein Alters Notch-1 Expression and Neurogenesis in Mouse Embryonic Stem Cells and in the Hippocampus of Transgenic Mice." *Journal of*

Neuroscience 28 (16): 4250–60. https://doi.org/10.1523/JNEUROSCI.0066-08.2008.

Czaniecki, Christopher, Tammy Ryan, Morgan G. Stykel, Jennifer Drolet, Juliane Heide, Ryan Hallam, Shalandra Wood, et al. 2019. "Axonal Pathology in HPSC-Based Models of Parkinson's Disease Results from Loss of Nrf2 Transcriptional Activity at the Map1b Gene Locus." *Proceedings of the National Academy of Sciences of the United States of America* 116 (28): 14280–89. https://doi.org/10.1073/pnas.1900576116.

D'Ambra, Eleonora, Davide Capauto, and Mariangela Morlando. 2019. "Exploring the Regulatory Role of Circular RNAs in Neurodegenerative Disorders." *International Journal of Molecular Sciences* 20 (21): 5–11. https://doi.org/10.3390/ijms20215477.

Dansie, Lorraine E., and Iryna M. Ethell. 2011. "Casting a Net on Dendritic Spines: The Extracellular Matrix and Its Receptors." *Developmental Neurobiology* 71 (11): 956–81. https://doi.org/10.1002/dneu.20963.

Dashtipour, Khashayar, Ali Tafreshi, Charles Adler, Thomas Beach, Xin Chen, Geidy Serrano, Stephanie Tashiro, and Charles Wang. 2017. "Hypermethylation of Synphilin-1, Alpha-Synuclein-Interacting Protein (SNCAIP) Gene in the Cerebral Cortex of Patients with Sporadic Parkinson's Disease." *Brain Sciences* 7 (7). https://doi.org/10.3390/brainsci7070074.

Dauer, W, and S Przedborski. 2003. "Parkinson's Disease: Mechanisms and Models." *Neuron* 39 (6): 889–909. https://www.ncbi.nlm.nih.gov/pubmed/12971891.

Dauer, William, and Serge Przedborski. 2003. "Parkinson's Disease: Mechanisms and Models." *Neuron* 39 (6): 889–909. https://doi.org/10.1016/S0896-6273(03)00568-3.

Davidson, W S, A Jonas, D F Clayton, and J M George. 1998. "Stabilization of Alpha-Synuclein Secondary Structure upon Binding to Synthetic Membranes." *The Journal of Biological Chemistry* 273 (16): 9443–49. https://doi.org/10.1074/jbc.273.16.9443.

Derrien, Thomas, Rory Johnson, Giovanni Bussotti, Andrea Tanzer, Sarah Djebali, Hagen Tilgner, Gregory Guernec, et al. 2012. "The GENCODE v7 Catalog of Human Long Noncoding RNAs: Analysis of Their Gene Structure, Evolution, and Expression." *Genome Research* 22 (9): 1775–89. https://doi.org/10.1101/gr.132159.111.

Desplats, Paula, Brian Spencer, Elizabeth Coffee, Pruthul Patel, Sarah Michael, Christina Patrick, Anthony Adame, Edward Rockenstein, and Eliezer Masliah. 2011. "α-Synuclein Sequesters Dnmt1 from the Nucleus: A Novel Mechanism for Epigenetic Alterations in Lewy Body Diseases." *Journal of Biological Chemistry* 286 (11): 9031–37. https://doi.org/10.1074/jbc.C110.212589.

Devi, Latha, and Hindupur K. Anandatheerthavarada. 2010. "Mitochondrial Trafficking of APP and Alpha Synuclein: Relevance to Mitochondrial Dysfunction in Alzheimer's and Parkinson's Diseases." *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease* 1802 (1): 11–19. https://doi.org/10.1016/j.bbadis.2009.07.007.

Dierssen, M., and G. J. A. Ramakers. 2006. "Dendritic Pathology in Mental Retardation: From Molecular Genetics to Neurobiology." *Genes, Brain and Behavior* 5 (SUPPL. 2): 48–60. https://doi.org/10.1111/j.1601-183X.2006.00224.x.

Diogenes, M. J., R. B. Dias, D. M. Rombo, H. Vicente Miranda, F. Maiolino, P. Guerreiro, T. Nasstrom, et al. 2012. "Extracellular Alpha-Synuclein Oligomers Modulate Synaptic Transmission and Impair LTP Via NMDA-Receptor Activation." *Journal of Neuroscience* 32 (34): 11750–62. https://doi.org/10.1523/JNEUROSCI.0234-12.2012.

Dong, Xiaoyu, and Shuyan Cong. 2019. "The Emerging Role of Micrornas in Polyglutamine Diseases." *Frontiers in Molecular Neuroscience*. Frontiers Media S.A. https://doi.org/10.3389/fnmol.2019.00156.

Dorval, Véronique, Wim Mandemakers, Francis Jolivette, Laetitia Coudert, Rachid Mazroui, Bart De Strooper, and Sébastien S. Hébert. 2014. "Gene and MicroRNA Transcriptome Analysis of Parkinson's Related LRRK2 Mouse Models." *PLoS ONE* 9 (1): 1–10. https://doi.org/10.1371/journal.pone.0085510.

Doxakis, Epaminondas. 2010. "Post-Transcriptional Regulation of α-Synuclein Expression by Mir-7 and Mir-153." *Journal of Biological Chemistry* 285 (17): 12726–34. https://doi.org/10.1074/jbc.M109.086827.

Dragicevic, E., J. Schiemann, and B. Liss. 2015. "Dopamine Midbrain Neurons in Health and Parkinson's Disease: Emerging Roles of Voltage-Gated Calcium Channels and ATP-Sensitive Potassium Channels."

Neuroscience. Elsevier Ltd. https://doi.org/10.1016/j.neuroscience.2014.10.037.

Duda, Johanna, Christina Pötschke, and Birgit Liss. 2016. "Converging Roles of Ion Channels, Calcium, Metabolic Stress, and Activity Pattern of Substantia Nigra Dopaminergic Neurons in Health and Parkinson's Disease." *Journal of Neurochemistry* 139 (October): 156–78. https://doi.org/10.1111/jnc.13572.

Duffy, David C., J. Cooper McDonald, Olivier J.A. Schueller, and George M. Whitesides. 1998. "Rapid Prototyping of Microfluidic Systems in Poly(Dimethylsiloxane)." *Analytical Chemistry* 70 (23): 4974–84. https://doi.org/10.1021/ac980656z.

Dupont, Cathérine, Randall Armant, and Carol A. Brenner. 2016. "Epigenetics: Definition, Mechanisms and Clinical Perspective Cathérine." *Stem Cell Research & Therapy* 27 (1): 351–57. https://doi.org/10.1055/s-0029-1237423.Epigenetics.

Durbeej, Madeleine, Michael D. Henry, Maria Ferletta, Kevin P. Campbell, and Peter Ekblom. 1998. "Distribution of Dystroglycan in Normal Adult Mouse Tissues." *Journal of Histochemistry and Cytochemistry* 46 (4): 449–57. https://doi.org/10.1177/002215549804600404.

Ebrahimi-Fakhari, Darius, Ippolita Cantuti-Castelvetri, Zhanyun Fan, Edward Rockenstein, Eliezer Masliah, Bradley T. Hyman, Pamela J. McLean, and Vivek K. Unni. 2011. "Distinct Roles in Vivo for the Ubiquitin-Proteasome System and the Autophagy-Lysosomal Pathway in the Degradation of α-Synuclein." *Journal of Neuroscience* 31 (41): 14508–20. https://doi.org/10.1523/JNEUROSCI.1560-11.2011.

Ekmark-Lewén, Sara, Veronica Lindström, Astrid Gumucio, Elisabeth Ihse, Anish Behere, Philipp J. Kahle, Eva Nordström, et al. 2018. "Early Fine Motor Impairment and Behavioral Dysfunction in (Thy-1)-h[A30P] Alpha-Synuclein Mice." *Brain and Behavior* 8 (3): 1–14. https://doi.org/10.1002/brb3.915.

El-Agnaf, O. M. A. 2003. "α-Synuclein Implicated in Parkinson's Disease Is Present in Extracellular Biological Fluids, Including Human Plasma." *The FASEB Journal*, August, 1945–47. https://doi.org/10.1096/fj.03-0098fje.

Eliezer, D, E Kutluay, R Bussell, and G Browne. 2001. "Conformational Properties of Alpha-Synuclein in Its Free and Lipid-Associated States." *Journal of Molecular Biology* 307 (4): 1061–73. https://doi.org/10.1006/jmbi.2001.4538.

Emmanouilidou, Evangelia, Katerina Melachroinou, Theodoros Roumeliotis, Spiros D Garbis, Maria Ntzouni, Lukas H Margaritis, Leonidas Stefanis, and Kostas Vekrellis. 2010. "Cell-Produced Alpha-Synuclein Is Secreted in a Calcium-Dependent Manner by Exosomes and Impacts Neuronal Survival." *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience* 30 (20): 6838–51. https://doi.org/10.1523/JNEUROSCI.5699-09.2010.

Emmanouilidou, Evangelia, Georgia Minakaki, Maria V. Keramioti, Mary Xylaki, Evangelos Balafas, Margarita Chrysanthou-Piterou, Ismini Kloukina, and Kostas Vekrellis. 2016. "GABA Transmission via ATP-Dependent K + Channels Regulates α-Synuclein Secretion in Mouse Striatum." *Brain* 139 (3): 871–90. https://doi.org/10.1093/brain/awv403.

Emmanouilidou, Evangelia, Leonidas Stefanis, and Kostas Vekrellis. 2010. "Cell-Produced α-Synuclein Oligomers Are Targeted to, and Impair, the 26S Proteasome." *Neurobiology of Aging* 31 (6): 953–68. https://doi.org/10.1016/j.neurobiolaging.2008.07.008.

Enna, S J. 2007. "The GABA Receptors BT - The GABA Receptors." In *The GABA Receptors*, edited by S J Enna and Hanns Möhler, 1–21. Humana Press. https://doi.org/10.1007/978-1-59745-465-0_1.

Enright, Anton J., Bino John, Ulrike Gaul, Thomas Tuschl, Chris Sander, and Debora S. Marks. 2003. "MicroRNA Targets in Drosophila." *Genome Biology* 5 (1). https://doi.org/10.1186/gb-2003-5-1-r1.

Epple, Robert, Dennis Krüger, Tea Berulava, Gerrit Brehm, Rezaul Islam, Sarah Köster, and Andre Fischer. 2021. "The Coding and Small Non-Coding Hippocampal Synaptic RNAome." *Molecular Neurobiology*, February, 1–14. https://doi.org/10.1007/s12035-021-02296-y.

Fanciulli, A, and G K Wenning. 2015. "Multiple-System Atrophy." *N Engl J Med* 372 (14): 1375–76. https://doi.org/10.1056/NEJMc1501657.

Fang, Lishan, Junchao Cai, Baixue Chen, Shanshan Wu, Rong Li, Xiaonan Xu, Yi Yang, et al. 2015. "Aberrantly

Expressed MiR-582-3p Maintains Lung Cancer Stem Cell-like Traits by Activating Wnt/β-Catenin Signalling." *Nature Communications* 6 (October). https://doi.org/10.1038/ncomms9640.

Feng, Chien Wei, Zhi Hong Wen, Shi Ying Huang, Han Chun Hung, Chun Hong Chen, San Nan Yang, Nan Fu Chen, Hui Min Wang, Chung Der Hsiao, and Wu Fu Chen. 2014. "Effects of 6-Hydroxydopamine Exposure on Motor Activity and Biochemical Expression in Zebrafish (Danio Rerio) Larvae." *Zebrafish* 11 (3): 227–39. https://doi.org/10.1089/zeb.2013.0950.

Fernagut, Pierre Olivier, and Marie Françoise Chesselet. 2004. "Alpha-Synuclein and Transgenic Mouse Models." *Neurobiology of Disease* 17 (2): 123–30. https://doi.org/10.1016/j.nbd.2004.07.001.

Figueiredo-Pereira, Maria E, Patricia Rockwell, Thomas Schmidt-Glenewinkel, and Peter Serrano. 2014. "Neuroinflammation and J2 Prostaglandins: Linking Impairment of the Ubiquitin-Proteasome Pathway and Mitochondria to Neurodegeneration." *Frontiers in Molecular Neuroscience* 7 (January): 104. https://doi.org/10.3389/fnmol.2014.00104.

Fiore, Roberto, Sharof Khudayberdiev, Reuben Saba, and Gerhard Schratt. 2011. "MicroRNA Function in the Nervous System." In *Progress in Molecular Biology and Translational Science*, 1st ed., 102:47–100. Elsevier Inc. https://doi.org/10.1016/B978-0-12-415795-8.00004-0.

Fogarty, Matthew J. 2019. "Amyotrophic Lateral Sclerosis as a Synaptopathy." *Neural Regeneration Research* 14 (2): 189–92. https://doi.org/10.4103/1673-5374.244782.

Follenzi, Antonia, and Luigi Naldini. 2002. "[26] Generation of HIV-1 Derived Lentiviral Vectors." In , 346:454–65. https://doi.org/10.1016/S0076-6879(02)46071-5.

Frankel, Lisa B., Jiayu Wen, Michael Lees, Maria Høyer-Hansen, Thomas Farkas, Anders Krogh, Marja Jäättelä, and Anders H. Lund. 2011. "MicroRNA-101 Is a Potent Inhibitor of Autophagy." *EMBO Journal* 30 (22): 4628–41. https://doi.org/10.1038/emboj.2011.331.

Freichel, Christian, Manuela Neumann, Theresa Ballard, Veronika Müller, Marie Woolley, Laurence Ozmen, Edilio Borroni, et al. 2007. "Age-Dependent Cognitive Decline and Amygdala Pathology in α-Synuclein Transgenic Mice." *Neurobiology of Aging* 28 (9): 1421–35. https://doi.org/10.1016/j.neurobiolaging.2006.06.013.

Frick, Karyn M., and Stephanie M. Fernandez. 2003. "Enrichment Enhances Spatial Memory and Increases Synaptophysin Levels in Aged Female Mice." *Neurobiology of Aging* 24 (4): 615–26. https://doi.org/10.1016/S0197-4580(02)00138-0.

Fujiwara, Hideo, Masato Hasegawa, Naoshi Dohmae, Akiko Kawashima, Eliezer Masliah, S Goldberg, Jie Shen, Koji Takio, and Takeshi Iwatsubo. 2003. "α -Synuclein Is Phosphorylated in Synucleinopathy Lesions" 4 (February 2002). https://doi.org/10.1038/ncb748.

Funahashi, Yu, Yuta Yoshino, Kiyohiro Yamazaki, Yoko Mori, Takaaki Mori, Yuki Ozaki, Tomoko Sao, Shinichiro Ochi, Jun Ichi Iga, and Shu Ichi Ueno. 2017. "DNA Methylation Changes at SNCA Intron 1 in Patients with Dementia with Lewy Bodies." *Psychiatry and Clinical Neurosciences* 71 (1): 28–35. https://doi.org/10.1111/pcn.12462.

Fyfe, Ian. 2020. "MicroRNAs — Diagnostic Markers in Parkinson Disease?" *Nature Reviews Neurology*. Nature Research. https://doi.org/10.1038/s41582-019-0305-y.

Gámez-Valero, Ana, Jaume Campdelacreu, Dolores Vilas, Lourdes Ispierto, Ramón Reñé, Ramiro Álvarez, M Pilar Armengol, Francesc E Borràs, and Katrin Beyer. 2019. "Exploratory Study on MicroRNA Profiles from Plasma-Derived Extracellular Vesicles in Alzheimer's Disease and Dementia with Lewy Bodies." *Translational Neurodegeneration* 8 (1): 31. https://doi.org/10.1186/s40035-019-0169-5.

Gao, Yuan, Fen Liu, Lei Fang, Runlan Cai, Chuanjie Zong, and Yun Qi. 2014. "Genkwanin Inhibits Proinflammatory Mediators Mainly through the Regulation of MiR-101/MKP-1/MAPK Pathway in LPS-Activated Macrophages." *PLoS ONE* 9 (5). https://doi.org/10.1371/journal.pone.0096741.

Garcia-Reitböck, Pablo, Oleg Anichtchik, Arianna Bellucci, Mariangela Iovino, Chiara Ballini, Elena Fineberg, Bernardino Ghetti, et al. 2010. "SNARE Protein Redistribution and Synaptic Failure in a Transgenic Mouse Model of Parkinson's Disease." *Brain* 133 (7): 2032–44. https://doi.org/10.1093/brain/awq132.

Garcia, David M., Daehyun Baek, Chanseok Shin, George W. Bell, Andrew Grimson, and David P. Bartel. 2010.

"Weak Seed-Pairing Stability and High Target-Site Abundance Decrease the Proficiency of Lsy-6 and Other MicroRNAs." *Nature Structural and Molecular Biology* 18 (10): 1139–46. https://doi.org/10.1038/nsmb.2115.

Gebremedhin, Kibrom G., and David J. Rademacher. 2016. "Histone H3 Acetylation in the Postmortem Parkinson's Disease Primary Motor Cortex." *Neuroscience Letters* 627 (August): 121–25. https://doi.org/10.1016/j.neulet.2016.05.060.

Ghiglieri, Veronica, Valeria Calabrese, and Paolo Calabresi. 2018. "Alpha-Synuclein: From Early Synaptic Dysfunction to Neurodegeneration." *Frontiers in Neurology* 9 (MAY). https://doi.org/10.3389/fneur.2018.00295.

Giasson, Benoit I., John E. Duda, Shawn M. Quinn, Bin Zhang, John Q. Trojanowski, and Virginia M.-Y. Lee. 2002. "Neuronal α-Synucleinopathy with Severe Movement Disorder in Mice Expressing A53T Human α-Synuclein." *Neuron* 34 (4): 521–33. https://doi.org/10.1016/S0896-6273(02)00682-7.

Giasson, Benoit I., Ian V J Murray, John Q. Trojanowski, and Virginia M Y Lee. 2001. "A Hydrophobic Stretch of 12 Amino Acid Residues in the Middle of α-Synuclein Is Essential for Filament Assembly." *Journal of Biological Chemistry* 276 (4): 2380–86. https://doi.org/10.1074/jbc.M008919200.

Gibb, W R, and A J Lees. 1988. "The Relevance of the Lewy Body to the Pathogenesis of Idiopathic Parkinson's Disease." *J Neurol Neurosurg Psychiatry* 51 (6): 745–52. https://doi.org/10.1136/jnnp.51.6.745.

Gillardon, Frank, Matthias Mack, Wolfgang Rist, Cathrin Schnack, Martin Lenter, Tobias Hildebrandt, and Bastian Hengerer. 2008. "MicroRNA and Proteome Expression Profiling in Early-Symptomatic α-Synuclein(A30P)-Transgenic Mice" 2 (5): 697–705. https://doi.org/10.1002/prca.200780025.

Glanzer, J, K Y Miyashiro, J-Y Sul, L Barrett, B Belt, P Haydon, and J Eberwine. 2005. "RNA Splicing Capability of Live Neuronal Dendrites." *Proceedings of the National Academy of Sciences of the United States of America* 102 (46): 16859–64. https://doi.org/10.1073/pnas.0503783102.

Goers, J, A B Manning-Bog, A L McCormack, I S Millett, S Doniach, D A Di Monte, V N Uversky, and A L Fink. 2003. "Nuclear Localization of Alpha-Synuclein and Its Interaction with Histones." *Biochemistry* 42 (28): 8465–71. https://doi.org/10.1021/bi0341152.

Golgi, C. 1873. "Sulla Struttura Della Sostanza Grigia Del Cervello."

Gómez-Suaga, Patricia, José M. Bravo-San Pedro, Rosa A. González-Polo, J. M. Fuentes, and Mireia Niso-Santano. 2018. "ER-Mitochondria Signaling in Parkinson's Disease Review-Article." *Cell Death and Disease* 9 (3). https://doi.org/10.1038/s41419-017-0079-3.

Gonçalves, Susana, and Tiago Fleming Outeiro. 2013. "Assessing the Subcellular Dynamics of Alpha-Synuclein Using Photoactivation Microscopy." *Molecular Neurobiology* 47 (3): 1081–92. https://doi.org/10.1007/s12035-013-8406-x.

Grant, Seth G.N. 2012. "Synaptopathies: Diseases of the Synaptome." *Current Opinion in Neurobiology*. https://doi.org/10.1016/j.conb.2012.02.002.

Gresa-Arribas, Núria, Cristina Viéitez, Guido Dentesano, Joan Serratosa, Josep Saura, and Carme Solà. 2012. "Modelling Neuroinflammation In Vitro: A Tool to Test the Potential Neuroprotective Effect of Anti-Inflammatory Agents." *PLoS ONE* 7 (9). https://doi.org/10.1371/journal.pone.0045227.

Grutzendler, Jaime, Narayanan Kasthuri, and Wen-Biao Gan. 2002. "Long-Term Dendritic Spine Stability in the Adult Cortex." *Nature* 420 (6917): 812–16. https://doi.org/10.1038/nature01276.

Gu, Qin Hua, Danni Yu, Zhonghua Hu, Xing Liu, Yanqin Yang, Yan Luo, Jun Zhu, and Zheng Li. 2015. "MiR-26a and MiR-384-5p Are Required for LTP Maintenance and Spine Enlargement." *Nature Communications* 6 (April). https://doi.org/10.1038/ncomms7789.

Guardia-Laguarta, Cristina, Estela Area-Gomez, Eric A. Schon, and Serge Przedborski. 2015. "A New Role for α-Synuclein in Parkinson's Disease: Alteration of ER–Mitochondrial Communication." *Movement Disorders* 30 (8): 1026–33. https://doi.org/10.1002/mds.26239.

Gui, Ya Xing, Hai Liu, Li Shan Zhang, Wen Lv, and Xing Yue Hu. 2015. "Altered MicroRNA Profiles in Cerebrospinal Fluid Exosome in Parkinson Disease and Alzheimer Disease." *Oncotarget* 6 (35): 37043–53. https://doi.org/10.18632/oncotarget.6158.

Hague, K, P Lento, S Morgello, S Caro, and H Kaufmann. 1997. “The Distribution of Lewy Bodies in Pure Autonomic Failure: Autopsy Findings and Review of the Literature.” *Acta Neuropathol* 94 (2): 192–96. https://www.ncbi.nlm.nih.gov/pubmed/9255396.

Han, Kyung Ah, Woo Hyun Shin, Sungyeon Jung, Wongi Seol, Hyemyung Seo, Che Myong Ko, and Kwang Chul Chung. 2017. “Leucine-Rich Repeat Kinase 2 Exacerbates Neuronal Cytotoxicity through Phosphorylation of Histone Deacetylase 3 and Histone Deacetylation.” *Human Molecular Genetics* 26 (1): 1–18. https://doi.org/10.1093/hmg/ddw363.

Hayama, Tatsuya, Jun Noguchi, Satoshi Watanabe, Noriko Takahashi, Akiko Hayashi-Takagi, Graham C.R. Ellis-Davies, Masanori Matsuzaki, and Haruo Kasai. 2013. “GABA Promotes the Competitive Selection of Dendritic Spines by Controlling Local Ca 2+ Signaling.” *Nature Neuroscience* 16 (10): 1409–16. https://doi.org/10.1038/nn.3496.

He, Rong, Xiaoyun Xie, Linyue Lv, Yongqi Huang, Xianmin Xia, Xiaowu Chen, and Lei Zhang. 2017. “Comprehensive Investigation of Aberrant MicroRNAs Expression in Cells Culture Model of MnCl2-Induced Neurodegenerative Disease.” *Biochemical and Biophysical Research Communications* 486 (2): 342–48. https://doi.org/10.1016/j.bbrc.2017.03.041.

Heman-Ackah, Sabrina M., Martina Hallegger, Mahendra S. Rao, and Matthew J. A. Wood. 2013. “RISC in PD: The Impact of MicroRNAs in Parkinson’s Disease Cellular and Molecular Pathogenesis.” *Frontiers in Molecular Neuroscience* 6 (November): 1–17. https://doi.org/10.3389/fnmol.2013.00040.

Henderson-Smith, Adrienne, Kathleen M. Fisch, Jianping Hua, Ganqiang Liu, Eugenia Ricciardelli, Kristen Jepsen, Mathew Huentelman, et al. 2019. “DNA Methylation Changes Associated with Parkinson’s Disease Progression: Outcomes from the First Longitudinal Genome-Wide Methylation Analysis in Blood.” *Epigenetics* 14 (4): 365–82. https://doi.org/10.1080/15592294.2019.1588682.

Hering, Heike, and Morgan Sheng. 2001. “Dentritic Spines: Structure, Dynamics and Regulation.” *Nature Reviews Neuroscience* 2 (12): 880–88. https://doi.org/10.1038/35104061.

Herms, Jochen, and Mario M. Dorostkar. 2016. “Dendritic Spine Pathology in Neurodegenerative Diseases.” *Annual Review of Pathology: Mechanisms of Disease*. Annual Reviews Inc. https://doi.org/10.1146/annurev-pathol-012615-044216.

Hibino, Hiroshi, Atsushi Inanobe, Kazuharu Furutani, Shingo Murakami, Ian Findlay, and Yoshihisa Kurachi. 2010. “Inwardly Rectifying Potassium Channels: Their Structure, Function, and Physiological Roles.” *Physiological Reviews*. American Physiological Society Bethesda, MD. https://doi.org/10.1152/physrev.00021.2009.

Hodara, Roberto, Erin H Norris, Benoit I Giasson, Amanda J Mishizen-eberz, David R Lynch, Virginia M Lee, and Harry Ischiropoulos. 2004. “Functional Consequences of ⎵ -Synuclein Tyrosine Nitration” 279 (46): 47746–53. https://doi.org/10.1074/jbc.M408906200.

Horst, Camila Hillesheim, Franciele Schlemmer, Natália de Aguiar Montenegro, Ana Carolina Martins Domingues, Gabriel Ginani Ferreira, Cínthia Yara da Silva Ribeiro, Rafael Rocha de Andrade, Elaine Del Bel Guimarães, Simoneide Souza Titze-de-Almeida, and Ricardo Titze-de-Almeida. 2018. “Signature of Aberrantly Expressed MicroRNAs in the Striatum of Rotenone-Induced Parkinsonian Rats.” *Neurochemical Research* 43 (11): 2132–40. https://doi.org/10.1007/s11064-018-2638-0.

Hoss, Andrew G., Adam Labadorf, Thomas G. Beach, Jeanne C. Latourelle, and Richard H. Myers. 2016. “MicroRNA Profiles in Parkinson’s Disease Prefrontal Cortex.” *Frontiers in Aging Neuroscience* 8 (MAR): 1–8. https://doi.org/10.3389/fnagi.2016.00036.

Hoyer, Wolfgang, Dmitry Cherny, Vinod Subramaniam, and Thomas M. Jovin. 2004. “Impact of the Acidic C-Terminal Region Comprising Amino Acids 109-140 on ??-Synuclein Aggregation in Vitro.” *Biochemistry* 43 (51): 16233–42. https://doi.org/10.1021/bi048453u.

Hsu, Justin B., Chih Min Chiu, Sheng Da Hsu, Wei Yun Huang, Chia Hung Chien, Tzong Yi Lee, and Hsien Da Huang. 2011. “MiRTar: An Integrated System for Identifying MiRNA-Target Interactions in Human.” *BMC Bioinformatics* 12 (1): 300. https://doi.org/10.1186/1471-2105-12-300.

Hunsberger, Joshua G, Emily B Fessler, Fairouz L Chibane, Yan Leng, Dragan Maric, Abdel G Elkahloun, and De-

Maw Chuang. 2013. “Mood Stabilizer-Regulated MiRNAs in Neuropsychiatric and Neurodegenerative Diseases: Identifying Associations and Functions.” *Am J Transl Res*. Vol. 5. www.ajtr.org.

Huse, Jason T., Cameron Brennan, Dolores Hambardzumyan, Boyoung Wee, John Pena, Sara H. Rouhanifard, Cherin Sohn-Lee, et al. 2009. “The PTEN-Regulating MicroRNA MiR-26a Is Amplified in High-Grade Glioma and Facilitates Gliomagenesis in Vivo.” *Genes and Development* 23 (11): 1327–37. https://doi.org/10.1101/gad.1777409.

Ibanez, P, A M Bonnet, B Debarges, E Lohmann, F Tison, P Pollak, Y Agid, A Durr, and A Brice. 2004. “Causal Relation between Alpha-Synuclein Gene Duplication and Familial Parkinson’s Disease.” *Lancet* 364 (9440): 1169–71. https://doi.org/10.1016/S0140-6736(04)17104-3.

Ismail, Azza, Ke Ning, Abdulmonem Al-Hayani, Basil Sharrack, and Mimoun Azzouz. 2012. “PTEN: A Molecular Target for NeurodegeneratIve DIsorders.” *Translational Neuroscience*. Springer. https://doi.org/10.2478/s13380-012-0018-9.

Iwai, Akihiko, Eliezer Masliah, Makoto Yoshimoto, Nianfeng Ge, Lisa Flanagan, H.A Rohan de Silva, Agnes Kittel, and Tsunao Saitoh. 1995. “The Precursor Protein of Non-Aβ Component of Alzheimer’s Disease Amyloid Is a Presynaptic Protein of the Central Nervous System.” *Neuron* 14 (2): 467–75. https://doi.org/10.1016/0896-6273(95)90302-X.

Jakubowski, Jennifer L, and Viviane Labrie. 2017. “Epigenetic Biomarkers for Parkinson’s Disease: From Diagnostics to Therapeutics.” *Journal of Parkinson’s Disease* 7 (1): 1–12. https://doi.org/10.3233/JPD-160914.

Janezic, S., S. Threlfell, P. D. Dodson, M. J. Dowie, T. N. Taylor, D. Potgieter, L. Parkkinen, et al. 2013. “Deficits in Dopaminergic Transmission Precede Neuron Loss and Dysfunction in a New Parkinson Model.” *Proceedings of the National Academy of Sciences* 110 (42): E4016–25. https://doi.org/10.1073/pnas.1309143110.

Jendroska, Klaus, Andrew J. Lees, Werner Poewe, and Susan E. Daniel. 1996. “Amyloid β-Peptide and the Dementia of Parkinson’s Disease.” *Movement Disorders* 11 (6): 647–53. https://doi.org/10.1002/mds.870110609.

Jenner, P, H R Morris, T W Robbins, M Goedert, J Hardy, Y Ben-Shlomo, P Bolam, D Burn, J V Hindle, and D Brooks. 2013. “Parkinson’s Disease--the Debate on the Clinical Phenomenology, Aetiology, Pathology and Pathogenesis.” *J Parkinsons Dis* 3 (1): 1–11. https://doi.org/10.3233/JPD-130175.

Jensen, Poul Henning, Henrik Hager, Morten S. Nielsen, Peter Højrup, Jørgen Gliemann, and Ross Jakes. 1999. “α-Synuclein Binds to Tau and Stimulates the Protein Kinase A-Catalyzed Tau Phosphorylation of Serine Residues 262 and 356.” *Journal of Biological Chemistry* 274 (36): 25481–89. https://doi.org/10.1074/jbc.274.36.25481.

Jenuwein, Thomas. 2001. “Translating the Histone Code.” *Science* 293 (5532): 1074–80. https://doi.org/10.1126/science.1063127.

Jia, Erteng, Ying Zhou, Zhiyu Liu, Liujing Wang, Tinglan Ouyang, Min Pan, Yunfei Bai, and Qinyu Ge. 2020. “Transcriptomic Profiling of Circular RNA in Different Brain Regions of Parkinson’s Disease in a Mouse Model.” *International Journal of Molecular Sciences* 21 (8): 1–25. https://doi.org/10.3390/ijms21083006.

Jia, Xiaojian, Feng Wang, Ying Han, Xuewen Geng, Minghua Li, Yu Shi, Lin Lu, and Yun Chen. 2016. “MiR-137 and MiR-491 Negatively Regulate Dopamine Transporter Expression and Function in Neural Cells.” *Neuroscience Bulletin* 32 (6): 512–22. https://doi.org/10.1007/s12264-016-0061-6.

Jin, Hong, Heng Wu, Gregory Osterhaus, Jianning Wei, Kathleen Davis, Di Sha, Eric Floor, Che Chang Hsu, Richard D. Kopke, and Jang Yen Wu. 2003. “Demonstration of Functional Coupling between γ-Aminobutyric Acid (GABA) Synthesis and Vesicular GABA Transport into Synaptic Vesicles.” *Proceedings of the National Academy of Sciences of the United States of America* 100 (7): 4293–98. https://doi.org/10.1073/pnas.0730698100.

Jin, Huajun, Arthi Kanthasamy, Anamitra Ghosh, Yongjie Yang, Vellareddy Anantharam, and Anumantha G. Kanthasamy. 2011. “α-Synuclein Negatively Regulates Protein Kinase Cδ Expression to Suppress Apoptosis in Dopaminergic Neurons by Reducing P300 Histone Acetyltransferase Activity.” *Journal of*

Neuroscience 31 (6): 2035–51. https://doi.org/10.1523/JNEUROSCI.5634-10.2011.

Jin, Yi, Zujian Chen, Xiqiang Liu, and Xiaofeng Zhou. 2013. "Evaluating the MicroRNA Targeting Sites by Luciferase Reporter Gene Assay." *Methods in Molecular Biology* 936: 117–27. https://doi.org/10.1007/978-1-62703-083-0_10.

Jing, Zhi Guan, Toyoki Maeda, Masahiro Sugano, Jun Ichi Oyama, Yoshihiro Higuchi, Tomokazu Suzuki, and Naoki Makino. 2008. "A Percentage Analysis of the Telomere Length in Parkinson's Disease Patients." *Journals of Gerontology - Series A Biological Sciences and Medical Sciences* 63 (5): 467–73. https://doi.org/10.1093/gerona/63.5.467.

Johnston, Daniel, Brian R. Christie, Andreas Frick, Richard Gray, Dax A. Hoffman, Lalania K. Schexnayder, Shigeo Watanabe, and Li Lian Yuan. 2003. "Active Dendrites, Potassium Channels and Synaptic Plasticity." *Philosophical Transactions of the Royal Society B: Biological Sciences* 358 (1432): 667–74. https://doi.org/10.1098/rstb.2002.1248.

Jonas, Stefanie, and Elisa Izaurralde. 2015. "Towards a Molecular Understanding of MicroRNA-Mediated Gene Silencing." *Nature Reviews Genetics* 16 (7): 421–33. https://doi.org/10.1038/nrg3965.

Jowaed, Ahmad, Ina Schmitt, Oliver Kaut, and Ullrich Wüllner. 2010. "Methylation Regulates Alpha-Synuclein Expression and Is Decreased in Parkinson's Disease Patients' Brains." *Journal of Neuroscience* 30 (18): 6355–59. https://doi.org/10.1523/JNEUROSCI.6119-09.2010.

Kahle, Philipp J. 2007. "α-Synucleinopathy Models and Human Neuropathology: Similarities and Differences." *Acta Neuropathologica* 115 (1): 87–95. https://doi.org/10.1007/s00401-007-0302-x.

Kahle, Philipp J, Manuela Neumann, Laurence Ozmen, Veronika Müller, Helmut Jacobsen, Alice Schindzielorz, Masayasu Okochi, et al. 2000. "Subcellular Localization of Wild-Type and Parkinson's Disease-Associated Mutant α-Synuclein in Human and Transgenic Mouse Brain." *Journal of Neuroscience* 20 (17): 6365–73. https://doi.org/10.1523/jneurosci.20-17-06365.2000.

Karampetsou, Mantia, Mustafa T. Ardah, Maria Semitekolou, Alexia Polissidis, Martina Samiotaki, Maria Kalomoiri, Nour Majbour, Georgina Xanthou, Omar M.A. El-Agnaf, and Kostas Vekrellis. 2017. "Phosphorylated Exogenous Alpha-Synuclein Fibrils Exacerbate Pathology and Induce Neuronal Dysfunction in Mice." *Scientific Reports* 7 (1): 1–18. https://doi.org/10.1038/s41598-017-15813-8.

Kiefer, Amy K., Joyce Y. Tung, Chuong B. Do, David A. Hinds, Joanna L. Mountain, Uta Francke, and Nicholas Eriksson. 2013. "Genome-Wide Analysis Points to Roles for Extracellular Matrix Remodeling, the Visual Cycle, and Neuronal Development in Myopia." Edited by Greg Gibson. *PLoS Genetics* 9 (2): e1003299. https://doi.org/10.1371/journal.pgen.1003299.

Kim, Eun Kyung, and Eui Ju Choi. 2010. "Pathological Roles of MAPK Signaling Pathways in Human Diseases." *Biochimica et Biophysica Acta - Molecular Basis of Disease*. Elsevier. https://doi.org/10.1016/j.bbadis.2009.12.009.

Kim, Eunjoon, Scott Naisbitt, Yi Ping Hsueh, Anuradha Rao, Adam Rothschild, Ann Marie Craig, and Morgan Sheng. 1997. "GKAP, a Novel Synaptic Protein That Interacts with the Guanylate Kinase- like Domain of the PSD-95/SAP90 Family of Channel Clustering Molecules." *Journal of Cell Biology* 136 (3): 669–78. https://doi.org/10.1083/jcb.136.3.669.

Kim, Eunjoon, and Morgan Sheng. 2004. "PDZ Domain Proteins of Synapses." *Nature Reviews Neuroscience*. Nat Rev Neurosci. https://doi.org/10.1038/nrn1517.

Kirik, Deniz, Carl Rosenblad, Corinna Burger, Cecilia Lundberg, Teit E. Johansen, Nicholas Muzyczka, Ronald J. Mandel, and Anders Björklund. 2002. "Parkinson-Like Neurodegeneration Induced by Targeted Overexpression of α-Synuclein in the Nigrostriatal System." *The Journal of Neuroscience* 22 (7): 2780–91. https://doi.org/10.1523/JNEUROSCI.22-07-02780.2002.

Kirilyuk, Alexander, Mika Shimoji, Jason Catania, Geetaram Sahu, Nagarajan Pattabiraman, Antonio Giordano, Christopher Albanese, et al. 2012. "An Intrinsically Disordered Region of the Acetyltransferase P300 with Similarity to Prion-Like Domains Plays a Role in Aggregation." *PLoS ONE* 7 (11): 48243. https://doi.org/10.1371/journal.pone.0048243.

Klaassen, Remco V., Jasper Stroeder, Françoise Coussen, Anne Sophie Hafner, Jennifer D. Petersen, Cedric

Renancio, Leanne J.M. Schmitz, et al. 2016. “Shisa6 Traps AMPA Receptors at Postsynaptic Sites and Prevents Their Desensitization during Synaptic Activity.” *Nature Communications* 7: 1–12. https://doi.org/10.1038/ncomms10682.

Koch, J. C., F. Bitow, J. Haack, Z. Hedouville, J. N. Zhang, L. Tönges, U. Michel, et al. 2015. “Alpha-Synuclein Affects Neurite Morphology, Autophagy, Vesicle Transport and Axonal Degeneration in CNS Neurons.” *Cell Death and Disease* 6 (7): e1811–e1811. https://doi.org/10.1038/cddis.2015.169.

Kontopoulos, Eirene, Jeffrey D. Parvin, and Mel B. Feany. 2006. “A-Synuclein Acts in the Nucleus To Inhibit Histone Acetylation and Promote Neurotoxicity.” *Human Molecular Genetics* 15 (20): 3012–23. https://doi.org/10.1093/hmg/ddl243.

Kramer, M L, and W J Schulz-Schaeffer. 2007. “Presynaptic Alpha-Synuclein Aggregates, Not Lewy Bodies, Cause Neurodegeneration in Dementia with Lewy Bodies.” *J Neurosci* 27 (6): 1405–10. https://doi.org/10.1523/JNEUROSCI.4564-06.2007.

Krüger, R, W Kuhn, T Müller, D Woitalla, M Graeber, S Kösel, H Przuntek, J T Epplen, L Schöls, and O Riess. 1998. “Ala30Pro Mutation in the Gene Encoding Alpha-Synuclein in Parkinson’s Disease.” *Nature Genetics* 18 (2): 106–8. https://doi.org/10.1038/ng0298-106.

Kume, K., M. Kikukawa, H. Hanyu, Y. Takata, T. Umahara, H. Sakurai, H. Kanetaka, K. Ohyashiki, J. H. Ohyashiki, and T. Iwamoto. 2012. “Telomere Length Shortening in Patients with Dementia with Lewy Bodies.” *European Journal of Neurology* 19 (6): 905–10. https://doi.org/10.1111/j.1468-1331.2011.03655.x.

Kuznetsova, Maria, Carey Wilson, Anthony J. Hannan, and Thibault Renoir. 2020. “How the Enriched Get Richer? Experience-Dependent Modulation of MicroRNAs and the Therapeutic Effects of Environmental Enrichment.” *Pharmacology Biochemistry and Behavior*. Elsevier Inc. https://doi.org/10.1016/j.pbb.2020.172940.

Laemmli, U. K. 1970. “Cleavage of Structural Proteins during the Assembly of the Head of Bacteriophage T4.” *Nature* 227 (5259): 680–85. https://doi.org/10.1038/227680a0.

Larsen, K. E., Y. Schmitz, M. D. Troyer, E. Mosharov, P. Dietrich, A. Z. Quazi, M. Savalle, et al. 2006. “-Synuclein Overexpression in PC12 and Chromaffin Cells Impairs Catecholamine Release by Interfering with a Late Step in Exocytosis.” *Journal of Neuroscience* 26 (46): 11915–22. https://doi.org/10.1523/JNEUROSCI.3821-06.2006.

Lau, Lonneke ML de, and Monique MB Breteler. 2006. “Epidemiology of Parkinson’s Disease.” *Lancet Neurology*. Elsevier. https://doi.org/10.1016/S1474-4422(06)70471-9.

Laviola, Giovanni, Anthony J. Hannan, Simone Macrì, Marcello Solinas, and Mohamed Jaber. 2008. “Effects of Enriched Environment on Animal Models of Neurodegenerative Diseases and Psychiatric Disorders.” *Neurobiology of Disease*. Neurobiol Dis. https://doi.org/10.1016/j.nbd.2008.05.001.

Lázaro, Diana F., Maria Angeliki S. Pavlou, and Tiago Fleming Outeiro. 2017. “Cellular Models as Tools for the Study of the Role of Alpha-Synuclein in Parkinson’s Disease.” *Experimental Neurology* 298 (December): 162–71. https://doi.org/10.1016/j.expneurol.2017.05.007.

Lee, H J, S Patel, and S J Lee. 2005. “Intravesicular Localization and Exocytosis of Alpha-Synuclein and Its Aggregates.” *J Neurosci* 25 (25): 6016–24. https://doi.org/10.1523/JNEUROSCI.0692-05.2005.

Lee, Yoontae, Rodney C. Samaco, Jennifer R. Gatchel, Christina Thaller, Harry T. Orr, and Huda Y. Zoghbi. 2008. “MiR-19, MiR-101 and MiR-130 Co-Regulate ATXN1 Levels to Potentially Modulate SCA1 Pathogenesis.” *Nature Neuroscience* 11 (10): 1137–39. https://doi.org/10.1038/nn.2183.

Lees, A J, E Tolosa, and C W Olanow. 2015. “Four Pioneers of L-Dopa Treatment: Arvid Carlsson, Oleh Hornykiewicz, George Cotzias, and Melvin Yahr.” *Mov Disord* 30 (1): 19–36. https://doi.org/10.1002/mds.26120.

Lehmann, Sabrina M., Christina Krüger, Boyoun Park, Katja Derkow, Karen Rosenberger, Jan Baumgart, Thorsten Trimbuch, et al. 2012. “An Unconventional Role for MiRNA: Let-7 Activates Toll-like Receptor 7 and Causes Neurodegeneration.” *Nature Neuroscience* 15 (6): 827–35. https://doi.org/10.1038/nn.3113.

Leng, Yan, and De Maw Chuang. 2006. “Endogenous α-Synuclein Is Induced by Valproic Acid through Histone Deacetylase Inhibition and Participates in Neuroprotection against Glutamate-Induced Excitotoxicity.”

Journal of Neuroscience 26 (28): 7502–12. https://doi.org/10.1523/JNEUROSCI.0096-06.2006.

Leonhardt, Heinrich, Andrea W. Page, Heinz Ulrich Weier, and Timothy H. Bestor. 1992. "A Targeting Sequence Directs DNA Methyltransferase to Sites of DNA Replication in Mammalian Nuclei." *Cell* 71 (5): 865–73. https://doi.org/10.1016/0092-8674(92)90561-P.

Lesage, S, M Anheim, F Letournel, L Bousset, A Honore, N Rozas, L Pieri, et al. 2013. "G51D Alpha-Synuclein Mutation Causes a Novel Parkinsonian-Pyramidal Syndrome." *Ann Neurol* 73 (4): 459–71. https://doi.org/10.1002/ana.23894.

Lévi, Sabine, R. Mark Grady, Michael D. Henry, Kevin P. Campbell, Joshua R. Sanes, and Ann Marie Craig. 2002. "Dystroglycan Is Selectively Associated with Inhibitory GABAergic Synapses but Is Dispensable for Their Differentiation." *Journal of Neuroscience* 22 (11): 4274–85. https://doi.org/10.1523/jneurosci.22-11-04274.2002.

Levy, Aaron D., Mitchell H. Omar, and Anthony J. Koleske. 2014. "Extracellular Matrix Control of Dendritic Spine and Synapse Structure and Plasticity in Adulthood." *Frontiers in Neuroanatomy*. Frontiers Media S.A. https://doi.org/10.3389/fnana.2014.00116.

Lewis, Benjamin P., I. Hung Shih, Matthew W. Jones-Rhoades, David P. Bartel, and Christopher B. Burge. 2003. "Prediction of Mammalian MicroRNA Targets." *Cell* 115 (7): 787–98. https://doi.org/10.1016/S0092-8674(03)01018-3.

Li, Dongsheng, Hongqi Yang, Jianjun Ma, Sha Luo, Siyuan Chen, and Qi Gu. 2018. "MicroRNA-30e Regulates Neuroinflammation in MPTP Model of Parkinson's Disease by Targeting Nlrp3." *Human Cell* 31 (2): 106–15. https://doi.org/10.1007/s13577-017-0187-5.

Li, Hong Tao, Hai Ning Du, Lin Tang, Jun Hu, and Hong Yu Hu. 2002. "Structural Transformation and Aggregation of Human α-Synuclein in Trifluoroethanol: Non-Amyloid Component Sequence Is Essential and β-Sheet Formation Is Prerequisite to Aggregation." *Biopolymers* 64 (4): 221–26. https://doi.org/10.1002/bip.10179.

Li, Jia Yi, Markus Plomann, and Patrik Brundin. 2003. "Huntington's Disease: A Synaptopathy?" *Trends in Molecular Medicine* 9 (10): 414–20. https://doi.org/10.1016/j.molmed.2003.08.006.

Li, Jiali, Dan Jiang, Xueshan Xiao, Shiqiang Li, Xiaoyun Jia, Wenmin Sun, Xiangming Guo, and Qingjiong Zhang. 2015. "Evaluation of 12 Myopia-Associated Genes in Chinese Patients with High Myopia." *Investigative Ophthalmology and Visual Science* 56 (2): 722–29. https://doi.org/10.1167/iovs.14-14880.

Li, Li, Hui Liu, Haijing Song, Yingsong Qin, Ying Wang, Ming Xu, Chaoxia Liu, Jin Gao, and Shen Sun. 2017. "Let-7d MicroRNA Attenuates 6-OHDA-Induced Injury by Targeting Caspase-3 in MN9D Cells." *Journal of Molecular Neuroscience* 63 (3–4): 403–11. https://doi.org/10.1007/s12031-017-0994-x.

Li, Lingzhi, and Luen Tang Bor. 2005. "Environmental Enrichment and Neurodegenerative Diseases." *Biochemical and Biophysical Research Communications*. Academic Press Inc. https://doi.org/10.1016/j.bbrc.2005.05.162.

Li, Yang I., Garrett Wong, Jack Humphrey, and Towfique Raj. 2019. "Prioritizing Parkinson's Disease Genes Using Population-Scale Transcriptomic Data." *Nature Communications* 10 (1): 1–10. https://doi.org/10.1038/s41467-019-08912-9.

Lim, Ee Wei, Dag Aarsland, Dominic Ffytche, Raquel Natalia Taddei, Daniel J. van Wamelen, Yi Min Wan, Eng King Tan, and Kallol Ray Chaudhuri. 2019. "Amyloid-β and Parkinson's Disease." *Journal of Neurology*. Dr. Dietrich Steinkopff Verlag GmbH and Co. KG. https://doi.org/10.1007/s00415-018-9100-8.

Lippi, Giordano, Catarina C Fernandes, Laura A Ewell, Danielle John, Benedetto Romoli, Giulia Curia, Seth R Taylor, et al. 2016. "MicroRNA-101 Regulates Multiple Developmental Programs to Constrain Excitation in Adult Neural Networks." *Neuron* 92 (6): 1337–51. https://doi.org/10.1016/j.neuron.2016.11.017.

Liu-Yesucevitz, L., G. J. Bassell, A. D. Gitler, A. C. Hart, E. Klann, J. D. Richter, S. T. Warren, and B. Wolozin. 2011. "Local RNA Translation at the Synapse and in Disease." *Journal of Neuroscience* 31 (45): 16086–93. https://doi.org/10.1523/jneurosci.4105-11.2011.

Liu, Gang, Jian Ping Zhou, and Ming Dong. 2019. "Down-Regulation of MiR-543 Expression Increases the Sensitivity of Colorectal Cancer Cells to 5-Fluorouracil through the PTEN/PI3K/AKT Pathway." *Bioscience*

Reports 39 (3). https://doi.org/10.1042/BSR20190249.

Liu, Hui, Christos Koros, Timo Strohäker, Claudia Schulte, Maria Bozi, Stefanos Varvaresos, Alain Ibáñez de Opakua, et al. 2021. "A Novel SNCA A30G Mutation Causes Familial Parkinson's Disease." *Movement Disorders*. https://doi.org/10.1002/mds.28534.

Liu, Shuang, Yong Zhang, Hong Bian, and Xiaohong Li. 2016. "Gene Expression Profiling Predicts Pathways and Genes Associated with Parkinson's Disease." *Neurological Sciences* 37 (1): 73–79. https://doi.org/10.1007/s10072-015-2360-5.

Liu, Ying, and Zuneng Lu. 2018. "Long Non-Coding RNA NEAT1 Mediates the Toxic of Parkinson's Disease Induced by MPTP/MPP+ via Regulation of Gene Expression." *Clinical and Experimental Pharmacology and Physiology* 45 (8): 841–48. https://doi.org/10.1111/1440-1681.12932.

Livak, Kenneth J., and Thomas D. Schmittgen. 2001. "Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2-ΔΔCT Method." *Methods* 25 (4): 402–8. https://doi.org/10.1006/meth.2001.1262.

Long, Justin M., and Debomoy K. Lahiri. 2011. "MicroRNA-101 Downregulates Alzheimer's Amyloid-β Precursor Protein Levels in Human Cell Cultures and Is Differentially Expressed." *Biochemical and Biophysical Research Communications* 404 (4): 889–95. https://doi.org/10.1016/j.bbrc.2010.12.053.

Long, Justin M., Balmiki Ray, and Debomoy K. Lahiri. 2014. "MicroRNA-339-5p down-Regulates Protein Expression of β-Site Amyloid Precursor Protein-Cleaving Enzyme 1 (BACE1) in Human Primary Brain Cultures and Is Reduced in Brain Tissue Specimens of Alzheimer Disease Subjects." *Journal of Biological Chemistry* 289 (8): 5184–98. https://doi.org/10.1074/jbc.M113.518241.

Lugli, Giovanni, John Larson, Michael P. Demars, and Neil R. Smalheiser. 2012. "Primary MicroRNA Precursor Transcripts Are Localized at Post-Synaptic Densities in Adult Mouse Forebrain." *Journal of Neurochemistry* 123 (4): 459–66. https://doi.org/10.1111/j.1471-4159.2012.07921.x.

Lugli, Giovanni, John Larson, Maryann E. Martone, Ying Jones, and Neil R. Smalheiser. 2005. "Dicer and EIF2c Are Enriched at Postsynaptic Densities in Adult Mouse Brain and Are Modified by Neuronal Activity in a Calpain-Dependent Manner." *Journal of Neurochemistry* 94 (4): 896–905. https://doi.org/10.1111/j.1471-4159.2005.03224.x.

Lugli, Giovanni, Vetle I Torvik, John Larson, and Neil R Smalheiser. 2008. "Expression of MicroRNAs and Their Precursors in Synaptic Fractions of Adult Mouse Forebrain." *Journal of Neurochemistry* 106 (2): 650–61. https://doi.org/10.1111/j.1471-4159.2008.05413.x.

Luk, Kelvin C., Victoria M. Kehm, Bin Zhang, Patrick O'Brien, John Q. Trojanowski, and Virginia M.Y. Lee. 2012. "Intracerebral Inoculation of Pathological α-Synuclein Initiates a Rapidly Progressive Neurodegenerative α-Synucleinopathy in Mice." *The Journal of Experimental Medicine* 209 (5): 975–86. https://doi.org/10.1084/jem.20112457.

Mahul-Mellier, Anne Laure, Johannes Burtscher, Niran Maharjan, Laura Weerens, Marie Croisier, Fabien Kuttler, Marion Leleu, Graham W. Knott, and Hilal A. Lashuel. 2020. "The Process of Lewy Body Formation, Rather than Simply α-Synuclein Fibrillization, Is One of the Major Drivers of Neurodegeneration." *Proceedings of the National Academy of Sciences of the United States of America* 117 (9): 4971–82. https://doi.org/10.1073/pnas.1913904117.

Mallet, Luc, Valérie Mesnage, Jean Luc Houeto, Antoine Pelissolo, Jérôme Yelnik, Cécile Behar, Marcella Gargiulo, et al. 2002. "Compulsions, Parkinson's Disease, and Stimulation." *Lancet* 360 (9342): 1302–4. https://doi.org/10.1016/S0140-6736(02)11339-0.

Manning-Bog, Amy B., Alison L. McCormack, Jie Li, Vladimir N. Uversky, Anthony L. Fink, and Donato A. Di Monte. 2002. "The Herbicide Paraquat Causes Up-Regulation and Aggregation of α-Synuclein in Mice: Paraquat and α-Synuclein." *Journal of Biological Chemistry* 277 (3): 1641–44. https://doi.org/10.1074/jbc.C100560200.

Manyam, Bala V. 1982. "Low CSF γ-Aminobutyric Acid Levels in Parkinson's Disease: Effect of Levodopa and Carbidopa." *Archives of Neurology* 39 (7): 391–92. https://doi.org/10.1001/archneur.1982.00510190009002.

Marín, Oscar. 2012. “Interneuron Dysfunction in Psychiatric Disorders.” *Nature Reviews Neuroscience*. Nature Publishing Group. https://doi.org/10.1038/nrn3155.

Maroteaux, L, J T Campanelli, and R H Scheller. 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. https://www.ncbi.nlm.nih.gov/pubmed/3411354.

Marques, Tainá M., H. Bea Kuiperij, Ilona B. Bruinsma, Anouke van Rumund, Marjolein B. Aerts, Rianne A.J. Esselink, Bas R. Bloem, and Marcel M. Verbeek. 2017. “MicroRNAs in Cerebrospinal Fluid as Potential Biomarkers for Parkinson’s Disease and Multiple System Atrophy.” *Molecular Neurobiology* 54 (10): 7736–45. https://doi.org/10.1007/s12035-016-0253-0.

Martin, Kelsey C., and Anne Ephrussi. 2009. “MRNA Localization: Gene Expression in the Spatial Dimension.” *Cell*. Cell. https://doi.org/10.1016/j.cell.2009.01.044.

Martínez-Cué, Carmen, and Noemí Rueda. 2020. “Cellular Senescence in Neurodegenerative Diseases.” *Frontiers in Cellular Neuroscience*. Frontiers Media S.A. https://doi.org/10.3389/fncel.2020.00016.

Marui, Wami, Eizo Iseki, Toshiki Nakai, Satoshi Miura, Masanori Kato, Kenji Uéda, and Kenji Kosaka. 2002. “Progression and Staging of Lewy Pathology in Brains from Patients with Dementia with Lewy Bodies.” *Journal of the Neurological Sciences* 195 (2): 153–59. https://doi.org/10.1016/S0022-510X(02)00006-0.

Masliah, E., E. Rockenstein, I. Veinbergs, M. Mallory, M. Hashimoto, A. Takeda, Y. Sagara, A. Sisk, and L. Mucke. 2000. “Dopaminergic Loss and Inclusion Body Formation in Alpha-Synuclein Mice.” *Science* 287 (February): 1265–69.

Mason, D M, N Nouraei, D B Pant, K M Miner, D F Hutchison, K C Luk, J F Stolz, and R K Leak. 2016. “Transmission of Alpha-Synucleinopathy from Olfactory Structures Deep into the Temporal Lobe.” *Mol Neurodegener* 11 (1): 49. https://doi.org/10.1186/s13024-016-0113-4.

Mattick, John S. 2003. “Challenging the Dogma: The Hidden Layer of Non-Protein-Coding RNAs in Complex Organisms.” *BioEssays* 25 (10): 930–39. https://doi.org/10.1002/bies.10332.

Mayo, M C, and Y Bordelon. 2014. “Dementia with Lewy Bodies.” *Semin Neurol* 34 (2): 182–88. https://doi.org/10.1055/s-0034-1381741.

McKenna, D, and J Peever. 2017. “Degeneration of Rapid Eye Movement Sleep Circuitry Underlies Rapid Eye Movement Sleep Behavior Disorder.” *Mov Disord* 32 (5): 636–44. https://doi.org/10.1002/mds.27003.

Mcmahon, Mari, Afshin Samali, and X Eric Chevet. 2017. “Regulation of the Unfolded Protein Response by Noncoding RNA.” *Am J Physiol Cell Physiol* 313: 243–54. https://doi.org/10.1152/ajpcell.00293.2016.-Cells.

McNeill, Thomas H., Sally A. Brown, Jose A. Rafols, and Ira Shoulson. 1988. “Atrophy of Medium Spiny I Striatal Dendrites in Advanced Parkinson’s Disease.” *Brain Research* 455 (1): 148–52. https://doi.org/10.1016/0006-8993(88)90124-2.

Meredith, Gloria E., and David J. Rademacher. 2011. “MPTP Mouse Models of Parkinson’s Disease: An Update.” *Journal of Parkinson’s Disease*. https://doi.org/10.3233/JPD-2011-11023.

Merlo, Daniela, Inmaculada Cuchillo-Ibañez, Rosanna Parlato, and Gerhard Rammes. 2016. “DNA Damage, Neurodegeneration, and Synaptic Plasticity.” *Neural Plasticity* 2016: 1–2. https://doi.org/10.1155/2016/1206840.

Miñones-Moyano, Elena, Sílvia Porta, Georgia Escaramís, Raquel Rabionet, Susana Iraola, Birgit Kagerbauer, Yolanda Espinosa-Parrilla, Isidre Ferrer, Xavier Estivill, and Eulàlia Martí. 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. https://doi.org/10.1093/hmg/ddr210.

Mizuno, Hideya, and Ayako Taketomi. 2018. “MicroRNA-101 Inhibits the Expression of Rhes, a Striatal-Enriched Small G-Protein, at the Post-Transcriptional Level in Vitro.” *BMC Research Notes* 11 (1): 528. https://doi.org/10.1186/s13104-018-3654-5.

Mograbi, Karla De Michelis, Ana Carolini Ferreira de Castro, Jainny Aniely Rocha de Oliveira, Patrick Jean Barbosa Sales, Luciene Covolan, Eliane Aparecida Del Bel, and Albert Schiaveto de Souza. 2017. “Effects of GABAa Receptor Antagonists on Motor Behavior in Pharmacological Parkinson’s Disease Model in

Mice.” *Physiological Reports* 5 (6). https://doi.org/10.14814/phy2.13081.

Mohite, Ganesh M., Rakesh Kumar, Rajlaxmi Panigrahi, Ambuja Navalkar, Nitu Singh, Debalina Datta, Surabhi Mehra, et al. 2018. “Comparison of Kinetics, Toxicity, Oligomer Formation, and Membrane Binding Capacity of α-Synuclein Familial Mutations at the A53 Site, Including the Newly Discovered A53V Mutation.” *Biochemistry* 57 (35): 5183–87. https://doi.org/10.1021/acs.biochem.8b00314.

Moolman, Donna L., Ottavio V. Vitolo, Jean Paul G. Vonsattel, and Michael L. Shelanski. 2004. “Dendrite and Dendritic Spine Alterations in Alzheimer Models.” *Journal of Neurocytology* 33 (3): 377–87. https://doi.org/10.1023/B:NEUR.0000044197.83514.64.

Mor, Danielle E, Elpida Tsika, Joseph R Mazzulli, Neal S Gould, Hanna Kim, Malcolm J Daniels, Shachee Doshi, et al. 2017. “Dopamine Induces Soluble a -Synuclein Oligomers and Nigrostriatal Degeneration.” *Nature Publishing Group*, no. September. https://doi.org/10.1038/nn.4641.

Morales, M., and D. H. Root. 2014. “Glutamate Neurons within the Midbrain Dopamine Regions.” *Neuroscience*. Elsevier Ltd. https://doi.org/10.1016/j.neuroscience.2014.05.032.

Mosharov, E. V. 2006. “ -Synuclein Overexpression Increases Cytosolic Catecholamine Concentration.” *Journal of Neuroscience* 26 (36): 9304–11. https://doi.org/10.1523/jneurosci.0519-06.2006.

Mostany, Ricardo, James E. Anstey, Kerensa L. Crump, Bohumil Maco, Graham Knott, and Carlos Portera-Cailliau. 2013. “Altered Synaptic Dynamics during Normal Brain Aging.” *Journal of Neuroscience* 33 (9): 4094–4104. https://doi.org/10.1523/JNEUROSCI.4825-12.2013.

Mouradian, M Maral. 2012. “Neurobiology of Disease MicroRNAs in Parkinson ’ s Disease.” *Neurobiology of Disease* 46 (2): 279–84. https://doi.org/10.1016/j.nbd.2011.12.046.

Mullin, S, and A Schapira. 2015. “The Genetics of Parkinson’s Disease.” *Br Med Bull* 114 (1): 39–52. https://doi.org/10.1093/bmb/ldv022.

Nakamura, Ken, Venu M. Nemani, Farnaz Azarbal, Gaia Skibinski, Jon M. Levy, Kiyoshi Egami, Larissa Munishkina, et al. 2011. “Direct Membrane Association Drives Mitochondrial Fission by the Parkinson Disease-Associated Protein α-Synuclein.” *Journal of Biological Chemistry* 286 (23): 20710–26. https://doi.org/10.1074/jbc.M110.213538.

Narayanan, Vijaya, and Suzanne Scarlata. 2001. “Membrane Binding and Self-Association of α-Synucleins.” *Biochemistry* 40 (33): 9927–34. https://doi.org/10.1021/bi002952n.

Navarro-Sánchez, Luis, Beatriz Águeda-Gómez, Silvia Aparicio, and Jordi Pérez-Tur. 2018. “Epigenetic Study in Parkinson’s Disease: A Pilot Analysis of DNA Methylation in Candidate Genes in Brain.” *Cells* 7 (10): 150. https://doi.org/10.3390/cells7100150.

Nemani, Venu M, Wei Lu, Victoria Berge, Ken Nakamura, Bibiana Onoa, Michael K. Lee, Farrukh a Chaudhry, Roger a Nicoll, and Robert H Edwards. 2010. “Increased Expression of α-Synuclein Reduces Neurotransmitter Release by Inhibiting Synaptic Vesicle Reclustering after Endocytosis.” *Neuron* 65 (1): 66–79. https://doi.org/10.1016/j.neuron.2009.12.023.

Neudorfer, O, N Giladi, D Elstein, A Abrahamov, T Turezkite, E Aghai, A Reches, B Bembi, and A Zimran. 1996. “Occurrence of Parkinson’s Syndrome in Type I Gaucher Disease.” *QJM* 89 (9): 691–94. https://doi.org/10.1093/qjmed/89.9.691.

Neumann, Manuela, Philipp J. Kahle, Benoit I. Giasson, Laurence Ozmen, Edilio Borroni, Will Spooren, Veronika Müller, et al. 2002. “Misfolded Proteinase K-Resistant Hyperphosphorylated α-Synuclein in Aged Transgenic Mice with Locomotor Deterioration and in Human α-Synucleinopathies.” *Journal of Clinical Investigation*. https://doi.org/10.1172/JCI200215777.

Neuner, Johanna, Saak V. Ovsepian, Mario Dorostkar, Severin Filser, Aayush Gupta, Stylianos Michalakis, Martin Biel, and Jochen Herms. 2014. “Pathological α-Synuclein Impairs Adult-Born Granule Cell Development and Functional Integration in the Olfactory Bulb.” *Nature Communications* 5 (May). https://doi.org/10.1038/ncomms4915.

Nithianantharajah, Jess, and Anthony J. Hannan. 2006. “Enriched Environments, Experience-Dependent Plasticity and Disorders of the Nervous System.” *Nature Reviews Neuroscience*. Nat Rev Neurosci. https://doi.org/10.1038/nrn1970.

Obergasteiger, Julia, Giulia Frapporti, Peter P. Pramstaller, Andrew A. Hicks, and Mattia Volta. 2018. “A New Hypothesis for Parkinson’s Disease Pathogenesis: GTPase-P38 MAPK Signaling and Autophagy as Convergence Points of Etiology and Genomics.” *Molecular Neurodegeneration*. BioMed Central Ltd. https://doi.org/10.1186/s13024-018-0273-5.

Oh, Won Chan, Stefano Lutzu, Pablo E. Castillo, and Hyung Bae Kwon. 2016. “De Novo Synaptogenesis Induced by GABA in the Developing Mouse Cortex.” *Science* 353 (6303): 1037–40. https://doi.org/10.1126/science.aaf5206.

Oishi, Maho, Kenji Yamashiro, Masahiro Miyake, Yumiko Akagi-Kurashige, Kyoko Kumagai, Isao Nakata, Hideo Nakanishi, et al. 2013. “Association between ZIC2, RASGRF1, and SHISA6 Genes and High Myopia in Japanese Subjects.” *Investigative Ophthalmology and Visual Science* 54 (12): 7492–97. https://doi.org/10.1167/iovs.13-12825.

Okano, Masaki, Daphne W Bell, Daniel A Haber, and En Li. 1999. “DNA Methyltransferases Dnmt3a and Dnmt3b Are Essential for De Novo Methylation and Mammalian Development.” *Cell* 99 (3): 247–57. https://doi.org/10.1016/S0092-8674(00)81656-6.

Ouberai, Myriam M., Juan Wang, Marcus J. Swann, Celine Galvagnion, Tim Guilliams, Christopher M. Dobson, and Mark E. Welland. 2013. “α-Synuclein Senses Lipid Packing Defects and Induces Lateral Expansion of Lipids Leading to Membrane Remodeling.” *Journal of Biological Chemistry* 288 (29): 20883–95. https://doi.org/10.1074/jbc.M113.478297.

Paik, Seung R, Hyun-ju Shin, Ju-hyun Lee, Chung-soon Chang, Jongsun Kim, Wolfgang Hoyer, Dmitry Cherny, Vinod Subramaniam, and Thomas M. Jovin. 2004. “Impact of the Acidic C-Terminal Region Comprising Amino Acids 109-140 on ??-Synuclein Aggregation in Vitro.” *Biochemistry* 43 (51): 16233–42. https://doi.org/10.1021/bi048453u.

Paiva, Isabel, Gaurav Jain, Diana F. Lázaro, Kristina Gotovac Jerčić, Thomas Hentrich, Cemil Kerimoglu, Raquel Pinho, et al. 2018. “Alpha-Synuclein Deregulates the Expression of COL4A2 and Impairs ER-Golgi Function.” *Neurobiology of Disease* 119: 121–35. https://doi.org/10.1016/j.nbd.2018.08.001.

Pamudurti, Nagarjuna Reddy, Osnat Bartok, Marvin Jens, Reut Ashwal-Fluss, Christin Stottmeister, Larissa Ruhe, Mor Hanan, et al. 2017. “Translation of CircRNAs.” *Molecular Cell* 66 (1): 9-21.e7. https://doi.org/10.1016/j.molcel.2017.02.021.

Pan-Montojo, F, M Schwarz, C Winkler, M Arnhold, G A O’Sullivan, A Pal, J Said, et al. 2012. “Environmental Toxins Trigger PD-like Progression via Increased Alpha-Synuclein Release from Enteric Neurons in Mice.” *Sci Rep* 2: 898. https://doi.org/10.1038/srep00898.

Parajuli, Laxmi Kumar, Ken Wako, Suiki Maruo, Soichiro Kakuta, Tomoyuki Taguchi, Masashi Ikuno, Hodaka Yamakado, Ryosuke Takahashi, and Masato Koike. 2020. “Developmental Changes in Dendritic Spine Morphology in the Striatum and Their Alteration in an A53t α-Synuclein Transgenic Mouse Model of Parkinson’s Disease.” *ENeuro* 7 (4): 1–14. https://doi.org/10.1523/ENEURO.0072-20.2020.

Parihar, Mordhwaj S., and Gregory J. Brewer. 2010. *Amyloid-ꞵ as a Modulator of Synaptic Plasticity*. *Journal of Alzheimer’s Disease*. Vol. 22. https://doi.org/10.3233/JAD-2010-101020.

Park, Goonho, Jieqiong Tan, Guillermina Garcia, Yunyi Kang, Guy Salvesen, and Zhuohua Zhang. 2016. “Regulation of Histone Acetylation by Autophagy in Parkinson Disease.” *Journal of Biological Chemistry* 291 (7): 3531–40. https://doi.org/10.1074/jbc.M115.675488.

Pasanen, P, L Myllykangas, M Siitonen, A Raunio, S Kaakkola, J Lyytinen, P J Tienari, M Poyhonen, and A Paetau. 2014. “Novel Alpha-Synuclein Mutation A53E Associated with Atypical Multiple System Atrophy and Parkinson’s Disease-Type Pathology.” *Neurobiol Aging* 35 (9): 2180 e1-5. https://doi.org/10.1016/j.neurobiolaging.2014.03.024.

Pasquinelli, Amy E., Brenda J. Reinhart, Frank Slack, Mark Q. Martindale, Mitzi I. Kuroda, Betsy Maller, David C. Hayward, et al. 2000. “Conservation of the Sequence and Temporal Expression of Let-7 Heterochronic Regulatory RNA.” *Nature* 408 (6808): 86–89. https://doi.org/10.1038/35040556.

Patt S, Gertz H J, Gerhard L, and Cervós-Navarro J. 1991. “Pathological Changes in Dendrites of Substantia Nigra Neurons in Parkinson’s Disease: A Golgi Study - PubMed.” Histol Histopathol . 1991.

https://pubmed.ncbi.nlm.nih.gov/1725760/.

Paumier, K L, K C Luk, F P Manfredsson, N M Kanaan, J W Lipton, T J Collier, K Steece-Collier, et al. 2015. “Intrastriatal Injection of Pre-Formed Mouse Alpha-Synuclein Fibrils into Rats Triggers Alpha-Synuclein Pathology and Bilateral Nigrostriatal Degeneration.” *Neurobiol Dis* 82: 185–99. https://doi.org/10.1016/j.nbd.2015.06.003.

Pavlou, Maria Angeliki S, Raquel Pinho, Isabel Paiva, and Tiago Fleming Outeiro. 2016. “The Yin and Yang of a -Synuclein-Associated Epigenetics in Parkinson ’ s Disease,” 1–9. https://doi.org/10.1093/brain/aww227.

Paxinou, E, Q Chen, M Weisse, B I Giasson, E H Norris, S M Rueter, J Q Trojanowski, V M Lee, and H Ischiropoulos. 2001. “Induction of Alpha-Synuclein Aggregation by Intracellular Nitrative Insult.” *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience* 21 (20): 8053–61. https://doi.org/10.1515/9781400870738-009.

Perez, R G, E Lin, J J Liu, F Guo, M J Zigmond, and J C Waymire. 2002. “A Role for Alpha-Synuclein in the Regulation of Dopamine Biosynthesis.” *J Neurosci* 22 (8): 3090–99.

Picconi, Barbara, Giovanni Piccoli, and Paolo Calabresi. 2012. “Synaptic Dysfunction in Parkinson’s Disease.” *Advances in Experimental Medicine and Biology* 970: 553–72. https://doi.org/10.1007/978-3-7091-0932-8_24.

Pickrell, Alicia M., and Richard J. Youle. 2015. “The Roles of PINK1, Parkin, and Mitochondrial Fidelity in Parkinson’s Disease.” *Neuron*. Cell Press. https://doi.org/10.1016/j.neuron.2014.12.007.

Pinho, Raquel, Isabel Paiva, Kristina Gotovac Jerčić, Luis Fonseca-Ornelas, Ellen Gerhardt, Christiane Fahlbusch, Paula Garcia-Esparcia, et al. 2019. “Nuclear Localization and Phosphorylation Modulate Pathological Effects of Alpha-Synuclein.” *Human Molecular Genetics* 28 (1): 31–50. https://doi.org/10.1093/hmg/ddy326.

Poewe, W, K Seppi, C M Tanner, G M Halliday, P Brundin, J Volkmann, A E Schrag, and A E Lang. 2017. “Parkinson Disease.” *Nat Rev Dis Primers* 3: 17013. https://doi.org/10.1038/nrdp.2017.13.

Polymeropoulos, M H, C Lavedan, E Leroy, S E Ide, A Dehejia, A Dutra, B Pike, et al. 1997. “Mutation in the Alpha-Synuclein Gene Identified in Families with Parkinson’s Disease.” *Science (New York, N.Y.)* 276 (5321): 2045–47. https://doi.org/10.1126/science.276.5321.2045.

Postuma, R B, D Berg, M Stern, W Poewe, C W Olanow, W Oertel, J Obeso, et al. 2015. “MDS Clinical Diagnostic Criteria for Parkinson’s Disease.” *Mov Disord* 30 (12): 1591–1601. https://doi.org/10.1002/mds.26424.

Pozo, Karine, and Yukiko Goda. 2010. “Unraveling Mechanisms of Homeostatic Synaptic Plasticity.” *Neuron*. Neuron. https://doi.org/10.1016/j.neuron.2010.04.028.

Praag, Henriette van, Gerd Kempermann, and Fred H. Gage. 2000. “Neural Consequences of Enviromental Enrichment.” *Nature Reviews Neuroscience* 1 (3): 191–98. https://doi.org/10.1038/35044558.

Prendergast, James G.D., Pin Tong, David C. Hay, Susan M. Farrington, and Colin A.M. Semple. 2012. “A Genome-Wide Screen in Human Embryonic Stem Cells Reveals Novel Sites of Allele-Specific Histone Modification Associated with Known Disease Loci.” *Epigenetics and Chromatin* 5 (1). https://doi.org/10.1186/1756-8935-5-6.

Price, A, C Manzoni, M R Cookson, and P A Lewis. 2018. “The LRRK2 Signalling System.” *Cell Tissue Res* 373 (1): 39–50. https://doi.org/10.1007/s00441-017-2759-9.

Quake, S. R., and A. Scherer. 2000. “From Micro- to Nanofabrication with Soft Materials.” *Science*. American Association for the Advancement of Science. https://doi.org/10.1126/science.290.5496.1536.

Radley, JJ, HM Sisti, J Hao, AB Rocher, T McCall, P.R Hof, B.S McEwen, and J.H Morrison. 2004. “Chronic Behavioral Stress Induces Apical Dendritic Reorganization in Pyramidal Neurons of the Medial Prefrontal Cortex.” *Neuroscience* 125 (1): 1–6. https://doi.org/10.1016/j.neuroscience.2004.01.006.

Ravanidis, Stylianos, Anastasia Bougea, Dimitra Karampatsi, Nikolaos Papagiannakis, Matina Maniati, Leonidas Stefanis, and Epaminondas Doxakis. 2021. “Differentially Expressed Circular <scp>RNAs</Scp> in Peripheral Blood Mononuclear Cells of Patients with Parkinson’s Disease.” *Movement Disorders*, January, mds.28467. https://doi.org/10.1002/mds.28467.

Reza-Zaldivar, Edwin Estefan, Mercedes Azucena Hernández-Sápiens, Benito Minjarez, Ulises Gómez-Pinedo,

Victor Javier Sánchez-González, Ana Laura Márquez-Aguirre, and Alejandro Arturo Canales-Aguirre. 2020. "Dendritic Spine and Synaptic Plasticity in Alzheimer's Disease: A Focus on MicroRNA." *Frontiers in Cell and Developmental Biology* 8 (May): 255. https://doi.org/10.3389/fcell.2020.00255.

Roberts, Bradley M., Natalie M. Doig, Katherine R. Brimblecombe, Emanuel F. Lopes, Ruth E. Siddorn, Sarah Threlfell, Natalie Connor-Robson, et al. 2020. "GABA Uptake Transporters Support Dopamine Release in Dorsal Striatum with Maladaptive Downregulation in a Parkinsonism Model." *Nature Communications* 11 (1): 1–17. https://doi.org/10.1038/s41467-020-18247-5.

Roberts, Justin T., and Glen M. Borchert. 2017. "Computational Prediction of MicroRNA Target Genes, Target Prediction Databases, and Web Resources." *Methods in Molecular Biology* 1617: 109–22. https://doi.org/10.1007/978-1-4939-7046-9_8.

Rocha, Emily M., Briana De Miranda, and Laurie H. Sanders. 2018. "Alpha-Synuclein: Pathology, Mitochondrial Dysfunction and Neuroinflammation in Parkinson's Disease." *Neurobiology of Disease* 109: 249–57. https://doi.org/10.1016/j.nbd.2017.04.004.

Rodrigues, Eva F, Ramona Langohr, Hedieh Shahpasandzadeh, Thales Ribeiro, Diana F La, Jochen Klucken, Marcos D Pereira, et al. 2014. "Systematic Comparison of the Effects of Alpha-Synuclein Mutations on Its Oligomerization and Aggregation." Edited by Daniel Kaganovich. *PLoS Genetics* 10 (11): e1004741. https://doi.org/10.1371/journal.pgen.1004741.

Rosen, Zev B., Stephanie Cheung, and Steven A. Siegelbaum. 2015. "Midbrain Dopamine Neurons Bidirectionally Regulate CA3-CA1 Synaptic Drive." *Nature Neuroscience* 18 (12): 1763–71. https://doi.org/10.1038/nn.4152.

Roser, Anna Elisa, Lucas Caldi Gomes, Rashi Halder, Gaurav Jain, Fabian Maass, Lars Tönges, Lars Tatenhorst, Mathias Bähr, André Fischer, and Paul Lingor. 2018. "MiR-182-5p and MiR-183-5p Act as GDNF Mimics in Dopaminergic Midbrain Neurons." *Molecular Therapy - Nucleic Acids* 11 (June): 9–22. https://doi.org/10.1016/j.omtn.2018.01.005.

Roshan, Reema, Shruti Shridhar, Mayuresh A. Sarangdhar, Arpita Banik, Mrinal Chawla, Manali Garg, Vijay Pal Singh, and Beena Pillai. 2014. "Brain-Specific Knockdown of MiR-29 Results in Neuronal Cell Death and Ataxia in Mice." *RNA* 20 (8): 1287–97. https://doi.org/10.1261/rna.044008.113.

Rott, Ruth, Raymonde Szargel, Vered Shani, Haya Hamza, Mor Savyon, Fatimah Abd Elghani, Rina Bandopadhyay, and Simone Engelender. 2017. "SUMOylation and Ubiquitination Reciprocally Regulate α-Synuclein Degradation and Pathological Aggregation." *Proceedings of the National Academy of Sciences* 114 (50): 13176–81. https://doi.org/10.1073/pnas.1704351114.

Roush, Sarah, and Frank J. Slack. 2008. "The Let-7 Family of MicroRNAs." *Trends in Cell Biology*. https://doi.org/10.1016/j.tcb.2008.07.007.

Rybak-Wolf, Agnieszka, Christin Stottmeister, Petar Glažar, Marvin Jens, Natalia Pino, Mor Hanan, Mikaela Behm, et al. 2014. "Circular RNAs in the Mammalian Brain Are Highly Abundant, Conserved, and Dynamically Expressed." *Molecular Cell* 58 (5): 870–85. https://doi.org/10.1016/j.molcel.2015.03.027.

Sa sa Peter, Authors, Bastiaan HA Urbanus, Remco V Klaassen, Sabine Spijker, August B Smit, Chris I De Zeeuw, Sa sa Peter, et al. 2020. "AMPAR Auxiliary Protein SHISA6 Facilitates Purkinje Cell Synaptic Excitability and Procedural Memory Formation Article AMPAR Auxiliary Protein SHISA6 Facilitates Purkinje Cell Synaptic Excitability and Procedural Memory Formation." https://doi.org/10.1016/j.celrep.2020.03.079.

Saika, Reiko, Hiroshi Sakuma, Daisuke Noto, Shuhei Yamaguchi, Takashi Yamamura, and Sachiko Miyake. 2017. "MicroRNA-101a Regulates Microglial Morphology and Inflammation." *Journal of Neuroinflammation* 14 (1): 1–10. https://doi.org/10.1186/s12974-017-0884-8.

Saito, Y, A Kawashima, N N Ruberu, H Fujiwara, S Koyama, M Sawabe, T Arai, et al. 2003. "Accumulation of Phosphorylated Alpha-Synuclein in Aging Human Brain." *J Neuropathol Exp Neurol* 62 (6): 644–54. https://www.ncbi.nlm.nih.gov/pubmed/12834109.

Salta, Evgenia, and Bart De Strooper. 2012. "Non-Coding RNAs with Essential Roles in Neurodegenerative." *The Lancet Neurology* 11 (2): 189–200. https://doi.org/10.1016/S1474-4422(11)70286-1.

Sang, Qiuling, Xiaoyang Liu, Libo Wang, Ling Qi, Wenping Sun, Weiyao Wang, Yajuan Sun, and Haina Zhang.

2018. “CircSNCA Downregulation by Pramipexole Treatment Mediates Cell Apoptosis and Autophagy in Parkinson’s Disease by Targeting MiR-7.” *Aging* 10 (6): 1281–93. https://doi.org/10.18632/aging.101466.

Sato, Shigeru, Yoshihiro Omori, Kimiko Katoh, Mineo Kondo, Motoi Kanagawa, Kentaro Miyata, Kazuo Funabiki, et al. 2008. “Pikachurin, a Dystroglycan Ligand, Is Essential for Photoreceptor Ribbon Synapse Formation.” *Nature Neuroscience* 11 (8): 923–31. https://doi.org/10.1038/nn.2160.

Satz, Jakob S., Adam P. Ostendorf, Shangwei Hou, Amy Turner, Hajime Kusano, Jane C. Lee, Rolf Turk, et al. 2010. “Distinct Functions of Glial and Neuronal Dystroglycan in the Developing and Adult Mouse Brain.” *Journal of Neuroscience* 30 (43): 14560–72. https://doi.org/10.1523/JNEUROSCI.3247-10.2010.

Schaser, Allison J, Valerie R Osterberg, Sydney E Dent, Teresa L Stackhouse, Colin M Wakeham, Sydney W Boutros, Leah J Weston, et al. 2019. “Alpha-Synuclein Is a DNA Binding Protein That Modulates DNA Repair with Implications for Lewy Body Disorders.” *Scientific Reports* 9 (1): 10919. https://doi.org/10.1038/s41598-019-47227-z.

Schechter, Meir, Jessica Grigoletto, Suaad Abd-Elhadi, Hava Glickstein, Alexander Friedman, Geidy E. Serrano, Thomas G. Beach, and Ronit Sharon. 2020. “A Role for α-Synuclein in Axon Growth and Its Implications in Corticostriatal Glutamatergic Plasticity in Parkinson’s Disease.” *Molecular Neurodegeneration* 15 (1): 24. https://doi.org/10.1186/s13024-020-00370-y.

Scheffold, Annika, Inge R. Holtman, Sandra Dieni, Nieske Brouwer, Sarah Fee Katz, Billy Michael Chelliah Jebaraj, Philipp J. Kahle, et al. 2016. “Telomere Shortening Leads to an Acceleration of Synucleinopathy and Impaired Microglia Response in a Genetic Mouse Model.” *Acta Neuropathologica Communications* 4 (1): 1–17. https://doi.org/10.1186/s40478-016-0364-x.

Schell, H., T. Hasegawa, M. Neumann, and P. J. Kahle. 2009. “Nuclear and Neuritic Distribution of Serine-129 Phosphorylated α-Synuclein in Transgenic Mice.” *Neuroscience*. https://doi.org/10.1016/j.neuroscience.2009.03.002.

Schell, Heinrich, Cindy Boden, André Maia Chagas, and Philipp J. Kahle. 2012. “Impaired C-Fos and Polo-Like Kinase 2 Induction in the Limbic System of Fear-Conditioned α-Synuclein Transgenic Mice.” Edited by Huaibin Cai. *PLoS ONE* 7 (11): e50245. https://doi.org/10.1371/journal.pone.0050245.

Schiemann, J, F Schlaudraff, V Klose, M Bingmer, S Seino, P J Magill, K A Zaghloul, G Schneider, B Liss, and J Roeper. 2012. “K-ATP Channels in Dopamine Substantia Nigra Neurons Control Bursting and Novelty-Induced Exploration.” *Nat Neurosci* 15 (9): 1272–80. https://doi.org/10.1038/nn.3185.

Schindelin, Johannes, Ignacio Arganda-Carreras, Erwin Frise, Verena Kaynig, Mark Longair, Tobias Pietzsch, Stephan Preibisch, et al. 2012. “Fiji: An Open-Source Platform for Biological-Image Analysis.” *Nature Methods*. Nature Publishing Group. https://doi.org/10.1038/nmeth.2019.

Schirinzi, Tommaso, Graziella Madeo, Giuseppina Martella, Marta Maltese, Barbara Picconi, Paolo Calabresi, and Antonio Pisani. 2016. “Early Synaptic Dysfunction in Parkinson’s Disease: Insights from Animal Models.” *Movement Disorders* 31 (6): 802–13. https://doi.org/10.1002/mds.26620.

Schwienbacher, Christine, Luisa Foco, Anne Picard, Eloina Corradi, Alice Serafin, Jörg Panzer, Stefano Zanigni, et al. 2017. “Plasma and White Blood Cells Show Different MiRNA Expression Profiles in Parkinson’s Disease.” *Journal of Molecular Neuroscience* 62 (2): 244–54. https://doi.org/10.1007/s12031-017-0926-9.

Scott, David A, Iustin Tabarean, Yong Tang, Anna Cartier, Eliezer Masliah, and Subhojit Roy. 2010. “A Pathologic Cascade Leading to Synaptic Dysfunction in -Synuclein-Induced Neurodegeneration.” *Journal of Neuroscience* 30 (24): 8083–95. https://doi.org/10.1523/JNEUROSCI.1091-10.2010.

Shioya, M., S. Obayashi, H. Tabunoki, K. Arima, Y. Saito, T. Ishida, and J. Satoh. 2010. “Aberrant MicroRNA Expression in the Brains of Neurodegenerative Diseases: MiR-29a Decreased in Alzheimer Disease Brains Targets Neurone Navigator 3.” *Neuropathology and Applied Neurobiology* 36 (4): 320–30. https://doi.org/10.1111/j.1365-2990.2010.01076.x.

Sims, Robert J, Kenichi Nishioka, and Danny Reinberg. 2003. “Histone Lysine Methylation: A Signature for Chromatin Function.” *Trends in Genetics : TIG* 19 (11): 629–39. https://doi.org/10.1016/j.tig.2003.09.007.

Singh, Abhishek, and Dwaipayan Sen. 2017. “MicroRNAs in Parkinson’s Disease.” *Experimental Brain Research*. Springer Verlag. https://doi.org/10.1007/s00221-017-4989-1.

Singleton, A B, M Farrer, J Johnson, A Singleton, S Hague, J Kachergus, M Hulihan, et al. 2003. “Alpha-Synuclein Locus Triplication Causes Parkinson’s Disease.” *Science* 302 (5646): 841. https://doi.org/10.1126/science.1090278.

Skipper, L, K Wilkes, M Toft, M Baker, S Lincoln, M Hulihan, O A Ross, M Hutton, J Aasly, and M Farrer. 2004. “Linkage Disequilibrium and Association of MAPT H1 in Parkinson Disease.” *Am J Hum Genet* 75 (4): 669–77. https://doi.org/10.1086/424492.

Smalheiser, Neil R, Giovanni Lugli, Vetle I Torvik, Nathan Mise, Rieko Ikeda, and Kuniya Abe. 2008. “Natural Antisense Transcripts Are Co-Expressed with Sense MRNAs in Synaptoneurosomes of Adult Mouse Forebrain.” *Neuroscience Research* 62 (4): 236–39. https://doi.org/10.1016/j.neures.2008.08.010.

Snead, David, and David Eliezer. 2014. “A-Synuclein Function and Dysfunction on Cellular Membranes.” *Experimental Neurobiology* 23 (4): 292–313. https://doi.org/10.5607/en.2014.23.4.292.

Song, C., A. Kanthasamy, H. Jin, V. Anantharam, and A. G. Kanthasamy. 2011. “Paraquat Induces Epigenetic Changes by Promoting Histone Acetylation in Cell Culture Models of Dopaminergic Degeneration.” *NeuroToxicology* 32 (5): 586–95. https://doi.org/10.1016/j.neuro.2011.05.018.

Sousa, N, NV Lukoyanov, MD Madeira, O.F.X. Almeida, and M.M. Paula-Barbosa. 2000. “Reorganization of the Morphology of Hippocampal Neurites and Synapses after Stress-Induced Damage Correlates with Behavioral Improvement.” *Neuroscience* 97 (2): 253–66. https://doi.org/10.1016/S0306-4522(00)00050-6.

Sousa, V. L., S. Bellani, M. Giannandrea, M. Yousuf, F. Valtorta, J. Meldolesi, and E. Chieregatti. 2009. “α -Synuclein and Its A30P Mutant Affect Actin Cytoskeletal Structure and Dynamics.” *Molecular Biology of the Cell* 20 (16): 3725–39. https://doi.org/10.1091/mbc.e08-03-0302.

Specht, Christian G., Cezar M. Tigaret, Georg F. Rast, Agnes Thalhammer, York Rudhard, and Ralf Schoepfer. 2005. “Subcellular Localisation of Recombinant α- and γ-Synuclein.” *Molecular and Cellular Neuroscience* 28 (2): 326–34. https://doi.org/10.1016/j.mcn.2004.09.017.

Spillantini, M G. 1999. “Parkinson’s Disease, Dementia with Lewy Bodies and Multiple System Atrophy Are Alpha-Synucleinopathies.” *Parkinsonism & Related Disorders* 5 (4): 157–62. https://www.ncbi.nlm.nih.gov/pubmed/18591134.

Spillantini, M G, R A Crowther, R Jakes, N J Cairns, P L Lantos, and M Goedert. 1998. “Filamentous Alpha-Synuclein Inclusions Link Multiple System Atrophy with Parkinson’s Disease and Dementia with Lewy Bodies.” *Neurosci Lett* 251 (3): 205–8. https://doi.org/10.1016/s0304-3940(98)00504-7.

Spillantini, Maria Grazia, and Michel Goedert. 2006. “The α-Synucleinopathies: Parkinson’s Disease, Dementia with Lewy Bodies, and Multiple System Atrophy.” *Annals of the New York Academy of Sciences* 920 (1): 16–27. https://doi.org/10.1111/j.1749-6632.2000.tb06900.x.

Spillantini, Maria Grazia, Marie Luise Schmidt, Virginia M.-Y. Lee, John Q Trojanowski, Ross Jakes, and Michel Goedert. 1997. “α-Synuclein in Lewy Bodies.” *Nature* 388 (6645): 839–40. https://doi.org/10.1038/42166.

Spires-Jones, TL, and BT Hyman. 2015. “The Intersection of Amyloid Beta and Tau at Synapses in Alzheimer’s Disease.” *Neuron* 82 (4): 756–71. https://doi.org/10.1016/j.neuron.2014.05.004.

Steward, O., and E. M. Schuman. 2001. “Protein Synthesis at Synaptic Sites on Dendrites.” *Annual Review of Neuroscience*. Annu Rev Neurosci. https://doi.org/10.1146/annurev.neuro.24.1.299.

Strahl, Brian D., and C. David Allis. 2000. “The Language of Covalent Histone Modifications.” *Nature*. Nature. https://doi.org/10.1038/47412.

Sturm, Edith, and Nadia Stefanova. 2014. “Multiple System Atrophy: Genetic or Epigenetic?” *Experimental Neurobiology* 23 (4): 277. https://doi.org/10.5607/en.2014.23.4.277.

Subramanian, Aravind, Pablo Tamayo, Vamsi K. Mootha, Sayan Mukherjee, Benjamin L. Ebert, Michael A. Gillette, Amanda Paulovich, et al. 2005. “Gene Set Enrichment Analysis: A Knowledge-Based Approach for Interpreting Genome-Wide Expression Profiles.” *Proceedings of the National Academy of Sciences of*

the United States of America 102 (43): 15545–50. https://doi.org/10.1073/pnas.0506580102.

Sugeno, Naoto, Sandra Jäckel, Aaron Voigt, Zinah Wassouf, Julia Schulze-Hentrich, and Philipp J Kahle. 2016. "α-Synuclein Enhances Histone H3 Lysine-9 Dimethylation and H3K9me2-Dependent Transcriptional Responses." *Scientific Reports* 6 (1): 36328. https://doi.org/10.1038/srep36328.

Sun, Xiaoyan, Zhenhua Song, Yawei Si, and Jin Hui Wang. 2018. "MicroRNA and MRNA Profiles in Ventral Tegmental Area Relevant to Stress-Induced Depression and Resilience." *Progress in Neuro-Psychopharmacology and Biological Psychiatry* 86 (August): 150–65. https://doi.org/10.1016/j.pnpbp.2018.05.023.

Sur, Cyrille, Keith A. Wafford, David S. Reynolds, Karen L. Hadingham, Frances Bromidge, Alison Macaulay, Neil Collinson, et al. 2001. "Loss of the Major GABAa Receptor Subtype in the Brain Is Not Lethal in Mice." *Journal of Neuroscience* 21 (10): 3409–18. https://doi.org/10.1523/jneurosci.21-10-03409.2001.

Sutton, Michael A., and Erin M. Schuman. 2006. "Dendritic Protein Synthesis, Synaptic Plasticity, and Memory." *Cell* 127 (1): 49–58. https://doi.org/10.1016/j.cell.2006.09.014.

Swaminathan, M., C. Fung, D. I. Finkelstein, J. C. Bornstein, and J. P.P. Foong. 2019. "α-Synuclein Regulates Development and Function of Cholinergic Enteric Neurons in the Mouse Colon." *Neuroscience* 423 (December): 76–85. https://doi.org/10.1016/j.neuroscience.2019.10.029.

Szegő, Éva M., Antonio Dominguez-Meijide, Ellen Gerhardt, Annekatrin König, David J. Koss, Wen Li, Raquel Pinho, et al. 2019. "Cytosolic Trapping of a Mitochondrial Heat Shock Protein Is an Early Pathological Event in Synucleinopathies." *Cell Reports* 28 (1): 65-77.e6. https://doi.org/10.1016/j.celrep.2019.06.009.

Taketo, M., and T. Yoshioka. 2000. "Developmental Change of GABA(A) Receptor-Mediated Current in Rat Hippocampus." *Neuroscience* 96 (3): 507–14. https://doi.org/10.1016/S0306-4522(99)00574-6.

Takeuchi, Masakazu, Yutaka Hata, Kazuyo Hirao, Atsushi Toyoda, Mina Irie, and Yoshimi Takai. 1997. "SAPAPs." *Journal of Biological Chemistry* 272 (18): 11943–51. https://doi.org/10.1074/jbc.272.18.11943.

Tanaka, Tetsuya, Shingo Haneda, Kazuhiko Imakawa, Senkiti Sakai, and Kentaro Nagaoka. 2009. "A MicroRNA, MiR-101a, Controls Mammary Gland Development by Regulating Cyclooxygenase-2 Expression." *Differentiation* 77 (2): 181–87. https://doi.org/10.1016/j.diff.2008.10.001.

Tatenhorst, Lars, Katrin Eckermann, Vivian Dambeck, Luis Fonseca-Ornelas, Hagen Walle, Tomás Lopes da Fonseca, Jan C. Koch, et al. 2016. "Fasudil Attenuates Aggregation of α-Synuclein in Models of Parkinson's Disease." *Acta Neuropathologica Communications* 4: 39. https://doi.org/10.1186/s40478-016-0310-y.

Tatura, Roman, Theo Kraus, Armin Giese, Thomas Arzberger, Malte Buchholz, Günter Höglinger, and Ulrich Müller. 2016. "Parkinson's Disease: SNCA-, PARK2-, and LRRK2- Targeting MicroRNAs Elevated in Cingulate Gyrus." *Parkinsonism and Related Disorders* 33 (December): 115–21. https://doi.org/10.1016/j.parkreldis.2016.09.028.

Tenreiro, Sandra, Katrin Eckermann, and Tiago F Outeiro. 2014. "Protein Phosphorylation in Neurodegeneration : Friend or Foe ? AGGREGATION" 7 (May): 1–30. https://doi.org/10.3389/fnmol.2014.00042.

Teskey, G. Campbell, Marie-H. Monfils, Greg Silasi, and Bryan Kolb. 2006. "Neocortical Kindling Is Associated with Opposing Alterations in Dendritic Morphology in Neocortical Layer V and Striatum from Neocortical Layer III." *Synapse* 59 (1): 1–9. https://doi.org/10.1002/syn.20215.

Teychenné, Paul F., Michael G. Ziegler, C. Raymond Lake, and S. J. Enna. 1982. "Low CSF GABA in Parkinsonian Patients Who Respond Poorly to Therapy or Suffer from the 'On-off' Phenomenon." *Annals of Neurology* 11 (1): 76–79. https://doi.org/10.1002/ana.410110113.

Thomas, Paul D., Anish Kejariwal, Nan Guo, Huaiyu Mi, Michael J. Campbell, Anushya Muruganujan, and Betty Lazareva-Ulitsky. 2006. "Applications for Protein Sequence-Function Evolution Data: MRNA/Protein Expression Analysis and Coding SNP Scoring Tools." *Nucleic Acids Research* 34 (WEB. SERV. ISS.). https://doi.org/10.1093/nar/gkl229.

Tiedge, Henri, and Jürgen Brosius. 1996. "Translational Machinery in Dendrites of Hippocampal Neurons in Culture." *Journal of Neuroscience* 16 (22): 7171–81. https://doi.org/10.1523/jneurosci.16-22-07171.1996.

Titze-de-Almeida, Ricardo, and Simoneide Souza Titze-de-Almeida. 2018. “MiR-7 Replacement Therapy in Parkinson’s Disease.” *Current Gene Therapy* 18 (3): 143–53. https://doi.org/10.2174/1566523218666180430121323.

Tofaris, G. K., H. T. Kim, R. Hourez, J.-W. Jung, K. P. Kim, and A. L. Goldberg. 2011. “Ubiquitin Ligase Nedd4 Promotes -Synuclein Degradation by the Endosomal-Lysosomal Pathway.” *Proceedings of the National Academy of Sciences* 108 (41): 17004–9. https://doi.org/10.1073/pnas.1109356108.

Tofaris, George K., Robert Layfield, and Maria Grazia Spillantini. 2001. “α-Synuclein Metabolism and Aggregation Is Linked to Ubiquitin-Independent Degradation by the Proteasome.” *FEBS Letters* 509 (1): 22–26. https://doi.org/10.1016/S0014-5793(01)03115-5.

Tokue, Moe, Kanako Ikami, Seiya Mizuno, Chiyo Takagi, Asuka Miyagi, Ritsuko Takada, Chiyo Noda, et al. 2017. “SHISA6 Confers Resistance to Differentiation-Promoting Wnt/β-Catenin Signaling in Mouse Spermatogenic Stem Cells.” *Stem Cell Reports* 8 (3): 561–75. https://doi.org/10.1016/j.stemcr.2017.01.006.

Tönges, Lars, Tobias Frank, Lars Tatenhorst, Kim A. Saal, Jan C. Koch, Éva M. Szego, Mathias Bähr, Jochen H. Weishaupt, and Paul Lingor. 2012. “Inhibition of Rho Kinase Enhances Survival of Dopaminergic Neurons and Attenuates Axonal Loss in a Mouse Model of Parkinson’s Disease.” *Brain* 135 (11): 3355–70. https://doi.org/10.1093/brain/aws254.

Trachtenberg, Joshua T., Brian E. Chen, Graham W. Knott, Guoping Feng, Joshua R. Sanes, Egbert Welker, and Karel Svoboda. 2002. “Long-Term in Vivo Imaging of Experience-Dependent Synaptic Plasticity in Adult Cortex.” *Nature* 420 (6917): 788–94. https://doi.org/10.1038/nature01273.

Tsuchida, Takeyuki, Tatsuo Mano, Kagari Koshi-Mano, Taro Bannai, Tomoyasu Matsubara, Satoshi Yamashita, Toshikazu Ushijima, et al. 2018. “Methylation Changes and Aberrant Expression of FGFR3 in Lewy Body Disease Neurons.” *Brain Research* 1697 (October): 59–66. https://doi.org/10.1016/j.brainres.2018.06.017.

Ueda, Kenji, Hisashi Fukushima, Eliezer Masliah, Y U Xia, Akihiko Iwai, Makoto Yoshimoto, Deborah A C Otero, Jun Kondo, Yasuo Ihara, and Tsunao Saitoh. 1993. “Molecular Cloning of CDNA Encoding an Unrecognized Component of Amyloid in Alzheimer Disease (Neurodegeneration/Chaperone/Amyloid P/A4 Protein/Neuritic Plaque).” *Proc. Natl. Acad. Sci. USA* 90 (December): 11282–86. https://www.pnas.org/content/pnas/90/23/11282.full.pdf.

Ungerstedt, Urban. 1968. “6-Hydroxy-Dopamine Induced Degeneration of Central Monoamine Neurons.” *European Journal of Pharmacology* 5 (1): 107–10. https://doi.org/10.1016/0014-2999(68)90164-7.

Urbizu, Aintzane, and Katrin Beyer. 2020. “Epigenetics in Lewy Body Diseases: Impact on Gene Expression, Utility as a Biomarker, and Possibilities for Therapy.” *International Journal of Molecular Sciences* 21 (13): 1–31. https://doi.org/10.3390/ijms21134718.

Uversky, Vladimir N., Jie Li, and Anthony L. Fink. 2001. “Evidence for a Partially Folded Intermediate in Alpha-Synuclein Fibril Formation.” *The Journal of Biological Chemistry* 276 (14): 10737–44. https://doi.org/10.1074/jbc.M010907200.

Uversky, Vladimir N, Jie Li, Kiowa Bower, and Anthony L Fink. 2002. “Synergistic Effects of Pesticides and Metals on the Fibrillation of a -Synuclein : Implications for Parkinson ’ s Disease” 23: 527–36.

Valente, E M, P M Abou-Sleiman, V Caputo, M M Muqit, K Harvey, S Gispert, Z Ali, et al. 2004. “Hereditary Early-Onset Parkinson’s Disease Caused by Mutations in PINK1.” *Science* 304 (5674): 1158–60. https://doi.org/10.1126/science.1096284.

Valera, Elvira, Brian Spencer, Jennifer Mott, Margarita Trejo, Anthony Adame, Michael Mante, Edward Rockenstein, et al. 2017. “MicroRNA-101 Modulates Autophagy and Oligodendroglial Alpha-Synuclein Accumulation in Multiple System Atrophy.” *Frontiers in Molecular Neuroscience* 10 (October): 1–13. https://doi.org/10.3389/fnmol.2017.00329.

Vallerga, Costanza L., Futao Zhang, Javed Fowdar, Allan F. McRae, Ting Qi, Marta F. Nabais, Qian Zhang, et al. 2020. “Analysis of DNA Methylation Associates the Cystine–Glutamate Antiporter SLC7A11 with Risk of Parkinson’s Disease.” *Nature Communications* 11 (1): 1–10. https://doi.org/10.1038/s41467-020-15065-

7.

Vasconcellos, L F, and J S Pereira. 2015. “Parkinson’s Disease Dementia: Diagnostic Criteria and Risk Factor Review.” *J Clin Exp Neuropsychol* 37 (9): 988–93. https://doi.org/10.1080/13803395.2015.1073227.

Vekrellis, Kostas, Hardy J Rideout, and Leonidas Stefanis. 2004. “Neurobiology of α-Synuclein.” *Molecular Neurobiology* 30 (1): 001–022. https://doi.org/10.1385/MN:30:1:001.

Vekrellis, Kostas, Maria Xilouri, Evangelia Emmanouilidou, Hardy J Rideout, and Leonidas Stefanis. 2011. “Pathological Roles of α-Synuclein in Neurological Disorders.” *The Lancet. Neurology* 10 (11): 1015–25. https://doi.org/10.1016/S1474-4422(11)70213-7.

Vicente Miranda, Hugo, and Tiago Fleming Outeiro. 2010. “The Sour Side of Neurodegenerative Disorders: The Effects of Protein Glycation.” *Journal of Pathology* 221 (1): 13–25. https://doi.org/10.1002/path.2682.

Vilar, Marçal, Hui-Ting Chou, Thorsten Lührs, Samir K Maji, Dominique Riek-Loher, Rene Verel, Gerard Manning, Henning Stahlberg, and Roland Riek. 2008. “The Fold of Alpha-Synuclein Fibrils.” *Proceedings of the National Academy of Sciences of the United States of America* 105 (25): 8637–42. https://doi.org/10.1073/pnas.0712179105.

Vilardo, Elisa, Christian Barbato, Maria Teresa Ciotti, Carlo Cogoni, and Francesca Ruberti. 2010. “MicroRNA-101 Regulates Amyloid Precursor Protein Expression in Hippocampal Neurons.” *Journal of Biological Chemistry* 285 (24): 18344–51. https://doi.org/10.1074/jbc.M110.112664.

Vilas, D, A Iranzo, E Tolosa, I Aldecoa, J Berenguer, I Vilaseca, C Marti, et al. 2016. “Assessment of Alpha-Synuclein in Submandibular Glands of Patients with Idiopathic Rapid-Eye-Movement Sleep Behaviour Disorder: A Case-Control Study.” *Lancet Neurol* 15 (7): 708–18. https://doi.org/10.1016/S1474-4422(16)00080-6.

Villalba, Rosa M., Abraham Mathai, and Yoland Smith. 2015. “Morphological Changes of Glutamatergic Synapses in Animal Models of Parkinson’s Disease.” *Frontiers in Neuroanatomy*. Frontiers Research Foundation. https://doi.org/10.3389/fnana.2015.00117.

Villalba, Rosa M., and Yoland Smith. 2010. “Striatal Spine Plasticity in Parkinson’s Disease.” *Frontiers in Neuroanatomy*. Frontiers Media SA. https://doi.org/10.3389/fnana.2010.00133.

Volles, M. J., S. J. Lee, J. C. Rochet, M. D. Shtilerman, T. T. Ding, J. C. Kessler, and P. T. Lansbury. 2001. “Vesicle Permeabilization by Protofibrillar α-Synuclein: Implications for the Pathogenesis and Treatment of Parkinson’s Disease.” *Biochemistry* 40 (26): 7812–19. https://doi.org/10.1021/bi0102398.

Volpicelli-Daley, L A, K C Luk, T P Patel, S A Tanik, D M Riddle, A Stieber, D F Meaney, J Q Trojanowski, and V M Lee. 2011. “Exogenous Alpha-Synuclein Fibrils Induce Lewy Body Pathology Leading to Synaptic Dysfunction and Neuron Death.” *Neuron* 72 (1): 57–71. https://doi.org/10.1016/j.neuron.2011.08.033.

Waller, Rachel, Matthew Wyles, Paul R. Heath, Mbombe Kazoka, Helen Wollff, Pamela J. Shaw, and Janine Kirby. 2018. “Small RNA Sequencing of Sporadic Amyotrophic Lateral Sclerosis Cerebrospinal Fluid Reveals Differentially Expressed MiRNAs Related to Neural and Glial Activity.” *Frontiers in Neuroscience* 11 (JAN): 731. https://doi.org/10.3389/fnins.2017.00731.

Wang, Chuan-Jie, Yi Wu, Qun Zhang, Ke-Wei Yu, and Yu-Yang Wang. 2019. “An Enriched Environment Promotes Synaptic Plasticity and Cognitive Recovery after Permanent Middle Cerebral Artery Occlusion in Mice.” *Neural Regeneration Research* 14 (3): 462. https://doi.org/10.4103/1673-5374.245470.

Wang, Fushun, Tifei Yuan, Alfredo Pereira, Alexei Verkhratsky, and Jason H. Huang. 2016. “Glial Cells and Synaptic Plasticity.” *Neural Plasticity*. Hindawi Limited. https://doi.org/10.1155/2016/5042902.

Wang, Sheng, Xuan Zhang, Yuanyuan Guo, Han Rong, and Tiebang Liu. 2017. “The Long Noncoding RNA HOTAIR Promotes Parkinson’s Disease by Upregulating LRRK2 Expression.” *Oncotarget* 8 (15): 24449–56. https://doi.org/10.18632/oncotarget.15511.

Wang, Xinghui, Qianqian Chen, Sheng Yi, Qianyan Liu, Ruirui Zhang, Pan Wang, Tianmei Qian, and Shiying Li. 2019. “The MicroRNAs Let-7 and MiR-9 down-Regulate the Axon-Guidance Genes Ntn1 and Dcc during Peripheral Nerve Regeneration.” *Journal of Biological Chemistry* 294 (10): 3489–3500. https://doi.org/10.1074/jbc.RA119.007389.

Welch, Jeffrey M., Dongqing Wang, and Guoping Feng. 2004a. “Differential MRNA Expression and Protein

Localization of the SAP90/PSD-95-Associated Proteins (SAPAPs) in the Nervous System of the Mouse." *Journal of Comparative Neurology* 472 (1): 24–39. https://doi.org/10.1002/cne.20060.

———. 2004b. "Differential MRNA Expression and Protein Localization of the SAP90/PSD-95-Associated Proteins (SAPAPs) in the Nervous System of the Mouse." *Journal of Comparative Neurology* 472 (1): 24–39. https://doi.org/10.1002/cne.20060.

Wersinger, Christophe, and Anita Sidhu. 2003. "Attenuation of Dopamine Transporter Activity by α-Synuclein." *Neuroscience Letters* 340 (3): 189–92. https://doi.org/10.1016/S0304-3940(03)00097-1.

Williamson, Roger A., Michael D. Henry, Karla J. Daniels, Ronald F. Hrstka, Jane C. Lee, Yoshihide Sunada, Oxana Ibraghimov-Beskrovnaya, and Kevin P. Campbell. 1997. "Dystroglycan Is Essential for Early Embryonic Development: Disruption of Reichert's Membrane in Dag1-Null Mice." *Human Molecular Genetics* 6 (6): 831–41. https://doi.org/10.1093/hmg/6.6.831.

Winner, Beate, Martin Regensburger, Sebastian Schreglmann, Leah Boyer, Iryna Prots, Edward Rockenstein, Michael Mante, et al. 2012. "Role of -Synuclein in Adult Neurogenesis and Neuronal Maturation in the Dentate Gyrus." *Journal of Neuroscience* 32 (47): 16906–16. https://doi.org/10.1523/JNEUROSCI.2723-12.2012.

Witwer, Kenneth W., and Marc K. Halushka. 2016. "Toward the Promise of MicroRNAs – Enhancing Reproducibility and Rigor in MicroRNA Research." *RNA Biology*. Taylor and Francis Inc. https://doi.org/10.1080/15476286.2016.1236172.

Woodcock, D.M., P.J. Crowther, and W.P. Diver. 1987. "The Majority of Methylated Deoxytidines in Human DNA Are Not in the CpG Dinucleotide." *Biochemical and Biophysical Research Communications* 145: 888–94. http://www.nature.com/articles/s41467-019-10101-7.

Wright, Kevin M., Krissy A. Lyon, Haiwen Leung, Daniel J. Leahy, Le Ma, and David D. Ginty. 2012. "Dystroglycan Organizes Axon Guidance Cue Localization and Axonal Pathfinding." *Neuron* 76 (5): 931–44. https://doi.org/10.1016/j.neuron.2012.10.009.

Wu, Qihui, Hajime Takano, Dawn M. Riddle, John Q. Trojanowski, Douglas A. Coulter, and Virginia M.-Y. Lee. 2019. "α-Synuclein (ASyn) Preformed Fibrils Induce Endogenous ASyn Aggregation, Compromise Synaptic Activity and Enhance Synapse Loss in Cultured Excitatory Hippocampal Neurons." *The Journal of Neuroscience* 39 (26): 5080–94. https://doi.org/10.1523/jneurosci.0060-19.2019.

Wu, Xiaojuan, Xingjun Meng, Feng Tan, Zhigang Jiao, Xiuping Zhang, Huichun Tong, Xiaoliang He, Xiaodong Luo, Pingyi Xu, and Shaogang Qu. 2019. "Regulatory Mechanism of MiR-543-3p on GLT-1 in a Mouse Model of Parkinson's Disease." *ACS Chemical Neuroscience* 10 (3): 1791–1800. https://doi.org/10.1021/acschemneuro.8b00683.

Wu, Yuanming, Wengang Wang, Ana Díez-Sampedro, and George B. Richerson. 2007. "Nonvesicular Inhibitory Neurotransmission via Reversal of the GABA Transporter GAT-1." *Neuron* 56 (5): 851–65. https://doi.org/10.1016/j.neuron.2007.10.021.

Xu, Xiaonan, Chengle Zhuang, Zimu Wu, Hongyan Qiu, Haixia Feng, and Jun Wu. 2018. "LincRNA-P21 Inhibits Cell Viability and Promotes Cell Apoptosis in Parkinson's Disease through Activating α -Synuclein Expression." *BioMed Research International* 2018. https://doi.org/10.1155/2018/8181374.

Xue, Gang, Gang Wang, Qianqian Shi, Hui Wang, Bo Min Lv, Min Gao, Xiaohui Niu, and Hong Yu Zhang. 2020. "Interpreting the Dynamic Pathogenesis of Parkinson's Disease by Longitudinal Blood Transcriptome Analysis." *BioRxiv*. https://doi.org/10.1101/2020.10.26.356204.

Yamada, Masahito, Yoshiaki Wada, Hiroshi Tsukagoshi, EI Ichi Otomo, and Michio Hayakawa. 1988. "A Quantitative Golgi Study of Basal Dendrites of Hippocampal CAl Pyramidal Cells in Senile Dementia of Alzheimer Type." *Journal of Neurology, Neurosurgery and Psychiatry* 51 (8): 1088–90. https://doi.org/10.1136/jnnp.51.8.1088.

Yan, Qi, Chengming Zhu, Shouhong Guang, and Xuezhu Feng. 2019. "The Functions of Non-Coding RNAs in RRNA Regulation." *Frontiers in Genetics* 10 (April). https://doi.org/10.3389/fgene.2019.00290.

Yeung, Rigil K., Zheng Hua Xiang, Shui Ying Tsang, Rui Li, Timothy Y.C. Ho, Qi Li, Chok King Hui, Pak Chung Sham, Ming Qi Qiao, and Hong Xue. 2018. "Gabrb2-Knockout Mice Displayed Schizophrenia-like and Comorbid

Phenotypes with Interneuron-Astrocyte-Microglia Dysregulation." *Translational Psychiatry* 8 (1): 128. https://doi.org/10.1038/s41398-018-0176-9.

Yin, Guo Nan, Ho Won Lee, Je Yoel Cho, and Kyoungho Suk. 2009. "Neuronal Pentraxin Receptor in Cerebrospinal Fluid as a Potential Biomarker for Neurodegenerative Diseases." *Brain Research* 1265 (April): 158–70. https://doi.org/10.1016/j.brainres.2009.01.058.

Yoshino, Yuta, Bhaskar Roy, and Yogesh Dwivedi. 2021. "Differential and Unique Patterns of Synaptic MiRNA Expression in Dorsolateral Prefrontal Cortex of Depressed Subjects." *Neuropsychopharmacology* 46 (5): 900–910. https://doi.org/10.1038/s41386-020-00861-y.

You, H, L L Mariani, G Mangone, D Le Febvre de Nailly, F Charbonnier-Beaupel, and J C Corvol. 2018. "Molecular Basis of Dopamine Replacement Therapy and Its Side Effects in Parkinson's Disease." *Cell Tissue Res* 373 (1): 111–35. https://doi.org/10.1007/s00441-018-2813-2.

Yuan, Li Lian, and Xixi Chen. 2006. "Diversity of Potassium Channels in Neuronal Dendrites." *Progress in Neurobiology*. Pergamon. https://doi.org/10.1016/j.pneurobio.2006.03.003.

Zaja-Milatovic, S., D. Milatovic, A. M. Schantz, J. Zhang, K. S. Montine, A. Samii, A. Y. Deutch, and T. J. Montine. 2005. "Dendritic Degeneration in Neostriatal Medium Spiny Neurons in Parkinson Disease." *Neurology* 64 (3): 545–47. https://doi.org/10.1212/01.WNL.0000150591.33787.A4.

Zaqout, Sami, and Angela M. Kaindl. 2016. "Golgi-Cox Staining Step by Step." *Frontiers in Neuroanatomy* 10 (March). https://doi.org/10.3389/fnana.2016.00038.

Zarranz, J J, J Alegre, J C Gomez-Esteban, E Lezcano, R Ros, I Ampuero, L Vidal, et al. 2004. "The New Mutation, E46K, of Alpha-Synuclein Causes Parkinson and Lewy Body Dementia." *Ann Neurol* 55 (2): 164–73. https://doi.org/10.1002/ana.10795.

Zhao, C. L., H. A. Cui, and X. R. Zhang. 2021. "MiR-543-5p Inhibits Inflammation and Promotes Nerve Regeneration through Inactivation of the NF-KB in Rats after Spinal Cord Injury." *European Review for Medical and Pharmacological Sciences* 23: 39–46. https://doi.org/10.26355/eurrev_201908_18626.

Zhu, Jinwei, Qingqing Zhou, Yuan Shang, Hao Li, Mengjuan Peng, Xiao Ke, Zhuangfeng Weng, et al. 2017. "Synaptic Targeting and Function of SAPAPs Mediated by Phosphorylation-Dependent Binding to PSD-95 MAGUKs." *Cell Reports* 21 (13): 3781–93. https://doi.org/10.1016/j.celrep.2017.11.107.

Zhu, Qing-Yuan, Qin Liu, Jian-Xia Chen, Ke Lan, and Bao-Xue Ge. 2010. "MicroRNA-101 Targets MAPK Phosphatase-1 To Regulate the Activation of MAPKs in Macrophages." *The Journal of Immunology* 185 (12): 7435–42. https://doi.org/10.4049/jimmunol.1000798.

Zigoneanu, Imola G., Yoo Jeong Yang, Alexander S. Krois, Md Emdadul Haque, and Gary J. Pielak. 2012. "Interaction of α-Synuclein with Vesicles That Mimic Mitochondrial Membranes." *Biochimica et Biophysica Acta - Biomembranes* 1818 (3): 512–19. https://doi.org/10.1016/j.bbamem.2011.11.024.

Zongaro, Samantha, Renate Hukema, S. D'Antoni, Laetitia Davidovic, Pascal Barbry, Maria Vincenza Catania, Rob Willemsen, Bernard Mari, and Barbara Bardoni. 2013. "The 3' UTR of FMR1 MRNA Is a Target of MiR-101, MiR-129-5p and MiR-221: Implications for the Molecular Pathology of FXTAS at the Synapse." *Human Molecular Genetics* 22 (10): 1971–82. https://doi.org/10.1093/hmg/ddt044.

Züchner, S., J. R. Wendland, A. E. Ashley-Koch, A. L. Collins, K. N. Tran-Viet, K. Quinn, K. C. Timpano, et al. 2009. "Multiple Rare SAPAP3 Missense Variants in Trichotillomania and OCD." *Molecular Psychiatry*. Nature Publishing Group. https://doi.org/10.1038/mp.2008.83.

Curriculum Vitae

Personal Information

Full name:	Maria Xylaki
Date/ Place of birth:	17 May 1991/ Chania, Crete, Greece
Nationality:	Greek

Academic Qualification

Sep 2017 – June 2021	**Ph.D. Student** Ph.D. Programme "Cellular and Molecular Physiology of the Brain", Göttingen Graduate School for Neurosciences, Biophysics and Molecular Biosciences, Georg August University Göttingen **Ph.D. Thesis in Prof. Dr. Outeiro´s lab** Department of Experimental Neurodegeneration, University Medical Center Göttingen *"The role of RNA in synapse physiology and neurodegeneration in models of Parkinson's disease"*
Nov 2014 – Nov 2016	**M.Sc. in Molecular Medicine - Specialty in Neurobiology** Interdepartmental MSc Programme in Molecular Medicine, University of Athens **M.Sc. Thesis in Dr. Vekrellis' lab** Laboratory of Neurodegenerative Diseases, Center of Basic Research, Biomedical Research Foundation of the Academy of Athens (BRFAA) *"Effect of phospholipase A2 inhibitors in α-synuclein mediated toxicity and inflammation in vitro and in vivo"*
Oct 2009 – Apr 2014	**B.Sc. in Chemistry** Department of Chemistry, School of Science, University of Athens, Greece **B.Sc. thesis in Prof. Dr. Kokotos' lab** Laboratory of Organic Chemistry, Department of Chemistry, School of Sciences, University of Athens, Greece *"Synthesis of Vorinostat and study of the catalytic synthesis of amides"*