From THE DEPARTMENT OF PHYSIOLOGY AND PHARMACOLOGY
Karolinska Institutet, Stockholm, Sweden

STRIATAL GLUTAMATERGIC NEUROTRANSMISSION AND PLASTICITY IN PARKINSON'S DISEASE AND AGING

Mona Nouhi

Karolinska Institutet

Stockholm 2018

Published by Karolinska Institutet.
Printed by Eprint AB 2018
Cover illustration by Owen Mortensen

ISBN 978-91-7831-049-4

Striatal glutamatergic neurotransmission and plasticity in Parkinson's disease and aging

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

Mona Nouhi

Principal Supervisor:
Associate professor Karima Chergui
Karolinska Institutet
Department of Physiology and Pharmacology
Division of Molecular Neurophysiology

Co-supervisor(s):
Professor Per Svenningsson
Karolinska Institutet
Department of Clinical Neuroscience
Division of Translational Neuropharmacology

Dr. Xiaoqun Zhang
Karolinska Institutet
Department of Clinical Neuroscience
Division of Translational Neuropharmacology

Opponent:
Associate professor Tomas Björklund
Lund University
Department of Experimental Medical Science
Division of Molecular Neuromodulation

Examination Board:
Associate professor André Fisahn
Karolinska Institutet
Department of Neurobiology, Care Sciences and Society
Division of Neurogeriatrics

Associate professor Kent Jardemark
Karolinska Institutet
Department of Physiology and Pharmacology
Division of Translational Pharmacology

Professor Robert Fredriksson
Uppsala University
Department of Pharmaceutical Biosciences
Division of Molecular Neuropharmacology

To whom I have no words to express my love and gratitude for

To whom I owe everything

To my mom

A functionally “normal” brain is a changing brain, a brain whose capacity and mechanisms of change are shifting appropriately from one time point in life to another

Lindsay Oberman

ABSTRACT

Parkinson's disease (PD) is a devastating neurodegenerative disorder with aging as the main risk factor. PD is characterized by severe movement disturbances but is also associated with non-motor symptoms. Affected motor functions in PD are due to alteration in the basal ganglia circuitry as a consequence of loss of dopaminergic neurons which project to the striatum, an important part of basal ganglia. One important mechanism controlling the neuronal activity within striatum and hence motor functions and learning is synaptic plasticity. Synaptic plasticity in striatum is highly dependent on efficient interactions between two neurotransmitters, dopamine and glutamate. Long term potentiation (LTP) form of synaptic plasticity in striatum is dependent on glutamatergic neurotransmission through NMDA receptors. As a result of progressive degeneration of the dopaminergic neurons in the substantia nigra, the glutamatergic neurotransmission is altered. This alteration directly affects LTP as it has been shown in different animal models of PD that LTP is lost in striatum of PD models.

Synaptic plasticity and it's mechanisms of induction in experimental settings has been studied extensively for over a century, especially in hippocampus. Studying plasticity in striatum has been much more complicated due to cellular heterogeneity and random distribution of different cell types within striatum. Also, how different types of plasticity in the principal projection neurons are modulated by dopamine and other modulatory neurotransmitters in striatum is not clear. Moreover, experimental settings and different protocols for induction of synaptic plasticity in striatum in both in-vivo and in-vitro conditions results in different outcomes and effects the direction of the plasticity.

This thesis aimed to study how glutamatergic neurotransmission and LTP are affected in striatum upon dopaminergic degeneration and with aging. In paper I of this thesis we investigated the difference in using different electrophysiological recording conditions in induction of LTP in dorsolateral part of striatum using same induction protocol (high frequency stimulation). Based on our results we establish that high frequency stimulation induces opposing forms of dopamine-dependent synaptic plasticity in the striatum depending on recording method. We also conclude that cell-attached and field potential recordings can be useful methods studying LTP in striatum as they do not alter the intracellular milieu of the neurons. In paper II we studied the effect of a positive allosteric modulator of NMDA receptors containing GluN2D/2C, CIQ, on synaptic plasticity and behavioral deficits in a mouse model of PD. We demonstrated that by using CIQ we can rescue the lost LTP in 6-OHDA lesion model of PD and improve forelimb-use asymmetry. As aging is the main risk factor for developing PD in Paper III we investigated the effect of aging on LTP and the effect of CIQ on LTP, because we had shown beneficial effect of this compound on LTP in a PD model. Our result demonstrates that LTP is lost in the striatum of aged mice; however this loss does not share same mechanisms as seen in PD and LTP is not rescued by CIQ.

In conclusion the findings presented in this thesis help to better understand and study the mechanisms of synaptic plasticity in striatum under in-vitro experimental procedures. Our findings also suggest that targeting GluN2D containing NMDA receptors might have potential therapeutic implications for intervention in Parkinson's disease.

LIST OF SCIENTIFIC PAPERS

I. Skiteva O, Yao N, **Nouhi M** and Chergui K. (2017) High frequency stimulation induces LTD of AMPA receptor-mediated postsynaptic responses and LTP of synaptically-evoked firing in the dorsolateral striatum. *Neuroscience Letters* 14;666:11-16

II. **Nouhi M**, Zhang X, Yao N and Chergui K. (2017) CIQ, a positive allosteric modulator of GluN2C/D-containing N-methyl-d-aspartate receptors, rescues striatal synaptic plasticity deficit in a mouse model of Parkinson's disease. *CNS Neuroscience &Therapeutics* 24(2):144-153

III. **Nouhi M**, Yao N and Chergui K (2018) A positive allosteric modulator of GluN2C/D-containing NMDA receptors fails to rescue impaired striatal synaptic plasticity in aged mice. *Manuscript*

CONTENTS

LIST OF ABBREVIATIONS

AADC	Aromatic amino acid decarboxylase
ABD	Agonist-binding domain
Ach	Acethylcholine
ADP	Adenosine diphosphate
AMPA-R	α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors
ATP	Adenosine triphosphate
BG	Basal ganglia
Chl	Cholinergic interneuron
DA	Dopamine
DBS	Deep brain stimulation
D1R	Dopamine type 1 receptor
D2R	Dopamine type 2 receptor
EPSCs	Excitatory postsynaptic currents
ER	Endoplasmic reticulum
fEPSP/PS	Field excitatory postsynaptic potential/population spike
FSI	Fast spiking interneurons
GP	Globus pallidus
GPe	Globus pallidus external segment
GPi	Globus pallidus internal segment
HFS	High frequency stimulation
iGluRs	Ionotropic glutamate receptors
IPSCs	Inhibitory postsynaptic currents
LB	Lewy body
L-DOPA	L-3,4-dihydroxyphenylalanine/levodopa
LRRK-2	Leucine-rich repeat kinase 2
LTD	Long-term depression
LTP	Long-term potentiation
mGluRs	Metabotropic glutamate receptors
MPTP	1-Methyl-4-phenyl-1, 2, 5, 6-tetrahydropyridine

MSN	Medium spiny neuron
mtDNA	Mitochondrial DNA
NMDA-R	N-methyl-D-aspartate receptors
NTD	N-terminal domain
Nurr-1	Nuclear receptor related 1 protein
PAMs	Positive allosteric modulators
PD	Parkinson’s disease
PLTS	Persistent and low-threshold spike interneurons
ROS	Reactive oxygen species
SN	Substantia nigra
SNc	Substantia nigra pars compacta
SNr	Substantia nigra pars reticulate
STN	Subthalamic nucleus
TH	Tyrosine hydroxylase
6-OHDA	6-hydroxydopamine
α-syn	Alpha-synuclein

1 INTRODUCTION

1.1 PARKINSON'S DISEASE

Parkinson's disease (PD) is the second most common neurodegenerative disorder after Alzheimer's disease (Lee and Gilbert 2016, Michel, Hirsch et al. 2016). James Parkinson first described PD back in 1817 (Wood-Kaczmar, Gandhi et al. 2006). In the "essay on the shaking palsy" he describes the main clinical features of PD as: rigidity, bradykinesia and resting tremor that are considered as the key motor symptoms (Przedborski 2017). PD is foremost a sporadic disease with less than 10% inherited cases and age as the main risk factor as the average age of onset is 60 of years (Tysnes and Storstein 2017) (Michel, Hirsch et al. 2016). For example mutations in the two autosomal dominant genes; Leucine-rich repeat kinase-2 (LRRK2) and Alpha-synuclein (α-Syn) are associated with a rare form of familial PD. Also, Parkin and DJ-1 are the two identified autosomal recessive genes which when mutated can give rise to PD (Kalia and Lang 2015).

Two decades later after the first description of PD by James Parkinson, Trétiakoff reports the neuropathological alterations associated with PD (Przedborski 2017). In PD the ability to control voluntary movements is lost as a consequence of profound alterations in the functional organization of a group of subcortical nuclei, the basal ganglia (BG) (Afifi 2003). PD is a progressive disease in which the clinical symptoms are manifested as a result of degeneration of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNpc), one of the nuclei that compose the basal ganglia. Motor symptoms of PD are manifested when dopamine neuronal loss reaches 50-60% and after 70-80% loss of dopamine terminals in the striatum (Masilamoni and Smith 2018). This loss of DA neurons in SNpc results in a typical depigmentation of this brain region as neurons of SNpc contain high amount of neuromelanin (Przedborski 2017). Additionally presence of intraneuronal proteinaceous cytoplasmic inclusions termed "Lewy bodies" (LB) is another pathological hallmark of PD (Lees, Hardy et al. 2009). Neurodegeneration and LB formation is not solely limited to BG but also are present in other brain regions such as hippocampus and locus coreleus. This spread of disease pathology to different brain structures may explain the development of non-motor symptoms ;which often precede the motor symptoms; such as cognitive impairments; sleep disorders, fatigue and psychiatric symptoms among others (Przedborski 2017).

Although to date the mechanisms that lead to neurodegeneration and inclusion formation are still unclear, several pathways and mechanisms have been suggested as potential candidates. These pathways are believed to be altered pararllely and in different stages of the disease or as a cascade of events. Mitochondrial dysfunctions, protein homeostasis, inflammation and environmental toxins alongside the genetic factors mentioned above are described as pathogenesis in PD. Also it has been suggested that neuroinflammation which is prompted by dopamine cell loss may in fact trigger further neuronal degeneration and hence progression of

the disease (Przedborski 2017). Nonetheless the outcome of alteration in these pathways and final degeneration of DA-neurons cause pathological changes in neurotransmission in the basal ganglia thalamocortical-motor circuit (Wood-Kaczmar, Gandhi et al. 2006, Surmeier, Guzman et al. 2010, Surmeier and Sulzer 2013). Thereby it is believed that the loss of DA-neurons, and hence the affected mechanisms controlling the motor circuit such as plasticity, account for the majority of the motor symptoms (Schirinzi, Madeo et al. 2016). The chain of events and mechanisms between dopamine loss and manifestation of motor symptoms however remain unclear.

1.2 ETIOLOGY OF PD

To date the etiology of PD remains unknown and can only be explained by a combination of environmental and genetic risk factors which together interact and increase the risk of developing PD (Bartels and Leenders 2009). Based on epidemiological studies it has been shown that men have overall higher incidence of PD than women (Wirdefeldt, Adami et al. 2011). Environmental factors such as exposure to neurotoxin 1-Methyl-4-phenyl-1, 2, 5, 6-tetrahydropyridine (MPTP) has been also shown to increase risk of developing PD (Mursaleen and Stamford 2016). MPTP is a byproduct of synthesis of synthetic heroin which causes loss of nerve cells through blocking complex in the mitochondrial electron transport chain (Mursaleen and Stamford 2016). Other environmental risk factors include exposure to pesticides, heavy metals and rural living and use of well water among others (Mursaleen and Stamford 2016). Also head trauma, infection, inflammation and diabetes are some of pre-existing medical conditions that in some studies have been reported to correlate with increased risk of developing PD (Mursaleen and Stamford 2016). Familial components have been studied in monozygotic and dizygotic twins were genetic and environmental risk factors can be studied. Based on these studies late-onset PD is mostly caused by environmental factors and for early-onset PD the important factor is the genetics (Mursaleen and Stamford 2016). 11 genes have been linked to PD; most of the genes have been shown to be involved in the oxidative response pathway, mitochondrial function, and vesicle trafficking and protein degradation pathway with autosomal dominant or autosomal recessive inheritance (Swanson, Sesso et al. 2009, Mursaleen and Stamford 2016). Neuroprotective factors associated with lifestyle such as exercise, Uric acid, nicotine, caffeine, estrogen and calcium modulators have been shown to slow down or minimize risk of developing PD (Swanson, Sesso et al. 2009).

1.3 PATHOGENESIS OF PD

Observation of "Lewy bodies" as the hallmark of Parkinson's disease was first reported by Fritz Heinrich Lewy back in 1912. Postmortem studies confirm presence of Lewy bodies in

almost all regions of the brain from PD patients both with sporadic and familial form of PD (Breydo, Wu et al. 2012). These inclusions are mainly composed of toxic misfolded aggregations of the protein α-synuclein, exclusively in neurons. Additionally, inclusions "Lewy neutritis" are composed of α-synuclein and are also hallmarks of PD pathophysiology. The exact function of α-synuclein is not known, however under normal physiological conditions this protein is mainly located at synaptic terminals, modulating vesicle docking to presynaptic terminal and vesicle release and has protective effects against nerve injury, protecting nerve terminals (Vekrellis, Xilouri et al. 2011, Dehay, Bourdenx et al. 2015). Based on genetic studies, autosomal dominant early-onset PD in some cases are due to missense mutations in the gene (SNCA) encoding α-synuclein (Breydo, Wu et al. 2012). Also, non-genetic factors such as post-translational modifications in form of phosphorylation at sites were under physiological conditions are not or nitration may lead to missfolding of this protein (Dehay, Bourdenx et al. 2015). Another triggering factor for missfolding of α-synuclein can be due to higher expression levels of the protein itself due to alteration in the SNCA transcriptional regulatory mechanisms or in chaperone-mediated autophagy and clearance of the extra number of the protein. Thus, an imbalance or alteration in the levels of synthesis and degradation of this protein may cause an increase in aggregation and oligomerization of α-synuclein into inclusions (Vekrellis, Xilouri et al. 2011). Lewy bodies and Lewy neutritis formation and accumulation disturb normal cellular machinery and functions through targeting different pathways and compartments. For example, various studies confirm impairment of mitochondrial complex I and V activity due to overexpression of oligomeric aggregates of α-synuclein. This in turn may lead to neuronal cell death by indirectly triggering release of reactive oxygen species (Vekrellis, Xilouri et al. 2011). Additionally, neurotoxic effects of α-synuclein overexpression on Ca^{2+} homeostasis have been proposed as another mechanism. One hypothesis is that overexpression of α-synuclein may cause cell death by increasing levels of calcium and proton leaking from lysosomes into cytosol by increasing lysosomal permeability (Post, Lieberman et al. 2018). Importantly dopamine neurons have been shown to be more sensitive and vulnerable to higher levels of α -synuclein compared to other neurons even though non-dopaminergic neuronal loss due to overexpression of α-synuclein is also detected in PD. This is believed to be due to highly branched axons of these neurons and synapses, resulting in a higher bioenergetics demand which can lead to mitochondrial oxidative stress (Pacelli, Giguere et al. 2015). This is confirmed by the observation that striatal DA terminals are lost earlier during the progression of the disease before dopamine cell bodies are degenerated (Kordower, Olanow et al. 2013). How α-synuclein is released into the extracellular space from one neuron is not confirmed but one possible mechanism may be through exocytosis along with secretory vesicles and eventually spreading through a cell-to-cell transmission to other cells and brain regions (Beyer, Domingo-Sabat et al. 2009, Vekrellis, Xilouri et al. 2011).

1.4 SPREAD OF THE DISEASE

According to postmortem investigation comparing healthy and Parkinson's disease human brains staining for α-synuclein inclusions, tracing the spread of inclusions, PD pathology is described to be affecting different parts of the brain in six stages commonly known as Braak staging hypothesis (Goedert, Spillantini et al. 2013). Commonly Lewy bodies emerge first in the olfactory bulb and vagus nerve (stage 1) from there via the pons (stage 2) to the midbrain (stage 3) and to the basal prosencaphalon and mesocortex (stage 4) and finally to the neocortex (stages 5 and 6) (Braak, Ghebremedhin et al. 2004). Stages 1-3 are presymptomatic stages and the symptomatic phase starts after stage 3, when the extend of dopaminergic neuronal loss is great (Dexter and Jenner 2013). It is at this stage which motor symptoms of Parkinson's disease are manifested. Nevertheless, as predicted from the wide spreading of the disease pathology before reaching basal ganglia, non-motor symptoms emerge as Lewy bodies affect non-dopaminergic nuclei of the brain as well (Dexter and Jenner 2013). "For example, constipation as a common NMS is associated with neuronal loss and the presence of Lewy bodies in the dorsal motor nucleus of the vagus, which provides parasympathetic innervation to the stomach and intestine" (Dexter and Jenner 2013). However, this hypothesis is challenged by scientists who demonstrate that Lewy pathology and distribution in different brain regions are very sparse and cell type specific (Surmeier, Obeso et al. 2017). For example, according to some recent works by independent groups it has been shown that GABAergic neurons independently of the brain region studied are never affected by Lewy pathology (Kingsbury, Bandopadhyay et al. 2010, Surmeier, Obeso et al. 2017). In conclusion the theory of PD acting as a Prion-like disease and spread of the pathology in a cell-to-cell fashion resulting in neuronal dysfunction and eventually neurodegeneration needs further investigation and to date it remains ambiguous (Surmeier, Obeso et al. 2017).

1.5 PATHOPHYSIOLOGY OF PD

Mechanisms leading to dopaminergic cell death and development and progression of Parkinson's disease remains to date for most part unknown. Great amount of research is being done worldwide targeting this question which will lead to identification of better symptomatic treatments or hopefully cure of this neurodegenerative disorder. Several mechanisms and cellular alterations have been proposed to be involved as a cascade of events which eventually cause neuronal cell death. Studies done in humans and animal models of PD both in genetic cases and sporadic PD have led to several well confirmed theories of which some are briefly described below.

1.5.1 Mitochondrial dysfunction

Mitochondrion is responsible to produce energy in form of adenosine triphosphate (ATP) for the survival of cells. ATP is produced in the inner membrane of the mitochondria as the end product of electrons flowing down the electron transport chain, generating energy which is used by ATP synthase to through oxidative phosphorylation produce ATP from adenosine diphosphate (ADP). Reactive oxygen species (ROS) are byproducts of oxidative phosphorylation, which under normal conditions are kept under regulated levels by the action of antioxidant proteins (Puspita, Chung et al. 2017). Increased levels of ROS production due to ex. dysfunction of electron transport chain can lead to oxidative stress with severe effects on the overall cell machinery and survival. Several observations have linked oxidative stress due to dysfunction of mitochondria to neuronal degeneration in PD (both in sporadic and familial), specially dopamine neurons which are more susceptible to oxidative stress (Subramaniam and Chesselet 2013). Postmortem studies on brains from PD patients strongly confirm reduction in activity or protein levels of mitochondria complex in substantia nigra, frontal cortex and striatum (Mizuno, Ohta et al. 1989, Schapira, Cooper et al. 1990, Parker, Parks et al. 2008, Subramaniam and Chesselet 2013). More importantly studying genetic studies have confirmed that several of the PD related genes identified encode proteins which are directly linked to mitochondrial function. Mutations in genes; PINK1, Parkin, DJ-1 and LRRK2 are all linked to mitochondrial dysfunction associated with familial PD (Surmeier, Guzman et al. 2010). For example, alterations in mitochondria autophagy due to mutations in PINK1 and Parkin results in accumulation of damaged mitochondria as the proteins encoded by these genes are involved in repair and autophagic mechanisms (Michel, Hirsch et al. 2016, Puspita, Chung et al. 2017). Moreover, mitochondrial DNA (mtDNA) is especially vulnerable to oxidative stress as they lack protection by histone proteins compared to nuclear DNA. mtDNA encode proteins involved in the electron transport chain and thus any mutation caused by excessive ROS production to mtDNA directly effects the ATP production machinery which in turn leads to production of even more ROS and thereby a negative loop causing cell death (Puspita, Chung et al. 2017). Environmental toxins such as MPTP mentioned previously and the pesticide Rotenone (mitochondrial complex I inhibitor) are also directly linked to oxidative stress and damaging for mitochondria hemostasis and increased risk of developing PD (Puspita, Chung et al. 2017). In conclusion directly or indirectly mitochondrial dysfunction and ROS production have a toxic effect on individual neurons and ultimately pathology of sporadic and familial PD.

1.5.2 Neuroinflammation

Neuroinflammatory mechanisms are involved in various neurodegenerative disorders. However, whether inflammatory responses are triggered and activated due to neuronal degeneration or whether inflammatory processes are part of the dysfunctional toxic pathways

which may partly lead to progression of the disease is not known. Microglial activation and astrocytic reaction are strongly linked to neuropathology of PD (Hirsch and Hunot 2009). Several post-mortem studies have demonstrated activated microglial cells in the substantia nigra of patients with PD compared to healthy controls (McGeer, Itagaki et al. 1988, Banati, Daniel et al. 1998). Also, involvement of astrocytes was shown based on the observation that astrocyte density in SN of PD patients is lower than in areas not affected by the disease. Lower astrocyte density may lead to a less effective clearance of the surrounding milieu of affected neurons from reactive free radicals and hence might be a triggering factor in progression of the disease (Damier, Hirsch et al. 1993). Neurodegeneration caused by activation of inflammatory processes insert the toxic effect through mediating oxidative stress and damage on dopaminergic neurons and adjacent environment. For example, microglial activation which is increased in substantia nigra of PD patients and animal models of PD, may lead to production of toxic amounts of oxygen and nitrogen-derived products (O^{2-} and NO free radicals). Through this process recognized as oxidative burst, NO and O^{2-} react and the highly reactive species peroxynitrite ($ONOO^-$) is produced causing further toxic oxidative reactions and damage to enzymes such as Tyrosine hydroxylase (TH) (Przedborski, Chen et al. 2001). Other consequences of highly activated microglial cells in substantia nigra are mediated through release of cytotoxic inflammatory compounds. Proinflammatory cytokines such as TNF could through direct binding to cell surface receptors on dopaminergic neurons activate proapoptotic pathways. Also, it has been shown that NO free radicals can potentiate production of TNF by microglial and astrocyte cells and hence creating a damaging cycle (Hirsch and Hunot 2009). Nevertheless, as mentioned whether neuroinflammation is the cause or consequence of neurodegeneration needs to be established to understand the origin and cause of this disease.

1.5.3 Dysfunction of the Autophagy-Lysosome system

One of the mechanisms used by cells for degrading dysfunctional proteins or organelles is the autophagy-lysosome pathway. This system is highly regulated and also can affect apoptosis (Kenney and Benarroch 2015). In this process structures called autophagosomes which contain the cytosolic components for removal, transport the contain to lysosomes. This process can be considered as a recycling pathway, as the metabolites produced after degradation are once again used by the cell to produce new compartments or energy (Boya, Reggiori et al. 2013). Dysfunction of this pathway has been associated with PD and α-synuclein pathology. Based on a recent work studying dopamine neurons in brain tissues from PD patients researchers have found a decreased lysosomal expression in DA neurons compared to controls which was also shown to be associated with a higher α-synculein expression (Chu, Dodiya et al. 2009). In addition, PD-related genes such as DJ-1 and LRRK2 are also indirectly coupled to this pathway as mutations in these two genes result in accumulation of autophagosomes and reduced lysosomal enzyme activity respectively

(Michel, Hirsch et al. 2016). Also, loss of autophagy gene Atg7 which is involved in formation of autophagosome was demonstrated to result in accumulation of α-synuclein in the presynaptic terminal, resulting in enhanced levels of this protein and hence PD pathology (Friedman, Lachenmayer et al. 2012). In conclusion failure in removal of excessive and dysfunctional organelles and proteins in particular α-synuclein by the autophagy-lysosome pathway creates a negative feedback loop influencing progression of PD.

1.5.4 Calcium Homeostasis

Dopamine neurons have an autonomous pacemaking activity which is calcium dependent and which helps maintaining a constant basal dopamine innervation/tone of the striatum. Thus, proper functioning of dopamine neurons is partly dependent on regulated levels of calcium as both lower or higher levels of calcium than the physiological level can be crucial to normal rhythmic activity of these neurons. To maintain these rhythmic activity dopamine neurons, sustain an elevated intracellular concentration of calcium through L-type voltage-dependent Ca^{2+} channels. Dopamine neurons have low calcium buffering capacity and small elevation in cytosolic calcium levels can cause a metabolic demand and intersects with mitochondrial oxidative stress. Thus, calcium levels are constantly kept under regulated concentration using ATP dependent pumps to pump back calcium across plasma membrane (Chan, Gertler et al. 2009). L-type Ca^{2+} channels (specifically Cav1.2 and Cav 1.3) are considered to be the main source of elevated calcium levels in dopamine neurons and hence blockers of these receptors such as Isradipine have shown to be protective against mitochondrial stress and helpful in PD (Michel, Hirsch et al. 2016). Also, different mutations in proteins involved in PD and dopamine neurons may lead to enhanced pacemaking activity in DA neurons and thereby increased Ca^{2+} influx and demand on mitochondria and ER. Overexpression of A53T mutant α-Synuclein and mutation in DJ-1 both result in over activity of dopamine neurons. Also, mutation to PINK1 has shown to cause overload of calcium in mitochondria due to dysfunction of calcium efflux mechanism from mitochondria due to the mutation. One other speculated route to increased levels of calcium is through increased activity of N-methyl-D-aspartate (NMDA) receptors due to increased glutamatergic inputs and over activity of subthalamic nucleus (Michel, Hirsch et al. 2016). In conclusion disruption in levels of calcium entry into dopamine neurons or efflux or increased activity of dopamine neurons result in enhanced levels of calcium and higher energy demand and thereby stress on mitochondria capacity which may trigger mechanisms involved in cell death pathway and hence dopamine neurodegeneration (Michel, Hirsch et al. 2016).

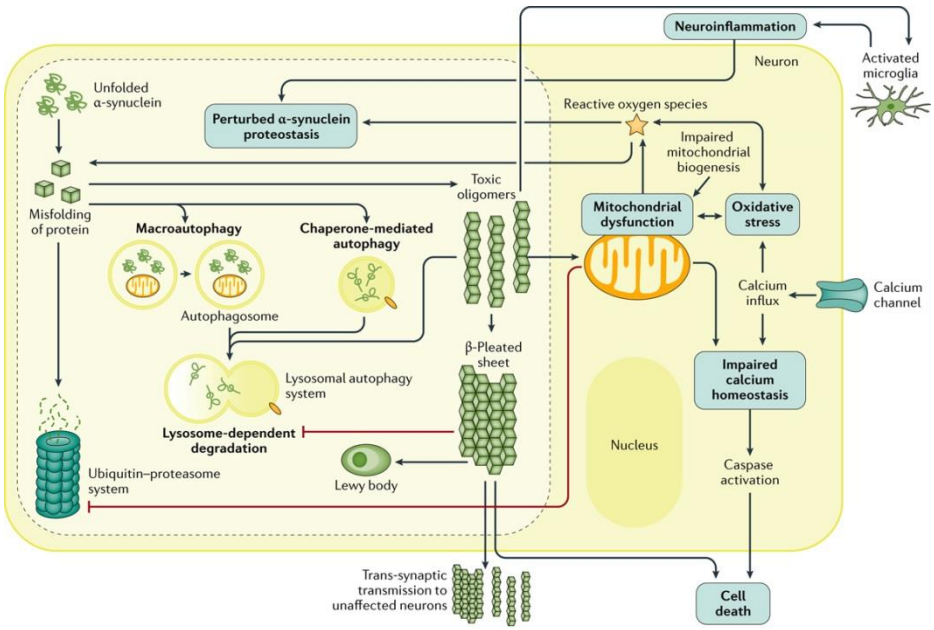

Figure 1: Molecular pathways involved in pathogenesis of Parkinson's disease. Modified from (Poewe, Seppi et al. 2017).

1.6 TREATMENT OF PD

Treatment of Parkinson's disease is unfortunately limited to symptomatic relief and currently there is no treatment halting or slowing the progress of the disease and neurodegeneration. One of the key treatments of PD since its discovery to date is the dopamine replacement therapy using L-3, 4-dihydroxyphenylalanine (L-DOPA). L-DOPA was first discovered by Arvid Carlsson and colleagues back in 1950's (Schulz, Hausmann et al. 2016). L-DOPA is the precursor of dopamine and which can be taken orally and cross the blood-brain barrier. Once in the brain, L-DOPA is decarboxylated to dopamine and mediates its action through postsynaptic dopamine receptors (Dorszewska, Prendecki et al. 2014). Efficacy of L-DOPA treatment is achieved in a greater extend together with using enzyme inhibitors such as inhibitors of the peripheral aromatic amino acid decarboxylase (AADC) to prevent breakdown of L-DOPA before reaching the brain (Dorszewska, Prendecki et al. 2014, Taddei, Spinnato et al. 2017). This golden standard treatment of motor symptoms of PD over long-term normally causes side effects such as increased toxicity and inflammatory responses and most importantly dyskinesia as the therapeutic effect of it is lost (Dorszewska, Prendecki et al. 2014). Mechanisms leading to L-DOPA induced dyskinesia are not known and thus alternative or combinational therapies are necessary to reduce both motor and non-motor symptoms of PD. One such alternative approach is using dopamine agonists acting directly on dopamine receptors. Dopamine agonist, Apomorphine (D1 and D2- like agonist) was the first agonist demonstrating positive effects on PD symptoms (Brooks 2000). Other dopamine agonist developed are mostly D2-like receptor agonist but commonly show beneficial effect

in early stages of PD with less motor complications which allows L-DOPA treatment to be started at the later stages of the disease (You, Mariani et al. 2018).

Deep brain stimulation (DBS) of subthalamic nucleus is used as a non-pharmacological treatment at the later stages of the disease for reducing tremors associated with PD (Schulz, Hausmann et al. 2016). Using this method, a continuous high frequency electric stimulation is delivered to SNc were the activity is increased, however the exact mechanism in which DMS inserts its positive effects is still not known (Pires, Teixeira et al. 2017). Nevertheless as the disease progress life quality of PD patients are highly affected due to non-motor symptoms associated with progression of the disease and thus needs to be combated with combination of different pharmacological treatments (Masilamoni and Smith 2018). Non-dopaminergic treatments, targeting glutamatergic neurotransmission through NMDA and mGlu receptors and the cholinergic system are other targets which together with dopamine replacement therapy can combat some of the symptoms of PD (Finlay and Duty 2014).

1.7 ANIMAL MODELS OF PARKINSON'S DISEASE

The exact disease processes and molecular mechanisms of cell death are still not clear and under investigation, but thanks to various animal models, we have now greater insight into PD pathogenesis. Animal models of PD can be divided into two groups. One is the classical neuro-toxin based or pathogenic models and the other is the more modern genetic models or etiologic models (Bezard, Yue et al. 2013). The ideal PD model should reflect the core pathology hallmarks of the disease as well as progressive developmental and behavioral phenotypes of the disease. Unfortunately none of the different models recapitulate all aspects of the disease and each may reflect a mechanism or pathway involved in the progression of the disease. Thus studying multiple models can give a better understanding of the disease (Bezard, Yue et al. 2013).

1.7.1 Neurotoxin model

6-hydroxydopamine (6-OHDA) is the hydroxylated analogue of the neurotransmitter dopamine. 6-OHDA does not cross the blood-brain barrier and therefore is injected unilaterally into the brain in the nigro-striatal tract (Duty and Jenner 2011). Following its injection, 6-OHDA is taken up into the dopaminergic neurons via the dopamine transporter, DAT. Although the exact mechanism underlying 6-OHDA-toxicity is still not clear, current understanding is that, once inside dopaminergic neurons, 6-OHDA initiates degeneration through a combination of oxidative stress and mitochondrial respiratory dysfunction (Duty and Jenner 2011). As a result of the injection, a70% striatal dopamine depletion is detected within 2 weeks. To confirm DA-loss, the levels of the enzyme tyrosine hydroxylase (TH;

rate-limiting step of DA biosynthesis) are measured in the postmortem brains. 6-OHDA model is an acute model thereby it does not reflect the progressive development of the disease and also no Lewy-bodies are developed (Bezard, Yue et al. 2013). However it does reflect biochemical, neurochemical and neurophysiological features of the disease as the nigro-striatal tract is degenerated by the lesion.

1.7.2 Leucine-rich repeat kinase 2 (LRRK2)

Autosomal transmissions of mutations in LRRK2 gene is both linked to familial form of PD and even sporadic PD. LRRK2 is a large multidomain-containing protein that is localized to membranous structures (Biskup, Moore et al. 2006). LRRK2 is involved in several cellular functions such as neurotransmission, endocytosis and neuronal outgrowth and guidance. Mutations associated with PD correspond to the GTPase and kinase domains (Biskup and West 2009). Studies have demonstrated an increase in the activity of the kinase domain upon LRRK2 mutation, causing neurotoxicity in PD. Most of the current LRRK2 transgenic mice have abnormalities in the nigrostriatal system; such as decreased DA release and uptake or late behavioral deficits, which are DA responsive. DA neurons, however, do not neurodegenerate. These abnormalities probably represent some of the earliest neuronal dysfunctions caused by LRRK2 mutation, making animal models with mutations in the LRRK2 gene ideal for studying early pathogenic events in PD. Indeed, one advantage of these models is the age-dependent decrease in striatal DA content (Schirinzi, Madeo et al. 2016).

1.7.3 Orphan nuclear receptor Nurr-1

Nurr-1 belongs to the family of ligand-activated transcription factors called nuclear receptors (Decressac, Volakakis et al. 2013). Studies have reported an involvement of Nurr1 in the development of midbrain DA neurons. Nurr-1 is also highly expressed in mature DA neurons in the adult brain and deficiency is associated with cellular changes that resemble early stages of PD. Nurr-1 function seems to be perturbed in patients with PD and in rodents. Interestingly, Nurr1 expression is down regulated in postmortem human brain tissue; this decreased Nurr1 expression might underlie decreased production of DA, and DA-neuron degeneration (Decressac, Volakakis et al. 2013, Kadkhodaei, Alvarsson et al. 2013). Nurr-1 knockout mice display age-dependent morphological, biochemical, and behavioral phenotypes that resemble the progressive degeneration observed in early stages of PD (Decressac, Volakakis et al. 2013).

1.8 BASAL GANGLIA

Basal ganglia (BG) are composed of four main nuclei which are interposed between the cortex and the thalamus. The main task of this group of nuclei is to modulate movement execution by processing the signals that arise from the cortex, and producing an output signal that returns to the cortex, through thalamus. BG is also involved in non-motor functions, including cognition (Bar-Gad and Bergman 2001). The BG is divided along a dorsolateral/ventromedial axis into two functionally different divisions. The dorsal part of BG is composed of four different, interconnected nuclei: the neostriatum, the globus pallidus (GP), the substantia nigra (SN) and the subthalamic nucleus (STN). In higher vertebrates the neostriatum is divided by the internal capsule to putamen and caudate nucleus. GP is also composed of two major units, the external (GPe) and internal (GPi, also called entopeduncular nucleus). Also SN is composed of two-subunits: the pars compacta (SNc) and pars reticulata (SNr). These sections are part of the dorsal BG and are responsible for the motor and associative functions. The ventral part of BG is associated with limbic functions and is composed of two different nuclei (nucleus accumbens and ventral pallidum) and VTA and parts of the dorsal BG (medial part of STN and SN) (Tepper, Abercrombie et al. 2007).

1.8.1 Macrocircuit of the basal ganglia

The striatum receives inputs from different brain regions but most importantly from cortex, thalamus and SN. Excitatory/glutamatergic inputs arrive from somatosensory and motor cortex and from anterior and ventral lateral thalamic nuclei (Wilson 2007). In addition striatum receives projection from other brain regions, which are important for modulation of the glutamatergic inputs to the striatum. Midbrain projections to striatum arise from dopaminergic neurons of SNc. Moreover some smaller projections from raphe nucleus and locus coeruleus account for the serotonergic and noradrenergic innervation of striatum, respectively(Afifi 2003). According to the standard model of BG, these converged inputs are passed through the two principal pathways (direct and indirect) from striatum to the two output nuclei: GPi and SNr. The direct pathway transmits the information as an inhibitory signal directly to SNr /GPi. However in the indirect pathway, information is sent to the output nuclei via GPe and STN. Neurons of the output nuclei are also GABAergic, thus the net effect of projections through direct pathway is inhibition of the output nuclei and disinhibition by the indirect pathway (Bolam, Hanley et al. 2000, Bar-Gad and Bergman 2001, Shipp 2017). The functional outcome of the direct pathway is an increase in movements as the thalamus is disinhibited for initiation of appropriate movement. Conversely, the indirect pathway increases the excitatory effects of STN neurons on the SNr, thereby inhibiting thalamus and reducing locomotion or suppressing competing movements (Kravitz, Freeze et al. 2010). In Parkinson's disease, over activity in the indirect pathway and inhibition of the direct pathway are associated with hypokinesia (Shipp 2017). A third route

of entry to the basal ganglia has been described but much less studied; the hyperdirect pathway of the corticosubthalamic projections. According to this system, STNreceives direct excitatory cortical and thalamic inputs, which further are transmitted to GPi/SNr (Mathai and Smith 2011).

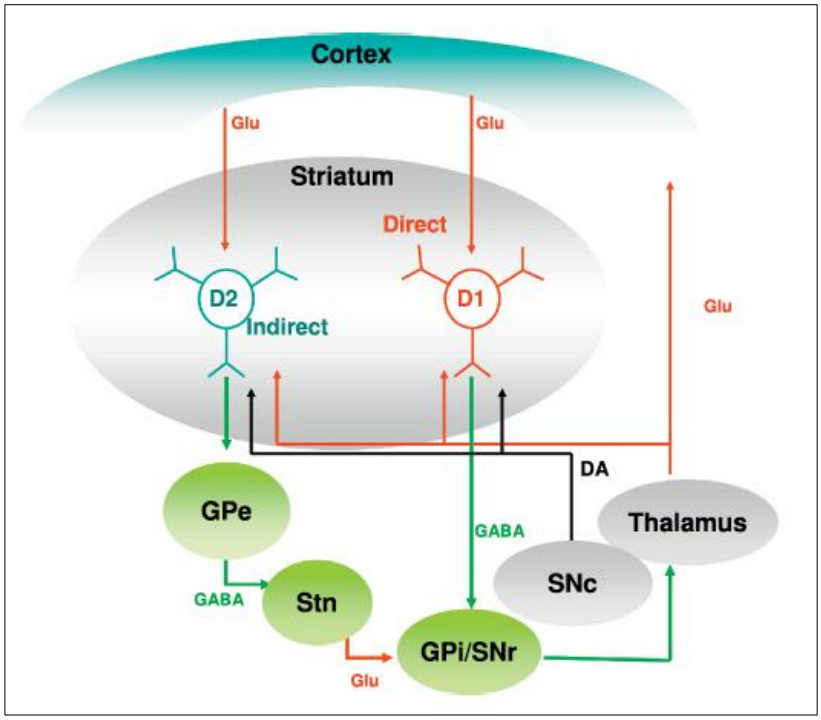

Figure 2: Simplified model of macrocircuit of the basal ganglia. Glutamatergic inputs to the striatum from cortex and thalamus are sent to the output nuclei; GPi/SNr, through the direct and indirect pathways and from there back to cortex via thalamus. Dopaminergic inputs to the striatum arrive from SNc. Modified from (Cerovic, d'Isa et al. 2013).

1.8.2 The Striatum

Striatum is mainly composed of GABAergic neurons and interneurons and is the main recipient of cortical and thalamic inputs to the basal ganglia (Cheatwood, Corwin et al. 2005). The principal projection neurons of the striatum are the GABAergic medium spiny neurons (MSNs). MSNs account for around 90-95% of the total neuronal population of the striatum, receiving the excitatory inputs to basal ganglia on their dendritic spines (Afifi 2003, Tepper, Abercrombie et al. 2007). MSNs fire sparsely and require coordinated excitatory synaptic inputs to spike. In vivo, MSNs have two states of excitability. In the up state, MSNs rest at a depolarized membrane potential (-70 to -40 mV); thereby it is more likely that they fire action potentials upon increased activity of many convergent corticostriatal inputs, compared to the hyperpolarized down state (-61 to -94mV) (Stern, Jaeger et al. 1998, Mallet, Le Moine et al. 2005, Wilson 2007). MSNs have extensive local axon collaterals that project to other MSNs

as well as interneurons of the striatum (Tepper, Koos et al. 2004, Venance, Glowinski et al. 2004). MSNs give rise to the two principal pathways (direct/indirect MSNs), which are important for action selection. These different subpopulations express different dopamine receptors and neuropeptides (Kita and Kitai 1988). Direct pathway MSNs, express low affinity dopamine type 1 receptors (D1R) and also express substance P and dynorphin. Indirect pathway MSNs, express high affinity dopamine type 2 receptor (D2R) and the neuropeptide encephalin (Izzo, Graybiel et al. 1987). However recent studies have confirmed co-expression of both type 1 and 2 dopamine receptors in a proportion of MSNs (Gittis and Kreitzer 2012, Calabresi, Picconi et al. 2014, Lim, Kang et al. 2014). MSNs are also innervated by the dopaminergic inputs from SNc and ventral tegmental area (VTA). Dopaminergic neurons project onto neck of the spines or dendritic shaft, in a close interaction with glutamatergic synapses (Calabresi, Pisani et al. 1997). Dopamine modulates the response and synaptic strength of corticostriatal projections to MSNs (Tepper, Abercrombie et al. 2007). As mentioned, direct and indirect MSNs express different classes of dopamine receptors. Dopamine receptors are G-protein coupled receptors, linked to different intracellular signaling pathways and thereby can produce different responses upon their activation (Girault 2012). Thus, dopamine has opposite modulatory effects on these neurons: dMSNs are activated and iMSNs inhibited by dopamine (Surmeier, Ding et al. 2007, Calabresi, Picconi et al. 2014).

Cortical and thalamic inputs to the striatum also project onto striatal interneurons (Tepper, Abercrombie et al. 2007). Striatal interneurons comprise 5-10% of all striatal neurons. Most striatal interneurons are GABAergic and some are cholinergic. GABAergic interneurons can be divided into two groups: fast-spiking (FSI) and persistent and low-threshold spike interneurons (PLTS). Neurochemically FSIs can be identified by their expression of calcium-binding protein parvalbumin. FSIs display brief action potentials with large, rapidly peaking spike afterhyperpolarization. Moreover FSIs are electrotonically coupled due their expression of gap junctions (Tepper, Koos et al. 2004). FSIs are considered to be important for a feedforward inhibition onto MSNs since they are activated earlier and at a lower threshold than MSNs (Koos and Tepper 2002). Thus FSIs create a GABAergic network to control spike timing in MSNs (Tepper, Koos et al. 2004, Wilson 2007). PLTS interneurons express somatostatin and neuropeptide Y. They also regulate MSNs through their inhibitory projections onto somata and dendrites of MSNs. However this projection onto MSNs was shown to be of much lower density than for example FSIs. But PLTS are considered important for providing neurotransmitters such as NPY, SOM and NO and thereby modulating striatal circuitry. Recently it was shown that PLTS interneurons might project onto and inhibit tonic firing of other interneurons such as cholinergic interneurons (Gittis, Nelson et al. 2010, Gittis and Kreitzer 2012). Cholinergic interneurons (Chl) have very large somata and are considered to be tonically active based on in-vivo recordings. They represent only 1-2% of the total striatal neuronal population and provide the major source of acetylcholine (Ach). Despite being few in numbers, they greatly influence the local striatal circuit with their dense projections throughout the striatum (Kravitz, Freeze et al. 2010). They

also have the characteristic long- lasting after hyperpolarization phase, broad action potentials, fire single spikes and have a resting membrane potential around -60mV (Oswald, Oorschot et al. 2009). Chl interneurons receive direct excitatory inputs mainly from thalamus but also from cortex (Tepper, Abercrombie et al. 2007, Lim, Kang et al. 2014). Both classes of MSNs and other striatal interneurons express receptors for acetylcholine. MSNs express mainly postsynaptic M1 muscarinic receptors and thereby are excited upon activation by acetylcholine. In contrast presynaptic terminals of corticostriatal neurons express M2 receptors, leading to inhibition of neurotransmitter release. This dual action of cholinergic interneurons can be explained by the burst-pause pattern, which is characteristic for these interneurons (Ding, Guzman et al. 2010). Thalamic inputs cause a burst of spikes in these interneurons, which leads to transient inhibition of the corticostriatal inputs. Meanwhile the pause phase creates a time window during which striatopallidal MSNs become more responsive to cortical inputs. This response pattern of the cholinergic interneurons is considered important for salient stimuli and suppression of ongoing motor activity (Ding, Guzman et al. 2010, Lim, Kang et al. 2014). GABA released by MSNs may also influence excitability of Chl interneurons. It has been shown by optogenetic studies that MSNs evoke inhibitory postsynaptic currents (IPSCs) in Chl interneurons, suggesting a direct synapse between MSNs and these interneurons. Also, dopamine acting through D2 and D5 receptors expressed on Chl interneurons inhibits autonomous spiking of Chl interneurons and thereby release of acetylcholine (Tritsch and Sabatini 2012, Lim, Kang et al. 2014, Wang, Zhang et al. 2014).

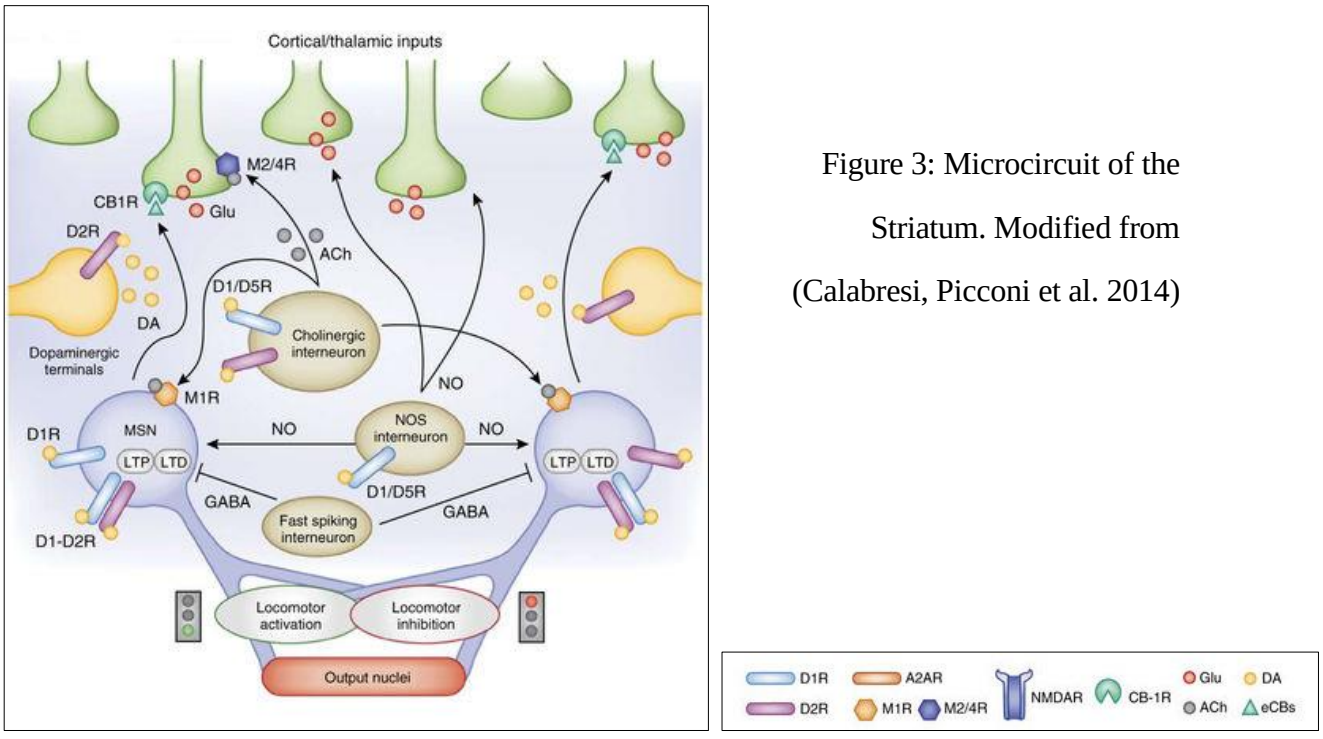

Figure 3: Microcircuit of the Striatum. Modified from (Calabresi, Picconi et al. 2014)

1.9 SYNAPTIC PLASTICITY

One of the most important and well-studied mechanisms for learning and memory is experience-dependent modification in the efficacy of synaptic connections, also referred to as synaptic plasticity. Plasticity can be short or long lasting and may either lead to suppression or potentiation of a synapse. In the striatum long-term potentiation (LTP) and long-term depression (LTD) are strongly associated with motor learning and associative memory processes. Plasticity at the excitatory corticostriatal synapses onto MSNs and striatal interneurons has been studied for many years but yet there is a great controversy regarding types of plasticity observed by different researchers. MSNs undergo both types of plasticity upon repetitive activation of the excitatory cortical inputs. Indeed striatal plasticity is dependent on excitatory inputs, but also nigrostriatal dopaminergic modulation of these inputs is a key player in induction of plasticity (Wickens 2009).

LTD induced by high-frequency stimulation (HFS) of corticostriatal fibers onto MSNs was the first reported type of plasticity observed in-vitro in the striatum. This form of LTD is NMDA-receptor independent; however it requires activation of glutamate metabotropic receptors (particularly mGluR1) and co-activation of both D1 and D2 dopamine receptors. Sufficient levels of postsynaptic intracellular Ca^{2+} (influx through voltage sensitive calcium channels) and activation of Ca^{2+} dependent protein kinases are also necessary for LTD. Also, for LTD to be induced, HFS stimulation needs to be paired with membrane depolarization and action potential discharges of the postsynaptic neurons (Calabresi, Pisani et al. 1997). Endocannabinoids released postsynaptically have also been reported to act as retrograde messengers acting on presynaptic CB1-receptors to reduce glutamate release and thereby inducing LTD (Lerner and Kreitzer 2011). It is largely agreed that LTD does occur at synapses on iMSNs, however independent studies report differential results regarding LTD at dMSNs and it still remains a controversial topic, which needs further investigation (Wickens 2009, Gardoni and Bellone 2015).

On the other hand LTP at corticostriatal synapses is NMDA receptor dependent (Pisani, Centonze et al. 2005). However controversy regarding induction of LTP is great and also less characterized. Initially based on brain slice recordings, it was indicated that the same induction protocol as used for LTD, induces LTP but in Mg^{2+} free extracellular solution allowing removal of voltage-dependent blockade of NMDA receptors channel (Calabresi, Pisani et al. 1997). Later, researchers could demonstrate presence of LTP in the in-vivo recordings thereby challenging the necessity of removal of Mg^{2+} from extracellular medium (Kreitzer and Malenka 2008, Lovinger 2010). What is commonly agreed on is that dopamine and D1 receptor activation and not D2 is necessary for LTP induction in dorsal striatum. This difference is mainly described by the postreceptor pathways, which are coupled to these receptors. Activation of D1 receptors exerts a positive modulation on the intracellular cascade, by acting on adenylate cyclase and cAMP formation that in turn activates protein kinase A and activation and phosphorylation of dopamine and cAMP-regulated

phosphoprotein of 32kDa (DARPP-32). Phosphorylation of DARPP-32 eventually leads to phosphorylation of NMDA receptors and hence their activation, as well as an increase in surface expression of AMPA and NMDA receptors (Cepeda and Levine 2012, Cerovic, d'Isa et al. 2013). In general, both LTD and LTP share a common process which involves modulation of the direction of plasticity by cAMP/PKA pathway but with opposite fashion. MSNs are biased towards LTD during low PKA activity levels and towards LTP with increased activity of PKA and thereby enhanced NMDA receptor signaling (Lerner and Kreitzer 2011). Additionally Ach, another modulatory neurotransmitter in striatum, is believed to contribute to induction of LTP. As during pause in the tonic firing of cholinergic interneurons, the inhibitory action of Ach on M2- muscarinic receptors on the presynaptic terminals of corticostriatal neurons is reduced and glutamate release is increased (Calabresi, Centonze et al. 2000, Pisani, Bernardi et al. 2007, Surmeier and Graybiel 2012, Gardoni and Bellone 2015). Also Ach acts directly on pre-synaptic nicotinic receptors on the axon terminals of the dopaminergic fibers in the striatum, causing an increase in DA release upon HFS, again favoring induction of LTP (Surmeier and Graybiel 2012). Importantly D1 receptor activation per se also causes an increase in cAMP levels and activation of PKA and eventually through the action of DARPP-32 leads to increased opening of L-type Ca^{2+} channels on MSNs, bringing MSNs to a more excitable state, favoring induction of LTP (Girault 2012, Gardoni and Bellone 2015).

Variability in the ability to induce LTP at corticostriatal synapses by different researchers highlights the importance of other factors involved and contributing to this form of plasticity. Other G protein-coupled receptors such as A2A receptors expressed in dendritic spines of MSNs also regulate and influence the direction of plasticity in striatum through interaction with CB1 and dopamine receptors. As for LTP in the indirect pathway MSNs, many studies demonstrate the need of A2A receptor signaling for induction of LTP which was confirmed even in the absence of dopamine in dopamine-depleted mice (Lopez de Maturana and Sanchez-Pernaute 2010).

Thus the final polarity of long-term modifications at these corticostriatal synapses may be influenced by: phasic activity (up and down state) of MSNs, other neurotransmitter systems (particularly Ach and dopamine), age of the animal, experimental conditions and precise striatal sub-region (Partridge, Tang et al. 2000, Calabresi, Galletti et al. 2007). As for sub-divisions of striatum, most reports on adult mice indicate LTP in dorsomedial striatum and a developmental switch of plasticity in dorsolateral from LTP to LTD (Di Filippo, Picconi et al. 2009, Wickens 2009, Girault 2012). Difference in type of synaptic plasticity observed in different sub-regions of dorsal striatum (medial and lateral portion), highlights the specific roles of each section. This because of receiving inputs from different areas of cortex and thalamus resulting in different learning and memory paradigms (Partridge, Tang et al. 2000).

1.10 NMDA RECEPTORS

Glutamate is the most important and the main excitatory neurotransmitter in the nervous system. Glutamate acts on different classes of membrane receptors, including ionotropic (iGluRs) and metabotropic glutamate receptors (mGluRs). iGluRs are cation-permeable ion channels and can further be divided into three groups: N-methyl-D-aspartate (NMDA) receptors, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors and kainate receptors. Since the discovery of NMDA receptors and identification of their major functional roles in excitatory synaptic transmission, neuronal plasticity and in many neurological disorders; much attention has been focused on studying the exact role of these receptors based on their subunit composition both in physiological condition but also disease. NMDA receptors are plastic complexes, composed of several homologous subunits that depending on the CNS regions, cell type and development stage can vary greatly. Also, NMDA receptors are mobile, present both in the pre and postsynaptic sites and at extrasynaptic sites and therefore they can regulate synaptic strength (Furukawa, Singh et al. 2005). NMDA receptors are heterotetrameric assemblies of the obligatory subunit GluN1 and a combination of GluN2 and/or GluN3 subunits. Due to alternative splicing of one single gene encoding GluN1, eight different GluN subunits exist. Additionally 4 different GluN2 (A-D) and two different GluN3 (A and B) subunits exist; however these are encoded by six different genes. The expression pattern and distribution of the different subunits are highly regulated during development. The only subunit, which is ubiquitously expressed throughout the brain and both during embryonic stage and adulthood, is the GluN1. In the adult brain the expression of GluN2A and GluN2B are highest in brain structures such as Hippocampus, Striatum and Cortex, suggesting a role in synaptic function and plasticity. However as the GluN2A expression peaks the GluN2D decreases in the adult CNS and becomes restricted to the diencephalon and mesencephalon. GluN2C in the adult brain is mostly expressed in the cerebellum (Paoletti and Neyton 2007, Paoletti 2011) (Mullasseril, Hansen et al. 2010, Traynelis, Wollmuth et al. 2010).

All NMDA receptor subunits share the same membrane topology with a large extracellular N-terminus, 3 transmembrane segments (pore domain) and a cytoplasmic C-terminus. The extracellular part is composed of two domains. First the N-terminal domain (NTD), which is important for subunit assembly and also serve as a binding site for allosteric modulators such as Zn^{+}. The second domain is the agonist-binding domain (ABD). NMDA receptors require binding of both Glycine and Glutamate for activation. Glycine binds to ABD in GluN1 and GluN3. Glutamate binds to ABD in GluN2. The transmembrane domain makes the ion-pore/channel and is important for ion- selectivity. Also characteristic for NMDA receptors is the voltage sensitive blockade of the ion-pore by Mg^{2+} ion when in the inactive state. The C-terminal domain is involved in receptor trafficking and coupling of the receptor to intracellular signaling complexes (Paoletti 2011). Yet each subunit displays different properties thereby the subunit composition of each NMDA receptor subtype directly influences receptor biophysics. Single channel conductance, Mg^{2+} blockade and Ca^{2+}

permeability are the three most important permeation properties, which are directly linked to the subunit composition. Different NMDA receptor ensembles with different subunit composition can be expressed in the same neuron with different levels of calcium permeability (Sucher, Awobuluyi et al. 1996). For example, NMDA receptors containing GluN2B and GluN2A display high sensitivity to Mg^{2+} blockade and also generate channels with large conductance. While GluN2D and GluN2C show the opposite. Also Ca^{2+} permeability is higher in GluN2A and 2B than GluN2C or 2D. Gating property is also very much determined by the subunits. In the classical GluN1/GluN2 NMDA receptors, GluN1 is the Glycine sensitive subunit and GluN2 provides sensitivity to Glutamate. Also GluN1/GluN2A receptors have a higher open probability than GluN1/GluN2B and even GluN1/GluN2D. Also GluN2C and GluN2D have a more instable open state, reflected by the weaker channel mean open time (Regan, Romero-Hernandez et al. 2015). The deactivation kinetic of the GluN2 subunits also are different, with GluN2A being the fastest and GluN2D having the slowest deactivation kinetic (Hackos and Hanson 2017). In brief NMDA receptors after a brief pulse of glutamate released into the synaptic cleft, following an action potential in the presynaptic terminal are slowly activated. NMDA receptor requires adequate depolarization of the postsynaptic membrane (AMPA receptor mediated) for removal of Mg^{2+}ions (which block the receptor pore at resting membrane potential) from the pore and entry of cation ions. Thus, individual inputs do not activate NMDA receptors and several pulses in a short time window or high frequency inputs are required (Blanke and VanDongen 2009, Vyklicky, Korinek et al. 2014).

As mentioned NMDA receptors are mobile and plastic which makes them highly responsive to synaptic events and neuronal activity or sensory experiences. Changes in NMDA receptor composition and number of receptors are rapid and can have an overall effect on the neuronal networks/circuit. Also NMDA receptor composition and function are subjected to various disease pathology. Thus as the NMDAR-mediated transmission contributes to various aspects of neural circuit function, long-term changes of the NMDA receptors may have important functional implications for information processing and brain function (Picconi, Ghiglieri et al. 2008, Paoletti 2011, Paoletti, Bellone et al. 2013).

1.10.1 Modulators of NMDA receptors

NMDA receptors are extensively studied due to their involvement in different neurodegenerative disorders as well as stroke and traumatic brain injury among others. Hypofunction and hyperfunction of NMDA receptors have been associated with Schizophrenia and stroke respectively (Ogden and Traynelis 2011, Hackos and Hanson 2017). Targeting NMDA receptors using agonists and antagonist as therapeutic approach for many neurological conditions has many times failed due to severe side-effects and toxicity accompanying the beneficial outcomes. For example, antiparkinsonian drugs Amantadine and

its derivative Memantine, both antagonists of NMDA receptors have shown side-effects such as loss of appetite, blurred vision, dizziness, hallucination, insomnia, confusion and muscle pain (Olivares, Deshpande et al. 2012). Therefor subunit specific modulators and antagonists of these receptors are studied as they can act as therapeutic compounds to regulate NMDA receptor function and hence the downstream affected pathways or overall synaptic strength and transmission affected in various disorders of the nervous system. Subunit specific modulators of NMDA receptors can indirectly influence receptor function and activity with less side effects. This is since both negative and positive modulators bind to other regions than the ligand binding sites (Burnell, Irvine et al. 2018). Most relevant to this thesis are the positive allosteric modulators (PAMs) of NMDA receptors. PAMs are compounds which enhance receptor activity in presence of glutamate and glycine. One important advantage of PAMs in their selectivity is that they can enhance activity of weakly activated NMDA receptors in comparison to NMDAR agonists which target all receptors and thus might cause excitotoxicity (Burnell, Irvine et al. 2018). One such compound is CIQ ((3-chlorophenyl) (6, 7-dimethoxy-1-((4-methoxyphenoxy) methyl)-3, 4-dihydroisoquinolin 2(1H)-yl) methanone) (Mullasseril, Hansen et al. 2010).

CIQ is a newly identified selective positive allosteric modulator of NMDA receptors containing GluN2C or GluN2D subunits. To enhance receptor activity CIQ does not alter agonist EC50 or deactivation kinetic and does not have any agonist activity (Hackos and Hanson 2017). Instead CIQ increases the opening frequency of the NMDA receptor and thus giving a longer window for neurotransmission. CIQ acts through binding to the M1 transmembrane helix domain of GluN2 subunit as mutations in this region of the NMDA receptor alters the regulatory effect of CIQ and channel open probability (Ogden and Traynelis 2013, Wang, Brown et al. 2017). Effect of CIQ in potentiating NMDA current responses is reversible as shown by patch-clamp recordings before and after wash-out of CIQ (Mullasseril, Hansen et al. 2010). There is little data available which have investigated the effect of CIQ on different brain functions and behavior. Ogden et al., studied involvement of NMDA receptors containing GluN2C subunit in amygdala in fear learning and extinction learning in mice. Based on their data they demonstrated involvement of GluN2C/D subunits in fear learning and a positive effect of CIQ on fear acquisition and retention (Ogden, Khatri et al. 2014). Additionally, another study investigated the role of NMDA receptors containing GluN2D subunit on synaptic activity in subthalamic nucleus. Based on their data it was suggested that NMDA receptors containing GluN2B and GluN2D mediated NMDA component of EPSCs recorded from neurons in this region and CIQ enhance spike rates (Swanger, Vance et al. 2015). The effect of systematic administration of CIQ on schizophrenia-like behavior was studied by another group, in which they could demonstrate reversal/attenuation of these phenotypes (prepulse inhibition) (Suryavanshi, Ugale et al. 2014). Finally, by using CIQ researchers have shown presence of GluN2D in hippocampal CA interneurons and that NMDA component recorded from these interneurons is potentiated by using CIQ (Perszyk, DiRaddo et al. 2016).

1.11 SYNAPTIC ALTERATIONS IN AGING

Aging is the main risk factor for developing many neurodegenerative disorders such as PD and Alzheimer's disease. Normal aging alters molecular, morphological and hence functional biological processes of the brain. Alteration in neuronal volume, axonal degeneration, loss of synapses and synaptic plasticity are some of the consequences of normal, healthy aging but which can also become part of a cascade of events leading to pathological changes leading to development of various age-related disorders (Salvadores, Sanhueza et al. 2017).

In dorsal striatum, processes such as synaptic plasticity necessary for regulating motor coordination and the overall information flow in basal ganglia is disturbed as an effect of aging (Wang 2008). As for PD in normal aging levels of dopamine are decreased even though the level of dopamine loss is much greater in PD but yet this loss inserts an alteration in nigrostriatal DA neuron function (Salvatore, Apparsundaram et al. 2003). Dopamine loss in PD is a consequence of dopamine neuron degeneration, however recent studies show that in normal aging dopamine depletion due to decline in synthesis is the major cause of reduction and not dopaminergic cell loss (Darbin 2012, Rodriguez, Rodriguez-Sabate et al. 2015). Also, as a result of aging there is a reduction in expression levels of dopamine type 1 (DA1) receptors hence direct effect on LTP (Magnusson 1998, Nouhi, Zhang et al. 2018). Interestingly it has been shown that healthy aged individuals are not responsive to dopamine replacement therapy in comparison to PD patients. This was demonstrated to be due to reduced activity in the DOPA-decarboxylase (DDC) enzyme converting the precursor L-DOPA to dopamine (Darbin 2012). This loss of dopamine and its receptors directly affects the regulatory dopaminergic inputs to the direct and indirect MSNs and eventually imbalance in the two pathways controlling movements (Darbin 2012).

Moreover, loss of LTP at corticostriatal synapses can be partly explained by age-related reduction in number of physical synapses, altered expression of NMDA receptors and the downstream intracellular signaling pathways being affected as a consequence of aging (Magnusson 1998). Changes in NMDA receptors numbers and/or function (lower level of glutamate in synaptic cleft, reduction in NMDAR binding sites and age-related reduction in neuronal excitability in CNS) and hence effect on modulating dopamine release may be of importance to be considered as factors influencing absence of LTP in aged striatum (Zhang and Chergui 2015). Thus, a change in the overall NMDA receptor expression level and change in the subunit composition of these receptors together with the altered modulatory effect of NMDA evoked dopamine release in striatum may be a reason for altered synaptic plasticity in this region. Also, overall in CNS there is a decreased neuronal excitability (Zhang and Chergui 2015). This is explained by the observation that aged neurons have a more hyperpolarized state due to enhanced activation of Ca^{2+}-activated K^{+} channels and thereby in a state far from threshold for activation of NMDA receptors (Akopian and Walsh 2006). Moreover, there is some evidence for alterations in the cholinergic neurotransmission, with a reduced activity of choline acetyltransferase and thus alteration in the cholinergic

system modulating the glutamatergic inputs on the medium spiny neurons (MSNs) of striatum (Bergado and Almaguer 2002).

1.12 SYNAPTIC ALTERATIONS IN PD

Studies performed in striatum of both 6-OHDA-lesion and genetic models of PD, where extend of the DA-neuronal loss is still small or ongoing demonstrate profound changes in striatal synaptic transmission. The alteration in synaptic transmission in turn leads to other more functional mechanisms of the basal ganglia circuit being affected. Eventually, the imbalance in this system, starting from DA-loss to which is believed to be the main trigger, will lead to development of PD and its symptoms (Gardoni and Bellone 2015). One hypothesis explaining early pathology in development of PD and dopamine degeneration is the retrograde degeneration of the distal dopaminergic axons before loss of cell bodies (Salvadores, Sanhueza et al. 2017).

Dopamine depletion has a direct effect on both projection neurons of striatum but also interneurons, mainly cholinergic interneurons. Both dopamine from substantia nigra pars compacta and the glutamatergic inputs from cortex terminate onto the dendritic spines of MSNs. These co-localized inputs along with dopamine and NMDA receptor integration on spines of MSNs result in a direct cross-talk, information processing and modulation between the two signaling pathways. Thus, dopamine depletion has a direct effect on the glutamatergic signaling onto MSNs and downstream mechanisms (Vastagh, Gardoni et al. 2012). For example one major alteration is the imbalance in the firing rate of iMSNs and dMSNs and thereby imbalance in the two principal pathways of the basal ganglia (Gittis and Kreitzer 2012). The result of this imbalance is enhanced activity and output of the indirect pathway. Also, an increased activity in the output nuclei of basal ganglia is also detected. This increase in the firing rate is also associated with a disruption of information processing in the basal ganglia circuit and the signal being sent back to the cortex to regulate movement. Also in the dorsal striatum, there is a loss of dendritic spines on MSNs which is also directly correlated with the level of dopamine neurodegeneration (Villalba and Smith 2018). Moreover dopamine loss causes a change in the synapses between interneurons and MSNs and also within different neuronal population in the striatum (Surmeier and Graybiel 2012). As it has been shown there is a weakened collateral projection between MSNs in the striatum of PD models studied. Also both PLTS and FS interneurons tend to double their projections onto dMSNs and iMSNs respectively. Cholinergic interneurons are also less modulated by GABAergic tones and their firing and release of Ach is increased (Gittis and Kreitzer 2012). Another important alteration seen in different models of PD, is the subunit change in NMDA receptors and hence the consequent alteration in the glutamatergic neurotransmission mediated by these receptors (Gardoni, Ghiglieri et al. 2010). More specifically this alteration is observed in NMDA receptors on MSNs and cholinergic interneurons, which in turn has

shown to affect both dopamine release and synaptic transmission in striatum of the 6-OHDA model of PD (Gardoni and Bellone 2015). Loss of corticostriatal synaptic plasticity as a consequence of altered activity of different neuronal populations has also been observed in other models of PD. And as this is a key mechanism regulating motor control, this loss may have a direct impact on the disease phenotypes observed in PD (Calabresi, Galletti et al. 2007, Kreitzer and Malenka 2008, Bagetta, Ghiglieri et al. 2010).

2 AIMS

Loss of dopamine neurons and thereby dopamine input to the striatum in Parkinson's disease have profound effects on the overall synaptic transmission, synaptic plasticity and different neurotransmitter systems in both striatum and basal ganglia as part of the brain modulating motor movements. The overall aim of this study has been to investigate whether glutamatergic neurotransmission and plasticity are affected in striatum of a mouse model of Parkinson's disease and with aging as aging is the main risk factor for developing Parkinson's disease.

Specific aims of individual projects are listed below.

Paper I: to study the mechanisms of induction of LTP in the striatum using different electrophysiological recording methods.

Paper II: to investigate whether pharmacological manipulation of NMDA receptors containing GluN2D subunit can restore LTP in the striatum and behavioral deficits observed in a mouse model of Parkinson's disease.

Paper III: to study how aging affects synaptic plasticity in the striatum of aged mice.

3 MATERIAL AND METHODS

Animals

Animals used in all experiments were male C57Bl/6 mice age 4-11 weeks from Janvier Labs, France or Envigo, Holland. Aged mice used in study III were 20 months old and from Charles River, Germany. Animals were acclimatized to the new environment for at least 5 days upon arrival from the distributor before participating in experiments. All mice were maintained on a 12:12 hour's light/dark cycle and had free access to food and water. All efforts were made to minimize animal suffering and number of animals used for each set of experiments. All experiments were approved by the local ethical committee (Stockholms norra djurförsöksetiska nämnd).

6-OHDA lesion model of Parkinson's disease

We used unilateral 6-hydroxydopamine (6-OHDA) lesion model of Parkinson's disease in study I and II. To generate this model, the neurotoxin 6-OHDA was stereotactically injected in the substantia nigra pars compacta to produce degeneration of dopaminergic neurons and dopamine depletion of the striatum. To do so mice were first anesthetized with a single intraperitoneal (i.p) injection of 80 mg/kg ketamine and 5 mg/kg xylazine. After placement in a stereotaxic frame, 3 g of 6-OHDA dissolved in 0.01% ascorbic acid solution was injected over 2 minutes into substantia pars compacta of the right hemisphere. The coordinates for injection were AP: −3 mm; ML: −1.1 mm; and DV: −4.5 mm relative to bregma and the dural surface. Mice were allowed to recover from the surgery for 1- 3 weeks before they were used for electrophysiological or behavioral experiments.

Brain slice preparation

Mice underwent cervical dislocation followed by decapitation. Coronal corticostriatal brain slices (300 or 400 μm thick) were prepared with a microslicer (VT 1000S; Leica Microsystem, Heppenheim, Germany) in oxygenated (95% O2 + 5% CO2) artificial cerebrospinal fluid (aCSF) containing (in mM): NaCl (126), KCl (2.5), NaH2PO4 (1.2), MgCl2 (1.3), CaCl2 (2.4), glucose (10) and NaHCO3 (26) pH 7.4. For brain slices used in patch-clamp experiments the slices were prepared in a sucrose-based aCSF containing NaCl (15.9), KCl (2), NaH2PO4 (1), Sucrose (219.7), MgCl2 (5.2), CaCl2 (1.1), glucose (10) and NaHCO3 (26).

Slices were incubated for at least 1 hour, at 32°C in oxygenated (95% O2 + 5% CO2) artificial cerebrospinal fluid (aCSF) containing (in mmol/L): (126) NaCl, (2.5) KCl, (1.2) NaH2PO4, (1.3) MgCl2, (2.4) CaCl2, (10) glucose, and (26) NaHCO3, pH 7.4. For patch-

clamp experiments slices were incubated in a modified oxygenated aCSF containing (in mM): NaCl (126), KCl (2.5), NaH2PO4 (1.2), MgCl2 (4.7), CaCl2 (1), glucose (10) and NaHCO3 (23.4).Slices were transferred to a recording chamber and were continuously perfused with oxygenated aCSF at 28°C.

Electrophysiology in brain slices

Extracellular field potential recording

Extracellular field potentials were recorded using a glass micropipette filled with aCSF positioned on the slice surface in the dorsolateral part of the striatum. These synaptic responses were evoked by stimulation pulses applied every 15 seconds to the brain slice through a concentric bipolar stimulating electrode (FHC, Bowdoinham, ME) placed near the recording electrode on the surface of the slice. Single stimuli (0.1 ms duration) were applied at an intensity yielding 50%- 60% maximal response as assessed by a stimulus/response curve established, by measuring the amplitude of the field excitatory postsynaptic potentials/population spikes (fEPSP/PSs) evoked by increasing stimulation intensities. These fEPSP/PSs were mediated by glutamate acting on AMPA receptors. After 20 minutes stable baseline recording, high frequency stimulation (HFS) was used to induce LTP of the fEPSP/PS. HFS consisted of 100- Hz trains of 1- second duration repeated 4 times with a 10-second inter- train interval. Signals were amplified 500 or 1000 times via an Axopatch 200B or a GeneClamp 500B amplifier (Axon Instruments), acquired at 10 kHz and filtered at 2 kHz. Data were acquired and analyzed with the pClamp 9 or pClamp 10 software (Axon Instruments, Foster City CA, USA). Data are expressed as percent of the baseline response measured for each slice during the 10 minutes preceding the start of perfusion with drugs or HFS.

Whole-cell patch clamp and cell attached recording

Whole-cell patch-clamp and cell-attached recordings of medium spine neurons (MSNs) of the dorsolateral part of the striatum were made with patch electrodes (3-5 MΩ) filled with a potassium gluconate-based intracellular solution containing (in mM): D-gluconic acid potassium salt (120), KCl (20), HEPES (10), EGTA (10), MgCl2 (2), CaCl2 (1), ATP-Mg (2), GTPNa3 (0.3), pH = 7.3. AMPA receptor mediated excitatory postsynaptic currents (AMPAR-EPSCs) were evoked every 15s by electrical stimulation of the slice through a patch electrode filled with aCSF placed near the recorded neuron. Cell-attached recordings of MSNs were performed with patch electrodes (5-8 MΩ). A patch electrode filled with aCSF was placed near the recorded neuron. The position of this stimulation electrode and the stimulation intensity were adjusted to obtain stable synaptically-evoked spiking of a success

rate < 40% and a latency > 2.5 ms, evoked every 15s. HFS was applied with same protocol as for field recording after stable baseline.

A slice or neuron was considered to show long term synaptic plasticity if we observed a change in the response, relative to baseline, which was > 25% (voltage-clamp), > 20% (field recording), and doubled (cell-attached) 30 min or 1 h after HFS.

Behavioral test

Cylinder test

Forelimb-use asymmetry is one of the main motor impairments induced in the 6-OHDA lesion model of PD (Grealish, Mattsson et al. 2010, Glajch, Fleming et al. 2012). To assess this impairment and the effect of different treatments on this behavior the cylinder test was used in study II. One week after lesioning the mice with 6-OHDA, mice were injected with either vehicle or CIQ and, 90 minutes after the first (acute treatment) and the seventh injections (sub-chronic treatment), were placed in a transparent glass cylinder (13 cm diameter, 24 cm height) to examine forelimb-use asymmetry. When placed in the cylinder, mice explore the novel environment in the cylinder by standing on the hindlimbs and with forelimbs against the cylinder wall. We counted the number of times the mice touched the wall of the cylinder with their left forepaw (contralateral to the lesion) and right forepaw (ipsilateral to the lesion) during 5 minutes to evaluate forelimb- use asymmetry. Data were presented as the number of contralateral touches as a percentage of the total touches.

Western immunoblotting

Following 6-OHDA lesioning of mice the levels of tyrosine hydroxylase (TH) as a measurement of dopamine neuron loss were measured using western blot experiments (WB). Also, in study III levels of GluN2D and GluN1subunit of NMDA receptors and GluR1 subunit of AMPA receptors were measured in slices collected from aged mice. The detail description of the experimental procedure is described in paper III. In brief, striatum was dissected from brain slices and frozen in -20°C. Samples were processed in 1% sodium dodecyl sulfate (SDS) and boiled. Protein concentration was measured using standard protein assay kit (bicnichoninic acid protein assay) and equal amounts of protein were separated by SDS-polyacrylamide gel electrophoresis. Proteins were transferred to a nitrocellulose membrane and blocked with 5% (w/v) dry milk followed by incubation with primary antibodies and later with secondary antibodies. After washing the membranes immunoreactive bands were detected with BIO-RAD ChemiDoc MP imaging system. The levels of proteins were normalized for the value of ß-actin.

4 PRESENT INVESTIGATIONS

The constituent studies have focused on understanding the mechanism of long-term potentiation in the striatum of aged and parkinsonian mice. Also, by manipulating NMDA receptors, how normal levels of activity can be restored in the dopamine-denervated striatum.

Paper I

High frequency stimulation induces LTD of AMPA receptor-mediated postsynaptic responses and LTP of synaptically-evoked firing in the dorsolateral striatum

The discrepancy in inducing synaptic plasticity in striatum using high frequency stimulation is in large due to different experimental settings such as different recording solutions, usage of pharmacological blockers and area of stimulation. We examined the ability of HFS to induce synaptic plasticity using same protocol but with three different electrophysiological recording methods: whole-cell voltage clamp of MSNs, cell attached recording of MSNs and extracellular recordings of fEPSP/PS. In this paper we could demonstrate that under physiological concentration of Mg^{2+} and without addition of pharmacological blockers, HFS induces two opposing forms of synaptic plasticity in the striatum, i.e. LTD of AMPAR-EPSCs and LTP of synaptically-evoked firing in MSNs as well as of the fEPSP/PS. Also, our results demonstrate that the intensity of stimulation applied during single pulses; recording baseline and post-HFS are important for induction of LTP of the fEPSP/PS. This was observed comparing different stimulation intensities in their ability to increase fEPSP/PS after HFS, and we found that only intermediate intensities potentiate fEPSP/PS. LTP is mediated by D1R which require higher levels of dopamine for activation compared to D2R. Data obtained from whole cell recordings show LTD of AMPAR-mediated responses which may be explained by low stimulation intensities used in this type of recording and hence lower levels of dopamine released which are not sufficient to stimulate D1R and induce LTP. Based on our results cell attached recordings and field potential recordings are of advantage for studying LTP in striatum.

Paper II

CIQ, a positive allosteric modulator of GluN2C/D-containing N-methyl-d-aspartate receptors, rescues striatal synaptic plasticity deficit in a mouse model of Parkinson's disease

Physiological and pathophysiological processes involving NMDA receptors are highly dependent on the subunit composition of these receptors. The expression pattern of GluN2 subunit in striatum is altered in mouse models of PD. We previously had reported that by enhancing the activity of NMDA receptors that contain the GluN2D subunit using positive allosteric modulator of this subunit dopamine release can be enhanced in the partially

dopamine-depleted striatum. In this study we examined the ability of CIQ, a positive allosteric modulator of NMDA receptors containing GluN2C/2D subunits to rescue loss of LTP and forelimb-use asymmetry in the 6-OHDA lesion mouse model of PD. Using field potential recordings in the dorsolateral striatum we observed rescue of the impaired LTP in lesion striatal slices after i.p injection of a single dose of CIQ. LTP was unaffected in control slices after single i.p CIQ injection. Lower dose of CIQ administrated daily for 7 days (chronic) also restored LTP in the dopamine-depleted striatum. LTP was unaffected in the intact striatal slices. Forelimb-use asymmetry is a motor impairment observed in mice receiving a unilateral 6-OHDA lesion of the striatum. We tested the mice using a cylinder test and we demonstrated that mice receiving vehicle show a greater asymmetry after chronic treatment compared to mice receiving a chronic treatment with CIQ. Thus, CIQ acting on GluN2D subunit of NMDA receptors in striatum has the potential to reverse forelimb-use asymmetry in the 6-OHDA lesion mice. This effect of CIQ is likely mediated by acting and potentiating the upregulated levels of GluN2D expressed in the medium spiny neurons of the lesioned striatum. The shift from expression of GluN2B to GluN2D in MSNs in the lesion striatum results in a lower conductance and calcium permeability of NMDA receptors and hence lower excitability and loss of LTP. Therefore, CIQ administrated systematically enhances the activity of NMDA receptors in the lesion striatum back towards normal levels and thereby by applying HFS long term potentiation is rescued.

Paper III

A positive allosteric modulator of GluN2C/D-containing NMDA receptors fails to rescue impaired striatal synaptic plasticity in aged mice

Aging is the main risk factor for developing PD. As a consequence of aging many physiological processes are altered, which could become a risk factor for developing various diseases such as neurodegenerative disorders. PD and aging in many aspects share same pathophysiological pattern in the basal ganglia and striatum. Dopamine loss is observed upon aging and hence motor symptoms that are developed mimic the symptoms in PD. Thus, in this study we aimed to study LTP in striatum of aged mice as LTP is crucial in regulating the motor pathway in the basal ganglia. Also, we investigated whether CIQ can have the same positive effect as seen in PD in study II on plasticity in striatum of aged mice. We observed loss of LTP in dorsolateral portion of the striatum of aged mice compared to young mice and no effect of CIQ on LTP in aged striatum. The loss of LTP in striatal slices from aged mice is most likely due to significant loss of dopamine and also AMPA receptors as confirmed with western blot experiments. However, in contrast to 6-OHDA lesion mice the levels of GluN2D subunit of NMDA receptors were not significantly different than aged mice as shown with our western blot experiments. This might explain why we observed no effect of CIQ on LTP in aged striatal slices.

5 GENERAL CONCLUSIONS

This thesis has been aimed to better understand the mechanism of synaptic plasticity in the striatum as the brain region involved in modulating movements. Plasticity during development and later in life is necessary for organized nervous system circuitry, establishment of functional networks, functional and structural adaptation to external stimuli and learning and memory formation amongst others (96). In PD and also as a result of normal aging synaptic plasticity in striatum is lost. Yet there is little known about mechanism of induction of synaptic plasticity in striatum and controversy regarding types of plasticity which are inducible under experimental settings are great. Both PD and aging result in motor impairments such as bradykinesia (slowness of movements). Manifestation of motor symptoms in PD and upon healthy aging are possibly due to loss of dopamine and altered neurotransmission and plasticity in basal ganglia. Loss of LTP in striatum can also be due to alteration in the glutamatergic neurotransmission and NMDA receptors upon dopaminergic neurodegeneration. This is of importance in attempts to identify alternative/complementary therapeutic targets to dopamine replacement therapy for PD. Results obtained from the studies included in this thesis have led to the following conclusions:

I. Our findings described in paper I demonstrate that, a stimulation protocol, which is commonly used to induce synaptic plasticity in various brain regions, induces opposing forms of plasticity in striatum. HFS induces LTD of pure AMPA responses but induces LTP of the firing in projection neurons in corticostriatal brain slices. The polarity of plasticity therefore, depends on electrophysiological recording method used. Also we could demonstrate that stimulation intensity is of importance in the abbility of the different methods to induce LTP. Lower levels of dopamine are released under low stimulation intensities which is not sufficient for induction of LTP. Also importantely we could based on our results confirm that under normal levels (physiological) of Mg^{2+} and without blocking GABA, LTP can be induced as there is a great contreversy regarding these experimental conditions. Based on our results we conclude that methods that do not alter the intracellular milieu of the recorded neurons such as cell attached and field potential recordings that also induce LTP of synaptically evoked firing can be useful for future studies.

II. GluN2 subunit of NMDA receptors determine the functional and pharmacological properties of NMDA receptors. Also, they are of terapeutic importance for managing motor symptoms of PD. We could confirm that by using a positive allosteric modulator of GluN2C/2D containing NMDA receptors which rescued lost

LTP in dopamine-depleted striatal slices. More importantely forelimb-use asymmetry the common motor phenotype upon 6-OHDA lesioning of striatum was reduced upon a chronic treatment with CIQ. The positive effect of CIQ on LTP and the behavioral impairment is most likely mediated due to upregulation of GluN2D in MSNs of the dopamine-depleted striatum. Based on our previous results and the current data obtained in this thesis we suggest GluN2D containing NMDA receptors as a potential target for developing antiparkinsonian drugs.

III. As aging is the main risk factor for developing PD, there are similarities between phatogenesis of PD and normal aging. Based on our results glutamatergic synaptic transmission is increased in aged mice but this is not due to altered glutamate release from presynaptic terminals. CIQ did not have any effect on LTP in the aged striatum, this might be explained by our results showing that levels of GluN2D are not affected due to aging. Our findings demonstrate that loss of LTP in dorsolateral striatum in aged mice can be due to loss of domapine which was reduced in the aged striatal slices to same levels as in PD. The level of TH in our experiments were much more reduced than previous published studies. This is important when studying aging and its consequences since previous studies confim that aging is complex and diverse between and within individuals of the same species (Rodriguez, Rodriguez-Sabate et al. 2015). Even though DA levels were reduced to same levels as seen in PD models, NMDA receptor subunit composition were not altered as seen in 6-OHDA lesion model of PD. These results show that other mechanisms are responsible for the loss of LTP in aged striatum than alteration in NMDA receptors and transmission.

6 FUTURE PERSPECTIVES

We are living in a world with growing population and ever increasing life expectancy. This will inevitably lead to a drastic increase in the incidence of many, universal age related neurological disorders such as Parkinson's disease. So, every individual living in a country with high life expectancy will be in one way or another affected by the increasing risk of developing an age related disorder. If we put it this way, this is not just a number of clinical diagnosis being made, this is you or a loved one losing basic functions like the ability to move or even remembering the most basic things. According to WHO Parkinson's disease is the third most common neurological disorder after epilepsy and Alzheimer's disease (and other dementias). PD results in long-term disability and significant loss of quality of life. It does not only affect the motor movements but also cognition and the mental health which are more devastating to some PD patients. The need for research in this field is hence enormous. The contributions that researchers do today are to understand and identify how the disease pathology is being triggered and developed as to date this is unknown. To be able to treat this disease and halt neurodegeneration the cause of the disease must be identified. Also a great effort and research is directed towards finding therapeutic targets and compounds which can help the patients in the different stages of the disease and symptoms. Research presented in this thesis is a miniscule contribution toward better understanding how PD affects the networks controlling movements and what/where to target to be able to rescue some of the lost mechanisms and functions within this network.

7 ACKNOWLEDGEMENTS

I remember August 2007, during the orientation week for the newly admitted biomedical students at the Uppsala University we attended a tour of the Rudbeck laboratory and talked to researchers about their work as an inspiration for possible future careers after finishing the program. I left the building after the visit, being extremely excited and feeling uplifted, I called my mom telling her that I am convinced that I have chosen the right university program and that the future and what I want to do is even more clear to me and this is exactly what I wanted. Here I am now, almost 11 years later and I am at the end of one road but beginning of another. The journey has not been easy and has had many up and downs but I am happy and grateful for these years, the experiences, challenges, new possibilities and the people whom I have got to know and people whom have made this journey possible and more joyful at times when it has been difficult.

I would like to thank my supervisor **Karima Chergui**, for supervising me during my PhD education and to share with me of her knowledge and experience. Life lesson I learned from you early during my PhD that I will always keep in mind is to work hard and it will sooner or later be paid off despite failures along the way.

Thanks to my Co-supervisors **Per Svenningsson** and **Xiaoqun Zhang** for their inputs, guidance and collaboration during this time.

Many thanks to **Håkan Westerblad** and **Ernst Brodin** for being there whenever I needed extra support and guidance.

Thanks to my mentor **Gilad Silberberg**, for always being positive and encouraging me to look forward and sharing with me of his experiences in academia.

Thanks to members of Molecular Neurophysiology group: **Ning**, **Olga** and **Giacomo**. Thanks **Ning** for the nice collaboration in all the projects, you have always been very supportive and lending a helping hand whenever needed. **Olga**, we had a nice trip to Washington together and I got to know you better, it was really fun rearranging the office together with you. **Giacomo**, you are a friend more than a colleague to me. Thanks for all the nice talks and laughter's we have shared at work and fun times outside work and the trips that I will never forget, especially Sri Lanka. Sorry for needing to hear me talk too much about the "same subject" ;-) in times, but I am convinced that these last 2 years would have been much more difficult for me without you.

To my Co-PhD student representatives **Michaela**, **Alex**, **Chiara** and **Delilah** thanks for all the PhD events that we organized together and talks about our lives as PhD students. **Chiara** good luck with rest of your PhD, don't work so much and give the flies some break ;-). **Delilah**, you will be great at your thesis defence, no stress and good luck for your next step in your career, it will be great for sure. **Alex**, I am so happy and lucky for having you as a

friend. You have always sent encouraging and loving messages at the times when I have needed them the most and you must know that I have always appreciated them. Thanks for the friendship and times we share outside work. You are always welcome to our place whenever you lock yourself outside again ;-).

To my fellow PhD-students at FyFa **Alexandra**, **Resti**, **Milana**, **Maria**, **Petter**, **Paula**, **Sara**, **Maarten** and **Nilesh** thanks for all the funny and memorable moments created around lunch and fika breaks. I am happy to have got to know you all!

To my NS&B friend **Anna** I found a true friend after the 8 weeks in Woods Hole and for that I am so happy. Thanks for coming to my PhD defence and party. So incredible that we have been present in each other's important life events.

To my dear friends **Manizheh**, **Mastoureh**, **Yaser** and **Shahin** thanks for all the lunch gatherings that have continued until dinner and late evenings and of course the talks. Special thanks to **Manizheh** for all the coffee breaks and updates and talks during the last years. Talking to you even when not at work has become a habit for me that I happily would continue with.

Merima, **Yanara** and **Johanna**, my dear friends from the very beginning of this journey! **Johanna**, thanks for all the nice conversations far away over skype or close, it's always with a positive energy that I continue my day after talking to you. **Yanara**, I have got to know you more and more over the past few years and I am happy for this. I enjoy our tjejkvällar together with you a lot and hope for many more in future after the stresses for our thesis defences are over. **Merima**, we tend to argue a lot sometimes but you know that this is part of being really really good friends and having known each other for too long time ;-) Thanks for the friendship and all the moments we have shared.

Thanks to my uncle **daie Ehsan** for being more than an uncle for me! For all the support in my private life and all the love!

To my grandmother and grandfather, **maman Effat** and **baba Javad**, your love, kindness, care and support for me warms my heart. Knowing that you are there and wish all the best for me gives me strength.

To my brothers, **Amin** and **Ahad**, I guess we are very much alike despite all ;-) you as with me don't show your love verbally but your love and support is transparent through the way you care for me and always help and support me in all the different everyday matters like **Ahad** helping me to move more than several times between different cities in Sweden and abroad and also in Stockholm. **Amin**, I know I can always count on you when I need advice on various different matters and you do your best to help me and sorry if I don't always show gratitude.

My brother **Ali**, you were with me in every moment of writing this thesis as you left us on the day when I started writing the first line of this thesis. Your life brought joy and wisdom for us

as your family. Your place will never be replaced in our heart but hopefully we will eventually learn how to live with this great empty space that you left behind as you leave this earthly life.

Thanks to my love, my fiancé **Erik**, you came to my life in the most difficult time during my PhD and from the very beginning even before knowing that much about PhD life and the challenges accompanying research in academia; you have supported me and been the stable source of comfort and calm that I have turned to. I am so happy to have you as my life partner and "behtarin doost". I look forward to our long vacation after my defence and many many more years together with you.

My deepest gratitude to my mom **Maman,** whom without, none of this would have been possible. For your unconditional love I am forever grateful and simply cannot return all that you have done for me and my brothers.

8 REFERENCES

Afifi, A. K. (2003). "The basal ganglia: a neural network with more than motor function." Semin Pediatr Neurol **10**(1): 3-10.

Akopian, G. and J. P. Walsh (2006). "Pre- and postsynaptic contributions to age-related alterations in corticostriatal synaptic plasticity." Synapse **60**(3): 223-238.

Bagetta, V., V. Ghiglieri, C. Sgobio, P. Calabresi and B. Picconi (2010). "Synaptic dysfunction in Parkinson's disease." Biochem Soc Trans **38**(2): 493-497.

Banati, R. B., S. E. Daniel and S. B. Blunt (1998). "Glial pathology but absence of apoptotic nigral neurons in long-standing Parkinson's disease." Mov Disord **13**(2): 221-227.

Bar-Gad, I. and H. Bergman (2001). "Stepping out of the box: information processing in the neural networks of the basal ganglia." Curr Opin Neurobiol **11**(6): 689-695.

Bartels, A. L. and K. L. Leenders (2009). "Parkinson's disease: the syndrome, the pathogenesis and pathophysiology." Cortex **45**(8): 915-921.

Bergado, J. A. and W. Almaguer (2002). "Aging and synaptic plasticity: a review." Neural Plast **9**(4): 217-232.

Beyer, K., M. Domingo-Sabat and A. Ariza (2009). "Molecular pathology of Lewy body diseases." Int J Mol Sci **10**(3): 724-745.

Bezard, E., Z. Yue, D. Kirik and M. G. Spillantini (2013). "Animal models of Parkinson's disease: limits and relevance to neuroprotection studies." Mov Disord **28**(1): 61-70.

Biskup, S., D. J. Moore, F. Celsi, S. Higashi, A. B. West, S. A. Andrabi, K. Kurkinen, S. W. Yu, J. M. Savitt, H. J. Waldvogel, R. L. Faull, P. C. Emson, R. Torp, O. P. Ottersen, T. M. Dawson and V. L. Dawson (2006). "Localization of LRRK2 to membranous and vesicular structures in mammalian brain." Ann Neurol **60**(5): 557-569.

Biskup, S. and A. B. West (2009). "Zeroing in on LRRK2-linked pathogenic mechanisms in Parkinson's disease." Biochim Biophys Acta **1792**(7): 625-633.

Blanke, M. L. and A. M. J. VanDongen (2009). Activation Mechanisms of the NMDA Receptor. Biology of the NMDA Receptor. A. M. Van Dongen. Boca Raton (FL).

Bolam, J. P., J. J. Hanley, P. A. Booth and M. D. Bevan (2000). "Synaptic organisation of the basal ganglia." J Anat **196 (Pt 4)**: 527-542.

Boya, P., F. Reggiori and P. Codogno (2013). "Emerging regulation and functions of autophagy." Nat Cell Biol **15**(7): 713-720.

Braak, H., E. Ghebremedhin, U. Rub, H. Bratzke and K. Del Tredici (2004). "Stages in the development of Parkinson's disease-related pathology." Cell Tissue Res **318**(1): 121-134.

Breydo, L., J. W. Wu and V. N. Uversky (2012). "Alpha-synuclein misfolding and Parkinson's disease." Biochim Biophys Acta **1822**(2): 261-285.

Brooks, D. J. (2000). "Dopamine agonists: their role in the treatment of Parkinson's disease." J Neurol Neurosurg Psychiatry **68**(6): 685-689.

Burnell, E. S., M. Irvine, G. Fang, K. Sapkota, D. E. Jane and D. T. Monaghan (2018). "Positive and Negative Allosteric Modulators of N-Methyl-d-aspartate (NMDA) Receptors: Structure-Activity Relationships and Mechanisms of Action." J Med Chem.

Calabresi, P., D. Centonze, P. Gubellini, G. A. Marfia, A. Pisani, G. Sancesario and G. Bernardi (2000). "Synaptic transmission in the striatum: from plasticity to neurodegeneration." Prog Neurobiol **61**(3): 231-265.

Calabresi, P., F. Galletti, E. Saggese, V. Ghiglieri and B. Picconi (2007). "Neuronal networks and synaptic plasticity in Parkinson's disease: beyond motor deficits." Parkinsonism Relat Disord **13 Suppl 3**: S259-262.

Calabresi, P., B. Picconi, A. Tozzi, V. Ghiglieri and M. Di Filippo (2014). "Direct and indirect pathways of basal ganglia: a critical reappraisal." Nat Neurosci **17**(8): 1022-1030.

Calabresi, P., A. Pisani, D. Centonze and G. Bernardi (1997). "Synaptic plasticity and physiological interactions between dopamine and glutamate in the striatum." Neurosci Biobehav Rev **21**(4): 519-523.

Cepeda, C. and M. S. Levine (2012). "Dopamine-NMDA receptor interactions: twenty years later." Dev Neurosci **34**(1): 2-4.

Cerovic, M., R. d'Isa, R. Tonini and R. Brambilla (2013). "Molecular and cellular mechanisms of dopamine-mediated behavioral plasticity in the striatum." Neurobiol Learn Mem **105**: 63-80.

Chan, C. S., T. S. Gertler and D. J. Surmeier (2009). "Calcium homeostasis, selective vulnerability and Parkinson's disease." Trends Neurosci **32**(5): 249-256.

Cheatwood, J. L., J. V. Corwin and R. L. Reep (2005). "Overlap and interdigitation of cortical and thalamic afferents to dorsocentral striatum in the rat." Brain Res **1036**(1-2): 90-100.

Chu, Y., H. Dodiya, P. Aebischer, C. W. Olanow and J. H. Kordower (2009). "Alterations in lysosomal and proteasomal markers in Parkinson's disease: relationship to alpha-synuclein inclusions." Neurobiol Dis **35**(3): 385-398.

Damier, P., E. C. Hirsch, P. Zhang, Y. Agid and F. Javoy-Agid (1993). "Glutathione peroxidase, glial cells and Parkinson's disease." Neuroscience **52**(1): 1-6.

Darbin, O. (2012). "The aging striatal dopamine function." Parkinsonism Relat Disord **18**(5): 426-432.

Decressac, M., N. Volakakis, A. Bjorklund and T. Perlmann (2013). "NURR1 in Parkinson disease--from pathogenesis to therapeutic potential." Nat Rev Neurol **9**(11): 629-636.

Dehay, B., M. Bourdenx, P. Gorry, S. Przedborski, M. Vila, S. Hunot, A. Singleton, C. W. Olanow, K. M. Merchant, E. Bezard, G. A. Petsko and W. G. Meissner (2015). "Targeting alpha-synuclein for treatment of Parkinson's disease: mechanistic and therapeutic considerations." Lancet Neurol **14**(8): 855-866.

Dexter, D. T. and P. Jenner (2013). "Parkinson disease: from pathology to molecular disease mechanisms." Free Radic Biol Med **62**: 132-144.

Di Filippo, M., B. Picconi, M. Tantucci, V. Ghiglieri, V. Bagetta, C. Sgobio, A. Tozzi, L. Parnetti and P. Calabresi (2009). "Short-term and long-term plasticity at corticostriatal synapses: implications for learning and memory." Behav Brain Res **199**(1): 108-118.

Ding, J. B., J. N. Guzman, J. D. Peterson, J. A. Goldberg and D. J. Surmeier (2010). "Thalamic gating of corticostriatal signaling by cholinergic interneurons." Neuron **67**(2): 294-307.

Dorszewska, J., M. Prendecki, M. Lianeri and W. Kozubski (2014). "Molecular Effects of L-dopa Therapy in Parkinson's Disease." Curr Genomics **15**(1): 11-17.

Duty, S. and P. Jenner (2011). "Animal models of Parkinson's disease: a source of novel treatments and clues to the cause of the disease." Br J Pharmacol **164**(4): 1357-1391.

Finlay, C. and S. Duty (2014). "Therapeutic potential of targeting glutamate receptors in Parkinson's disease." J Neural Transm (Vienna) **121**(8): 861-880.

Friedman, L. G., M. L. Lachenmayer, J. Wang, L. He, S. M. Poulose, M. Komatsu, G. R. Holstein and Z. Yue (2012). "Disrupted autophagy leads to dopaminergic axon and dendrite degeneration and promotes presynaptic accumulation of alpha-synuclein and LRRK2 in the brain." J Neurosci **32**(22): 7585-7593.

Furukawa, H., S. K. Singh, R. Mancusso and E. Gouaux (2005). "Subunit arrangement and function in NMDA receptors." Nature **438**(7065): 185-192.

Gardoni, F. and C. Bellone (2015). "Modulation of the glutamatergic transmission by Dopamine: a focus on Parkinson, Huntington and Addiction diseases." Front Cell Neurosci **9**: 25.

Gardoni, F., V. Ghiglieri, M. Di Luca and P. Calabresi (2010). "Assemblies of glutamate receptor subunits with post-synaptic density proteins and their alterations in Parkinson's disease." Prog Brain Res **183**: 169-182.

Girault, J. A. (2012). "Integrating neurotransmission in striatal medium spiny neurons." Adv Exp Med Biol **970**: 407-429.

Gittis, A. H. and A. C. Kreitzer (2012). "Striatal microcircuitry and movement disorders." Trends Neurosci **35**(9): 557-564.

Gittis, A. H., A. B. Nelson, M. T. Thwin, J. J. Palop and A. C. Kreitzer (2010). "Distinct roles of GABAergic interneurons in the regulation of striatal output pathways." J Neurosci **30**(6): 2223-2234.

Glajch, K. E., S. M. Fleming, D. J. Surmeier and P. Osten (2012). "Sensorimotor assessment of the unilateral 6-hydroxydopamine mouse model of Parkinson's disease." Behav Brain Res **230**(2): 309-316.

Goedert, M., M. G. Spillantini, K. Del Tredici and H. Braak (2013). "100 years of Lewy pathology." Nat Rev Neurol **9**(1): 13-24.

Grealish, S., B. Mattsson, P. Draxler and A. Bjorklund (2010). "Characterisation of behavioural and neurodegenerative changes induced by intranigral 6-hydroxydopamine lesions in a mouse model of Parkinson's disease." Eur J Neurosci **31**(12): 2266-2278.

Hackos, D. H. and J. E. Hanson (2017). "Diverse modes of NMDA receptor positive allosteric modulation: Mechanisms and consequences." Neuropharmacology **112**(Pt A): 34-45.

Hirsch, E. C. and S. Hunot (2009). "Neuroinflammation in Parkinson's disease: a target for neuroprotection?" Lancet Neurol **8**(4): 382-397.

Izzo, P. N., A. M. Graybiel and J. P. Bolam (1987). "Characterization of substance P- and [Met]enkephalin-immunoreactive neurons in the caudate nucleus of cat and ferret by a single section Golgi procedure." Neuroscience **20**(2): 577-587.

Kadkhodaei, B., A. Alvarsson, N. Schintu, D. Ramskold, N. Volakakis, E. Joodmardi, T. Yoshitake, J. Kehr, M. Decressac, A. Bjorklund, R. Sandberg, P. Svenningsson and T. Perlmann (2013). "Transcription factor Nurr1 maintains fiber integrity and nuclear-encoded mitochondrial gene expression in dopamine neurons." Proc Natl Acad Sci U S A **110**(6): 2360-2365.

Kalia, L. V. and A. E. Lang (2015). "Parkinson's disease." Lancet **386**(9996): 896-912.

Kenney, D. L. and E. E. Benarroch (2015). "The autophagy-lysosomal pathway: General concepts and clinical implications." Neurology **85**(7): 634-645.

Kingsbury, A. E., R. Bandopadhyay, L. Silveira-Moriyama, H. Ayling, C. Kallis, W. Sterlacci, H. Maeir, W. Poewe and A. J. Lees (2010). "Brain stem pathology in Parkinson's disease: an evaluation of the Braak staging model." Mov Disord **25**(15): 2508-2515.

Kita, H. and S. T. Kitai (1988). "Glutamate decarboxylase immunoreactive neurons in rat neostriatum: their morphological types and populations." Brain Res **447**(2): 346-352.

Koos, T. and J. M. Tepper (2002). "Dual cholinergic control of fast-spiking interneurons in the neostriatum." J Neurosci **22**(2): 529-535.

Kordower, J. H., C. W. Olanow, H. B. Dodiya, Y. Chu, T. G. Beach, C. H. Adler, G. M. Halliday and R. T. Bartus (2013). "Disease duration and the integrity of the nigrostriatal system in Parkinson's disease." Brain **136**(Pt 8): 2419-2431.

Kravitz, A. V., B. S. Freeze, P. R. Parker, K. Kay, M. T. Thwin, K. Deisseroth and A. C. Kreitzer (2010). "Regulation of parkinsonian motor behaviours by optogenetic control of basal ganglia circuitry." Nature **466**(7306): 622-626.

Kreitzer, A. C. and R. C. Malenka (2008). "Striatal plasticity and basal ganglia circuit function." Neuron **60**(4): 543-554.

Lee, A. and R. M. Gilbert (2016). "Epidemiology of Parkinson Disease." Neurol Clin **34**(4): 955-965.

Lees, A. J., J. Hardy and T. Revesz (2009). "Parkinson's disease." Lancet **373**(9680): 2055-2066.

Lerner, T. N. and A. C. Kreitzer (2011). "Neuromodulatory control of striatal plasticity and behavior." Curr Opin Neurobiol **21**(2): 322-327.

Lim, S. A., U. J. Kang and D. S. McGehee (2014). "Striatal cholinergic interneuron regulation and circuit effects." Front Synaptic Neurosci **6**: 22.

Lopez de Maturana, R. and R. Sanchez-Pernaute (2010). "Regulation of corticostriatal synaptic plasticity by G protein-coupled receptors." CNS Neurol Disord Drug Targets **9**(5): 601-615.

Lovinger, D. M. (2010). "Neurotransmitter roles in synaptic modulation, plasticity and learning in the dorsal striatum." Neuropharmacology **58**(7): 951-961.

Magnusson, K. R. (1998). "The aging of the NMDA receptor complex." Front Biosci **3**: e70-80.

Mallet, N., C. Le Moine, S. Charpier and F. Gonon (2005). "Feedforward inhibition of projection neurons by fast-spiking GABA interneurons in the rat striatum in vivo." J Neurosci **25**(15): 3857-3869.

Masilamoni, G. J. and Y. Smith (2018). "Metabotropic glutamate receptors: targets for neuroprotective therapies in Parkinson disease." Curr Opin Pharmacol **38**: 72-80.

Mathai, A. and Y. Smith (2011). "The corticostriatal and corticosubthalamic pathways: two entries, one target. So what?" Front Syst Neurosci **5**: 64.

McGeer, P. L., S. Itagaki, B. E. Boyes and E. G. McGeer (1988). "Reactive microglia are positive for HLA-DR in the substantia nigra of Parkinson's and Alzheimer's disease brains." Neurology **38**(8): 1285-1291.

Michel, P. P., E. C. Hirsch and S. Hunot (2016). "Understanding Dopaminergic Cell Death Pathways in Parkinson Disease." Neuron **90**(4): 675-691.

Mizuno, Y., S. Ohta, M. Tanaka, S. Takamiya, K. Suzuki, T. Sato, H. Oya, T. Ozawa and Y. Kagawa (1989). "Deficiencies in complex I subunits of the respiratory chain in Parkinson's disease." Biochem Biophys Res Commun **163**(3): 1450-1455.

Mullasseril, P., K. B. Hansen, K. M. Vance, K. K. Ogden, H. Yuan, N. L. Kurtkaya, R. Santangelo, A. G. Orr, P. Le, K. M. Vellano, D. C. Liotta and S. F. Traynelis (2010). "A subunit-selective potentiator of NR2C- and NR2D-containing NMDA receptors." Nat Commun **1**: 90.

Mursaleen, L. R. and J. A. Stamford (2016). "Drugs of abuse and Parkinson's disease." Prog Neuropsychopharmacol Biol Psychiatry **64**: 209-217.

Nouhi, M., X. Zhang, N. Yao and K. Chergui (2018). "CIQ, a positive allosteric modulator of GluN2C/D-containing N-methyl-d-aspartate receptors, rescues striatal synaptic plasticity deficit in a mouse model of Parkinson's disease." CNS Neurosci Ther **24**(2): 144-153.

Ogden, K. K., A. Khatri, S. F. Traynelis and S. A. Heldt (2014). "Potentiation of GluN2C/D NMDA receptor subtypes in the amygdala facilitates the retention of fear and extinction learning in mice." Neuropsychopharmacology **39**(3): 625-637.

Ogden, K. K. and S. F. Traynelis (2011). "New advances in NMDA receptor pharmacology." Trends Pharmacol Sci **32**(12): 726-733.

Ogden, K. K. and S. F. Traynelis (2013). "Contribution of the M1 transmembrane helix and pre-M1 region to positive allosteric modulation and gating of N-methyl-D-aspartate receptors." Mol Pharmacol **83**(5): 1045-1056.

Olivares, D., V. K. Deshpande, Y. Shi, D. K. Lahiri, N. H. Greig, J. T. Rogers and X. Huang (2012). "N-methyl D-aspartate (NMDA) receptor antagonists and memantine treatment for Alzheimer's disease, vascular dementia and Parkinson's disease." Curr Alzheimer Res **9**(6): 746-758.

Oswald, M. J., D. E. Oorschot, J. M. Schulz, J. Lipski and J. N. Reynolds (2009). "IH current generates the afterhyperpolarisation following activation of subthreshold cortical synaptic inputs to striatal cholinergic interneurons." J Physiol **587**(Pt 24): 5879-5897.

Pacelli, C., N. Giguere, M. J. Bourque, M. Levesque, R. S. Slack and L. E. Trudeau (2015). "Elevated Mitochondrial Bioenergetics and Axonal Arborization Size Are Key Contributors to the Vulnerability of Dopamine Neurons." Curr Biol **25**(18): 2349-2360.

Paoletti, P. (2011). "Molecular basis of NMDA receptor functional diversity." Eur J Neurosci **33**(8): 1351-1365.

Paoletti, P., C. Bellone and Q. Zhou (2013). "NMDA receptor subunit diversity: impact on receptor properties, synaptic plasticity and disease." Nat Rev Neurosci **14**(6): 383-400.

Paoletti, P. and J. Neyton (2007). "NMDA receptor subunits: function and pharmacology." Curr Opin Pharmacol **7**(1): 39-47.

Parker, W. D., Jr., J. K. Parks and R. H. Swerdlow (2008). "Complex I deficiency in Parkinson's disease frontal cortex." Brain Res **1189**: 215-218.

Partridge, J. G., K. C. Tang and D. M. Lovinger (2000). "Regional and postnatal heterogeneity of activity-dependent long-term changes in synaptic efficacy in the dorsal striatum." J Neurophysiol **84**(3): 1422-1429.

Perszyk, R. E., J. O. DiRaddo, K. L. Strong, C. M. Low, K. K. Ogden, A. Khatri, G. A. Vargish, K. A. Pelkey, L. Tricoire, D. C. Liotta, Y. Smith, C. J. McBain and S. F. Traynelis (2016). "GluN2D-Containing N-methyl-d-Aspartate Receptors Mediate Synaptic Transmission in Hippocampal Interneurons and Regulate Interneuron Activity." Mol Pharmacol **90**(6): 689-702.

Picconi, B., V. Ghiglieri, V. Bagetta, I. Barone, C. Sgobio and P. Calabresi (2008). "Striatal synaptic changes in experimental parkinsonism: role of NMDA receptor trafficking in PSD." Parkinsonism Relat Disord **14 Suppl 2**: S145-149.

Pires, A. O., F. G. Teixeira, B. Mendes-Pinheiro, S. C. Serra, N. Sousa and A. J. Salgado (2017). "Old and new challenges in Parkinson's disease therapeutics." Prog Neurobiol **156**: 69-89.

Pisani, A., G. Bernardi, J. Ding and D. J. Surmeier (2007). "Re-emergence of striatal cholinergic interneurons in movement disorders." Trends Neurosci **30**(10): 545-553.

Pisani, A., D. Centonze, G. Bernardi and P. Calabresi (2005). "Striatal synaptic plasticity: implications for motor learning and Parkinson's disease." Mov Disord **20**(4): 395-402.

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.

Post, M. R., O. J. Lieberman and E. V. Mosharov (2018). "Can Interactions Between alpha-Synuclein, Dopamine and Calcium Explain Selective Neurodegeneration in Parkinson's Disease?" Front Neurosci **12**: 161.

Przedborski, S. (2017). "The two-century journey of Parkinson disease research." Nat Rev Neurosci **18**(4): 251-259.

Przedborski, S., Q. Chen, M. Vila, B. I. Giasson, R. Djaldatti, S. Vukosavic, J. M. Souza, V. Jackson-Lewis, V. M. Lee and H. Ischiropoulos (2001). "Oxidative post-translational modifications of alpha-synuclein in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of Parkinson's disease." J Neurochem **76**(2): 637-640.

Puspita, L., S. Y. Chung and J. W. Shim (2017). "Oxidative stress and cellular pathologies in Parkinson's disease." Mol Brain **10**(1): 53.

Regan, M. C., A. Romero-Hernandez and H. Furukawa (2015). "A structural biology perspective on NMDA receptor pharmacology and function." Curr Opin Struct Biol **33**: 68-75.

Rodriguez, M., C. Rodriguez-Sabate, I. Morales, A. Sanchez and M. Sabate (2015). "Parkinson's disease as a result of aging." Aging Cell **14**(3): 293-308.

Salvadores, N., M. Sanhueza, P. Manque and F. A. Court (2017). "Axonal Degeneration during Aging and Its Functional Role in Neurodegenerative Disorders." Front Neurosci **11**: 451.

Salvatore, M. F., S. Apparsundaram and G. A. Gerhardt (2003). "Decreased plasma membrane expression of striatal dopamine transporter in aging." Neurobiol Aging **24**(8): 1147-1154.

Schapira, A. H., J. M. Cooper, D. Dexter, J. B. Clark, P. Jenner and C. D. Marsden (1990). "Mitochondrial complex I deficiency in Parkinson's disease." J Neurochem **54**(3): 823-827.

Schirinzi, T., G. Madeo, G. Martella, M. Maltese, B. Picconi, P. Calabresi and A. Pisani (2016). "Early synaptic dysfunction in Parkinson's disease: Insights from animal models." Mov Disord **31**(6): 802-813.

Schulz, J. B., L. Hausmann and J. Hardy (2016). "199 years of Parkinson disease - what have we learned and what is the path to the future?" J Neurochem **139 Suppl 1**: 3-7.

Shipp, S. (2017). "The functional logic of corticostriatal connections." Brain Struct Funct **222**(2): 669-706.

Stern, E. A., D. Jaeger and C. J. Wilson (1998). "Membrane potential synchrony of simultaneously recorded striatal spiny neurons in vivo." Nature **394**(6692): 475-478.

Subramaniam, S. R. and M. F. Chesselet (2013). "Mitochondrial dysfunction and oxidative stress in Parkinson's disease." Prog Neurobiol **106-107**: 17-32.

Sucher, N. J., M. Awobuluyi, Y. B. Choi and S. A. Lipton (1996). "NMDA receptors: from genes to channels." Trends Pharmacol Sci **17**(10): 348-355.

Surmeier, D. J., J. Ding, M. Day, Z. Wang and W. Shen (2007). "D1 and D2 dopamine-receptor modulation of striatal glutamatergic signaling in striatal medium spiny neurons." Trends Neurosci **30**(5): 228-235.

Surmeier, D. J. and A. M. Graybiel (2012). "A feud that wasn't: acetylcholine evokes dopamine release in the striatum." Neuron **75**(1): 1-3.

Surmeier, D. J., J. N. Guzman, J. Sanchez-Padilla and J. A. Goldberg (2010). "What causes the death of dopaminergic neurons in Parkinson's disease?" Prog Brain Res **183**: 59-77.

Surmeier, D. J., J. A. Obeso and G. M. Halliday (2017). "Parkinson's Disease Is Not Simply a Prion Disorder." J Neurosci **37**(41): 9799-9807.

Surmeier, D. J., J. A. Obeso and G. M. Halliday (2017). "Selective neuronal vulnerability in Parkinson disease." Nat Rev Neurosci **18**(2): 101-113.

Surmeier, D. J. and D. Sulzer (2013). "The pathology roadmap in Parkinson disease." Prion **7**(1): 85-91.

Suryavanshi, P. S., R. R. Ugale, D. Yilmazer-Hanke, D. J. Stairs and S. M. Dravid (2014). "GluN2C/GluN2D subunit-selective NMDA receptor potentiator CIQ reverses MK-801-induced impairment in prepulse inhibition and working memory in Y-maze test in mice." Br J Pharmacol **171**(3): 799-809.

Swanger, S. A., K. M. Vance, J. F. Pare, F. Sotty, K. Fog, Y. Smith and S. F. Traynelis (2015). "NMDA Receptors Containing the GluN2D Subunit Control Neuronal Function in the Subthalamic Nucleus." J Neurosci **35**(48): 15971-15983.

Swanson, C. R., S. L. Sesso and M. E. Emborg (2009). "Can we prevent parkinson's disease?" Front Biosci (Landmark Ed) **14**: 1642-1660.

Taddei, R. N., F. Spinnato and P. Jenner (2017). "New Symptomatic Treatments for the Management of Motor and Nonmotor Symptoms of Parkinson's Disease." Int Rev Neurobiol **132**: 407-452.

Tepper, J. M., E. D. Abercrombie and J. P. Bolam (2007). "Basal ganglia macrocircuits." Prog Brain Res **160**: 3-7.

Tepper, J. M., T. Koos and C. J. Wilson (2004). "GABAergic microcircuits in the neostriatum." Trends Neurosci **27**(11): 662-669.

Traynelis, S. F., L. P. Wollmuth, C. J. McBain, F. S. Menniti, K. M. Vance, K. K. Ogden, K. B. Hansen, H. Yuan, S. J. Myers and R. Dingledine (2010). "Glutamate receptor ion channels: structure, regulation, and function." Pharmacol Rev **62**(3): 405-496.

Tritsch, N. X. and B. L. Sabatini (2012). "Dopaminergic modulation of synaptic transmission in cortex and striatum." Neuron **76**(1): 33-50.

Tysnes, O. B. and A. Storstein (2017). "Epidemiology of Parkinson's disease." J Neural Transm (Vienna) **124**(8): 901-905.

Wang, L., X. Zhang, H. Xu, L. Zhou, R. Jiao, W. Liu, F. Zhu, X. Kang, B. Liu, S. Teng, Q. Wu, M. Li, H. Dou, P. Zuo, C. Wang, S. Wang and Z. Zhou (2014). "Temporal components of cholinergic terminal to dopaminergic terminal transmission in dorsal striatum slices of mice." J Physiol **592**(16): 3559-3576.

Wang, T. M., B. M. Brown, L. Deng, B. D. Sellers, P. J. Lupardus, H. J. A. Wallweber, A. Gustafson, E. Wong, M. Volgraf, J. B. Schwarz, D. H. Hackos and J. E. Hanson (2017). "A novel NMDA receptor positive allosteric modulator that acts via the transmembrane domain." Neuropharmacology **121**: 204-218.

Wang, Y. (2008). "Differential effect of aging on synaptic plasticity in the ventral and dorsal striatum." Neurobiol Learn Mem **89**(1): 70-75.

Vastagh, C., F. Gardoni, V. Bagetta, J. Stanic, E. Zianni, C. Giampa, B. Picconi, P. Calabresi and M. Di Luca (2012). "N-methyl-D-aspartate (NMDA) receptor composition modulates dendritic spine morphology in striatal medium spiny neurons." J Biol Chem **287**(22): 18103-18114.

Vekrellis, K., M. Xilouri, E. Emmanouilidou, H. J. Rideout and L. Stefanis (2011). "Pathological roles of alpha-synuclein in neurological disorders." Lancet Neurol **10**(11): 1015-1025.

Venance, L., J. Glowinski and C. Giaume (2004). "Electrical and chemical transmission between striatal GABAergic output neurones in rat brain slices." J Physiol **559**(Pt 1): 215-230.

Wickens, J. R. (2009). "Synaptic plasticity in the basal ganglia." Behav Brain Res **199**(1): 119-128.

Villalba, R. M. and Y. Smith (2018). "Loss and remodeling of striatal dendritic spines in Parkinson's disease: from homeostasis to maladaptive plasticity?" J Neural Transm (Vienna) **125**(3): 431-447.

Wilson, C. J. (2007). "GABAergic inhibition in the neostriatum." Prog Brain Res **160**: 91-110.

Wirdefeldt, K., H. O. Adami, P. Cole, D. Trichopoulos and J. Mandel (2011). "Epidemiology and etiology of Parkinson's disease: a review of the evidence." Eur J Epidemiol **26 Suppl 1**: S1-58.

Wood-Kaczmar, A., S. Gandhi and N. W. Wood (2006). "Understanding the molecular causes of Parkinson's disease." Trends Mol Med **12**(11): 521-528.

Vyklicky, V., M. Korinek, T. Smejkalova, A. Balik, B. Krausova, M. Kaniakova, K. Lichnerova, J. Cerny, J. Krusek, I. Dittert, M. Horak and L. Vyklicky (2014). "Structure, function, and pharmacology of NMDA receptor channels." Physiol Res **63 Suppl 1**: S191-203.

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.

Zhang, X. and K. Chergui (2015). "Dopamine depletion of the striatum causes a cell-type specific reorganization of GluN2B- and GluN2D-containing NMDA receptors." Neuropharmacology **92**: 108-115.