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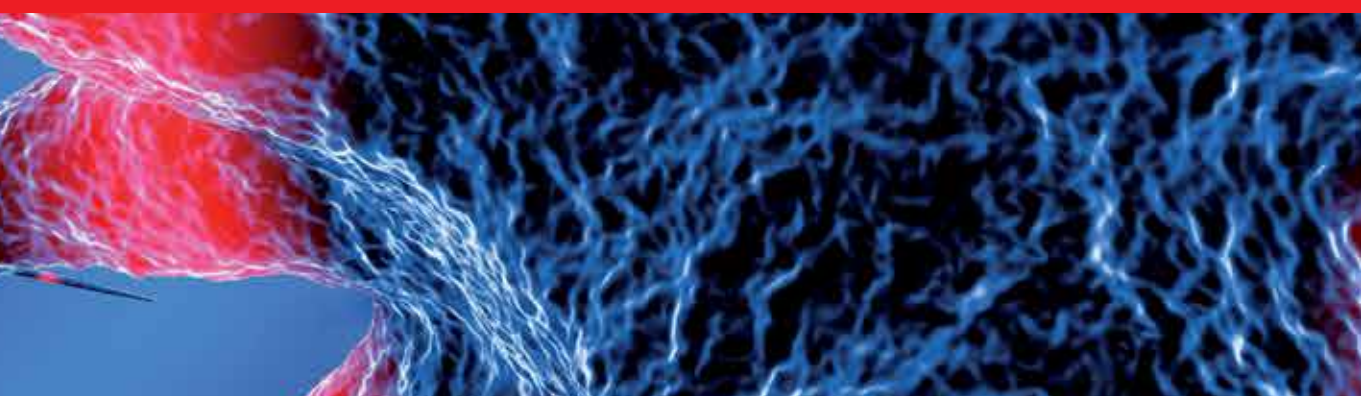
Dopamine
Health and Disease

Edited by Sarat Chandra Yenisetti



DOPAMINE - HEALTH AND DISEASE

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http://dx.doi.org/10.5772/intechopen.74360
Edited by Sarat Chandra Yenisetti
Assistant to the Editor(s):Limamanen Phom and Mohamad Ayajuddin

Contributors

Alexandros Monios, Lambrini Kourkouta, Petros Ouzounakis, Areti Tsaloglidou, Konstantinos Koukourikos, Konstantinos Tsaras, Christos Iliadis, Ioanna V. Papathanasiou, Katy Satué Ambrojo, María Marcilla, Juan Carlos Gardón Poggi, Maria Rosa Avila-Costa, José Luis Ordóñez-Librado, Ana Luisa Gutiérrez-Valdez, Javier Sanchez-Betancourt, Verónica Anaya-Martínez, Cesar Sanchez-Vazquez Del Mercado, Leonardo Reynoso-Erazo, Rocio Tron-Alvarez, Patricia Aley-Medina, Jesus Espinosa-Villanueva, Vianey Rodriguez-Lara, Lakshminarayanan Rajamani, Madhavi Srinivasan, Christina Poh Choo Sim, Jolanta Dorszewska, Katarzyna Wize, Wojciech Kozubski, Sarat Chandra Yenisetti

First published in London, United Kingdom, 2018 by IntechOpen
eBook (PDF) Published by IntechOpen, 2019
IntechOpen is the global imprint of INTECHOPEN LIMITED, registered in England and Wales, registration number: 11086078, The Shard, 25th floor, 32 London Bridge Street
London, SE19SG – United Kingdom
Printed in Croatia

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library

Additional hard and PDF copies can be obtained from orders@intechopen.com

Dopamine - Health and Disease
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p. cm.

Print ISBN 978-1-78984-269-2

Online ISBN 978-1-78984-270-8

eBook (PDF) ISBN 978-1-83881-792-3

Meet the editor

Dr. Sarat Chandra Yenisetti is a Professor and Head of the Drosophila Neurobiology Laboratory in the Department of Zoology, Nagaland University (Central), Nagaland, India. He obtained post-doctoral training in "modeling Parkinson's disease using *Drosophila*" from the University of Regensburg, Germany and the Neurogenetics Branch of the National Institute of Neurological Disorders and Stroke (NINDS), NIH, Bethesda, USA. His laboratory in India follows *Drosophila* approaches to understand dopaminergic neurodegeneration and identification of therapeutic targets, knowledge of which may help to reduce the burden of Parkinson's disease in humans. Dr. Sarat visited the USA, Japan, Germany, Taiwan, South Korea, the United Kingdom, Brazil, and Canada to participate in multiple academic assignments.

Contents

Preface

Neurons communicate with each other and with other tissues and organs with the help of certain chemicals, which eventually regulate our mood, behaviour, ability to perform, and many other biological processes. In the simple feel of excitement and restlessness, turbulence has an underlying magic of chemical basis! On the other hand, serenity and tranquility, too! That's it? Well, kind of…

Dopamine (DA) is a hormone and also a neurotransmitter, which performs a critical role in reward and movement control in the brain. Furthermore, it has a role to play in the modulation of behaviour and cognition: voluntary movement, motivation, inhibition of prolactin production, sleep, dreaming, mood, attention, working memory, and learning. DA also performs multiple other functions outside the brain. Regulating unrelated critical biological functions make this "feel-good" chemical a vital factor for sustaining life in both health and disease. Hence, it is important to understand the nature of this chemical so that we may address different conditions of human health and disease. This is the clear purpose behind *Dopamine - Health and Disease.*

The focus of the Drosophila Neurobiology Laboratory in Nagaland University, India, is to understand fundamental processes responsible for neurodegeneration under disease conditions. We ask basic questions relating to Parkinson's disease (PD), where dopaminergic neurodegeneration is the characteristic pathophysiological feature. In PD, dopaminergic neurodegeneration leads to depletion of brain dopamine levels. In other neurological disease conditions, such as schizophrenia and depression, dopamine has an important role to play. Therefore, knowledge relating to biological roles of dopamine and the other molecules it interacts with is critical to understand and develop therapeutic approaches to human neurodegenerative conditions. Hence, we have been motivated to be a part of this meaningful project.

Dopamine is an exciting molecule in biology due to the fundamental fact that it is critical to human health and wellbeing; the further biochemical modification of dopamine helps in formulating therapeutic strategies to human disease(s). This logic is reflected in the sectioning of the book into two parts: 1. Dopamine in Health and Disease and 2. Dopamine in Biomedical Research. The first part contains chapters relating to biological functions of dopamine in animal health in general and human wellbeing in particular. The second part comprises a chapter that discusses oxidative polymerization of dopamine which will assist in creating adhesive nano-coatings on multiple substrates and their role in biomedical research. Because these parts deal with approaches relating to human health and also to biomedical research, the book will appeal to basic scientists involved in biomedical research and also to technocrats. Because all these stories have a direct impact on our day-to-day life, anyone who has an eye for health and disease-related concepts will find this book a good read.

I take this opportunity to express gratitude to Ms. Maja Bozicevic, Author Service Manager of this project, for her constant and flawless support. I thank profusely Mr. Limamanen Phom and Mr. Mohamad Ayajuddin, assistant editors of the book, who are research scholars in my laboratory, for their assistance all along.

Further, I take this occasion to convey heartfelt gratefulness to IntechOpen for giving me a chance to edit a book on the most thrilling molecule, Dopamine.

I strongly believe that our humble effort will supplement scientific and non-scientific communities in stimulating a critical understanding of the biological purpose of the "ticklish" DA.

Sarat Chandra Yenisetti
Nagaland University (Central), India

Section 1

Dopamine in Health and Disease

Chapter 1

Introductory Chapter: "Feel Good" Chemical Dopamine - Role in Health and Disease

Sarat Chandra Yenisetti

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.81451

> *"People say, "I wish I had more motivation today, because then I would try something." But our thinking is backward. The way our brain works is that dopamine - the so-called feel-good chemical - is released the second we actually do something. So the motivation doesn't come before, it comes after."*
>
> *—Brendon Burchard*

1. Introduction

Dopamine (DA) (3,4-dihydroxyphenethylamine) is a member of the catecholamine family (a monoamine, an organic compound that has a catechol and a side-chain amine) of neurotransmitters in brain and is an antecedent to epinephrine (adrenaline) and norepinephrine (noradrenaline). DA is produced in the body (primarily by nervous tissue and adrenal glands) initially by the hydration of the amino acid tyrosine to DOPA by tyrosine hydroxylase and further by the decarboxylation of DOPA by aromatic-L-amino-acid decarboxylase. It is a key transmitter in the extrapyramidal system of the brain and crucial in synchronizing movement. A group of receptors (dopamine receptors) facilitates its function.

DA performs critical role in reward and movement control in the brain. Further, it has a function to play in modulation of behavior and cognition; voluntary movement, motivation, inhibition of prolactin production, sleep; dreaming; mood; attention; working memory; and learning. DA has multiple other functions outside the brain. In blood vessels, it impedes norepinephrine delivery and acts as a vasodilator (at endogenous concentrations); in the kidneys, it increases sodium evacuation and urine yield; in the pancreas, it diminishes insulin making; in the digestive system, it lessens gastrointestinal motility and guards intestinal mucosa; and in the immune system, it diminishes activity of lymphocytes. In the circulation, DA is primarily deposited in and transported by blood platelets [1]. Performing multiple unrelated critical biological functions makes this smart chemical, a "VVIP" for sustenance of life both in health and disease.

2. Synthesis, metabolism, and reuptake of dopamine

Tyrosine hydroxylase (TH) is a rate-limiting enzyme in the biosynthesis of DA and other catecholamines (**Figure 1**). Altering expression level of this critical enzyme, which eventually regulates the synthesis of DA, assists in developing promising therapeutic approaches and strategies to promote human health and address disease [2]. In presynaptic neurons,

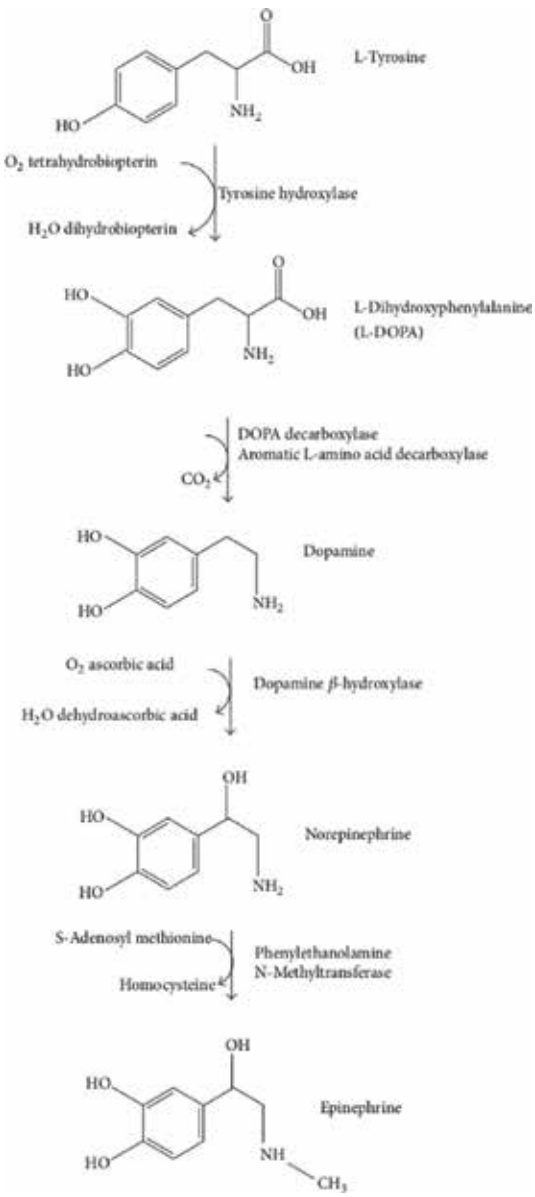

Figure 1. Catecholamine biosynthesis (reproduced with approval from Olguin et al. [8]).

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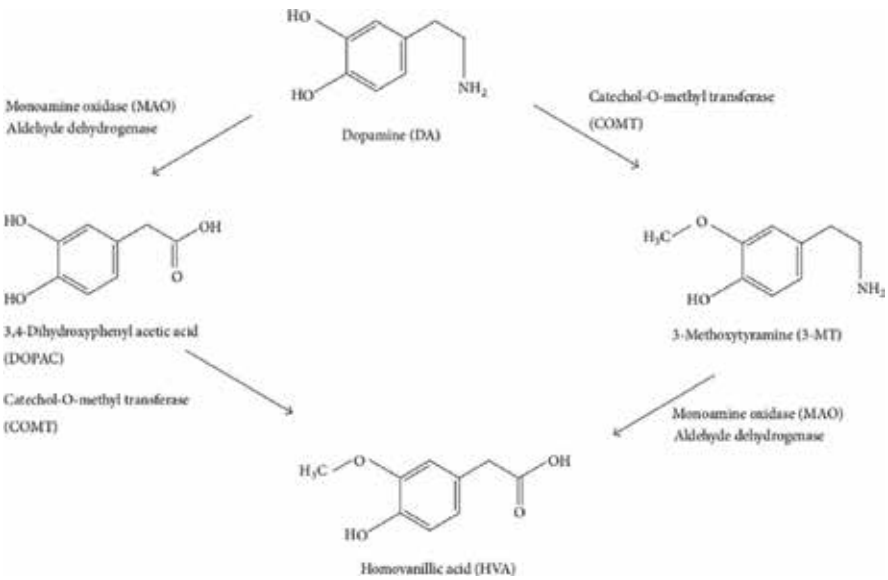

Figure 2. Dopamine metabolism (reproduced with approval from Olguin et al. [8]).

DA is encapsulated into synaptic vesicles and stored (this process is regulated by VMAT2 (vesicular monoamine transporter 2)). Later, synaptic vesicles release the DA into the synapse. Then, it binds either to presynaptic receptors (the signal can either inhibit (leading to inhibit the synthesis and release of neurotransmitters) or excite the cell) or to postsynaptic receptors. Once after the execution of function, DA is taken up into the presynaptic cell either by DAT (DA transporter) or by plasma membrane monoamine transporters (**Figure 2**).

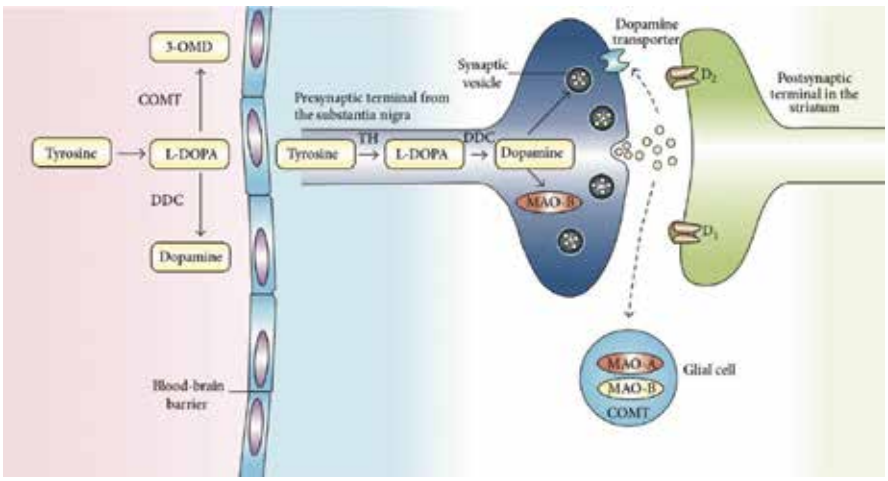

Figure 3. Dopamine release and reuptake process (reproduced with approval from Olguin et al. [8]).

COMT (catechol O-methyl transferase, principally expressed by glial cells) and MAO (has two isoforms A and B: monoamine oxidase A, primarily found in catecholaminergic neurons (e.g., neurons of *substantia nigra*) and monoamine oxidase B, chiefly found in astrocytes) are two critical enzymes responsible for breakdown of dopamine. COMT breaks down DA to 3-MT (3-methoxytyramine), which is subsequently reduced to HVA (homovanillic acid) by MAO. On the other hand, MAO converts DA to DOPAC (3,4-dihydroxyphenyl acetic acid), which is reduced by COMT to HVA and excreted out through urine (**Figure 3**). Therefore, inhibition of MAO can be a potential therapeutic strategy, which would decelerate the breakdown of DA and confer neuroprotection in neurodegenerative diseases like Parkinson's disease [3].

3. Dopamine: health and disease

DA acts upon to elicit feelings of enjoyment and bolstering, which motivates an animal to perform certain tasks repeatedly. In other words, reward system of the brain is strongly associated with "feel good" chemical DA. In certain areas of brain such as nucleus accumbens and prefrontal cortex, release of dopamine primarily due to fulfilling experiences such as food, drugs, physical exercise, sex, learning new tasks, figuring out unknown things, and neural stimuli correlated with these actions [4].

Parkinson's disease (PD) is a degenerative ailment causing tremor and motor impairment and is instigated by a loss of dopamine-secreting neurons in an area of the midbrain called *substantia nigra.* Dopamine agonists (drugs such as Mirapex (pramipexole), etc.) by mimicking dopamine bind and activate dopamine receptors, mirroring the activity of dopamine, leading to tricking the brain as if it has dopamine by conferring the biological functions of dopamine. Hence, these agonists are used as therapeutic agents in treating depression and Parkinson's disease. Replenishing dopamine levels through L-Dopa improves the PD symptoms, which is time-tested therapeutic strategy for PD [5].

There is evidence that schizophrenia comprises distorted levels of dopamine activity [6, 7], and most antipsychotic drugs utilized to cure this are DA antagonists, which reduce dopamine activity [8]. Therefore, antagonists have a significant therapeutic value in treating psychiatric conditions such as schizophrenia, which result due to overexcited dopamine organization. Attention-deficit hyperactivity disorder, bipolar disorder, and addiction are also characterized by defects in dopamine production or metabolism [9–12]. When present in sufficiently high levels, dopamine can be a neurotoxin (chemical that disorders neural tissue) and a metabotoxin (endogenously produced metabolite that causes adversarial health consequences at persistently elevated levels). Chronically high levels of dopamine are linked with neuroblastoma, Costello syndrome, leukemia, pheochromocytoma, aromatic L-amino acid decarboxylase deficiency, and Menkes disease (MNK). High levels of dopamine can lead to hyperactivity, insomnia, agitation and anxiety, depression, delusions, excessive salivation, nausea, and digestive problems [9].

4. Natural ways to boost brain dopamine levels for healthy living

Though DA is available in certain food materials, as it does not cross blood-brain barrier, it will not be available in brain. Therefore, other way of enhancing brain dopamine levels in a simple way is consuming foods containing the precursor of dopamine, tyrosine that can cross blood-brain barrier and enter brain where dopamine will be synthesized from tyrosine.

Foods such as bananas, eggs, avocados, almonds, fish, and chicken are rich in tyrosine. Recent studies substantiate the fact that certain bacteria (probiotics) in gut synthesize dopamine and influence mood and behavior. Velvet beans (*Mucuna pruriens*) contain significant levels of L-Dopa, the precursor molecule of dopamine, and assist in enhancing brain dopamine levels [13]. Studies illustrate that regular exercise [14] and listening to music [15] enhances the brain dopamine synthesis and also upregulates the number of dopamine receptors, which eventually helps for good mood and happy living.

5. Conclusion

The book "*Dopamine - Health and Disease*" focuses on multiple biological functions of dopamine relating to health and disease. This basic understanding is fundamental in developing and implementing therapeutic methods and strategies, which eventually contribute for promoting quality living of mortals. The authors' contributions lean toward the aspect that by taking advantage of fundamental understanding and knowledge relating to dopamine *per se* and its biological functions, how efforts can be made to translate the discoveries/innovations to promote human well-being, rather than from the perspective of hard-core scientific paper. Reader would appreciate this perspective as it directly influences the value of our lives.

Therefore, the very nature and purpose of the present endeavor aims at understanding the fundamental knowledge relating to dopamine and applying the same for supporting human life, which is very essence of biomedical research. At the same time, it is essential to realize the basic fact that practice of balanced lifestyle, meaning moderate consumption of food along with good physicomental (Yoga, Dahn, Shinshin-Toitsu-Do, etc.) and recreational activities, as practiced in ancient civilizations, can sustain the optimum levels of neurotransmitters and hormones and promote happy living. Here comes the importance of traditional/indigenous practices and knowledge of multiple age-old civilizations in promoting human health. The contents of this book fulfill the aim with which this project is initiated and moved on and successfully brought to a shape.

Acknowledgements

This work is supported by funding from Department of Biotechnology (DBT), India, grant numbers (BT/405/NE/U-Excel/2013 and BT/PR16868/NER/95/328/2015), and DST-SERB (Department

of Science and Technology-Science and Engineering Research Board), India, grant number (EMR/2016/002375).

Author details

Sarat Chandra Yenisetti

Address all correspondence to: yschandrays@rediffmail.com

Drosophila Neurobiology Laboratory, Department of Zoology, Nagaland University (Central), Lumami, Nagaland, India

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Chapter 2

Dopamine and Early Onset Parkinson's Disease

Katarzyna Wize, Wojciech Kozubski and
Jolanta Dorszewska

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.80400

Abstract

Parkinson's disease (PD) is divided into early-onset (EOPD) occurring at the age of fewer than 45 years of age and late-onset PD (LOPD) above 45 years of age. EOPD accounts for 5–10% of all the cases with PD. It is thought that occurrence in this age is connected with genetic factors, mutations in e.g. *PRKN, PINK1, DJ-1* and changes in proteins it is encoded. The loss of dopaminergic neurons in the nigrostriatal system leads to decreased dopamine (DA) concentrations. Pathogenic PD proteins may affect the DA level. The lower level of DA may be responsible for movement-related symptoms. EOPDs have a slower progression of the disease and a longer disorder duration but tend to develop dyskinesias and motor fluctuations earlier than LOPD. Currently, the diagnosis of PD is based on clinical criteria, supported neuroimaging like MRI or PET. Understanding the pathogenesis of the EOPD may be contributing to improving diagnostics and effectiveness of pharmacotherapy.

Keywords: molecular factors, dopamine, Parkinson's disease of early onset

1. Introduction

Parkinson's disease (PD) is one of the most common and spontaneous degenerative disease of the central nervous system (CNS) that is characterized by classical motor symptoms like bradykinesia, muscular rigidity, rest tremor, or postural instability [1]. It is estimated that approximately 5 million people worldwide suffer from PD. The frequency of disease increases with age; there are 1% of people older than 60 years and 5% of people over 85 years [2–4]. It seems that males suffer more often than females [5]. Furthermore, the estimates indicate that the number of PD patients will maintain increase trend because of population aging.

PD usually develops in the fifth or the sixth decade of life and is called late-onset PD (LOPD), but in a small group of patients, it is diagnosed even before the age of 40 years. The definition of early-onset PD (EOPD) is arbitrary. Some authors defined this disorder with an age of onset (AOO) below 40 years, others even below 50 years, but usually, it refers to age less than 45 [6, 7]. According to the literature data, 5–10% patients suffer from EOPD. EOPD can also be subdivided into the group called juvenile PD with AOO less than 21 years [8].

The main factor in PD pathology is loss or degeneration of dopaminergic neurons in the substantia nigra (SN). Although this disease was described more than 200 years ago, its cause is still not fully understood. It is considered that the pathogenesis depends on both genetic and environmental factors, but genetic changes are main causes in about 5–10% of the PD patients [9]. Some genes and its proteins associated with EOPD like *PRKN* gene and the Parkin protein or *PINK1* gene are identified.

The phenotype of PD is various and related to AOO. It includes classical motor symptoms and non-motor symptoms such as disorder of mood, cognitive, behavioral, sensory, and autonomic dysfunctions (e.g., orthostatic hypotension and urogenital dysfunction) [10]. Patients' characteristic of EOPD and LOPD is summarized in **Table 1**.The study of Wickremaratchi et al. [7] showed that features like tremor, rigidity, response to most common treatment, or presence of dystonia and dyskinesia's have linear changes (increasing or decreasing). However, dystonia demonstrates the highest risk of occurrence among EOPD and reduction among LOPD patients.

The proper diagnosis of PD is very important. Nowadays, there are a lot of neuroimaging methods that can be used to increase the accuracy of differential diagnosis, but none of them have been endorsed to routine use in clinical practice [11, 12].

Features	EOPD	LOPD
Mean age of onset (years)	44	72
Survival from onset (years)	27	10
Mean age at death (years)	71	82
Tremor at onset, only (%)	45	59
Bradykinesia and tremor at onset (%)	23	9
Bradykinesia at onset, only (%)	32	25
Postural instability at onset (%)	0	7

Table 1. Patients' characteristic of EOPD and LOPD [8].

2. Dopamine and pathogenesis of Parkinson's disease

Dopamine (DA) is the organic chemical of the catecholamine family and precursor for noradrenaline. It is synthesized in presynaptic neuron from tyrosine to L-dihydroxyphenylalanine (L-dopa) via tyrosine hydroxylase. Subsequently, aromatic amino acid decarboxylase removes a

carboxyl group, form neurotransmitter, which is packed into synaptic vesicles. DA is released into the synapse during stimulation, actives dopaminergic receptors, and evokes a response in the postsynaptic cell [13]. It plays a pivotal role in the generation of normal movements by transmission information from SN to the striatum, where movements are initiated and controlled facility and balance [14].

The pathomechanism of PD is progressive and subsequent degeneration of neurons in SN, which results in the decreased level of DA in the dopaminergic neurons. Further, there is also the presence of Lewy bodies (LBs), intracytoplasmic eosinophilic inclusion bodies, in others neurons in SN. The literature indicates that loss of 60–70% of dopamine neurons in SN is presented as PD motor symptoms [15]. Pathogenesis of PD involves both environmental and genetic factors. It is thought that the pathways involved in PD are impairment of cellular clearance pathways, protein aggregation, oxidative stress, mitochondria dysfunction, and neuroinflammation (**Figure 1**) [16–18].

α-Synuclein (ASN) is a major component of LB [19]. Aggregation of ASN is considered to be engaged in the pathogenesis of PD in consequence of the cellular clearance pathway like

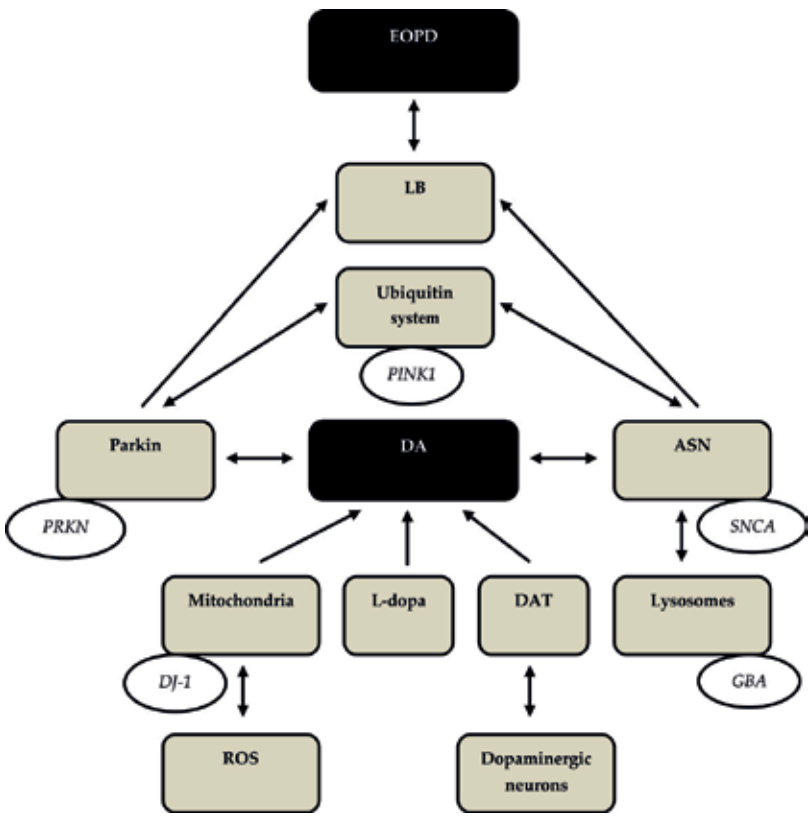

Figure 1. Association between DA in Parkinson's disease, EOPD and genetic and biochemical factors. EOPD—early-onset Parkinson's disease, LB—Lewy bodies, DA—dopamine, ASN—α-synuclein, L-dopa—L-dihydroxyphenylalanine, DAT—dopamine transporter, ROS—reactive oxygen species.

ubiquitin-proteasome and autophagy-lysosome [20]. The literature indicates that ASN modulates dopamine transporter (DAT) activity. DAT is responsible for removing DA from the synaptic cleft. It is showed that the polymorphisms in gene coding DAT (*DAT1)* are engaged in the detoxication mechanism and oxidative stress [21]. Membrane depolarization of DAT enhances plasma membrane ASN localization, which subsequently increases DA efflux [22]. The study of Mazzulli et al. [23] shows that the loss of lysosomal enzyme glucocerebrosidase (GBA) causes interference in protein degradation and accumulation of ASN, and GBA substrate is associated with the amyloid formation of purified ASN. On the other hand, GBA activity in neurons of PD brain is inhibited by ASN.

Oxidative stress is a disturbance in the balance between prooxidant and antioxidant homeostasis and production of reactive oxygen species (ROS) [24]. The main mitochondrial site of generation ROS is complex I [25]. There is a direct relationship between mitochondrial dysfunction and decreased activity of complex I among PD patients [26]. Moreover, it is known involvement of such genes like *PRKN, PINK1,* and *DJ1* in mitochondrial PD pathogenesis [18]. One of the causes of the increase of oxidative stress and ROS in dopaminergic neurons is self-oxidation of DA to quinones (DAQs).

DAQs are electrophilic species, very reactive toward cellular nucleophiles, which effect damage of cells. DAQs can bind to Parkin and promote its aggregation. Thus, this protein losses its function. It seems that DAQs are more responsible for inactivation of Parkin than ROS [14]. The study of Bisaglia et al. [27] shows that DAQs interact with ASN by inhibition of ASN fibrilization and stabilizing ASN/DAQ oligomers. It seems that DAQs can also modify the structure of DJ-1 through modifications in cysteine residues of its protein [28].

Kitada et al. [29] show that mutations in *PINK1* gene, which is associated with EOPD, and inactivation of encoded protein impair DA release. However, they do not alter the levels of DA, a number of dopaminergic neurons, DA synthesis, and levels of DA receptors. These results indicate that this impairment is sufficient to cause dysfunction of the nigrostriatal circuit by deficits in synaptic plasticity.

3. Genetic risk factors for early-onset Parkinson's disease

The etiology of EOPD is not completely explained. It seems that genetic factors, environmental factors, or both of them may play an important role in the pathogenesis of this disease. There have been identified several genes and their mutations associated with EOPD, but new loci are still being identified. Most of these genes are inherited autosomal recessive, for example, *PRKN, PINK1, or DJ1,* but some of them are associated with the autosomal dominant pattern, for example, *SNCA* [30].

3.1. *PRKN* gene

One of the most important genes involved in the pathogenesis of EOPD is *PRKN* (PARK2) that encodes 465 amino acid-long Parkin protein. Parkin is a part of multiprotein E3 ubiquitin

ligase complex and is involved in the regulation of mitochondrial quality control pathway and promotion selective autophagy of depolarized mitochondria (mitophagy) [31]. Moreover, overexpression of this protein leads to elevated expression of complex I subunits and decreased the accumulation of ROS [32]. Parkin interacts with other proteins such as PINK1, which promotes the mitochondrial translocation of Parkin [33]. There is also a suggestion about a role in DA utilization in human dopaminergic neurons by controlling the precision of dopaminergic neurotransmission and DA oxidation [34].

PRKN gene is located on chromosome 6q26 and consists of 12 exons. There are various data results about the frequency of mutations in *PRKN* that implies a possible role of the environment [35]. Some of them indicate that they are responsible for 9% of cases of EOPD, but others suggest even twice higher number—18% among patients with age of onset (AOO) before 45 years and 77% of those with AOO before 20 years. Mutations in *PRKN* gene are also more frequent in patients with a positive history than in sporadic cases [36, 37]. Pathogenic mutations in *PRKN* gene cause losing quality control pathway and accumulation of damaged mitochondria, what in consequence leads to elevation of ROS, cell death, and PD [31]. There have been identified more than 100 mutations in *PRKN* gene, which includes deletions, insertions point mutations, and large arrangements [38]. Some of them seem to be pathogenic like Q171X, R275W, G284R, or T425 N, but another likely to be non-pathogenic, for example, A82Q, L174 L, or L261 L [35, 39]. Hedrich et al. [40] indicate that R275W mutation is the most common point mutation in EOPD and is always combined with other changes in *PRKN* gene.

3.2. *PINK1* gene

Another gene which mutations are involved in the occurrence of EOPD is phosphate and tensin homolog (PTEN)-induced putative kinase 1 (*PINK1).* It is a 581 amino acid ubiquitously protein kinase, which includes a 34 amino acid mitochondrial targeting motif and a highly conserved protein domain (amino acids 156–509, exons 2–8) showing a high degree of homology to the serine/threonine kinases [38, 41]. It is widely expressed in human brain and plays a role in the mitochondrial response to oxidative stress, degradation of impaired mitochondria by activation this organelle's autophagy (mitophagy) by Parkin, and regulation of Parkin localization [42, 43]. Morais et al. [44] also show that modifications in *PINK1* may cause elevated ROS production and impaired DA release.

Mutations in *PINK1* (PARK6) gene are the second most common cause of AR EOPD [38]. *PINK1* is mapped to chromosome 1p36.12 and contains eight exons. There have been reported more than 100 *PINK1* gene mutations including large deletions, frame shift mutations, nonsense, or missense mutations, which cause loss of protein function [45]. It is considered that mutations in this gene are responsible for 14% of EOPD cases, but there is wide variation between different ethnic group [37, 46]. The study of Kilarski et al. [36] indicates that majority of mutations are homozygous and they are more common in Asian populations than in white patients or Latin American. One of the reported mutations in *PINK1* was Q456X in exon 7 by Bonifati et al. [46]. It is a nonsense mutation that results in a premature stop codon. The study of Siuda et al. [43] suggests that this mutation leads to complete loss of PINK1 at the RNA level in skin fibroblast derived from a patient, what causes dysfunction of Parkin. Other mutations

in this gene associated with EOPD and are likely to be pathogenic Y258X, R276X, M318 L, and A427E [39, 47, 48]. The literature data also indicate occurrence of such mutations that seems to be non-pathogenic or the significance is unknown in EOPD patients like R312R, A339T, D391D, G411S, T420 T, D525N, and S576S [39].

3.3. *DJ-1* gene

The third gene associated with EOPD is *DJ-1* (PARK7). It encodes a 189 amino acid-long protein, which is a mitochondrial peroxidase. DJ-1 protein has homodimeric structure, which is ubiquitously expressed in brain areas and also in peripheral tissues [49, 50]. The literature indicates multiple functions of this protein-like protection cells against oxidative stress, acting as a chaperone and protease or interactions with other known PD-proteins such as Parkin or PINK1 [51–53]. Moreover, it plays an important role in the maintenance of mitochondrial complex I activity and defense function against cytotoxicity induced by toxic ion metals like copper or mercury [53, 54]. DJ-1 protects against dopamine toxicity and control the vesicular sequestration of DA [55]. Mutations cause instability of a dimeric structure, which is physiological form, and lack of expression [45]. Modified proteins are not properly folded, unstable, and degraded by the proteasome what results in a reduction of neuroprotective function and antioxidative activity [38].

The *DJ-1* gene is located on chromosome 1p36.23 and contains eight exons, where first two of them are noncoding and alternatively spliced in mRNA [56]. The *DJ-1* gene mutations in EOPD are rarer than *PRKN* and *PINK1* mutations with overall frequency 0.4%, which increases with familial cases to 0.8% [36]. The *DJ-1* locus was identified in a Dutch family with AR EOPD [57] and that led to the identification of mutations in *DJ-1* gene of two families [56]. They have been identified in nucleotide substitutions like missense, truncating, spic-site mutations and also large deletions [58]. The study of Abou-Sleiman et al. [59] identified two mutations in *DJ-1.* The first one was homozygous M26I in an Ashkenazi Jewish patient, which causes substitution of methionine for isoleucine. The second was a substitution at codon 149 in which highly conserved polar aspartate residue exchanges to non-polar alanine (D149A). There have been found another mutation in EOPD like A104T [60] or L10P in Asian populations. The study of Guo et al. [61] also suggests that two identified mutations in the Italian population, D24A and F162 L, may cause PD in the case of presence in homozygous or compound heterozygous state with other mutations. The literature data indicate that there was a considerable reduction of DAT binding in the Turkish patient with an E64D mutation in the homozygous state. These results show a significant decline of presynaptic dopaminergic afferents [62]. Moreover, the clinically unaffected sister of EOPD patient (homozygous for E64D) had demonstrated reduction of DA uptake in comparison with a clinically unaffected brother, who has the heterozygous state for this mutation.

3.4. *GBA* gene

The *GBA* gene is mapped to chromosome 1q22 and encodes the lysosomal enzyme GBA. It is β-glucosidase that catalyzes the breakdown of glucose and ceramide, which are a precursor for glycosphingolipids and sphingomyelin occurring in nervous tissues [63, 64]. Mutations in *GBA*

gene play an important role in neurological disorder like PD. They account for 5% of all PD cases, but the frequency of occurrence is ranged from 10.7 to 31.3% of Ashkenazi Jewish patients with PD and from 2.3 to 9.4% in patients of other populations [65]. The most common mutation in the Ashkenazi Jewish is N370S, but in Caucasian populations are N370S and L444P. There have been also identified such mutations in EOPD as H255Q, E326K, D409H, or R329H [66]. The activity of this protein is decreased in heterozygous mutations in PD patients in comparison to non-mutated carriers [67]. It is suggested that they cause dysfunction of the autophagy-lysosome pathway, mainly impairment in macroautophagy and chaperone-mediated autophagy involved in accumulation, aggregation, and transmission of ASN [64].

Moreover, homozygous mutations in *GBA* gene leads to Gaucher's disease (GD), the most common lysosomal storage disorder due to deficiency of enzyme GBA [68]. The literature indicates that mutations of *GBA*, even in the heterozygous state, may be associated with this disorder [69]. Patients with GD have an increased risk of PD and parkinsonism features. It seems that there is no GD genetic variant linked with PD, but N370S is the most frequent mutation detected in American, European, and Ashkenazi Jewish population [65, 68].

3.5. *SNCA* gene

SNCA (PARK1 and PARK4) gene was the first gene ever identified as causal PD. It is an inherited autosomal dominant pattern and located to chromosome 4q22.1 [30]. *SNCA* gene encodes ASN, but the functions of its are still not completely understood. It is known that it is the main component of LB [19]. ASN reduces protein kinase C (PKC) activity, which is sensitive to oxidative stress and protects dopaminergic cells against apoptosis [70]. It can also regulate glucose levels by increasing tissue glucose uptake, modulate calmodulin activity, and act as a molecular chaperone and antioxidant by protecting dopaminergic neurons against oxidative stress [71–74]. Moreover, ASN can decrease the activity of tyrosine hydroxylase and thus regulates the production of DA and control its levels [75]. It also interacts with other proteins including Parkin or DAT by decreasing its activity [76].

One of the most common mutations in *SCNA* gene associated with EOPD is A53T. It was firstly identified in members of Contursi kindred and three families from Greece, but later A53T was also found, for example, in Sweden and Korean population [77–79]. They were also described in such mutations as A30P and E46K related to EOPD [37, 80].

4. The phenotype of early-onset Parkinson's disease

Patients with EOPD are characterized as younger AOO and longer disease duration than patients with LOPD [81]. Some symptoms vary among patients (**Table 2**), but classical motor symptoms are mainly affected.

EOPD with *PRKN* mutations is characterized by excellent response to L-dopa treatment and in consequence presence of dose-related fluctuations or dyskinesias after around 7 years of pharmacotherapy. The most common motor features are limb tremor and bradykinesia, but

Gene (locus)	Location	Selected mutations in EOPD patients	Inheritance	Clinical phenotype
PRKN (PARK2)	6q26	Q171X, R275W, G284R, T425N, A82Q, L174L, L261L	Recessive	Tremor, bradykinesia, urinary dysfunctions
PINK1 (PARK6)	1p36.12	Q456X, Y258X, R276X, M318L, A427E, R312R, A339T, D391D, G411S, T420T, D525N, S576S	Recessive	Foot dystonia, gait impairment, excellent L-dopa responsiveness
DJ-1 (PARK7)	1p36.23	D149A, A104T, L10P, D24A, F162 L, E64D	Recessive	Similar to *PRKN*
GBA	1q22	N370S, L444P, H255Q, E326K, D409H, R329H	Recessive	Mild Gaucher's symptoms, cognitive impairment
SNCA (PARK1, PARK4)	4q22.1	A53T, A30P, E46K	Dominant	Rigidity, rapid progression

Table 2. Genes implicated in EOPD and its clinical phenotype [30, 39].

there are also reported such as poor balance or freezing episodes. Patients with *PRKN* mutations have autonomic symptoms like urinary urgency (45%), impotence (28%), and orthostatic faintness (13%) [82]. They also have a lower frequency of excessive daytime sleepiness than general PD population, and insomnia is considered as a most common sleep problem [83]. The results of Mini-Mental State Examination score (MMSE) in *PRKN* patients are ranged 30–25; thus, cognitive functions are normal [82]. The study of Kim et al. [83] showed that the patients with two mutations have significantly younger AOO and longer duration of the disease in comparison to patients without *PRKN* mutations. Moreover, they can have a positive family history with PD and use a lower dose of L-dopa. Patients can also present psychiatric dysfunction like depression, psychosis, obsessive–compulsive disorder, or anxiety [84]. Some literature data indicate that *PRKN* mutation carriers and non-mutation carriers are clinically indistinguishable [85].

Most of EOPD patients with mutations in *PINK1* gene show typical symptoms of the disease resting tremor, rigidity, and bradykinesia. They have very good and sustained response to L-dopa treatment [46]. The Ibáñez et al. [86] study showed that even after 45 years of disease duration, the patient has a good response to L-dopa therapy. Moreover, there is a very slow progression of the disease and patients have no worsening for several decades. Siuda et al. [43] demonstrated two homozygous Q456X mutation carriers in a Polish family, who developed their first symptom, foot dystonia, in 16 and 27 years. Subsequently, patients suffered from progressive gait difficulties and had sensory symptoms in the lower limbs. It seems that disease onset in the lower limbs and early gait impairment can be characteristic for PD with *PINK1* mutations [86, 87]. Besides having classical motor symptoms, patients with *PINK1* mutations present L-dopa–responsiveness dystonia or restless leg syndrome (RLS) [81]. Cognitive impairment is rare and appears only in cases with a long duration of PD [86].

The phenotype of *DJ1*-related EOPD varies among mutations. Patients with the M26I mutation are characterized similar phenotype as *PRKN* mutation carriers. They have early leg dystonia before starting treatment and psychological disturbance, mainly anxiety [59]. The study of Hering et al. [62] showed that EOPD starts with slowing of movements and stiffness in the left

leg and arm among patient with identified novel E64D mutation. Moreover, the first features were sleep disturbances, depression, and speech difficulties. In the patient with bradykinesia, rigidity and postural tremor occurred only on the left side of the body, but there was no problem with cognition. The observation of Abbas et al. [88] indicate that the patient with missense mutation I105F found in exon 5 presented asymmetric onset, moderate L-dopa response, but no pyramidal features or dystonia. It seems that special feature in this patient was extreme motor restlessness to L-dopa. However, in the same study, it is demonstrated that homozygous R98Q variant is responsible for good L-dopa response and the treatment induces dyskinesia.

GBA mutation carriers have significantly younger AOO in comparison to non-carriers [89, 90]. Patients characterize of good or excellent response to L-dopa therapy and present a typical PD phenotype. Furthermore, some of them present impressive to subthalamic nucleus deep-brain stimulation. There are also cases of *GBA* patients that affect depression [90]. The study of Sato et al. [89] indicates that *GBA* mutation carriers have a positive history of PD in families. They present poorer motor progression, more often postural instability, persistent asymmetry, and responsive for L-dopa for more than 5 years [91]. According to the literature data, *GBA* mutations are associated with cognitive impairment, which is revealed by a lower MMSE score [92]. It is considered that patients with both GD and PD present mild Gaucher's symptoms [65].

The phenotype of *SNCA*-related EOPD consists of typical features for this type of the disease—asymmetric onset, good responsiveness for L-dopa in initial time, and early motor complications. The literature indicates that *SNCA* A53T mutation carriers with long-term PD present cognitive defects like dementia and average or inconsiderable in shorter term one. Besides, it was noted psychiatric syndromes, for example, depression, anxiety, dysautonomia, or olfaction impairments [93]. There can be observed numbness in the first of the disease, insomnia and occasional hypotensive attacks [94]. Whereas G51D carrier has phenotype differing from those with A53T. Patient characterizes the rapid progression of the disease, which consequently leads to loss of autonomy and death in few years. There were also noted manifested cognitive deterioration, visual hallucinations, and seizures [95]. The study of Somme et al. [96] shows that E46T mutation in early stages is also associated with a visual hallucination, sleep disorder, rigidity, and dementia.

5. Neuroimaging of early-onset Parkinson's disease

A lot of neuroimaging techniques are used to diagnose PD properly, follow the progress, and also get to know the neurobiology mechanism involved in revealing the disease. The most commonly used methods are magnetic resonance imaging (MRI), positron emission tomography (PET), single-photon emission computed tomography (SPECT), magnetic resonance spectroscopy (MRS), and transcranial sonography (TCS). There are also multimodal neuroimaging techniques that combine imaging with complementary modalities to increase the benefits of examination.

PET imaging is a technique using radiolabeled agents like ^{11}C, ^{18}F, and ^{15}O. It is more sensitive and presents a better special resolution in comparison to SPECT, which employs radioisotopes ^{123}I or ^{99m}Tc. It is thought that SPECT is cheaper, more widely available, and a valuable imaging modality for many PD applications [97]. It seems that Technetium^{99m}-labeled tropane derivative (^{99m}Tc-TRODAT-1) can be used to reveal dysfunction of dopaminergic system by binding DAT [98]. It was also showed that striatal DAT-binding potential was 34% lower among EOPD than LOPD patients [99]. The study of Shyu et al. [100] identified lower uptake of ^{99m}Tc-TRODAT-1 in the putamen, but normal in the caudal nucleus among patients with *PRKN* mutations in early stages of EOPD. There is more symmetrical loss demonstrated in both structures in the latter stages of the disease. However, the PET results of Nagasawa et al. [101] show that the function of presynaptic dopamine terminals does not correlate with PD severity and degrees of main symptoms.

MRS is a kind of magnetic resonance for identifying many endogenous compounds involved in the pathomechanism of PD like DA, γ-aminobutyric acid (GABA), and glutamate, so it gives an opportunity for probing biochemical systems [102, 103]. It allows research neurochemicals directly, without invasion and radiation exposure.

There is also another kind of resonance MRI in patients with EOPD. MRI creates images of the human body by detecting spin properties of nuclei [97]. MRI is not able to directly image dopaminergic neuronal loss, but it can provide complementary data to those obtained with nuclear tracer imaging [104]. The study of Wang et al. [105] shows that pathological asymmetry between both hemispheres in NG pathways in the early stage of EOPS using an MRI method.

TCS is another technique used in PD. It is a noninvasive, validated ultrasound method for demonstrating characteristic alterations of deep brain regions especially SN, but also lenticular nucleus (NL) or ventricles [106]. It is less expensive than the previously described tools, that is why it can be an important advantage of its application [97]. The literature indicates that TCS-MRI fusion allows analyzing SN and NL echogenicity as highly sensitive and specific markers for EOPD [107].

There are also multimodal imaging for imaging structure and metabolism like PET/CT. Using this method, the study of Shi et al. [108] shows the unequal radioactive distribution of ^{18}F-2-deoxy-D-glucose among patients with compound mutations in the *PRKN* gene. Moreover, the authors observed the reduction of ^{11}C-2 β-carbomethoxy-3 β-(4-fluorophenyl) tropane uptake in the caudal putamen.

6. Summary

The occurrence of EOPD is associated with molecular factors both genetic and biochemical ones. The presence of various genetic variants such as *PRKN* gene is associated with Parkin protein, the *PINK1* gene affecting the efficiency of the ubiquitin-proteasome system, the *DJ-1* gene linked with mitochondria, *GBA* gene connected with lysosomes and *SNCA* gene encoding ASN may accelerate revealing of PD. It seems that discovering the relationship

between genetic bases and protein parameters may lead to explain the causes of appearance PD depended of age. Furthermore, in the future, it could entail with bases for earlier diagnosis of EOPD and in consequence introduction of more effective pharmacotherapy.

Author details

Katarzyna Wize[1], Wojciech Kozubski[2] and Jolanta Dorszewska[1]*

*Address all correspondence to: dorszewskaj@yahoo.com

1 Laboratory of Neurobiology, Department of Neurology, Poznan University of Medical Sciences, Poznan, Poland

2 Chair and Department of Neurology, Poznan University of Medical Sciences, Poznan, Poland

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Chapter 3

Sleep and Health: Role of Dopamine

Kourkouta Lambrini, Ouzounakis Petros,
Papathanassiou Ioanna, Koukourikos Konstantinos,
Tsaras Konstantinos, Iliadis Christos,
Monios Alexandros and Tsaloglidou Areti

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.79476

Abstract

Introduction: Sleep is an important part of people's lives and proper sleep is a prerequisite for good health.

Purpose: The purpose of this chapter is to highlight the importance of sleep in the promotion of health, sleep-related patients, and dementia at various stages of the age of the immortal. It also refers to sleeping on Parkinson's disease and dopamine.

Material & methods: An extensive review of the relevant literature was performed via electronic databases (Medline, PubMed, Cinahl and Google Scholar) and Greek and international journals.

Results: Sleep is described as a special state of consciousness. It is composed of phases and is characterized as relatively unresponsive to the surrounding area. It is a periodic situation. The fall of consciousness during sleep provides time for the body systems to be reconstructed and renewed. Thus, sleep is a corrective mechanism that contributes to the regeneration of the person's normal and emotional state. It occurs cyclically, usually once a day. Sleep is divided into two types, known as REM (Rapid Eye Movement), and NREM (Non Rapid Eye Movement).

Conclusion: Sleep occupies about one third of our total lifetime and is a very important biological function. Its functional significance is related to the resting of brain function and to the proper functioning of memory and learning.

Keywords: sleep, health, disease, Parkinson's disease, dopamine

1. Introduction

Sleep is an important part of people's lives and proper sleep is a prerequisite for good health. People have a need for a steady sleeping period of about 7–8 hours, especially during the night [1].

The reasons that gave rise to the need for sleep and the way the sleep was incorporated into the biological cycle are one of the great mysteries of evolution. The only thing we know for sure is that our sleep is necessary in order to be able to work during the day, so its disorder in any way adversely affects our everyday lives. Physical, mental and social well-being, as well as protection from certain illnesses and accidents, depends on the quality and the quantity of sleep [2].

Sleep, therefore, is a basic necessity of the human body and at the same time a basic prerequisite of its good health, in order its normal functions to be carried out. As a result, any sleep disorder has a direct impact on the body function, reducing its performance [3].

Recognizing the significance of sleep for the human's health, the World Health Organization introduced 21st of March as World Sleep Day [4].

In this section, ancient Greeks' perceptions about sleep, its benefits and its importance to the well-being of people of all age groups as well as its importance to the patients, are mainly presented. At the same time, the measures and the ways of defending and promoting it are also highlighted.

2. Sleep concepts in antiquity

In ancient Greek Mythology, "Sleep" and "Death" are twin brothers, "wretched Gods" who lived in Tartarus, children of Night and Erebus [5].

The sleep was worshiped in the mainland of Greece. Significant centers of sleep's Worship were Epidaurus, Troizina and Olympia. He was a young man, handsome, with wings on his shoulders, who made the tired people being asleep. It was sometimes pictured as a handsome, young man who was seeding sweet dreams in the earth or he was sleeping in a bed, and some other times it was pictured as a demon with wings that was carrying a dead man to death. Indeed, it is said that he has made the leader of gods, Zeus, being asleep, despite his will, following pressure from the goddess Hera, who wanted to influence the evolution of the Trojan War [6].

One of their many children was Morpheus, the God of Dreams. He was the only god who could intervene in the dreams of kings and heroes. He was transferring gods' messages to the mortals in the form of dreams and he could take any human form himself and appear in dreams. He had the capacity to send images to people's dreams or visions, to shape them, and to form the beings that lived in them [7].

Of their other sons, Phobitoor was responsible for nightmares and he was taking animal or monster styles. Fantasos was creating surreal images by taking forms of objects such as stones or woods and Cecil was helping those dreams' aspects that portrayed reality by making dreams realistic.

God Hermes, one of the 12 Olympian Gods, was also the God of Sleep. The Romans, respectively, considered Somnus as God of Sleep [8]. The Greek philosophers expressed their views about the dreams and their interpretation in various ways. Empedocles, Plato, and Aristotle had particular approaches to the subject, based on the "clairvoyant's dreams", giving rational and not metaphysical interpretations [9].

Aristotle recognizes the preservation of living beings as the purpose of sleep claiming that all living organisms that are in move are intended to rest, fact beneficial and necessary. A living organism, according to Aristotle, cannot constantly be in action and it is not possible to have his senses in full operation incessantly. Based on this and since it is not possible for the same living being to be simultaneously in two opposite situations, the philosopher logically concludes that the animal from the situation of wakefulness passes to sleep [10].

He also determines sleep through the concept of wakefulness, posing it to the opposite function from that of sleep. These two functions take place in the same part of the body, in the place they are produced themselves. Furthermore, he believes that the way we can perceive awake man, in exactly the same way we perceive asleep one. Subsequently, he stresses the meaning of senses, always in relation to wakefulness and sleep, and states that someone who is awake, has his senses in use, as he perceives the external things and his internal movements, fact that does not happen to the person who is sleeping. Thus, he ends up linking these two passions (sleep–wakefulness) with the esthetic part of the soul, meaning the use of senses during the wakefulness period and their lack or weakness during sleep [11].

The approach of Heraclitus has contributed a lot to the sleep and dream issue. Heraclitus describes sleep as a temporary death of consciousness, where vision disappears and self-consciousness is lost. At this point dreams come about, but they do not dissolve the darkness of this conscious "Night", where the sleeping person is retreated to a place entirely subjective, in which he has no consciousness of his identity. During sleep the individual "touches" the "dead", while when he is awake, touches the man who is asleep.

Heraclelian philosophy generally rejects any idea of the objective dreaming, since it believes that the true nature of the beings is perceived exclusively by the mind [12].

Sleep was also involved with the art in many forms and often it is imprinted as eagle or butterfly's wings on the front, or with a horn from which the dreams are spreading [8]. In ancient art, sleep is portrayed as a naked young man, sometimes with beard and feathers on the head, or as an asleep on a bed of feathers with black curtains around, while Morpheus prevents any noises that could awaken him.

In the Kypselo's Ark, in Olympia, the two brothers, Sleep and Death, are depicted as little boys sleeping in their mother's arms, Death is painted in black tunic and Sleep is painted in white. In Sparta, his depiction is always accompanied by that of Death, and in the following years Death and Sleep merged into a deity [13].

2.1. Orphic hymn for the god of sleep

> *"Oh! The sleep, the king of all blessed gods and mortals, and all the animals that are fed by the broad earth, you are the only ruler of all, and come to all, and you can bind the bodies with bonds that are not made of copper.*

You release us from the cares and give us sweet relief from the labor. You make us a sacred consolation for all sorrows and you also bring us the preaching of death and you save our souls, because you are by nature the true brother of Lethe and Death. But, oh! Blessed god, please, I ask you to come together with sweetness and to save the mystics favorably for the divine works" [14].

3. Normal sleep

Sleep is described as a special state of consciousness. It is composed of phases and is characterized as relatively unresponsive to the surrounding area. It is a periodic situation. The fall of consciousness during sleep provides time for the body systems to be reconstructed and renewed. Thus, sleep is a corrective mechanism that contributes to the regeneration of the person's normal and emotional state. It occurs cyclically, usually once a day. Sleep is divided into two types, known as REM (Rapid Eye Movement), and NREM (Non Rapid Eye Movement) [15].

NEM sleep is referred to as calm sleep and its awakening becomes more difficult. It passes from four phases. In the second type, we distinguish four (4) additional phases, which follow a specific repeating pattern throughout its duration [16].

(a) ***Phase 1:*** The first phase is subjectively considered to be lighter than the others and is often seen as a transition from the state of alertness to sleep. The person wakes up much more easily, the heart and respiratory rate falls slightly. At this stage a progressive muscle relaxation takes place, the body deeply sinks into an unconsciousness stage and faint images associated with the world of dreams are apparent. Electroencephalographic waves are observed similar to those observed during wakefulness (alpha waves with a frequency of 9–12 cycles) [17].

(b) ***Phase 2:*** The second phase is characterized by light sleep. The heart and respiratory rate is decreasing, body temperature and metabolism are decreasing. The second phase lasts about 10–20 min and includes 50–55% of total sleep. The eyes begin to turn around slowly. The slightest noise can wake the sleeper. It is distinguished by an encephalogram showing the characteristic groups of cells called sleeping spindles.

(c) ***Phase 3:*** The third phase marks the onset of deep sleep. The person wakes up with difficulty and rarely moves. It takes about 15–30 min and includes 10% of our sleep time. Heart rate, blood pressure and body temperature are decreased. Beta waves (a frequency of a wave per second and five times wider than alpha waves) occur in the electroencephalogram [18].

(d) ***Phase 4:*** The fourth phase is characterized by deep sleep. The heart and respiratory rate falls to 20–30% lower than that of wakefulness. This phase lasts about 15–30 min and occupies 10% of sleep time. The person is quite loose, rarely moving and difficult to awaken. Blood pressure, heart rate and body temperature have reached the lowest values. It is said that this phase promotes the physical state of man. Delta waves appear in the encephalogram [19].

Every night, when the person is getting ready for sleep, the body temperature decreases, the breathing becomes slower, the muscles relax and the person begins to yawn. The yawning is a prolonged breath and acts as a protective device to provide the body with oxygen when a fall in breathing occurs during sleep [20].

After the pre-mentioned four phases (in the meantime, almost ninety (90) minutes have elapsed since the person fell asleep), suddenly in the encephalogram there is a completely different phase from the previous ones. Alpha waves reappear, and the brain suddenly has a great activity as if awake. Circulation and temperature are increased. Diagrams showing the activity of eye bulbs (called nystagmograms) show a significant effect. It is what is called the REM phase, the phase of traditional sleep, where dreams appear [21]. The person in this period turns to bed. During the night, each person usually dreams 90 min, divided into 5–6 phases of REM. The duration of this special phase tends to increase during the night. So the first phase, which generally appears around at midnight lasts 6–10 min, and the last, about 5 in the morning, lasts about 20 min [22].

REM sleep is referred to as paradoxical or active sleep. During it, effects from the sympathetic nervous system prevail. It is said that this type of sleep restores the individual's mental state, in particular the functions related to learning, psychological adaptation and memory. It reviews processes and events that happened during the day, as well as other accumulated information [23].

The body seems to be paralyzed while the temperature, blood flow and oxygen consumption in the brain is increased. Moreover, heart rhythm, blood pressure and heart rate are elevated, the levels of which touch those of wakefulness. The rate of breathing varies from very fast, to very slow with periods of apnea [24].

In particular, REM sleep and the 4th phase NREM are of particular interest. Selective loss of either or both types of sleep creates need for replenishment. Thus, the body the next night increases the percentage of sleep and covers the gaps. This process is called replenishment phenomenon (Rebound effect) [25].

The body function presents daily high and low periods of physical and mental activity, which is determined by the so-called biological clock or circadian rhythm. The fact that man performs his duties during the day and night is asleep, suggesting that the biological clock initially is synchronized with the natural environment [26].

The body function presents daily high and low periods of physical and mental activity, which is determined by the so-called biological clock or circadian rhythm. The fact that man performs his duties during the day and during the night is asleep, suggests that the biological clock is initially synchronized with the natural environment [27].

The biological rhythm of sleep is often synchronized with other body functions, such as changes in body temperature associated with sleep patterns. The maximum body temperature value occurs normally in the afternoon, decreases progressively and falls sharply as soon as the person falls asleep [32].

The typical total length of 24-hour sleeping time varies 10 times between the species from about 2 hours in the giraffe to 20 hours in the small brown bat, while in humans it lasts about 8 hours. Nighttime sleep usually occurs in humans and many other mammals, but in some mammals occurs during the light period, as in rodents [33].

All people are asleep, although everyone has different behaviors in sleep. Some people need about 7.5 hours to rest and others need less or more sleeping hours. Younger people require more sleep than older people. As long as a person stays awake, the faster he wants to fall asleep.

People are usually sleeping supine and having their eyes closed. This is not the case in some mammals that sleep with their eyes open, like the ox. Moreover, others sleep while hanging their limbs, like the bat, and others while standing, like horses [34].

The movement during sleep is relative. Some people during sleep walk or speak and the fish swim. In general, the response to endogenous and exogenous stimuli, decreases, is not removed, and this condition is reversible. Response to stimuli and reversibility are two characteristics that clearly differentiate sleep from death, coma and narcosis [35].

Sleep is also connected with a variety of physiological changes associated with breathing, heart function, muscle tone, temperature, hormone secretion and blood pressure. Data from various studies have shown that from 4 am, body temperature, blood pressure, plasma cortisol concentrations and adrenaline increase in order to prepare the individuals, when they wake up, to be ready for activity. The opposite happens as the night approaches. Plasma cortisol concentrations, mental processes and body temperature are progressively reduced to prepare the individual for sleep [36].

3.1. Utility of sleep

Sleep thus is a charging of our body's batteries so that we can cope with everyday life having new forces. The importance of sleep in maintaining our body is also confirmed by serious disturbances that are caused when we do not sleep. As regards the importance of sleep, there is no longer any doubt that proper sleep is essential to good health. Physical, mental and social well-being, as well as protection from certain illnesses and accidents, depends on the quality and quantity of sleep [37].

Night sleep should be no less than 6 hours and more than 9 hours. As for the quality of sleep, which is function of the occurrence time and the relaxing effect, it depends on the lack of noise, the appropriate temperature, light meals and physical exercise. The comfort and stability of the sleeping area, as well as the observance of regular hours, also regulates the smooth functioning of sleep. This amazing sleep function seems to fulfill two functions, neurobiological and psychological [38].

The first is associated with the excretion of waste products of metabolic processes, the possibility of curing the CNS, especially in neonates and premature, by eliminating a large number of nervous stimuli bombarding the brain every day. The second is, according to Freud, a feigned satisfaction of our desires, and allows the vengeful and destructive loads to be neutralized, allowing the processing of a particular thought with consequent better acclimatization in real life. By performing these two functions, we are able to overcome intact the stimuli that usually bombard us [36]. In addition to night sleep, human health is also affected by the habit of sleeping during the day, also known as siesta. This sleep is usually short-term, mostly common at midday hours, approximately 12 hours after the nadir of normal wakefulness [1].

Lunchtime sleep (siesta) is common in countries near the equator due to climate. It is also observed when there is a night sleep deficit, like in cultures and societies where dinner is taken late at night, night sleeping does not take place before midnight, and getting up from the bed is early in the morning. Daytime sleep is also observed in shift workers, those with

hypersomnia, and in the elderly [39]. Short sleep during the day has been associated with better health levels. However, in some people this habit may have negative effects, causing difficulties in night difficulties in night sleep and delayed alertness during afternoon awakening [21].

3.2. Factors affecting sleep

Many and various factors can affect and change the type of sleep, such as the following [40]:

(a) ***General factors:*** Various life events such as noisy entertainments, intensive exercises, examinations in school or other trials, stress or stress from pressing work, etc. are included. All the above mentioned factors can change the type of sleep quantitatively and qualitatively.

Changes in sleep can also be caused by environmental causes such as bed and sleeping changes, ventilation, lighting, or noise [33].

(b) ***Personality of the individual:*** People with chronic neurosis, depression and introversion are believed to have a characteristic type of sleep. Although the total amount of sleep is increased, these people mention that they do not feel rested. Some researchers believe that increased sleep is due to the fact that during wakefulness, psychological and emotional problems were not effectively addressed [21].

(c) ***Age:*** Infants sleep more than children and young people more than the elderly. Generally, total time is increased in childhood, decreases at young age, then, it is flattened to be stabilized at this point until the advanced age. As the age progresses the number of awakenings and the time of the proportion of time during phase, changes [41].

Age of sleep according to age									
Developmental stage	Newborn	Infant	Toddler	Preschool age	School age	Teenager	Young adults	Average age	Elderly
14–16 hours/day	14–16 hours/day	12–14 hours/day	11–12 hours/day	11 hours/day	10 hours /day	7–8 hours/day	7–8 hours/day	7 hours /day	6 hours/day

(d) ***Underlying disease:*** In a large number of diseases it is possible to observe changes in the type and amount of sleep. For example, conditions characterized by pain affect the person's mood for sleeping. Nocturia, a common symptom for the elderly, can change the type of sleep. But also arterial hypertension often causes morning awakening, accompanied by a feeling of fatigue [24].

The symptoms of people with duo dental ulcer are exacerbated due to increased gastric secretions during sleep. A large number of respiratory illnesses are also involved with sleep. Additionally, the intake of certain substances can alter the behavior of sleep. L-Tryptophan is a very basic amino acid which is found in a wide variety of foods. It is believed that it reduces the onset of sleep time. Due to this property, it was considered a natural hypnotic. Moreover, man's habit of drinking a glass of warm milk before bedtime has a scientific basis, because milk contains this amino acid [42].

(e) ***Medicines*****:** The effects and side effects of medications bring about changes in sleep. Indicatively, antihypertensive and diuretics are mentioned. The side effects of administering antihistamines and antihypertensive drugs are drowsiness, night-time awakening and gait. Their beneficial effect compensates for the side effects, which can be reduced either by developing tolerance or by choosing antihistamine that has fewer side effects than the reported ones [43].

Sedatives, antidepressants and barbiturates also suppress sleep and their effects on sleep are quite similar to those observed in alcohol. From both causes, the latency of sleep time is reduced, continuous sleep and total sleep time increases and during acute drug administration, mild suppression of REM sleep appears. Very few of them promote sleep and this happens for a very short time [44].

Changes in sleep may be caused by many drug and substance groups, such as alcohol, anticholinergics, anticonvulsants, antidepressants, antihistamines, opiates, stimulants or irritants, and opioids.

Antidepressant drugs may change the type of sleep indirectly due to underlying depression, which causes sleeping abnormalities and directly due to the drug effect on sleep. The most persistent effects of antidepressant drugs on sleep are the general suppression of REM sleep and the prolonged latency of REM sleep. Sudden discontinuation of antidepressant drugs can lead to a prolonged period of REM sleep replacement, and the person usually complains about tension, dread and reduction of sleep quality [45].

(f) ***Irritants:*** Substances like caffeine or nicotine are included and they can disturb sleep. A cup of coffee, chocolate or Coca-Cola keep the person awake for several hours due to caffeine. Nicotine is considered a milder irritant but heavy smokers may experience changes in sleep. All irritants increase latent sleep time. Continuous administration, as well as immediate discontinuation has a serious effect on sleep [45].

(g) ***Alcohol***: Alcohol in a small amount promotes sleep because it causes relaxation, and in large amounts inhibits sleep. The use of alcohol leads to increased snoring and aggravates sleep apnea. With the use of alcohol may appear sleepwalking, nocturnal enuresis and in many cases nightmares. When chronic alcoholics decide to abstain from alcohol, they often experience insomnia. Alcohol withdrawal time is characterized by a decrease in overall and continued sleep [46].

(h) ***Psychological stress*****:** People who face serious personal or other problems usually develop stress that increases the tension and inhibits sleep. Continuous stress helps in getting poor sleep habits such as excessive sleep and insomnia [47].

(i) ***Exercise and fatigue*****:** Moderate fatigue resulting from exercise or from a pleasant job usually ensures restful sleep. Conversely, excessive fatigue from debilitating or stressful work can cause sleeping difficulties [48].

3.3. Sleep deprivation

Well-documented studies report that after 10 or 20 hours of insomnia, signs of excessive fatigue, reddening of the eyes, some mistakes in perception are beginning to appear. We have

the illusion that the floor waves. There is a substance in the blood, the in dole, which belongs to the same family as the hallucinogen L.S.D. [49].

If an individual stays sleepless for 60 hours, he will experience symptoms such as reduction of neck reflex, hand tremor, nystagmus, clumsy movements, eyelid dropping, dysarthria, difficulty in concentrating, reduced facial movements, and his general appearance seems to be apathetic. The changes start on the third day with illusions and as the sleep deprivation continues, perceptual, cognitive and psychomotor capacity of the individual are reduced, while visual hallucinations appear [50].

After 90 hours of insomnia, we have the impression that our face is full of spiders and it is impossible to distinguish between dream and reality. The electroencephalogram reveals the presence of a "short-sighted" period, where the person, although awake, has the same cerebrovascular features of sleep (slow waves). An individual under these conditions becomes a real public danger and can respond with reactions totally disproportionate to insignificant things [51].

Recovery from sleep deprivation is accompanied by increased overall sleep time. The values of the amount and type of the different sleep phases are restored during the first night of recovery [24].

4. Sleep disorders

Sleep disorders affect not only sleep but also many more aspects of life. They are related to adverse effects on the quality of life and health status during the day. There are three (3) main types of sleep disorders related to the biological sleep clock [28]:

(a) ***Type of delay of the sleep phase*:** The person cannot move the time of sleep and wakes up earlier than usual, so he sleeps and wakes up slowly in relation to the existed social requirements. People with delayed sleep phase often report that they feel sleepy in the early hours but are more energetic and alert late in the evening [2, 28].

(b) ***Jet lag type*:** The cycle of sleep and activity for most people is synchronized with the pace of day and night at the geographical point where they live and work. Jet lag is due to the de-synchronization between the various rhythms of the organism and the environmental rhythms. The rhythm that is most affected is the cycle of sleep and activity, with the associated changes in physical and mental functions. Symptoms of this syndrome are somnolence, fatigue, difficulty of concentrating, and irritation during the day. People, despite their fatigue throughout the day, cannot sleep and their sleep is anxious. This syndrome resolves in 2–7 days, depending on the travel distance from east to west and temperamental sensitivity [21].

Many people think they can avoid symptoms by changing eating and sleeping times before traveling. Others also think that the onset of the syndrome is directly related to lack of sleep, so sleep is the solution itself. Special treatment is not required [29].

(c) ***Type of shift change*:** This disorder is due to people working on night shifts or frequent shift changes. Shifts disrupt the worker's biological rhythm. Rolling working time creates short- and long-term health effects. Effects include sleep disorders, cardiovascular

disease, gastrointestinal disturbances and aggravation of chronic conditions. Young people and teenagers tolerate rolling schedule, showing fewer symptoms than the elderly [30]. It accounts for 10% of shift workers, which necessarily include night shifts. People's sensitivity to program changes varies widely, and a respected number of people simply do not adapt to changing hours. These people should not be employed on such a timetable. In this case, things are therapeutically more complex, because it is not always easy to change the individual's job. This is a medication that induces vigilance 30–60 min before the job, which is combined with the treatment of insomnia that occurs when the person wishes to sleep during the day [31].

4.1. Effects of sleep disorders

Sleep disorders can have serious effects on memory, learning, cardiovascular, nervous system, reduced productivity, our social behavior, and general deterioration in quality of life [52].

(a) ***Physical effects:*** Inadequate sleep has a serious effect on physical health resulting in illnesses such as diabetes mellitus, hypertension, heart disease, osteoporosis, various inflammations and many forms of cancer, especially breast and colon cancer [53]. These health problems arise from the production by the body of stress-related hormones, causing hypertension, which in turn is one of the main causes of heart attacks. Inadequate sleep increases blood levels of interleukin, resulting in increased fever, fatigue and loss of appetite. People suffering from insomnia produce elevated levels of cortisol, which is directly related to health problems as already mentioned [54].

(b) ***Psychological effects:*** Sleep and mental mood are characterized by a two-way relationship. As long as sleep affects mental mood, equally mood affects sleep. The lack of sufficient or good sleep adversely affects mental health, resulting in mental disorders such as depression, anxiety, alcoholism [33].

(c) ***Social-economic effects:*** Sleep disorders are associated with a negative impact on social behavior related to deterioration in quality of life, reduced productivity, excessive use of health resources, etc. Furthermore, there are direct adverse economic consequences due to medical costs, medications, medical consultations, examinations, investigations and inpatient and out-patient hospitalization. Indirect consequences are also apparent due to absences from work and the overall efficiency of the individual throughout the day [55].

4.2. Sleep and diseases

To a large number of diseases, changes in the type and amount of sleep may occur. For example, conditions characterized by pain affect the person's mood for sleeping [56].

4.2.1. Sleep in Parkinson's disease

Parkinson's disease is an age-related disorder characterized by movement disorders such as stiffness of the body, slowing of movement, and trembling of limbs when they are not in use. In advanced stages it progresses to dementia and eventually death [57]. The main symptoms are caused by the loss of dopamine-secreting cells in the substantia nigra [58].

More than 96% of patients suffering from Parkinson will experience sleep disturbances during the course of the disease. They are due to the interaction of various factors, such as motor problems (stiffness), circadian rhythm changes in sleep–wake cycle, behavioral disorders in sleep REM, psychiatric problems (anxiety, depression, dementia), side effects of drugs. It should be noted that the treatment of Parkinson's disease among its side effects includes sleep disorders characterized by daytime sleepiness [59].

Apart from the breakdown of nighttime sleep of the reported causes, 15% of patients with Parkinson will develop sleepiness throughout the day during the course of the disease. It is a sudden advent of sleep, in an inappropriate environment, without warning and without the possibility of suspension. Daytime sleepiness may be due either to the progression of the disease or to the various disorders that interrupt nighttime sleep or to the side effects of anti-Parkinsonian drugs [60].

Regarding the treatment of sleep disorders in Parkinson's disease, it aims to treat each individual disorder separately. In each case it is personalized. The basic principle of treatment is not to use plethora of sedative and hypnotic drugs. The medication is aimed at regulating anti-Parkinsonian treatment to reduce the kinetic problems that disturb sleep [61].

Dopamine is an organic chemical that plays several important roles in the brain and body. Also it is an amine synthesized which is synthesized in the brain and kidneys. Therefore in the brain, dopamine functions as a neurotransmitter and send signals to other nerve cells. The brain includes several distinct dopamine pathways, one of them plays a major role in the motivational component of reward-motivated behavior [62, 63].

In particular, dopamine is an organic substance used by nerve cells to communicate with each other. Dopamine acts on receptors found in the immune system cells and all dopamine receptor subtypes are found in lymphocytes. Several diseases have been found to be associated with damage to dopamine system. Dopamine deficiency caused by Parkinson's disease is associated with reduced movement, fatigue, slowing or blurring of cognitive functions, stiffness, loss of initiative or mobilization, and aggressive behavior in competitive situations [64].

Dopamine is available as an intravenous drug that acts on the sympathetic nervous system, with an increase in heart rate and blood pressure. However, due to the fact that dopamine cannot cross the blood–brain barrier, dopamine given as a drug does not directly affect the central nervous system. To increase the amount of dopamine in the brain of patients with conditions such as Parkinson's disease and dystonia, L-DOPA (which is the dopamine precursor) is often prescribed, because it crosses the blood–brain barrier [65]. Although L-DOPA treatment cannot restore the dopamine cells that have been lost, but it causes the remaining cells to produce more dopamine, thereby compensating for the loss to at least some degree [66].

Some medications act as dopamine agonists and can treat its low levels (hypodopaminergic) as they are typically used to treat PD, attention deficit disorder, hyperactivity disorder, certain mucosal tumors (prolactinoma), and they can also be useful to restless legs syndrome (RLS) [61]. For the treatment of Parkinsonism drugs such as bromocriptine and pergolide are sometimes used, but in most cases L-DOPA appears to give the best trade-off between positive effects and negative side-effects [66]. The development of a dopamine dysregulation

syndrome is sometimes associated with dopaminergic medications, which involves the overuse of dopaminergic medication and medication-induced compulsive engagement in natural rewards like gambling and sexual activity [67].

Restless legs syndrome (RLS) is a common sensory-kinetic disorder characterized by abnormal sensations that appear initially at rest or during sleep, relieved by the movement of the affected limb. The pathophysiology of RLS remains unclear although the role of dopamine dysfunction and iron deficiency in the brain, have been suggested [68].

Symptoms include unpleasant sensations in the extremities, especially in tibia. They can appear on both legs, sometimes also offend the hands. Individuals who have the syndrome usually report symptoms described as chills, tingling, burning, pain, pulling or even as something creeping under the skin. Symptoms get worse when the patient rests and they are improved with the movement. Symptoms are usually getting worse in the evening and during the night, so these patients often have poor sleep quality and consequently often experience daytime sleepiness. It is noted that there is no cure for this syndrome [65].

Treatment with dopaminergic agonists relieves symptoms, but does not result in total healing [69]. Adherence to the hygiene rules of sleep is also important. At the same time, psychiatric help is sought if the disorder is due to a psychiatric problem [56].

It is also noted that Researchers from the University of Barcelona and the Centro de Investigacion Biomedica en Red de Enfermedades Neurodegenerativas (CIBERNED) in Spain has discovered a new function of the neurotransmitter dopamine in controlling sleep regulation. The act of Dopamine in the pineal gland is central to dictating the 'circadian rhythm' in humans -- the series of biological processes that enables brain activity to adapt to the time of the day [70]. The translation of the light signals from the pineal gland which is received by the retina into a language understandable to the rest of the body [71]. In conclusion, the formation of these heteromers is an effective mechanism to stop melatonin production when the day begins and to 'wake up' the brain. This new function of dopamine could be extremely useful when designing new treatments to help mitigate circadian rhythm disturbances, for example those related to jet lag, those found among people who work at night, and in cases of sleep disorders in general [72].

4.3. Obstructive sleep apnea and diabetes mellitus

Most patients with diabetes mellitus (SC) have insufficient sleep in duration and quality. On the other hand, short-term and poor-quality sleep seems to adversely affect glucose metabolism and is associated with an increased risk of developing AD1 [73].

Patients with diabetes mellitus (SC) suffer from obstructive sleep apnea very often. It is likely that AD increases the risk for development of obstructive sleep apnea, mainly through the mechanisms of inflammation and autonomic nervous system (ADN) dysfunction. Additionally, diabetic neuropathy is associated with increased sensibility of promectal chemoreceptors to CO2, and sensitivity of peripheral chemoreceptors decreases [74].

Obstructive sleep apnea syndrome (OSAS) is a disorder that is characterized by repetitive partial or complete closure of upper airway during sleep. Also, obesity is the most important risk factor for OSAS. Many case studies in the literature show that OSAS is associated with insulin resistance, glucose intolerance and type 2 diabetes, independently of shared risk factors [75].

4.4. Sleep in chronic kidney failure

Sleep disturbances are very common in patients with chronic kidney deficiency. Pathophysiology is complicated and may include a combination of factors such as fluid balance, anemia, cardiovascular function, concomitant diseases, medications, physique, psychosocial and demographic factors and everyday habits. Recognition and treatment of these disorders can improve the quality of life and reduce morbidity and mortality in this patient [76].

The most commonly reported complaints are insomnia, Restless Legs Syndrome, sleep apnea and excessive daytime sleepiness [77]:

(a) ***Insomnia:*** Insomnia is common in CKD patients. There is a reduction in total sleep time of 4.4–6 hours and fragmentation due to a high percentage of microalarms - awakenings resulting in sleep efficiency ranging from 66 to 85% [49].

(b) ***Drowsiness:*** Daily drowsiness is common in patients with chronic obstructive pulmonary disease and correlates with uremic levels and periodic limb syndrome.

(c) ***Sleep impaired sleep syndrome (SASY):*** The most common symptoms of SASY is daytime fatigue, depression, cognitive impairment, which can be mistakenly attributed to Chronic Kidney Deficiency or other similar situations, and thus to undergo a subdiagnosis of SASY in these patients [55].

(d) ***Restless legs/periodic movement syndrome (sleep apnea):*** A rate of up to 80% of patients with restless legs syndrome (sleep deprivation syndrome) has an increased number of stereotyped movements of the legs called periodic movement of the tip (PKA). These increased and often intense movements of the limbs can last from a few minutes to a few hours. As a result of this, the patient experiences a lot of wakes and awakenings which in turn disrupt the normal and sleeping function of the sufferer [78].

5. Sleep and age

Age plays an important role in sleep duration as well as the formation of its internal architecture. As a person grows up, average sleep time falls from 16 to 18 hours for infants to 8 hours for a 12-year old child, then 7.5 hours for people between 25 and 45 and 6.5 hours for the elderly. Alongside with age, two things increase: the latent time that sleep comes and alertness time after sleep begins, that is more awakenings and inability to sleep again take place [39].

5.1. Sleep during infancy

During the first weeks of the infant's life, awakening at regular times during day and night is considered completely normal. Infants usually sleep all day with few intervals. The sleep-awakening cycle includes sleep and waking up for feeding and diaper change. Infants usually have an irregular such cycle and sleep 10–18 hours a day [79]. In order to develop the right models of sleep, babies have to go to bed when they feel sleepy and not when they are already asleep. Moreover, they have to learn to sleep by themselves from their first months. At the same time exposure to the sun and playing under it can guarantee a quieter sleep at night.

A baby's sleep gets more normal from the 4th to the 6th month because later it gets more difficult. Sleep duration is determined by neurological maturation, temperament factors and the baby's emotional state. When the baby has a troubled sleep, a sleep steady schedule needs to be followed [80].

The infant needs to sleep in specific hours during day and night, in a specific environment and with the following characteristics [38]:

- In its cot or basket with a stable and not very soft mattress.
- Low lighting.
- Noise-free.
- With relaxing music over its bed.
- With one of the baby's favorite dollies.

In the first 3–6 months, even if the baby has its own room, it is more practical for the cot to be in the parents' bedroom, so that they can feed it as easily as possible. Room temperature needs to be between 18 and 22–23°C. Its pajamas have to be light, the bed linen to be a light feather or woolen duvet or sleeping bag in winter, and in summer a sheet or cotton blanket is enough [81].

If the baby finds it difficult to sleep at night then a series of specific actions before sleep take place, such as a bath, a tender hug, lullaby or kiss, so that the baby can connect sleep to a pleasant feeling and sleep faster.

When it wakes up and cries at night make sure to see if it is hungry or its diaper wet, so as to give it milk or change the diaper. If it is in colic pain, rub its belly with oil. When the first teeth start to grow, you can put some gel on its gums to relieve pain after consulting the doctor [38].

For infants up to 1 year old the 'sudden infant death syndrome' is the first cause of death. Diagnosis takes place after ruling out all other possible causes of death. Breathing or heartbeat problems during sleep could be partially the cause as well. Death comes after arterial pressure falls and heartbeat slows down gradually till it stops. Main risk factors include smoking or drug use by the mother during pregnancy and after labor, cold winter weather and a baby's face-down position during sleep [82].

5.2. Sleep in children

Children's sleep changes with age. Before the 3rd month of life they pass from alertness to sleep with REM sleep directly, whereas after their 3rd month with NREM, like adults. The REM sleep rate changes as well. For a newborn it is 50%, while gradually it falls to 20–25% until the child is 3 years old [65]. Normal sleep duration also changes with age. For newborns it is 16–18 hours, for infants 13–15, preschool age 12–13, school 11–12 and in adolescence 9 hours. Usual sleep start time in toddlers is 8–8:30 p.m., while for teenagers it is 11–11:30 [81].

Sleep is a vital part of children's healthy development and is related to their physical, cognitive, emotional and social growth. In most cases sleep disorders are temporary, without long-term results. For some children, however, they can be very important [36].

In children sleepiness due to lack of sleep manifests as lack of attention, hyperactivity or aggressiveness. Lack of attention then has consequences on memory and learning. Quite often parents do not mention their child's sleep problem to the pediatrician or do not see the relation between sleep disorders and behavior during daytime. Thus, in a routine visit to the doctor questions about sleep need to be asked [83].

In pre-school children parasomnias are common, for instance nightmares, talking through sleep or night terror. Their frequency gradually decreases during the first 10 years of life. Most common sleep disorders in children are [81].

5.2.1. Behavioristic insomnia

Two types of it are often present in the same child. In the first, the child resists verbally or postpones sleep claiming fear, or leaves the bed and goes to find the parents. If time is lost its sleep is inadequate. The second type is about continuous night awakenings. The child that is used to going to bed under certain circumstances, like feeding or rocking in parents' arms, cannot calm down if it wakes up and cannot go back to sleep without the parents there [38].

Treating behavioristic insomnia: If all other medical problems are ruled out, like belly pain, breathlessness, otitis, allergic rhinitis, atopic dermatitis, underlying neurological disease or pharmaceutical effect, then the following measures are taken [84]:

- Steady sleep routine, around the time pre-school children go to bed (around 8–8:30). This should start 20–45 min before desired sleeping and include a bath, clothes changing, story narration or a game or blanket.
- The child should be in bed before falling asleep and not after.
- For children who wake up at night 'systematic indifference' is followed, that is no help is given to sleep again at night, so as to eliminate the need for a parent to be present (graduated extinction).
- The parent leaves child's room before it falls asleep. Every time it wakes up and looks for them, they have to wait more and more before answering.
- A positive behavior needs to be strengthened through reward.

Research shows that interventions in behavior clinically improve 80% of children to a great degree. No child showed any side effects from these treatments, and there is also a great secondary benefit in improving the daily behavior, self-confidence and mental health of the child and the parents [85].

5.2.2. Parasomnias

These are undesirable natural events or experiences that occur during sleep, during sleep or awakening. They are considered benign phenomenon in children and – if not very common and intense – they do not affect the duration and quality of sleep. They may exist individually in a child or co-exist with neurological psychiatric or other problems. Often there is a similar background to one of the parents [79].

The most common parasomnias in pre-school children are [81]:

- *Conjunctive awakening*. It occurs in children less than 5 years of age, 2–3 hours after the onset of sleep (NREM sleep disorder). The child sits on the bed restless and crying, or grumbles, can say something like "go" or "no" and does not calm down with what the parents say. The episode lasts 10–30 min and then comes back. Confusing awakens do not show stereotypical motions, sweating or flushing [81].
- *Nightmare*. The typical age for night terrors is 4–12 years old. The child wakes up with intense crying, has the same behavior as confusing awakening, with the difference of the presence of disturbances from the autonomic nervous system, that is, it is sweaty, has tachycardia and flushing. He does not seem to listen to the parents, he can jump out of bed as if he wants to avoid a threat, and in the morning he does not remember the episode [86].
- *Sleepwalking*. It is a NREM sleep disorder, which is most often seen in children aged 8–12, and this is because many episodes occur in infancy (e.g. the child is getting up and going to find his or her parents, or just going around In his cradle) go unnoticed. In sleepwalking, the child gets up from the bed and walks through the house, may seem uncomfortable and run around, or do simple activities that seem to be deliberate, like going to the bathroom. Especially for *sleepwalking,* where there is a risk of injury, preventative security measures such as locking the front door, guard rails on the ladder, removal of sharp and fragile objects, as well as floor barriers, a low bed, etc. should be taken [87].
- *Talking through sleep*. This is not pathological. It is the most common of all disorders [81].
- *Tooth grinding*. Also a frequent disorder in which the child sheds or tightens his teeth to sleep. When it is systematic, there is risk of tooth decay [88].
- *Nightmares*. It is a disorder of REM sleep and occurs more often in the early morning hours when it is more abundant. These are unpleasant, disturbing or even disgusting dreams that awake the child. When he wakes up, he is fully alert and older children remember to describe what happened. It is short-lived and the child continues to sleep. Children with post-traumatic anxiety disorder have more nightmares [89].
- *Night urination*. These are episodes of urinary incontinence in sleep, which occur at least twice weekly in children over 5 years of age. The majority of children gain control of the bladder until this age. These episodes can occur in all stages of sleep. They are either primary, when there has never been a period without enuresis, or secondary, which recurs after a period of at least 6 months and in this case may be associated with infection, diabetes, sleep apnea or other disorders [86].

Treating parasomnias: It is usually enough for the parents to reassure the child or stay with him until the end of the episode, while using various behavioral techniques such as programmed awakening. Drug administration is limited to selected cases of very resistant forms or to children with severe neurodevelopment problems and is given for a short time [81].

5.2.3. Respiratory disorders in sleep

It is a range of disorders ranging from simple snoring to classic obstructive sleep apnea, sleep apnea, or central hyponatremia syndromes [82].

Moreover, 10–12% of children snore, but even this disorder, which is otherwise benign, may have neuropsychiatric effects such as more anxiety, attention deficit disorder, social problems and depression. The most frequent and most important of all respiratory disturbances of sleep is obstructive apnea. These are episodes of partial or complete obstruction at the air intake during sleep, resulting in a reduction in oxygen in the blood [90].

The most common causes are hypertrophic tonsils and adenoids (carnations), craniofacial abnormalities, obesity and neuromuscular diseases. These recurrent sleep obstructions often result in waking up and a decrease in deep and relaxing sleep. The child can snore, sleep with open mouth, and wake up often to get breath, sound like drowning, getting night terrors or enuresis, sleepwalk. During the day it presents drowsiness, distraction, reduced academic performance, hyperactivity, and over time may present hypertension [2, 90]. Depending on the underlying cause, obstructive sleep apnea is treated with weight loss, medication, surgery, even with sleeping apnea (CPAP) devices [87].

There is also a minority of cases that need to be investigated, such as when the child snores or has sleep apnea, presents secondary enuresis, and finally to exclude *epilepsy*. Seizures, especially nighttime spasms originating from the frontal lobe of the brain, may be misdiagnosed as parasomnias [81]. Particular features from the child's history may help to distinguish. Convulsions occur at any time of the night, are stereotyped, shorter or occur several times the same night. When it is difficult to distinguish, further investigation by electroencephalography and polyp's graphic study is recommended [87].

Moreover, *narcolepsy*, although considered unusual in children, is rather sub-diagnosed. It is a disorder characterized by chronic daytime sleepiness with sleeps episodes during the day (usually 3–5 episodes lasting 10–20 min) that occur more frequently during monotonous activity. Many adult patients with postnatal narcolepsy report having symptoms as children. Narcolepsy has a genetic basis, is a chronic disease and its treatment is only symptomatic [91].

Restless legs syndrome also in some children may be synonymous with "growth pains." It is a hereditary disorder, usually a family history. This is a kinetic sleep disorder, in which the person complains about a strange, disturbing, creepy sensation on his feet, like something is crawling, appearing in the evening and at night. Some patients experience improvement by iron administration. This annoyance is temporarily relieved by the movement of the legs and so the person feels the need to shake his legs. This movement prevents him from falling asleep or breaking his sleep, resulting in fatigue and drowsiness in a day [92].

Sleep disturbances have a significant impact on the quality of life of the child and the family and are often treated easily. This underlines the need for proper diagnosis. Parents should monitor the sleeping of their children and when they recognize an unusual sleep behavior need to consult their pediatrician [81].

5.3. Sleep in the old age

When talking about sleep disorders in the elderly, we mean those that affect the ability to initiate and maintain sleep, including excessive sleep duration.

The timing and amount of sleep change with age. Elderly people tend to sleep early, wake up earlier and tolerate less changes in the sleep–wake cycle. As the circadian rhythm varies with

age, fatigue tends to become more intense as the sleep time increases. When this happens, the person wakes up earlier and the cycle repeats itself. Sleep efficiency / sleep duration compared to bedtime, decreases from 95% during puberty to less than <75% during third age [24].

Restless sleep in the elderly is due to various factors. First of all, poor sleep hygiene habits. Also, a medical or mood disorder that is adversely affected by sleep is more likely to occur, and medications to treat them may cause sleep disturbances. In addition, the possibility of primary sleep disorders, such as sleep apnea that may aggravate disturbed sleep, is increasing. Finally, aging affects the functioning of the urinary bladder, circadian rhythm, or hormone secretion and body temperature. These factors may result in less rejuvenating and more disturbed sleep [21, 93].

There are some age-related changes in sleep, although sleep disorders in the elderly may be related to psychological stress and stimuli, such as:

(a) ***Insomnia:*** It is the difficulty in the occurrence and maintenance of sleep. It may be transient (a few days), short (1–3 weeks) and chronic (>3–4 weeks). Treatment of insomnia usually does not require immediate medication. If it is nevertheless necessary on the basis of an individualized assessment, the lowest effective dose of the safest medicinal product should be used. The causes of insomnia include any medical condition, many medications and psychiatric disorders such as anxiety, dementia, and depression [27].

(b) ***Sleepiness:*** In the elderly the drowsiness during the day is persistent, excessive and does not diminish with extra sleep. It may be due to a wide variety of possible causes such as hypoglycemia, hypothyroidism, aphthous hyperthyroidism, uremia, hepatic failure, hypercapnia, hydrocephalus, head trauma, increased intracranial pressure of any etiology, etc. [26].

(c) ***Parasomnias:*** These are movements and behaviors that occur during sleep. The parasomnias that may occur in the elderly include the restless legs syndrome and periodic movements of the limbs in sleep [21].

(d) ***Sleep apnea:*** It is the temporary interruption of breathing during sleep due to airway obstruction. To combat the above sleep disorders in the elderly, it is advisable to avoid drinking before bedtime, frequent change of the diaper for incontinence, and discussing the problem with the attendant attending the elderly person with sleeping problems [3].

6. Sleep in the hospital

Sleep of the patient is a vital need and its fulfillment is a nursing responsibility. Only addressing the sleep problem requires not only specific scientific knowledge, as was said at the outset, but also a combination of nursing procedures in the context of integrated nursing care. Often, the patient does not have enough sleep, and this has an unpleasant effect on his psychosomatic well-being and resistance to the disease, as well as on health rehabilitation. In general, deprivation of sleep to a serious degree may cause disorder of thought and behavior, melancholy [21, 94].

Preparing the patients for sleeping, ensuring adequate sleep and monitoring their condition at night are unique nursing responsibilities, as no other health care profession has this concern [95]. For effective nursing care of sleeping patients, nurses need specialized knowledge from many sciences, including [21]:

- The physiology of sleep.
- Sleep disturbances and pathology.
- The psychology of sleep.
- Pain and anxiety as causes of insomnia.
- The pharmacology of hypnotics.
- The theory of dreams.
- The nursing system, such as patient preparation for sleep, night care, etc.
- The art of communication, because with this the nurse will identify and solve the problems of the patient's sleep.
- Technical nursing measures to ensure physical comfort.

As the most common causes of insomnia in the hospital environment are considered [93]:

1.	Environmentally	• Noise • Temperature • Lighting
2.	Physically	• Pain • Discomfort • Thirst • Hunger • A full bladder
3.	Psychological	• Anxiety • Overstress • Melancholy • Anger

Surveys have also shown that the factors that disturb the sleep of patients include the following [21]:

- Noise created by staff e.g. Conversations, book browses, and more.
- Noise and other environmental disturbances e.g. Squeaking doors and wheeled vehicles, sudden strikes of objects, cardio scopes, rewinders, suction fans, sliding furniture, telephones, intense lighting etc.
- Nursing and treatment procedures e.g. taking vital signs, injections, individual care.

- Noises generated by other patients, such as conversation, coughing, vomiting, snoring.
- The pathological condition of illness, pain, fever, discomfort, bedtime, lack of private space, difficulty in oral communication such as endotracheal intubation or aphasia, fear of death, and so on.

The patient struggling to sleep in so many noises reaches the point of wondering whether sleep is permitted in the hospital. Nurses again, as a health care professional, with their own personal interest, "good art" and their scientific education, have to care for the patient's exercise, keeping them busy, rest and sleep; this must be the link, the true dimension of hospitalization [94].

More importantly, nurses' responsibility in terms of sleep advancement is to help the person at all stages of the disease to ensure adequate, calm and effective sleep [93].

Information about the sleep environment is whether the person sleeps alone or shares the room with another, the number of pillows and bedding him uses ventilation, lighting and noise. Also noted are the drugs and the type they use, if they are eating before bedtime, the type of food and drink they are used to, whether they are showering or bathing before eating. In particular, the person's views on rest and sleep time he considers necessary to operate at desired levels are considered [87].

- General nursing interventions [95]
  - Help the person recognize that he is exercising control over his type of sleep, and that he can achieve restful sleep by natural means such as noise avoidance, normal temperature, and reduced light.
  - Help identify the type of sleep, sleeping habits and pre-sleep habits.
  - Help distinguish or establish a type of sleep relaxing and comfortable for oneself.
  - Encourage patient to identify the factors that affect his sleep pattern.
  - Take leisure and activity types throughout the day, in the afternoon and in the evening when planning your nighttime sleep.
- Nursing interventions for daily program [69]
  - Encourage the person to actively participate in activities appropriate to his/her situation. Design rest periods and activity for the whole day.
  - Help patients detect the time of daytime sleep. When the sleep of the day is taken at the same time and for a planned duration it is beneficial.
  - Help to avoid frequent insomnia throughout the day. Serious sufferers and those undergoing surgery will have a greater number of short-term sleep during the day.
- Nursing night program interventions [94]
  - Help the person reduce their activities before bedtime.
  - Encourage you to define and perform your pre-sleep habits and help them adapt to the hospital environment.
  - Give a gentle rub, place a suitable bed, keep it dry and clean.

- Offer them the right reading or music.
- Know that stress is more common at night. Give opportunities to the sick person to talk to you about his interests and his fears. Suggest that he discuss it with a family member or a trusted friend of his.
- Include the painkiller in the patient's program and administer it before bedtime. Although these drugs may affect the type of sleep, relief from pain is of greater importance.
- Plan hospitalizations so as not to disturb sleep, such as avoiding diuretic or stimulant administration before bedtime.

7. Conclusions

Sleep occupies about one third of our total lifetime and is a very important biological function. Its functional significance is related to the resting of brain function and to the proper functioning of memory and learning. Sleep deprivation causes disturbance of attention, performance at work and emotion.

Therefore, sleep is essential for a smooth living. Its duration is satisfactory when we wake up rested and rejuvenated. The duration of sleep differs from person to person but is estimated at about 8 hours a day. With aging, it usually reduces its duration and many elderly people sleep 5–6 hours a day.

In order to have a normal sleep, it is good to respect our biological clock that is to try to sleep for about the same hour at night and to wake up at about the same time in the morning.

Author details

Kourkouta Lambrini[1]*, Ouzounakis Petros[2], Papathanassiou Ioanna[3], Koukourikos Konstantinos[4], Tsaras Konstantinos[5], Iliadis Christos[6], Monios Alexandros[7] and Tsaloglidou Areti[8]

*Address all correspondence to: laku1964@yahoo.gr

1 Professor, Nursing Department, Technological Educational Institute of Thessaloniki, Macedonia, Greece

2 RN, General Hospital of Alexandroupoli, Greece

3 Assistant Professor, Technological Educational Institute of Larissa Thessaly, Greece

4 Clinical Professor, Nursing Department, Technological Educational Institute of Thessaloniki, Macedonia, Greece

5 Assistant Professor, Technological Educational Institute of Larissa Thessaly, Athens, Greece

6 RN, Private Health Center of Thessaloniki, Macedonia, Greece

7 Biologist, 7th Gymnasium, Athens, Greece

8 Assistant Professor, Nursing Department, Technological Educational Institute of Thessaloniki, Macedonia, Greece

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Chapter 4

Manganese Inhalation Induces Dopaminergic Cell Loss: Relevance to Parkinson's Disease

Maria Rosa Avila-Costa, Ana Luisa Gutierrez-Valdez, Veronica Anaya-Martínez, José Luis Ordoñez-Librado, Javier Sanchez-Betancourt, Enrique Montiel-Flores, Patricia Aley-Medina, Leonardo Reynoso-Erazo, Jesús Espinosa-Villanueva, Rocío Tron-Alvarez and Vianey Rodríguez-Lara

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.79473

Abstract

Parkinson's disease (PD) experimental models are crucial in the assessment of possible therapies. Nevertheless, even though PD was one of the first neurodegenerative conditions to be modeled, there are limitations such as spontaneous recovery; lack of bilateral damage, which is a PD characteristic; animal intensive care after neurotoxin administration; and ultrastructural and biochemical nonspecific alterations but mostly the neurodegenerative time course observed in humans. In this chapter, we investigated the effects of divalent and trivalent manganese inhalation on rats and mice to obtain a novel PD animal model inducing bilateral and progressive dopaminergic cell death. We found that after 5 or 6 months of inhalation, there was more than 70% decrease in the number of TH-immunopositive neurons, and these alterations are correlated with an evident motor performance deficits manifested as akinesia, postural instability, and action tremor. More interesting is the fact that these alterations were reverted with L-DOPA treatment, implying that the motor alterations are associated with nigrostriatal dopaminergic innervation, postulating new light for the understanding of manganese neurotoxicity as an appropriate PD experimental model. Our results are contributing to the development of a suitable PD animal model, reproducible, sensitive, time-efficient, and readily applicable behavioral tests.

Keywords: Parkinson's disease experimental model, rodents, manganese inhalation, dopaminergic cell loss

1. Introduction

The typical motor symptoms of Parkinson's disease (PD) (akinesia, bradykinesia, rigidity, tremor, and postural abnormalities) are related to the loss of nigral dopaminergic cells and decay in caudate-putamen dopamine (DA) content that led to the introduction of DA replacement therapy [1]. Consequently, there has been a fundamental role for PD animal models in developing new approaches treating this disease, in innovative treatment strategies, and in understanding the nature of the pathogenic processes involved in the dopaminergic neuronal loss [1, 2].

Several models display many of the distinctive features of the disease; however, none resembles the complex chronic neurodegenerative features observed in human PD. 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and 6-hydroxydopamine (6-OHDA) are considered as neurotoxicants that rapidly and selectively kill dopaminergic neurons (in 1–3 days), while in PD patients, the disease is progressive [3].

Emborg [4] declares that a representative animal model must present pathology and behavioral manifestations that match the disease, involving its temporal path. The more the similarity of a model is to PD, the bigger the predictive strength for clinical efficacy will be.

The results regarding manganese (Mn) as an experimental PD model have been studied since its toxicity (commonly called manganism) shares neurological symptoms with numerous clinical disorders frequently described as "extrapyramidal motor system dysfunction," and, in particular, idiopathic PD [5–7]. Manganism is associated with high brain levels of Mn, primarily in those areas known to contain high concentrations of nonheme iron, particularly the striatum, globus pallidus (GP), substantia nigra compacta (SNc), and subthalamic nuclei [8].

There is some disagreement on the alterations induced by Mn; while some researchers reported that Mn alters nigrostriatal dopaminergic levels and produces a Parkinson-like disorder [9–12], other authors confirmed that Mn alterations are related to different aspects to those associated to PD in both etiology and pathology [13, 14] especially in the remarkable SNc dopaminergic cell conservation [15–19]. As stated by Calne et al. [5], Lu et al. [16], and others [20–22], the most important between these differences is the absence of clinical response to L-DOPA.

However, studies have reported ostensibly contradictory results on the dopaminergic effects of Mn (see Gwiazda et al. [23] and Guilarte [24] for review), including decrease [9, 25–28], increase [11, 29], both [30], or no modification [15, 31, 32] in SNc or striatum DA levels in Mn-exposed animals, probably indicating differences in exposure procedures on DA consequences. These inconsistencies might disclose changes in the route of exposure, magnitude, duration, Mn compound or concentration, experimental animals' species, age, etc., among investigations, revealing the complexity of Mn toxicity and suggesting that the features that cause the toxicity are not entirely recognized.

It appears that lesser dosages of Mn augmented DA and its metabolite concentrations, whereas the inverse was detected with more significant Mn concentrations [30, 32]. Similarly,

it has been proposed that higher Mn dosages can drastically accelerate DA and other catecholamine oxidation, which concomitantly intensify reactive oxygen species formation of [33–35].

It seems that both trivalent and divalent Mn can be carried to the CNS through the brain barriers [36, 37]. Mn^{2+} is transferred into brain choroidal epithelial and capillary endothelial cells through nramp2 (DMT-1) or by divalent cation transporter DCT-1 [38]. On the other hand, trivalent Mn bound to transferrin is transported across the brain barriers via the receptor-mediated endocytosis [37]. Mn is then liberated into the endothelial cells by endosomal acidification [21], then is transported to the abluminal cell exterior for release into the extracellular fluid. Finally, it is delivered to the glial cells and neurons, for usage and storage [39]. It has been demonstrated that Mn inhibits complex I in the mitochondria altering the oxidative phosphorylation process. Also, it appears that trivalent Mn is more effective in inhibiting complex I than divalent Mn [40–43] and accelerating ferrous iron oxidation. Mn^{3+} increased facility to provoke oxidative stress has been established in rats treated with either Mn chloride [$MnCl_2$ (Mn^{2+})] or Mn acetate [$Mn(OAc)_3$ (Mn^{3+})] [41]; these researchers state that 1–1000 μM $MnCl_2$ induced increased reactive oxygen species in striatum, while $Mn(OAc)_3$ produced comparable results at significantly lower dosages (1–100 μM). Therefore, the Mn valence and metabolism appear to determine its toxicity.

Thus, since it has been suggested that trivalent Mn is more effective in producing oxidative stress and divalent Mn requires Mn^{3+} to induce oxidation and that there is an interaction between the two Mn compounds, this study examines Mn^{2+}/Mn^{3+} mixture inhalation effects on rats and mice to produce a unique PD experimental model provoking SNc dopaminergic cell death, progressive and bilateral, associating those changes with motor alterations. Moreover, we sought to determine if after Mn inhalation the motor alterations improve with L-DOPA treatment to ensure that the alteration's origin is dopaminergic.

2. Methods

Animals: 45 CD-1 male mice weighing 33 ± 2 g and 45 male Wistar rats weighing 180 ± 10 g were individually housed in hanging plastic cages under controlled light conditions (12 h light/dark regime) and fed with Purina Rodent Chow and water ad libitum (except the days of reaching task evaluation). The animals were weighed daily. The experiment was done according to the NIH Guide for the Care and Use of Laboratory Animals (No. 80-23 1996), Guide for Care and Use of Laboratory Animals certificated by SAGARPA-Mexico (NOM-062-ZOO-1999, Mexico) and approved by the Institutional Committee of Animal Care (UNAM). We made all attempts to reduce the number of rodents used and their distress.

2.1. Motor behavior

Before Mn exposure, all rodents were taught and trained for motor performance. Assessment and training were accomplished through the lighted part of the cycle, at the same hour every day. For the reaching task, the animals were kept without food to 90% of average body weight

for 24 h and received controlled quantities of food pellets once a day to sustain body weight and deprivation state. Behavior analyses were conducted the days the animals did not inhale. Each animal was tested once a week, a different day for each test.

2.2. The reaching task

The mouse reaching box was 19.5 cm × 8 cm and 20 cm high. A 1-cm vertical slot ran up the front of the box. A 0.2-cm-thick plastic shelf was displayed 1.1 cm from the floor on the box front. The rat-reaching box was 30 cm × 15 cm and 20 cm high. As for the mice box, this one has a 1-cm wide and narrow opening that ran up the front of the box. About 20-mg food pellets were positioned near the slot. Animals were habituated for 1 week by introducing them in the cages for 10 min. Pellets were initially reachable on the box floor and then within a short distance on the shelf. Food pellets were progressively raised from the box floor and positioned beyond the shelf (1 cm) until the rodents were obligated to retrieve the pellet with their preferred forelimb. According to Whishaw et al. [44], the pronation of the paw medially allows the mouse/rat to catch the food pellet with the forelimb and not with their tongue. The animals were independently trained and permitted to grasp with their preferred forelimb the pellets [44]. Each animal grasped for 20 food pellets each trial during the evaluating period. A successful reach was scored when the animal was able to retrieve with its forelimb and eat a pellet. When the pellet was knocked off the shelf or pulled into the chamber and dropped through the floor grating were scored as a failure [45]. The qualitative evaluation comprised the analysis of the "reaching performance": the posture, limb extension, aim, paw supination-pronation during grasping, and the pellet released into the snout.

2.3. The beam-walking task

This test evaluates the rodents' skills to traverse a narrow beam (3 mm) to reach an enclosed safety platform [46]. The mice apparatus is constructed by an elevating surface of a 10 × 100 cm × 3 mm wood beam 75 cm above the floor with two supports by 15° inclination. Rat's beam measured 2 m long and was elevated to a height of 1 m above the ground with wood supports with 15° inclination. A home box is situated near the end of the beam. On training days (4 days), each mouse/rat was positioned at the start of the beam with no inclination (four tests each day). When the animals traversed the apparatus in 20 s, they performed two more trials with the beam inclined. Mice were allowed up to 60 s and rats 120 s to traverse the wooden beam. The latency to cross the beam was recorded for each trial.

Video recording: the different trials were recorded with a Sony camcorder. The video camera was placed orthogonally to the reaching box to analyze the animal's behavior. Demonstrative motionless captures were taken from the video recordings with the Final Cut Pro X for Mac.

Neurological evaluation: Tremor and bradykinesia were assessed by inspection of Mn-exposed compared with control animals during the performance of the two tests.

2.4. Manganese inhalation

Afterward, two groups were formed: one group was exposed to deionized water (control groups; n = 20), while the second group (n = 20) was exposed to the mixture of chloride ($MnCl_2$)

0.04 M and acetate ($Mn(OAc)_3$) 0.02 M (Sigma-Aldrich, Co. Mexico). Inhalations were done as described by Avila-Costa et al. [47]. The animals were positioned in an acrylic chamber. Mn exposure was accomplished in locked acrylic boxes (35 cm × 44 cm and 20 cm high) attached to an ultra-nebulizer (Shinmed, Taiwan), with 10 l/min constant flux. The ultra-nebulizer produces 0.5–5-μm range droplets. A vapor was placed on the other side of the box with a sodium bicarbonate mixture to trap the residual metal. During inhalations, the rats/mice were examined continuously for respiration frequency, regularity, and depth. The inhalation chamber was monitored continuously for oxygen levels, temperature, and Mn concentration.

Based on the results found in the behavioral evaluations, we sacrifice the animals after being exposed to 40 (mice) and 72 (rats) inhalations (5/6 months of exposure) under deep anesthesia with sodium pentobarbital lethal dose IP (0.2 mg). Thus, when evident motor alterations were observed, twenty mice/rats were sacrificed (ten controls and ten Mn-exposed), anesthetized with sodium pentobarbital, and perfused via the aorta with phosphate buffer saline (0.1 M pH 7.4) containing 4% paraformaldehyde. The brain was removed and positioned in fixative solution for 2 h and processed for tyrosine hydroxylase (TH) and NeuN immunocytochemistry (five control and five Mn-exposed brains).

Later, the rest of the animals continued the Mn inhalation. Five were treated orally with 7.5 mg/kg L-DOPA (Sinemet [Carbidopa-L-DOPA 25/250]) every day during 2 months, five were reserved for the equivalent time but with no treatment, and five controls were kept for the same time and then sacrificed for further analysis; the motor behavior performance was assessed every week.

Additionally, the fresh tissue of other 10 control and 10 exposed animals, after 40 inhalations (mice) and after 72 inhalations (rats), was obtained to determine the concentrations of DA by HPLC in the striatum, SNc, and GP.

2.5. Sample preparation and immunohistochemistry

Tissue samples were serially sectioned at a thickness of 50 μm on a vibrating microtome (Pelco 101, Ted Pella Inc., Mexico) within the mesencephalon for TH and GP and striatum for NeuN immunocytochemistry. TH (Chemicon International, Inc., CA, USA, 1:1000) and NeuN immunostaining (Chemicon International, International, Inc., CA, USA, 1:200) with the ABC detection technique (Vector Lab, MI, USA) was performed for the cell analysis. All images were captured using an Optiphot 2 Nikon microscope. Images were analyzed using ImageJ software. The number of TH+ cells was calculated rostrocaudally through the SNc and ventral tegmental area (VTA) in nearby segments. The SNc was manually delineated to trace the region of interest (ROI) at low magnification (4×). The TH-positive cell number was calculated at the level of the third cranial nerve, within a 100-mm counting area at 40× only within this defined ROI [48, 49]. NeuN cell count of striatum and GP was done using 40× objective in seven sections per animal at 0.70 anterior, 0.48 mm posterior to bregma for dorsomedial striatum, and 0.80 anterior and 0.92 mm posterior to bregma for ventrocaudal GP according to [50] for rats and at rostrocaudal levels 0.86 anterior to 0.50 mm posterior to bregma for dorsomedial striatum and 0.62 anterior to 0.98 mm posterior to bregma for ventrocaudal GP according to [51] for mice, in a 11,550 and 3300 mm^2 counting area, respectively. It should be noted that both dorsomedial striatum and ventrocaudal GP receive the maximum dopaminergic innervation [52, 53].

2.6. Mn concentrations

The Mn concentration in the inhaling box was calculated by placing a filter at the gap of the inhaling chamber during the whole inhalation time; the flow rate was constant (10 l/min). After each exposure, the filter was detached and weighed; the metal concentration was calculated with a graphite furnace atomic absorption spectrometer (Perkin Elmer Mod. 3110, CT, USA). We analyzed six filters for each inhalation [54]. At the end of the experiment, rat/mice serum Mn levels were also estimated by graphite furnace atomic absorption spectrometry.

2.7. Dopamine concentrations

SNc, striatal, and GP DA contents were obtained after 5 months, for mice, and after 6 months for rats of Mn inhalation as described by [55]. Briefly, five controls and five Mn-exposed mice and five controls and five Mn-exposed rats were anesthetized and decapitated, and with a stereoscopic microscope, the tree structures were obtained. The tissue was homogenized in perchloric acid with 100 μl per brain. Then, the tissue was centrifuged (300 PSI, 2 min, Airfuge centrifuge, Beckman, Fullerton, CA, USA) and the supernatants filtered (0.22-μm membranes, Millipore, Bedford, MA, USA). The resulted tissue was resuspended, and by Bradford method, we performed the protein determination as reported elsewhere [56]. DA levels in 10 μl of supernatant were determined through HPLC reverse phase system attached to an electrochemical detector (BAS; West Lafayette, IN, USA). Results were analyzed using the Peak II integration software (SRI Instruments; Torrance, CA, USA). DA concentration is shown as pg./μg protein.

2.8. Statistical analysis

Unpaired t-test was used to analyze the number of TH and NeuN-positive cells. Repeated measures ANOVA analyzed motor behavior tests; post hoc comparisons were performed with Tukey's test. Group differences were established as statistically significant when $p < 0.05$. Statistical analysis was done using GraphPad 7 for Mac Software (San Diego, CA).

3. Results

After 5 (mice)/6 (rats) months of exposure, neither clinical alterations nor significant weight changes were detected in the exposed animals compared with controls.

3.1. Manganese concentrations

The average Mn concentration detected in the chamber filters was of 2676 μg/m^3 during the whole experiment. The average Mn concentration in serum of exposed mice was 30 ± 5 μg/l; control mice serum concentration of Mn was 0.05–0.12 μg/l. The average Mn concentration in serum of exposed rats was 45 ± 5 μg/l; control rat's serum Mn concentration was of 0.05 ± 0.12 μg/l.

3.2. Single-pellet reaching task

The task includes the accomplishment of motor sequences, beginning with smelling a food pellet forward-facing the reaching slot, lifting the arm, adapting position to project the limb across the narrow slot to the food pellet, and taking the food (**Figure 1**).

Mice and rats were presented with 20 food pellets. **Figure 2** displays the success reaches throughout the experiment. Repeated measures ANOVA established a substantial effect of Mn-exposed groups since eight inhalations ($p < 0.001$). Mice/rats were similar in their skill to recover the pellets before Mn exposure, but Mn inhalation occasioned significant alterations in both number of successful recoveries ($p < 0.001$) and precision in both mice (**Figure 2A**) and rats (**Figure 2B**); however, with L-DOPA treatment, the animals recover their functioning when compared to the non-treated ones, like the control groups' performance ($p < 0.001$). Control animals were steady during the entire experiment and were notably better than Mn-exposed animals (**Figures 1** and **2AB**).

The qualitative assessment showed postural swing and deficiencies in limb extension (resulting in several shortened reaches), aim, and paw supination-pronation during grasping and release of the pellet into the slot (**Figure 3A–J**); both mice and rats exhibited unusual movements when recovering the food after Mn inhalation. The forelimb was frequently totally pronated and moves laterally over the food (**Figure 3F**, **G**, and **I**), or the animal hits at the pellet (**Figure 3I**); some mice/rats from Mn-exposed groups displayed such behavioral alterations that endured for the complete study.

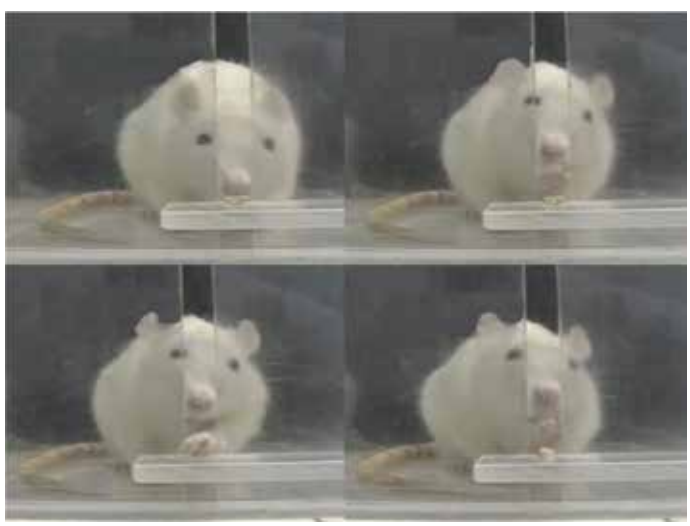

Figure 1. Characteristic pictures of a control animal taken during limb moving and withdrawal. The control animals moved their arm throughout the slot and opened their fingers; then, supinated their paw to take the food to the snout; and extended their digits to release the food into the mouth.

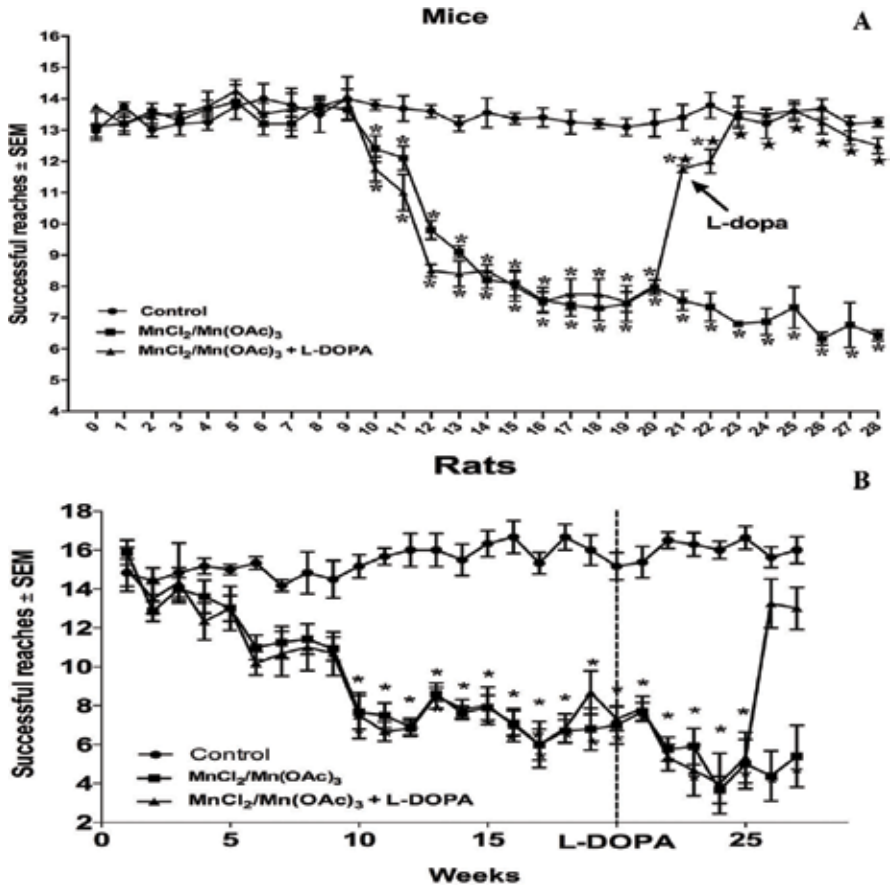

Figure 2. Reaching success scores (sum of food pellets taken out of 20; mean ± SEM) of control and Mn-exposed mice (A) and control and Mn-exposed rats (B) in the reaching task. The Mn-exposed group is impaired since week 12; note that L-DOPA treatment entirely reverses the alterations (*$p < 0.001$ vs. control group; repeated measures ANOVA).

The Mn-exposed groups are often incapable of accurately closing the digits around the pellet and dragging it to the slot without lifting the paw (**Figure 3F**, **G**, and **I**). These animals are also not capable of supinating the forelimb entirely and putting the mouth into the gap to recover the food with their tongue (**Figure 3J**). When the arm is withdrawn throughout the gap, Mn-exposed groups repeatedly turn their body and pursuit the food with the tongue instead of opening their fingers and introducing the food into the snout. The non-reaching forelimb is occasionally placed for support when recovering the food. Post-hoc analysis on the group's effect showed that at more Mn inhalations, success of retrievals was significantly lesser (**Figure 2**). These situations amazingly recover with L-DOPA treatment (**Figure 2A** and **B**). The treated animals adjust their posture and project the arm toward the food pellet, supinate and pronate the paw to obtain the food, close their digits, and drag the food to the snout; their motor performance with L-DOPA treatment was comparable to control groups (**Figures 1** and **2**).

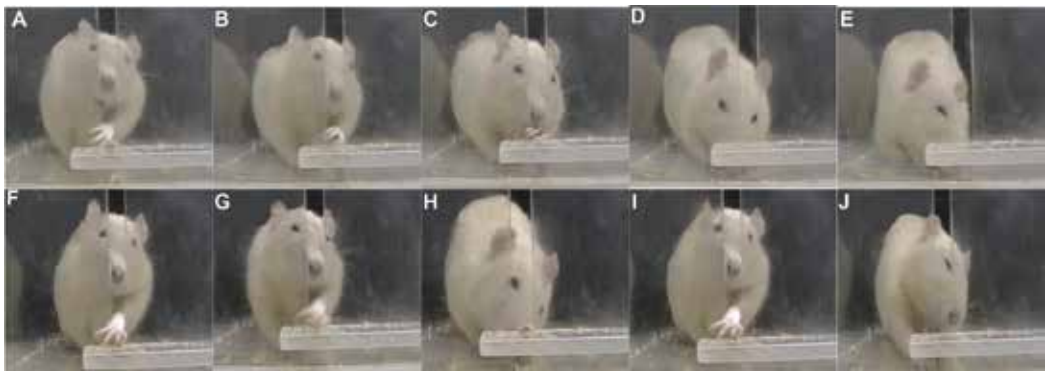

Figure 3. Illustrative still pictures of an exposed to Mn mouse (A–E) and a Mn-exposed rat (F–J). The animal approaches its forelimb by moving the elbow for the hand goes through the gap. As the arm moves closer to the food, the fingers open, and then the mouse pronates its forelimb by elbow adduction and rotates it around the wrist so that the hand is positioned on the top of the food. The pellet is grabbed by flexion of the fingers. The forelimb is withdrawn carrying the food. The animal lies on its hips to eat the pellet, which is secured by the hands. Frames (F–J) Mn-exposed rats displayed alterations characterized by severe postural modifications moving the forelimb obliquely throughout the gap making various small efforts without stretching the forelimb according to the midline of its body. The fingers are simultaneously adducted. The forelimb arises in front of the side or hits laterally, and the fingers do not take the food. The animal often pulls its forelimb across the gap and let fall the food to the floor cage chasing it with the tongue.

3.3. Beam-walking test

During the last day of evaluation before Mn exposure, we found no significant differences concerning the time in finishing the test for the controls and the Mn-exposed animals (ANOVA, $p > 0.05$). **Figure 4** depicts the mean of total time to traverse the beam. Mn-exposed mice (**Figure 4A**) and rats (**Figure 4B**) after 10 weeks of inhalation have a significant increase in the time to cross the beam compared with control groups; moreover, animals exhibit limb weakness, akinesia, postural instability, and action tremor. Mn-exposed mice have a significant reduction in the time taken to traverse the beam after two, four, six, and eight Mn inhalations (**Figure 4A**) proposing hyperactivity. Afterward there is a significant increase in the time to pass and a visible presence of freezing behavior time (data not shown), compared with control mice. As for the rats (**Figure 4B**) in the beam-walking test, Mn-exposed animals increased the execution at all-time points. While the control rats maintained an average of 20 s during the entire experiment, the Mn-exposed rats are slow and take more than 120 s to cross the beam after the tenth week (**Figure 4B**). This effect is completely reversed with L-DOPA treatment. Besides, all exposed animals also exhibited hind-limb weakness, delayed motor initiative (akinesia), postural instability, and action tremor. L-DOPA treatment reverted these motor alterations in both rats and mice.

3.4. Immunocytochemistry

3.4.1. TH immunocytochemistry

As for TH immunohistochemistry, mice (**Figure 5A**) exposed to 40 inhalations showed 67.58% decreased in the number of TH-immunopositive neurons in SNc compared to the control

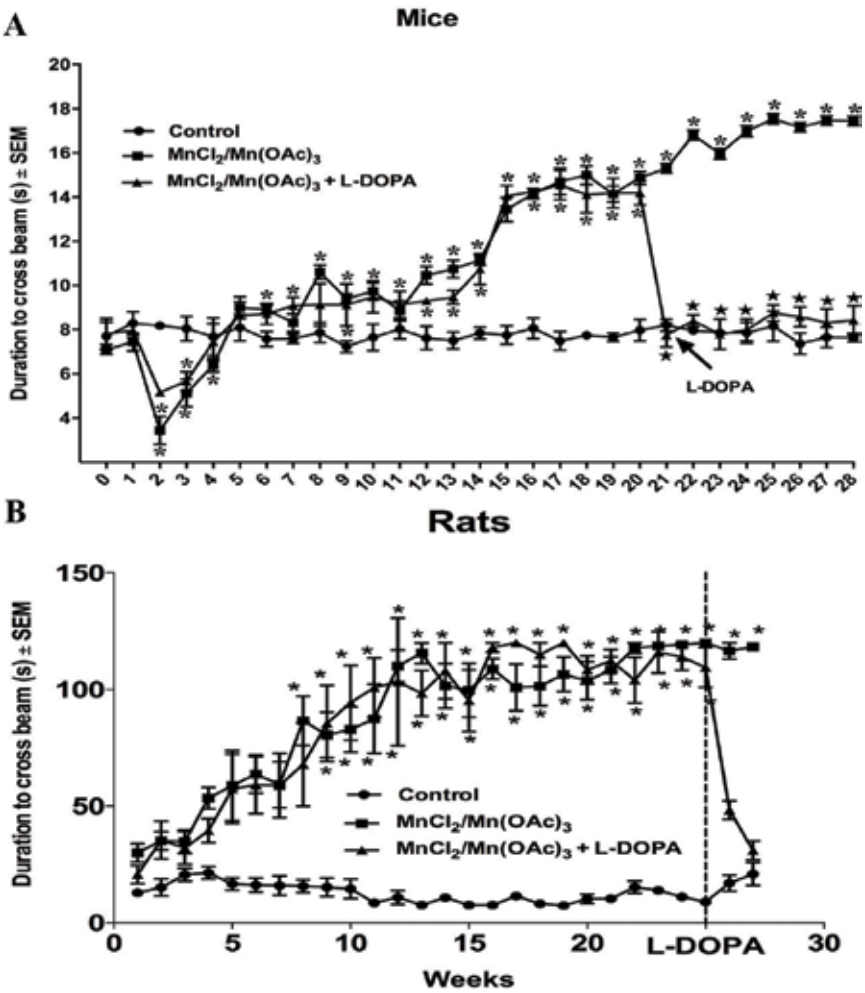

Figure 4. Mean latencies to traverse the beam (±SEM) before and after mice (A) and rats' (B) Mn inhalation and after L-DOPA treatment. It is notorious that after 2, 4, 6, and 8 of Mn inhalation, the mice significantly reduce the time to traverse the beam and afterward showed a significant increase in the time to cross the beam compared to controls. The Mn-exposed rats are significantly impaired since week 10. However, when the animals received the L-DOPA treatment, the time was reduced drastically resembling the values of the control group (*$p < 0.001$ vs. control group).

animals, while there was no loss of neurons in VTA of exposed animals compared to controls (**Figure 5A** and **6**). The rats showed a 75.9% loss in the number of TH immunoreactive neurons after 48 inhalations and, like mice, showed no neuronal loss in the VTA (**Figure 5B** and **6**).

3.4.2. NeuN immunocytochemistry

One of the required characteristics for animal models is the neuronal specificity for cerebral nuclei that are affected in humans, so to determine if the Mn mixture affects other brain structures, we performed anti-NeuN immunohistochemistry, a nuclear protein neuronal specific. In this respect, we found no significant loss in the number of neurons in any of the analyzed nuclei (data not shown).

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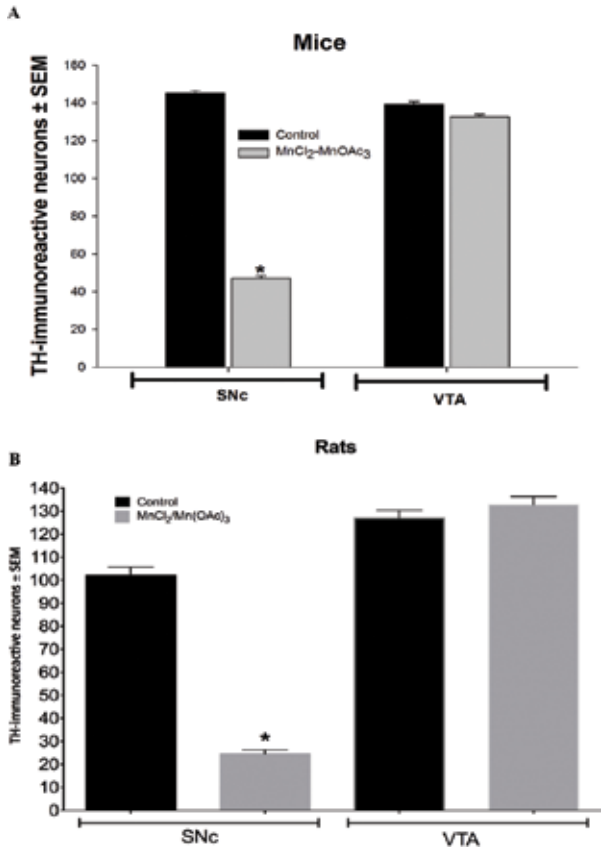

Figure 5. TH+ cell number from the SN) and VTA. The data are depicted as the mean ± standard error. A statistically significant diminution in TH+ cells was observed in the SNc (*$p < 0.05$ unpaired t-test) of Mn-exposed mice (A) and rats (B) compared to controls with no changes in the VTA.

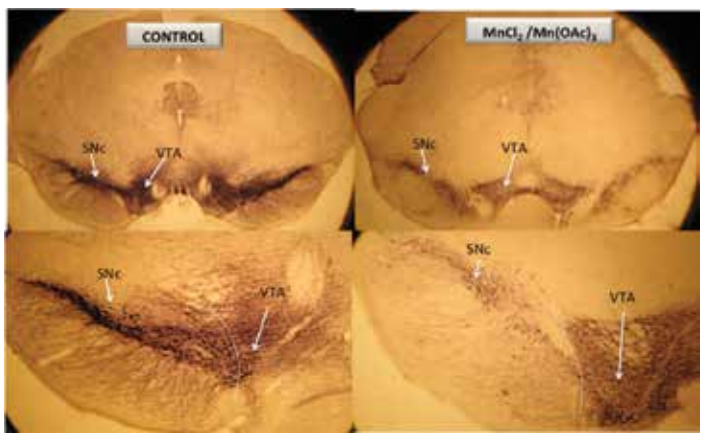

Figure 6. Characteristic TH+ immunostained from coronal sections comprising the SN and VTA of control and Mn-exposed animals showing the ROI which demonstrates the SNc area used for cell calculating. Note that the VTA contains many TH+ cells with no differences among groups and the SNc pronounced cell loss after Mn exposure (upper panel 4×, lower panel 10,000×).

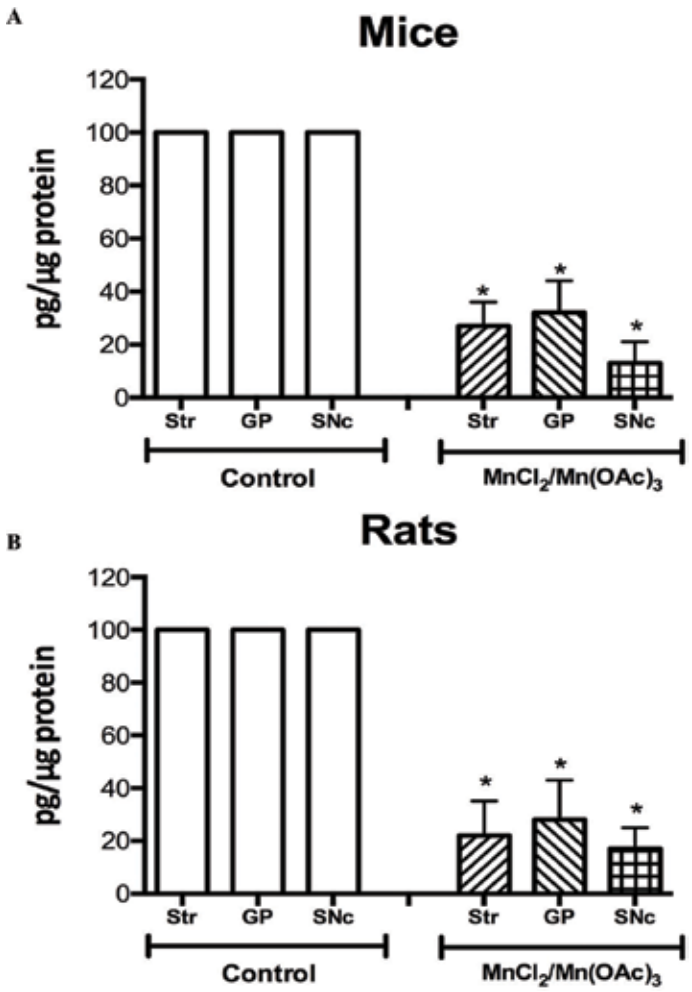

Figure 7. The decrease in dopamine concentrations in the striatum (str), GP, and SNc after 5 months (mice A) or 6 months (rats B) of Mn inhalation compared to controls. Contents are expressed as percentages, which were in pg/g of protein (*$p < 0.001$ vs. control group by one-way ANOVA with post hoc comparisons).

3.5. Dopamine concentrations

Figure 7 shows the change in DA content determined in the striatum (Str), GP, and SNc after 5 months (mice) or after 6 months (rats) of Mn inhalation compared to controls. The average content in the control mice was 96.545 ± 4.8820 and 28.008 ± 12.4500 pg/µg of protein for Mn-exposed mice; hence, DA content declines 71 and 76% for the rat's striatum.

4. Discussion

This research studied the fact that $MnCl_2$ mixed with $Mn(OAc)_3$ induces synergistic consequences by affecting the dopaminergic system reducing TH+ cell number in the SNc but not

in the VTA and reducing DA striatal, GP, and SNc levels, in both mice and rats. We found significant hyperactivity after the first weeks (2–8 inhalations) in mice and, afterward, evident reduction and alterations in locomotor activity; the motor changes improve drastically after L-DOPA treatment in both species. However, rats display different vulnerability to $MnCl_2$/$Mn(OAc)_3$ inhalation as they inhaled three times a week for 6 months. Nevertheless, regardless of the modified procedure, both species display notorious changes in motor behavior and a significant decrease in TH^+ cells in the SNc but not in VTA. Moreover, neither of the two species displayed neuronal death neither in the striatum nor the GP.

4.1. Motor performance alterations

4.1.1. Single-pellet reaching task

It has been demonstrated that skilled limb movements, such as the reach to grasp, display very similar motor components in humans and rodents [57, 58]. PD patients are often described as having poor manual skills that worsen as the disease progresses [59, 60]. These patients experience difficulties performing tasks requiring unilateral and bilateral arm movements and sequential and alternating limb movements [58]. In our results, mice and rats took the food from the ledge without raising the forelimb and either place the mouth into the gap to recover the food pellet with the tongue or turn their body and chase the food with the mouth. Those changes could comprise impairments to basal ganglia structures responsible for grasping movements [61]. Our results thus demonstrate that Mn-exposed animals have impairment in their success in retrieving food pellets probably due to dopaminergic cell loss.

4.1.2. Beam-walking test

Both rats and mice showed extremity coordination disturbances, step length, and motor performance. With longer inhalation times, the Mn-exposed groups display more trouble for climbing the wooden beam. The motor alterations observed here are similar with published results in which C57-treated MPTP exhibited impairments in limb coordination, step length, and motor performance after 2 weeks [62].

Qualitative examination showed that the groups which inhaled Mn mixture displayed postural instability, akinesia, hind-limb weakness, prolonged freezing behavior, and action tremor. According to this, Autissier et al. [9] described that subchronically orally exposed to Mn mice exhibited akinesia; this alteration was related with low striatal DA levels; Eriksson and coworkers [25] reported that 5 months after Mn exposure the animals developed akinesia, action tremor, and unsteady gait. The exposed animals lacked strength in lower and upper limbs, and the limb movements were uncoordinated. Furthermore, the stereotaxic injection of Mn^{3+} into the rat SNc altered the rearing behavior and the spontaneous activity [63, 64].

4.2. Immunocytochemistry

Rats and mice exposed to Mn showed severe loss of SNc TH-immunopositive cells, but not in VTA, GP, or striatum. Our results disagree with other reports which found no loss of dopaminergic neurons [11, 18, 19, 32, 65, 66] and loss of striatal and GP cells [15, 17, 19]. The disagreements concerning our results and the conclusions that describe no TH+ SNc cell death and GP

and striatal cell loss after Mn exposure might be due to at least three causes; first, the mixture of two Mn compounds, which, by far, no report includes such mixture of Mn compounds. Agreeing to Aschner [67], it appears that the Mn toxicity degree is about its oxidation state. As we mentioned above, divalent Mn might be oxidized to trivalent Mn by the superoxide anion [40], and because the electron transport chain in the mitochondria is recognized as the major superoxide producer in the cells, it is understood that the alterations induced by Mn are linked to its oxidation state [40]. It has been proposed that Mn^{3+} is more effective in producing cell damage [68] and Mn^{2+} needs the presence of Mn^{3+} to reach oxidation. Thus it seems that there is synergy between the two Mn states [43]. It also has been said that the brain is an important target of attack for transition metal ions, such as Mn, due to its abundant catecholamine concentration and the rapid oxidative metabolism catalyzed by these metals [69]. In this regard, it has been hypothesized that Mn interacts with catechols specific to dopaminergic neurons to rapidly deplete them and render such cells no longer viable [33, 40]. Thus, it is conceivable that Mn-induced DA oxidation results in the generation of reactive oxygen species, oxidative stress, and secondary cytotoxicity to dopaminergic neurons [40, 70, 71]. Numerous explanations have been proposed to clarify the vulnerability of dopaminergic cells to Mn, such as the lack of cellular antioxidant defenses by the accumulation of the metal [72] and the disruption of mitochondrial oxidative energy metabolism [73]. Second, the concentration of Mn obtained in the inhalation box (2676 mg/m^3) and the time of exposure (5 or 6 mo) are sufficient to produce motor and cell alterations. It has been suggested that Mn toxicity results, most often, from the chronic exposure to very high Mn dosage (>1 mg/m^3) [7] and after long-term exposure [23]. Third, apparently the exposure method determines the delivery of Mn to the brain [74, 75]. Roels et al. [75] explored Mn levels in rat brains after exposing them to either to $MnCl_2$ or MnO_2. These compounds were given intratracheally (inhalation) or intragastrically (oral). This study proposed was to achieve comparable Mn concentrations in the blood and to reach for low oral absorption of Mn vs. the higher rate of absorption from the lung. When the exposition was 1.22 mg $MnCl_2$/kg intratracheally once a week for 4 weeks, there was an increase in blood Mn concentration (68%), which also results in augmented Mn concentrations in the striatum (205%) and cortex (48%) when compared to control group. Oral $MnCl_2$ administration (24.3 mg $MnCl_2$/kg once weekly for 4 weeks) produced about the same blood Mn concentration (68% increase comparing to controls) as intratracheal Mn administration in the same form, but they did not find significant Mn increase in the striatum or cerebral cortex (22% increase versus controls). Therefore, inhaled Mn delivery seems to be more efficient than oral administration in increasing brain Mn levels.

Moreover, it is relevant to indicate that, while Mn exposure provoked important SNc dopaminergic cell death, it appears that the VTA dopaminergic cells are not affected. We do not have the facts yet to demonstrate whether this indicates Mn selectivity for the SNc dopaminergic neurons and not for the VTA cells. Nevertheless, it has been proposed that Mn gets into the neurons via the dopamine transporter (DAT) [76, 77] as in the case of some neurotoxins such as MPTP [78], 6-OHDA [79], Maneb, and Paraquat [26], where SNc cells are more vulnerable than VTA cells. It appears that SNc neurons and VTA exhibit different biochemistry, topography, and susceptibility to pathological processes [81], VTA has lesser DAT levels than the SNc [78, 80, 81]. Therefore it is conceivable that Mn gets into SNc cells via the significant volumes of DAT located in these neurons. Nevertheless, further research is required to settle this fact.

4.3. Dopamine concentrations

Several studies have shown that Mn accumulates in the basal ganglia, particularly in the GP, the NE, and the SNc which cause neurodegeneration; Mn chronic exposure can induce similar changes to those observed in PD [82]. Patients with this disease present rigidity, tremor, akinesia, and postural changes. These signs reflect the SNc dopaminergic neuronal loss [83]. In this disease, there is a threshold; the motor symptoms appear when DA depletion in the striatum is about 80%, and about 60% of SNc dopaminergic neurons are lost [84]. These results are consistent with our data, which show that after $MnCl_2/Mn(OAc)_3$ mixture inhalation, the number of TH-positive SNc neurons decreases to 63% (in mice) and 75% (in rats) and DA content decreases in the studied nuclei, which could explain the motor disturbances observed in the behavioral assessments. Thus, the significant reduction in the quantity of SNc TH+ neurons after $MnCl_2/Mn(OAc)_3$ exposure and the decrease of striatal DA concentrations described here explains the evident DA reduction and the parkinsonian symptoms. Therefore, we assume that the motor alterations are exclusively due to dopaminergic changes because L-DOPA was able to reverse those motor disturbances.

Some authors described that Mn-induced damage includes the GP [17, 19]; nevertheless, with our data, we can guarantee that the $MnCl_2/Mn(OAc)_3$ mixture inhalation also compromises the dopaminergic nigrostriatal pathway. With our results, we prove that L-DOPA treatment significantly recovers the motor performance alterations observed after Mn inhalation, implying that this motor change origin is dopaminergic. Furthermore, the alterations produced by the inhalation of Mn mixture compounds were sufficiently extensive to cause motor deficits such as tremor, rigidity, postural instability, and akinesia. And unlike the complete DA denervation produced by some neurotoxins such as 6-OHDA, which is the most frequently used model, the inhalation of $MnCl_2/Mn(OAc)_3$ leaves a considerable portion of the nigrostriatal projection unharmed. As in early and middle stages of PD, the presence of an intact, functioning sub-portion of the nigrostriatal system could allow L-DOPA treatment to be effective.

4.4. Differences among species

It is well established that different vulnerability to neurotoxins occur among species. So, the best PD experimental model MPTP, in rats, is not actuality used, and the implications of the data obtained from this model are debatable [85, 86]. Rats injected with MPTP doses comparable to those employed in mice do not show any significant dopaminergic neurodegeneration [86, 87]. Only injections of much higher doses of MPTP (multiple applications of 30–60 mg/kg body weight) cause significant dopaminergic cell loss in rats [88]. Remarkably, these rats must be therapeutically pretreated, with guanethidine, to prevent peripheral catecholamine release and extensive mortality [86]. These findings indicate that rats are somewhat insensitive to MPTP. Consequently, rats are not recommended for MPTP research, since rats fail to develop parkinsonian characteristics, as those observed, e.g., for monkeys and mice [89]. The apparent insensitivity of rats to MPTP toxicity may be related to a species-specific metabolism of MPTP and sequestration of MPP+, which could be different in rats compared to mice and monkeys [89]. And despite that MPTP in nonhuman primates and mice provokes a well animal PD model, a spontaneous recovery of parkinsonian

symptoms has been described in both monkeys [90] and mice [91] after MPTP administration, which causes concern to use this model for an assessment of long-term therapeutic effects. However, it has been reported that chronic administration of low doses of MPTP to macaques reproduces all the signs of PD and closely imitates the progressive nature [92]. Nonetheless, rodents are most commonly used over nonhuman primates since rodent models have the advantage that rats and mice are widely available. They have high reproductive rates and require reduced living space, simple feeding, and drinking schedules and low costs [93]. Moreover, because of the economic, logistic, and ethical constraints that are related to experimental research in primates, primate models of PD are used in relatively few laboratories worldwide [94].

Furthermore, 6-OHDA model has been extensively used in rats; only scarce studies using mice with 6-OHDA lesions have been published. In these studies, 6-OHDA was injected mainly either intrastriatally [95, 96] or intraventricularly, and the mice were subjected to relatively slight behavioral assessment [97]. Furthermore, Cenci and Lundblad [98] performed the stereotactic unilateral 6-OHDA injection in rats and mice and then treated them with L-DOPA and reported abnormal involuntary movements (AIMs); these researchers indicated that while rat and mice AIMs can be evaluated with the same parameters, there are important differences among the two species. Mice motor behavior is less articulate and faster than rats. It is, therefore, more challenging to determine mice normal and abnormal movements with 6-OHDA model. Additionally, Iancu et al. [99] stereotactically lesioned mice SNc; they got 53 well-lesioned animals out of 110 lesioned. The small amount of well-lesioned mice is probably due to the SNc size since in mice it is extremely small. The slight variances in the inhalation procedure between species that we found here are likely because rat Mn absorption is a fast saturable process probably mediated by a high-affinity system [100]. Consequently, the rats, although with the same Mn concentrations, required more inhalations per week for 6 months instead of 5. However, both species, cytological and behavioral alterations, were very similar.

5. Conclusion

Contrasting to MPTP and 6-OHDA PD models, where the alterations occur in a range of days or weeks, while PD in humans develops over decades [90], our PD experimental model induced by Mn inhalation seems to be a suitable model because the dopaminergic cell degeneration is bilateral and progressive and the variances among species are minimum.

It has been established [88] that an acceptable PD experimental model must have these features: (1) an average number of SNc dopaminergic cells at birth followed by a gradual selective loss of these cells in adulthood; (2) merely demonstrable and measurable motor alterations; (3) the model should be established at reasonably short time course to replicate the PD pathogenesis (about 3–6 months), which would allow for therapeutic substances and strategies assessment; and (4) Lewy bodies must be present. Hence, with our Mn inhalation model, we produce three of those characteristics. Nevertheless, further studies are needed to clarify if Mn exposure generates Lewy bodies and determine if the animals recover after the inhalation period.

Finally, the results from this research provided essential contributions toward a better understanding of the mechanisms involved in nigrostriatal degeneration in PD because it is highly feasible and adequately simulates the neuroanatomical, neurochemical, and some of the PD behavioral characteristics.

In brief, the results of this research suggest that the motor alterations induced by the inhalation of the combination of $MnCl_2/Mn(OAc)_3$ are related to nigrostriatal dopaminergic function, providing new light for the understanding of Mn neurotoxicity as an adequate PD experimental model.

Acknowledgements

This work was supported by the research grants from PAPIIT-DGAPA–UNAM PAPIIT-DGAPA IN215114, IN219617, and PAPCA-Iztacala UNAM 2016-2113. The authors thank Veronica Rodríguez Mata for her excellent photographic and technical assistance.

Conflict of interest

Authors declare that there is no conflict of interest.

Author details

Maria Rosa Avila-Costa[1]*, Ana Luisa Gutierrez-Valdez[1], Veronica Anaya-Martínez[1], José Luis Ordoñez-Librado[1], Javier Sanchez-Betancourt[1], Enrique Montiel-Flores[1], Patricia Aley-Medina[1], Leonardo Reynoso-Erazo[2], Jesús Espinosa-Villanueva[1], Rocío Tron-Alvarez[2] and Vianey Rodríguez-Lara[3]

*Address all correspondence to: nigraizo@unam.mx

1 Neuromorphology Lab, National Autonomous University of Mexico (UNAM), Tlalnepantla, Edo. Mex., Mexico

2 National Autonomous University of Mexico (UNAM), Health Education Project, Tlalnepantla, Edo. Mex., Mexico

3 Department of Cell Biology, Facultad de Medicina, Nacional University of Mexico (UNAM), Mexico City, Mexico

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Chapter 5

Physiology and Metabolic Anomalies of Dopamine in Horses: A Review

Katy Satué Ambrojo, Juan Carlos Gardon Poggi and
María Marcilla Corzano

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http://dx.doi.org/10.5772/intechopen.78569

Abstract

Dopamine (DA) is an important endogenous catecholamine that exerts generalized effects on both neuronal (as a neurotransmitter) and non-neuronal tissues (as an autocrine or paracrine agent). In the central nervous system (CNS), DA binds to specific membrane receptors present in neurons and plays a key role in the control of motor activity, learning, cognition, affectivity and attention. Horses can also present with hyper- and hypodopaminergic conditions, including stereotypic behaviors and pituitary pars intermedia dysfunction and Parkinsonian's syndrome, respectively. DA biosynthesis also occurs in peripheral tissues, and receptors in various organs such as the kidney, pancreas, lungs and blood vessels outside the CNS have been detected. DA emulates the actions related to the sympathetic nervous system (SNS), promoting the increase in heart rate, blood pressure, electrolyte balance and gastrointestinal (GI) motility. In fact, GI alterations in dopaminergic transmission have been directly or indirectly related to hypomotility and/or postoperative ileus (POI). On the other hand, there are physiological factors, such as breed, age, exercise and reproductive status that modify DA concentrations. In reproduction, the administration of DA antagonists in the middle/end of the spring and anestrus transition period advances the first ovulation of the year in mares. This chapter offers a brief description of the importance of DA as a neurotransmitter and peripheral hormone. Special attention is paid to: (1) functional alterations that occur in the brain and GI tract in various diseases and (2) current therapy to correct alterations in DA systems.

Keywords: dopamine, equine medicine, reproduction

1. Introduction

1.1. Biosynthesis, regulation, inactivation and degradation

Dopamine (DA) is synthesized in dopaminergic nerve terminals from the amino acid tyrosine. The majority of circulating tyrosine originates from dietary sources, but small amounts are derived from hydroxylation of phenylalanine by the liver enzyme phenylalanine hydroxylase. Hydrolysis of tyrosine to L-3,4 dihydroxyphenylalanine (L-DOPA) by the enzyme tyrosine hydroxylase (TH) and its subsequent decarboxylation by the enzyme DA decarboxylate leads to the formation of DA. The activity of TH is mainly controlled by the central nervous system (CNS) and the metabolic products of neurotransmitter synthesis (L-DOPA and DA) inhibit TH activity in brain tissue and minority by the catecholamines (serotonin, 5-HT), which act as regulatory factors through feedback mechanism [1, 2].

Although DA can be found in very different nerve pathways, there are four main dopaminergic nerve pathways that govern the synthesis and transmission of this catecholamine [3]:

- Mesolimbic (amygdala, hippocampus and prefrontal cortex). This pathway transmits DA from the ventral tegmental area (VTA) to the accumbens nucleus. VTA is located in the midbrain, while the accumulated nucleus is located in the limbic system.
- Mesocortical. This pathway transmits DA from VTA to the frontal and cerebral cortex.
- Nigrostriatal. This pathway transmits DA from the substantia nigra to the basal ganglia, specifically the striated nucleus. It is a neuronal pathway associated with motor control.
- Tuberoinfundibular. This pathway transmits DA from the middle hypothalamus to the infundibular region. The latter area connects different parts of the hypothalamus and the pituitary gland. It also controls the secretion of certain hormones, including prolactin (PRL) from the anterior pituitary gland. In the dopaminergic terminals the neurotransmitter is synthesized in the cytoplasm from where it can be released directly into the synaptic space or transported into the synaptic vesicles to be released by exocytosis. Once released into the synaptic space, the DA binds to the pre and post synaptic receptors.

Systemic DA is mainly derived from sympathetic nerve fibers, chromafine cells of the adrenal medulla, the gastrointestinal (GI) tract and neuroendocrine cells known as APUD (acronym for "amine precursor uptake and decarboxylation") [4]. These cell types are found in the kidney, pancreas, retina and peripheral leukocytes, among others, which are characterized by the synthesis of peptide hormones and amines with auto/paracrine functions [4–6]. It should be noted that some of these cells, such as those of the renal tubular epithelium, do not express the enzyme TH. Therefore, the synthesis of DA depends directly on the availability of L-DOPA and its transport into the cell, which increases in the presence of sodium [7]. In addition, the carotid body, an important peripheral chemo-receptor, releases DA under hypoxic conditions [8].

The dopaminergic receptors are grouped into two main families: D-1 and D-2. The first group, which includes subtypes D_1 and D_5, stimulate the activity of the adenylate cyclase enzyme and activate the protein kinase. The second group composed of subtypes D_2, D_3 and D_4 inhibits the

activity of this enzyme and alters the permeability of potassium channels [9]. In addition, when DA is present in high concentrations, it can act on adrenergic and serotoninergic receptors [7]. At the central level, D_1 receptors are widely expressed in the nigrostriatal, mesolimbic and mesocortical areas. While D_2 receptors are expressed in the stratum, black substance, hippocampus and hypothalamus, among others, highlighting their high concentration in the pituitary gland. In contrast, D_5, D_3 and D_4 receptors have lower levels, although they are also found in multiple brain regions. In general, at the peripheral level, DA receptors are found in the kidney, adrenal glands, sympathetic nodes, GI tract, blood vessels and heart [10]. Activation of the D_1 receptor causes dilation of the renal vasculature, heart, mesentery and brain, while the D_2 receptors inhibit secretion of aldosterone, PRL and renin. In addition, D_1 and D_2 receptors have been described in the ovarian cortex and corpus luteum (CL) and, to a lesser extent, in granulosa and teak cells [11] in the mare.

The degradation of the DA takes place in two phases. First, the enzyme monoamine oxidase (MAO) catalyzes its deamination, forming 3,4-dihydroxyphenylacetaldehyde (DOPAL). This aldehyde can be metabolized by aldehyde dehydrogenase to 3-4-dihydroxyphenylacetic acid (DOPAC) or by aldehyde reductase to 3,4-hydroxyphenylethanol (DOPET), resulting in its acid or alcoholic metabolite respectively. In addition, DOPAC can be inactivated by the enzyme catechol-O-methyltransferase (COMPT) which generates homovalinic acid (HVA). Both DA and its metabolites can be conjugated before urinary excretion by sulphation and glucuridation reactions [9].

1.2. Cellular effects of dopamine

1.2.1. Central nervous system and behavior

Dopaminergic neurons regulate important functions such as cognition, motor activity, vision, learning, pain perception, and sexual behavior, among others [4, 10, 12]. Several studies on horses have linked behavioral changes to changes in the central levels of DA. In fact, high concentrations of DA are associated with stereotypes such as shooting and bear dancing [3, 13], while decreased dopaminergic activity is accompanied by depression, lethargy and apathy [14]. In addition, there are racial variations in the expression of the dopaminergic D_4 receptor. This suggests their involvement in behavioral differences associated with the breed, such as alertness or curiosity [15].

On the other hand, DA controls circadian rhythms through the transport of light information in the retina and the synthesis of melatonin [4, 16]. In fact, DA can modify the synthesis of melatonin in the pineal gland by modulating the availability of 5-HT through its binding to DA-adrenergic receptors, D_4-α1 and D_4-β1 [17].

1.2.2. Endocrine system

As mentioned earlier, DA is a potent inhibitor of PRL secretion. In the presence of DA, the secretion of PRL is minimal. While when DA is absent, the rates of PRL secretion are high. PRL has self-regulating feedback on tuberous-infundibular DA neurons. Increased PRL concentrations due to lack of stimulation of the DA receptors in the lactotroposes cause a self-regulating feedback loop to the tuberous-infundibular DA neurons. These cells are activated to produce more DA, resulting in a reduction in prolactin secretion [18]. Melanotrophs of the pituitary

intermedia pars are innervated by periventricular hypothalamic periventricular dompaminergic neurons. The release of the neurotransmitter DA from these neurons causes tonic inhibition of the release of hormones from the surrounding melanotrophs. After release, DA binds to D_2 receptors in melanotropes that inhibit transcription of pro-opiomelanocortin (POMC) peptides, including adrenocorticotropic hormone (ACTH), α-melanocyte-stimulating hormone (α-MSH), and corticotrophin-like intermediate peptide (CLIP). Basic knowledge of the dopaminergic system is important to understand the pathogenesis and treatment of equine pituitary pars intermedia dysfunction (PPID). In fact, in horses with PPID, loss of inhibitory control of DA allows the cells of the intermediate pars to proliferate and produce and release higher levels of POMC protein derivatives [19].

1.2.3. Gastrointestinal system

DA plays an important role in the control of GI motility in horses. The agonist and antagonist receptors produce inhibitory (relaxation or inhibition of contractions) or excitatory (increased contractions, less frequently) effects on GI motility [20]. These effects are due to the fact that the D_1 receptor is mainly located in the effector cells (post-junctional) and the D_2 receptor is present both pre- and postjunctionally [21].

1.2.4. Renal function

The cells of the proximal tubule are the main source of DA synthesis, exerting natriuresis due to increased renal perfusion mediated by arteriolar vasodilatation and inhibition of tubular sodium reabsorption through the enzyme *sodium-potassium* adenosine triphosphatase (Na^+/K^+-ATPase) [22, 23]. For this reason, DA and its agonists are considered potential therapies for the treatment of renal hypotension, tubular obstruction, as they favor natriuresis and diuresis in horses. Although exogenous administration of DA does not significantly modify the fractionated excretion of sodium and potassium, it increases urine volume and decreases osmolarity [24]. In newborns, low-dose phenoldopam mesylate (D_1 agonist) increases urine output without causing systemic hemodynamic changes [25]. Additionally, stimulation of the D_1 and D_2 receptors promotes renin secretion and inhibits aldosterone. The administration of DA agonists such as bromocriptine inhibits the stimulation exerted by angiotensin II in Na^+/K^+-ATPase [26].

1.2.5. Cardiovascular system

Circulating DA, synthesized by the endothelial cells, alters the muscular contractility of the blood vessels. Thus, there is a negative correlation between this neurotransmitter and blood pressure [4]. However, exogenous administration of DA in horses has variable and dose-dependent effects depending on the general condition of the patient. Thus, infusion of high doses of DA increases blood pressure with an increased risk of arrhythmias [26]. However, there are no modifications at low doses (≤3 μg μg/kg/min) [24]. Under certain shock conditions, treatment with DA may increase blood pressure [27]. In fact, Trim et al. [28] demonstrated that infusion of DA in surgically operated endotoxic animals significantly improves

System	Functions	References
Central nervous	Motor control and movement (nigrostriatral pathway)	[4, 10, 12, 14, 16, 17]
	Behavioral effects: alertness, curiosity, cognition, learning and memory (mesolimbic pathway)	
	Modulation of circadian rhythms	
Endocrine	Mare:	[18, 29–32]
	• Modulation of reproductive seasonality (inhibition of pituitary PRL secretion - tonic inhibition on reproductive activity during seasonal anoestrus-tuberoinfundibular pathway)	[33, 34]
	• Regulation of luteal function	
	Stallion:	
	• Modulation of sperm viability, acrosomal integrity, capacitation and motility	
Gastrointestinal	Regulation of GI motility:	[20, 21]
	• Inhibitory effects (relaxation or inhibition of contractions)	
	• Excitatory effects (increased contractions, observed less frequently)	
Renal function	Increased renal perfusion and arteriolar vasodilatation	[22, 23]
	Inhibition of tubular sodium reabsorption (natriuresis)	[25, 26]
	Increase renin and inhibition aldosterone secretion	
Cardiovascular	Dose-dependent effects:	[26–28]
	High doses increases blood pressure and cardiac output	[24]
	Low doses: no modifications	
	Vasodilation (relaxation)	

Table 1. General functions of DA in the horse.

cardiovascular function. These studies have shown an increase in cardiac output and blood pressure. **Table 1** summarizes the main functions of DA in the horse.

2. Physiological modifications of dopamine in the horse

2.1. Age

Similar to PPID animals, aging decreases the concentrations of DA in the spinal cord of adult mares compared to pre-pubertal females [35]. In fact, the activity of the dopaminergic and serotonergic systems is reduced in these animals, decreasing plasma concentrations of DA and 5-HT [36, 37]. McFarlane et al. [14] verified that the number of dopaminergic nerve terminals in the periventricular intermediate peripheries and in the cell bodies associated with the hypothalamus is reduced in animals with PPID compared to healthy animals of the same age.

2.2. Breed

Podolak et al. [38] showed that Arabian horses have higher concentrations of DA at rest and after exercise compared to thoroughbred horses. In horses, the D_4 DA receptor gene (DRD_4) is found on chromosome 12, and two types of polymorphisms have been found. They are variable number of tandem repeats (VNTRs) consisting of 18 base pairs (six amino acids) and some single nucleotide polymorphisms (SNPs) in the exon region 3. One of these SNPs, G292A, was reported to be associated with horse personality. The *A* allele in G292A is associated with low curiosity and high vigilance in thoroughbred horses. A previous study reported that a Kiso horse, a native Japanese horse breed, had shorter repetitions in the VNTR region than thoroughbred horses [39]. However, it has not yet been verified whether the allele frequency of this polymorphism differs between races. However, samples from more breeds are needed to validate the differences in DRD4 in horses of different breeds.

2.3. Transport

In stallions, Medica et al. [40] observed an increase in plasma DA after 100 km transport, with a decrease after 300 km. This response is related to the process of adaptation during transport. This is due to the fact that neurotransmitters are necessary for maintaining the homeostatic process and balancing the effects of perceived stress during transport.

2.4. Seasonality

The concentrations of DA also vary with the season. In normal mares, the concentrations of DA in the cerebrospinal fluid are minimal in summer, medium in autumn and winter and maximum in winter anestrus [41]. However, this pattern is not maintained in ovariectomized females, suggesting the influence of gonads on dopaminergic seasonality [35]. Nevertheless, Haritou et al. [36] showed a decrease in plasma levels during the spring and early fall months in horses with PPID.

3. Clinical implications of domanine in horses

3.1. Hypothalamic: pituitary dysfunction

As noted earlier, knowledge of the dopaminergic system is important for understanding the pathogenesis and treatment of fescue equine toxicosis and PPID [19]. In mares grazing on land rich in *Acremonium coenophialum*, an endophytic fungus that grows on the stem, leaves, pods and seeds of the fescue, the alkaloid ergopeptine and, mainly, the ergovaline, appear to be responsible for most of the abnormalities associated with toxicosis in pregnant mares. The symptoms that characterize the clinical picture include, among others, an increase in gestation duration, abortion, birth of weak or dead foals, agalactia, thickening and retention of the placenta, and infertility [42, 43]. Because AD is the main inhibitor of PRL secretion, agalactia occurs first, because of the agonistic effect of ergopeptine on D_2 DA receptors. Second, ergoalkaloids inhibit ACTH secretion, reducing fetal cortisol, thereby reducing placental

progesterone (P_4) secretion. Third, these alkaloids reduce the binding of estrogens to tissues, raising serum estradiol levels. It is important to note that blood estrogen levels usually drop around parturition. Interaction between PRL, P_4 and estrogens plays an important role in preparing the mammary gland for lactation. Low levels of PRL and P_4 and high levels of estrogen induce agalactia prevent the development of the mammary gland. The gestation period may be prolonged due to blockage of the fetal corticotropin-releasing hormone (CRH) by ergopeptine. This fact causes the lack of production of ACTH and fetal cortisol and the prolongation of gestation in the affected mares. It is also hypothesized that placental anomalies are associated with vasoconstriction, edema, fibrosis and mucoid degeneration of the placental arteries secondary to anoxia [43]. The administration of bromocriptine (D_2 dopaminergic agonist) in ponies at the end of pregnancy leads to a decrease in plasma concentrations of PRL and P_4 and induces clinical signs similar to this type of poisoning [42]. In addition, exogenous DA receptor antagonists (domperidone and metoclopromine; D_2 receptor antagonists, sulpiride; D_2 and D_3 receptor antagonists, and phenothiazines; DA) receptor antagonists during the last 30 days of gestation may reverse the inhibitory effects of DA by increasing PRL secretion [14, 19], udder development and lactation. After parturiton, the same dose of domperidone, given twice daily for several consecutive days, stimulates milk production [43].

The intermediate pars of the pituitary gland receive direct innervation from the dopaminergic neurons of the periventricular nucleus of the hypothalamus. These axons project through the infundibular stem, travel along the periphery of the nerve pathway and then branch off into the intermediate pars where they end in the peach groves. At this site, the DA released by the periventricular nerve terminals interacts with the dopaminergic D_2 inhibitor receptors causing a decrease in hormone synthesis and release as well as an inhibition of cell division [44]. The lack of inhibition of DA may also result in multiclonal expansion. However, the neuronal cell bodies producing DA in lactotrophes are located in the arcuate nucleus while those of melanotrophs are in the periventricular nucleus. For this reason, a highly specific regional loss of these DA-producing cells may explain the monoclonal expansion of melanotrophs. In fact, there is good evidence that PPID is a neurodegenerative disorder characterized by a lack of dopaminergic input to melanotrophs in the intermediate pars [45].

PPID is one of the most common diseases of horses and ponies over 15 years of age. The pathological features of PPID are hypertrophy, hyperplasia and adenoma formation in the middle pars of the pituitary gland. Horses with PPID develop enlarged pituitary glands up to five times their normal weight. As the middle pars expand, it compresses the adjacent pituitary lobes and hypothalamus. This often results in a loss of periventricular dopaminergic nerve terminals and cellular bodies which decreases the concentrations of DA and DA-metabolite by eight times [14]. However, the intermediate pars remain active, secreting relatively large amounts of POMC derived peptides into the peripheral circulation (40-fold increase), α-MSH, ACTH, β-endorphin, β-lipotropin and CLIP. In fact, increased secretion of ACTH and CLIP results in hyperadrenocorticism [46].

While no initiating events causing neurodegeneration in PPID have been identified, evidence suggests that oxidative damage to dopaminergic neurons occurs in horses with PPID [14]. Oxidative stress results in the modification of cellular components including proteins, DNA

and lipids of the cell membrane due to excessive exposure to exogenous or endogenous sources of free radicals. This damage eventually leads to cell death or, in the case of neurons, to neurodegeneration. Dopaminergic neurons are particularly vulnerable to oxidative damage, since the metabolism of DA itself produces free radicals. Chronic oxidative stress is considered to be a factor in the development of other diseases associated with dopaminergic neurodegeneration, such as Parkinson's disease [47].

By immunohistochemical evaluation of pituitary and hypothalamic tissue, McFarlane et al. [48] showed that the immunoreactivity of TH is reduced in affected horses. This finding supports the role of dopaminergic neurodegeneration in PPID. In addition, immunohistochemical evaluation revealed an increase in the oxidative stress marker, 3-nitrotyrosine and in the nerve end protein, α-synnuclein, located in the intermediate pars of horses with this disease. These authors have also suggested a role for nitration of the overexpressed α-synuclein in the pathogenesis of neurodegeneration in PPID [47].

Loss of hypothalamic dopaminergic innervation appears to be an important mechanism for the development of PPID. For this reason, the use of DA agonists is a logical approach to treatment [47–49]. In fact, pergolide mesylate, in a daily dose (1 mg of PO/day h for 2 months, followed by 2 mg of PO/day for 4 months) is probably appropriate for most horses and ponies. This drug is a first-generation D_2 receptor agonist based on ergolinapara, restores dopaminergic inhibition of melanotrophs and regulates plasma ACTH [48]. A lower initial dose of 0.002 mg/kg body weight (range 0.002–0.01 mg/kg daily body weight) can also be calculated for small ponies or miniature horses. Systemic supplementation of DA or a DA agonist to horses with PPID results in a decrease of POMC peptides in plasma, ACTH and cortisol concentration.

Classic signs of PPID include hirsutism, polyuria/polyidipsia, lethargy, excessive sweating, loss of muscle mass, repeated infections, infertility, and bulging eyes as result of supraorbital fat redistribution [47]. However, insidiously onset chronic laminitis is the most significant clinical complication of PPID in horses (50%). PPID-induced laminitis is due to two factors: (1) alteration of helmet perfusion by excess catecholamines acting directly on vascular smooth muscle (vasoconstriction and limited blood flow) and (2) indirectly by overproduction of circulating cortisol causing insulin resistance. Castro et al. [50] showed that oral administration of domperidone at 1.1 and 5.5 mg/kg increased lamellar microvascular blood flow (LMBF). This effect begins 4 h after administration and the effect persisted for at least 8 h. Intravenous administration of 0.2 mg/kg domperidone increased the LMBF at 10 and 12 h after administration. In horses with laminitis, domperidone may be helpful in preventing vasoconstriction and reduction of LMBF. However, further research into the effects of the drug on horses with laminitis may be needed.

3.2. Behavioral alterations: stereotipies

The neurobiological consequences or regulations of equine stereotypes focus on neurotransmitter systems, specifically the serotoninergic and dopaminergic pathways. Various studies have reported that the DA and reward systems are the underlying mechanisms for the development of stereotypes [51, 52]. Stereotypes can act as a rewarding behavior and help the horse to fight in a suboptimal environment.

The different anatomical regions of the brain, the basal ganglia, have been identified as critical to the performance of stereotypes. Recent studies have focused on the striatum of the basal ganglia, which are related to neurophysiological processes during stereotyped activities. Basal ganglia have been identified as a critical region in relation to the performance of stereotypes [51]. DA is suggested as an activator and modulator of basal ganglia motor programs that reinforce behavior through a reward system. Neurological studies in cradle-biting horses have shown that the subtypes of D_1 and D_2 receptors in the nucleus accumbens were significantly higher and D_1 receptors in the caudate putamen (dorsomedial stratum) were significantly lower [51, 53]. Therefore, increased neural transmission within the striatal region of the basal ganglia appears to be associated with oral stereotypes, including crib biting [51].

Chronic stress resulting from weaning or lack of ability to carry out specific behavioral needs often resulting from living in a domestic environment can result in decreased or increased secretion of deoxyribonucleic acid. This fact develops depression or cradle bite, respectively. Depressed horses have little reaction to stimuli, and they can also fall into a state of learned helplessness. As a result, the horse makes no effort to learn, understand or give natural responses to the stimuli [51].

Because of the links between stress and DA, anxious horses may be more sensitive to environmental stressors. These factors such as restricted feeding or social isolation are common stressors faced by stable horses [54]. In animals kept under the same environmental conditions, behind this increased ability to respond to stress, anxious individuals may have a high striated DA compared to less anxious animals. This may allow the initiation of active coping in an attempt to gain control over the environment, similar to the elevated DA levels seen in the active coping DBA mouse strain [55]. A similar process can occur with anxious horses, as demonstrated by the increased rate of spontaneous blinking in these individuals [13].

From the neurobiological perspective of stereotypes, an alternative hypothesis is based on the activation of the mesoaccumbens pathway by highly motivated events. Highly motivated activity restrictions are known to initiate high dopaminergic transmission of mesoaccumbens. Therefore, the development of stereotypes can occur in environments where goal-directed behaviors are restricted [51]. Pharmacological treatment of these alterations focused especially on the neurotransmitters DA and serotonin, and opioid systems. The use of medications such as tryptophan, naloxone, naltrexone, dextromethorphan, acepromazine maleate, and clomipramine [56, 57] has been reported for the treatment of stereotypic behaviors.

On the other hand, chronic ingestion of yellow star thistle (*Centaurea solstitialis*) or Russian wolf mint (*Acroptilon repens*) causes nigropallidal encephalomalacia (NPE) in horses. Neurological signs are characterized by an abrupt onset of dystonia of the lips and tongue, inability to prehend food, depression and locomotor deficits. The transmission of DA plays an important role in four main pathways: nigrostriatal, mesolimbic, tuberous-infundibular and mesocortical. Lesions located within the substantia nigra pars reticulata, sparing the cell bodies of the dopaminergic neurons in the substantia nigra pars compacta, and in the rostral portion of the globus pallidus, with partial disruption of dopaminergic fibers passing through the globus pallidus. These findings indicate that equine NPE can serve as a large animal model of environmentally acquired toxic Parkinsonism. The clinical phenotype is directly attributable

to lesions in the globus pallidus and substantia nigra pars reticulata rather than to the destruction of dopaminergic neurons [58].

In horses experimentally infected with Westnile virus, central levels of DA are significantly decreased due to imbalance in expression of the enzyme TH and MAO. In these infected animals, TH and MAO decrease and increase, respectively. The decrease in dopaminergic activity, accentuated by the decrease in dopaminergic receptor expression, is associated with the characteristic clinical symptoms of the disease and resembles the motor alterations observed in Parkinson's disease in humans [59].

3.3. Hypomotility gastrointestinal

GI motility abnormalities in horses may be due to different conditions. These include equine herb disease, gastroduodenal ulceration, intraluminal obstruction or retention, excessive wall strain, strangulation obstruction, peritonitis, duodenitis, proximal jejunitis, colitis and postoperative ileus (POI) [60].

DA plays an important role in the control of GI motility in horses. Receptor agonists induce inhibitory (relaxation or inhibition of contractions) and excitatory (increased contractions, less frequently) effects in various portions of the GI tract [20]. These effects are possible since the D_1 receptor is mainly located in the effector cells (postjunctional) and the D_2 receptor is present both pre and postjunctional [21]. However, receptor antagonists affect intestinal motor activity from the stomach to the colon [61]. For the treatment of intestinal hypomotility in horses, prokinetic drugs such as D_1 and D_2 receptor antagonist and domperidone have been used as a competitive antagonist in peripheral D_2 receptors [62–64].

Metoclopramide is an antagonist of CNS and systemic D_2 dopaminergic receptors and blocks the inhibitory effect of DA on GI smooth muscle [65]. In a model of POI in horses, continuous infusion of metoclopramide restored coordinated gastroduodenal activity and GI transit. In a retrospective study also conducted in horses, the clinical use of metoclopramide (continuous infusion at 0.04 mg/kg body weight/h) after small bowel resection and anastomosis was evaluated. Although the horses treated in this study had decreased total volume, duration, and rate of gastric reflux, previously reported side effects were again noted [66]. In another study conducted on horses [67], metoclopramide increased contractility of the smooth muscle strips of the antrum pyloricus, duodenum and jejunum. When used as a pretreatment, metoclopramide has also been shown to improve gastric emptying in horses receiving endotoxin [68].

According to Nieto et al. [67], metoclopramide (0.2 mg/kg PO) improved jejunal motility, but there was no effect on cecal motility [61]. In addition, when evaluating gastric emptying, the researchers found that metoclopramide had less time needed to reach a peak than the control group. This suggests an improving effect of metoclopramide on gastric emptying. Complementary to these results, another *in vitro* study on equine smooth muscle strips derived from the pyloric antrum, proximal duodenum and mid-jejunum showed a significant increase in contractile amplitude of the muscle strips in the three locations, caused by metoclopramide. An interesting finding here is the observation that lower concentrations of the drug were needed in the proximal parts of the GI tract to obtain a response (10^{-9} M in the pyloric antrum compared to 10^{-5} M in the mid-jejunum). This may be because metoclopramide is believed to work by restoring gastroduodenal coordination.

However, due to motility disorders in horses such as POI and colic, the question arises whether metoclopramide can be considered a reliable drug in equine practice. The agent indeed has been found to be effective in cases of both natural and experimentally induced POIs [67, 69]. It has also been successful in the fight against experimentally induced colic [70]. However, the ability to cross the blood-brain barrier and cause serious central side effects should prompt professionals to use this drug with caution in equines. Recommended dosages include 0.125–0.250 mg/kg, diluted in 500 mL of polyionic solution for slow infusion (more than 60 min); 0.05 mg/kg (IM, four times daily); 0.1–0.25 mg/kg (SC, 3 or four times daily) or 5 mg/kg (PO, four times daily) [71].

Domperidone is a dopaminergic D_2 receptor antagonist present in both the center and periphery (including the GI tract) of the neural system [72]. Unlike metoclopramide, which crosses the blood-brain barrier easily, domperidone causes minimal extrapyramidal central side effects. This is because it interacts only slightly with central dopaminergic receptors. A recent study used oral administration of the drug at 1.1 and 5.5 mg/kg both in vivo and in vitro, the influence of domperidone therapy on gastric emptying, and motility of the intestinal tract in horses [64]. However, no effect was detected on the rate of gastric emptying at a dose of 1.1 mg/kg PO, which was previously effective in the treatment of fescue toxicosis in pregnant mares [73].

On the other hand, the higher dose of 5.5 mg/kg PO significantly increased the area under curve (AUC) and maximum concentrations (Cmax) in the acetaminophen test. Both test parameters have been postulated to increase gastric emptying. *In vitro* assembly of the same study showed no effect on the contractile response of the longitudinal and circular smooth muscle strips obtained from the duodenum, jejunum, ileum and equine colon (pelvic flexure) [50]. In addition, domperidone was found to decrease the contractile activity induced by DA of smooth muscle strips in the mid-jejunum. Therefore, more research is needed to elucidate the potential beneficial effects of domperidone *in vivo*, as well as to obtain more knowledge about its pharmacokinetic properties.

On the other hand, equine ileocolonic aganglionosis, also called lethal white colt syndrome (LWFS), is a severe congenital condition characterized by failed colonization of the neural crest in the caudal part of the small intestine and the entire large intestine. The LWFS, which is attributable to a mutation in the endothelin B receptor gene, results in intestinal akinesia and due reduction of colic in enteric neurons [74]. This evidence highlights the involvement of the dopaminergic system in the control of GI motility.

In addition, in horses under anesthesia with isoflurane, Dancker et al. [75] showed that DA increased cardiac output but decreased blood flow in the colon, as well as systemic vascular resistance and mean blood pressure compared to baseline values.

4. Role of dopamine in equine reproduction

4.1. Neuroendocrine basis

It is generally accepted that the primary controllers of gonadal function are various endocrine/paracrine mechanisms including the hypothalamic-pituitary-gonadal axis. However, there is evidence of additional components that control gonadal function. These additional elements

involve autonomic neuronal (catecholaminergic) activity and, possibly, endocrine-like effects produced by neurotransmitter chemicals secreted by non-neural ovarian cells [76, 77].

Earlier studies (reviewed in [78]) showed that decreased photoperiod during the fall and winter suppresses gonadotropin-releasing hormone (GnRH) secretion. This effect is mediated by changes in melatonin secretion from the pineal gland. Low levels of GnRH reduce gonadotropin secretion which in turn leads to reduced follicular growth and anovulation. The increase of the photoperiod during the spring induces a gradual increase of the hypothalamus-pituitary axis that allows the initiation of follicular growth and eventually ovulation [79].

Like other females with seasonal reproduction and relatively long gestation periods, the environmental control of reproduction in the mare is mainly through the photoperiod [80]. In addition to the photoperiod, environmental factors and associated neural pathways are involved in the neuroendocrine control of the mare's reproductive seasonality [81]. Therefore, the functions of several classical neurotransmitters, including opioid peptides, catecholamines and neuro-exciting amino acids and their relationship to their potential functions in regulating seasonal reproduction in mares, have been examined [81].

Substances released by catecholaminergic nerves can directly influence through their interaction with a variety of receptors in target tissue cells. They can also be internalized and converted to other catecholamines within the tissue to exert their action on other cells [77].

In the 1990s, some studies have reported on the role of DA in the control of reproduction in mares or stallions [82, 83]. In the mare, the concentration of DA in the CSF fluid is lower during the period of reproductive activity compared to the anovulatory period. In addition, it appears to be inversely correlated with plasma concentrations of luteinizing hormone (LH) [82]. Beyond seasonal variations, the concentrations of DA in the CSF appear to depend on the presence of gonads. Although ovariectomized mares show a seasonal variation in the secretion of LH, these females do not express a seasonal variation in the concentrations of DA in the CSF. This suggests a functional relationship between DA secretion and ovarian function [35].

On the other hand, there is also a regulatory function on seasonality due to the presence of synapses between dopaminergic and GnRH neurons in the median of pituitary eminence. In addition, suppression of these receptors increased LH during the anestrus period [84].

DA has been identified in the ovaries of laboratory experimental animals [85, 86], cows [87], sows [88] and mares [77]. The actions of DA are mediated through specific receptors found in the cell membrane. Five different DA receptors have been identified [84, 89], which according to their physiological, pharmacological and biochemical properties have been grouped into two general families: D_1 and D_2 [90]. Therefore, even within the same ovary, different functions can be controlled by the DA depending on whether it binds to D_1 or D_2 type receptors. Both types of receptors have been reported in ovarian tissues in mares [77].

It has been suggested that DA may act through the D_2 receptor to inhibit follicular growth [84]. This theory is based on the fact that dopaminergic antagonists such as sulpiride and domperidone have a positive effect on follicular growth in anovulatory mares. Also, in the fact that treatment with these antagonists does not increase FSH secretion in this type of

mares [29, 41, 84, 91]. These findings are supported by the observation that cortical samples appear to have a higher incidence of D_2 receptor mRNA compared to D_1 receptor mRNA [77].

DA is also present in equine follicular fluid. Higher concentrations of DA have been found in small follicles (<25 mm in diameter) compared to medium and large follicles. This suggests a role in early follicular recruitment. It has been reported that in mares during the breeding season, the mRNA of the DA D_1 and D_2 receptors is present in the germinal epithelium of the ovarian cortex and in some granular tissues of the antral follicles [91]. It was also hypothesized that the direct dopaminergic contribution in the ovary may affect follicular growth. It has also been described that the effects of DA on mare follicular growth can be mediated through the regulation of *follicle-stimulating hormone* (FSH) secretion. It has been observed that neither the amount of the message changes during seasonal fluctuations in the ovarian activity of the mare [91].

Like DA, PRL has also been shown to play a role in the ovarian function of the mare. DA acts to influence follicular dynamics by indirectly affecting the ovary and influencing circulating PRL concentrations [92, 93]. PRL is found in follicular fluid [94] and can be produced by granulosa cells [95]. PRL has been associated with seasonal follicular growth [83], ovulation [93] and CL [77, 96]. In mares, PRL levels are lower in autumn and winter compared to spring and summer when follicular activity resumes [92]. In addition, there is a positive correlation between PRL and follicular diameter during the spring transition in mares [83].

As mentioned earlier, PRL appears to be associated with follicular growth. PRL receptors (PRLr) are at the highest concentration in the antral follicles, where it has been shown that PRL is manufactured by granular cells [94]. Once PRL is produced, it is presumably accumulated in the follicular fluid, which suggests a paracrine/autocrine function for PRL within the follicle [95]. The DA is sent to the target organs through the dopaminergic nerves. In the pituitary gland, dopaminergic neurons of hypothalamic origin deliver DA to lactotrophs to inhibit PRL production. Dopaminergic nerves have also been shown to provide a source of DA to the equine ovary. Unlike other species, DA neurons in the mare's ovary do not appear to be associated with reproductive structures. DA neurons, as well as DA receptors in ovarian blood vessels, suggest a role for DA in the regulation of ovarian vascular compliance. But they can also serve as a method of local distribution of DA to vascularized reproductive structures. DA D_2 and PRLr are evenly distributed through the large and small luteal cells of CL [92]. The regulation of luteal function is not well described in the mare, but the PRL and DA appear to have some role [96].

4.2. Luteal function

DA also plays a role in the regulation of luteal function. For example, D_1 and D_2 DA receptor proteins have been detected in mares in CL [77]. In rats, DA produces stimulating effects on P_4 secretion from luteinized granular cells through interaction with D1 [97]. In the cow, DA has been reported to control luteal endocrine function [77]. In addition, in luteal tissue in mares, an increased incidence of gene expression of DA receptors was reported. Both types of receptors appear to be homogeneously distributed throughout the tissue [77].

In mares, systemic concentrations of DA increase from basal levels in summer to peak levels during the winter anestrus season [82]. During this seasonal change, but before detectable changes in the patterns of secretion of LH or FSH, the average P_4 concentrations during the luteal phase undergo a linear decrease [98–100]. This decrease in the function of CL can be regulated by DA in the ovary since DA results from a seasonal decrease in PRL [30]. The treatment of cycling mares during this transition period with the specific D_2 antagonist did not alter the seasonal change in luteal secretion of P_4, suggesting that this function may be under the control of D1 [30].

4.3. Pharmacologic control of reproduction in mares

4.3.1. Cyclicity

DA antagonists have been used of as an alternative of artificial photoperiod, progestogens, GnRH and its analogues, and gonadotropins to induce early cyclicity and ovulation in anovulatory mares [30]. However, differences in environmental factors, such as photoperiod and temperature, or stress have been recognized to exert an influence on the efficacy of these treatments [31].

The most commonly used DA antagonists in horses were sulpiride [29, 32] and domperidone [91]. Two other antagonists also used were fluphenazine [101] and perphenazine [102]. All these compounds have been documented to induce follicular growth or ovulation in seasonal anovulatory mares. However, there is a wide variation in the results between studies [84].

Domperidone has a high affinity for D_2 receptors and a half-life of about 7 h. It is metabolized predominantly in the liver and intestine [84]. It is usually given by mouth, but can also be given by injection. It is often used to treat agalactia and fescue toxicity. It is also increasingly popular for inducing cyclicality and follicular growth in anovulatory and transitional mares [84].

Sulfiride is selective for D2 receptors and also, although with some inconsistent results, has been used in the mare to induce cyclicality. Besognet et al. [103] described successful treatments using both a high dose (1.0 mg/kg body weight once daily) and a low dose (200 mg/mare). Daels et al. [104] used a dose of 0.5 mg/kg to decrease the time interval at first ovulation. However, other studies have shown no influence on the date of first ovulation [29]. Therefore, the results have been mixed, with some researchers reporting increased follicular development and earlier onset of ovarian cycles and others who have reported no effect [80].

The use of DA antagonists in noncyclic mares was mainly performed in the northern hemisphere and treatments generally began in January and February. A comparison between these studies indicates that the most favorable environmental conditions for mares coincide with an earlier response to treatment with DA antagonists [31]. For example, mares housed indoors and photo-stimulated ovulated earlier [104] compared to mares kept outdoors [83, 104]. When treatment with domperidone, for 12–17 days, in mares, kept outdoors and subjected to natural photoperiod began in April [105], treated females did not ovulate earlier compared to control females (31.6 days vs. 31.0 days). It should be noted that in this study, the treatment started with an average follicular diameter of 16.7 mm.

Information on the fertility of mares treated with DA antagonists is scarce and, in most cases, with a low number of animals. In these studies [103, 104] pregnancy rates of 57% (4/7) and 60%

(4/6) were reported at 18 and 14 days after ovulation, respectively. Mari et al. [106] reported a pregnancy rate of 40% (4/10) and 70% (7/10) for mares treated with sulpiride or domperidone, respectively, and all pregnant mares were foaled. Differences in efficacy in the use of DA antagonists may be due to the FSH secretion status of the anestro mare and the presence or absence of functional FSHr in the ovaries. A hypothesis of this assumption could be that the direct dopaminergic ovarian contribution may affect follicular growth through the regulation of FSHr populations [41].

DA also appears to be associated with some aspects of ovarian follicular growth in mares. DA antagonists have been able to stimulate follicular recruitment in anestrus mares [91, 104]. In opposition, the administration of the DA agonists delayed the spring transition to the breeding season [102]. However, the use of DA antagonists in mares during the breeding season did not change the timing of the estral cycle events [83].

If AD through the D_2 receptors inhibits ovarian follicular growth, blocking of these receptors in the preantral follicles of the anestrus mares by the DA antagonists would presumably interfere with inhibition and allow follicular development. However, follicular growth may occur only if other stimulating factors, such as FSH secretion and ovarian responsiveness, are present [29].

4.3.2. Lactation

DA is the most important factor inhibiting the release of prolactin. In mares, different DA D2 receptor antagonists have been used to prevent the plasma PRL decrease induced by ergot alkaloids [101]. This decrease in PRL is an agalactia cause in mares during the lactation period [107]. Therefore, D_2 DA antagonists may be used to restore lactation in affected mares.

Two different studies demonstrate that lactation can be induced successfully in the summer, in intact, cyclic mares using a D_2 DA antagonist sulpiride, after steroids treatment administered by vaginal sponges. Thus, Chavatte-Palmer et al. [108] have demonstrated the induction of lactation in non-pregnant cyclic mares. Therefore, is possible to use these mares as foster mothers for foals separated from their mother, for a short time after the birth [109].

In ovariectomized mares, the PRL secretion induced by the D_2 DA antagonist is lower than in treated intact mares; this fact indicates that ovarian steroids increased plasma PRL levels. Thus, when lactation is induced in mares with a D_2 DA antagonist to increase PRL, steroids secreted by the ovary are necessary. Therefore, treatment with exogenous steroid is not necessary in intact mares. So, this induction is possible at the end or the beginning of the breeding or foaling season [108].

4.4. Stallion

In stallions, Urra et al. [33] documented that DA acts as a physiological modulator of viability, capacitation and sperm motility. Indeed, the acrosome integrity and thyrosine phosphorylation is significantly reduced at high concentrations of this catecholamine in equine sperm. Bromocriptine (DA agonist) on PRL secretion and subsequently on gel-free seminal volume are consistent with the hypothesis that PRL is involved with the sexual stimulation-induced

rise in seminal volumes in stallions. The number of mounts, sperm concentration, motility, pH of gel-free semen, number of spermatozoa per ejaculate, and PRL concentration in gel-free semen were not affected by treatment of bromocriptine and sulpiride during period of sexual stimulation. The lack of effect of sulpiride (AD antagonist) treatment indicates that PRL alone does not mediate the effect of sexual stimulation on seminal volume [34].

5. Conclusion

This chapter analyzes the physiological mechanisms of secretion, regulation, cerebral functions and extracerebral self/paracrine of DA in equids, the physiological factors that modify the profile of DA, such as age, breed, exercise and reproductive status and the importance of DA in the reproductive seasonality in the mare. Likewise, the implication of DA and the effects exerted by the agonists and antagonists of the dopaminergic receptors used in equine clinic in PPID and stereotypies, microvascular blood flow of the hoof, fescue poisoning in pregnant mares and GI hypomotility have been described.

Author details

Katy Satué Ambrojo[1]*, Juan Carlos Gardon Poggi[2] and María Marcilla Corzano[1]

*Address all correspondence to: ksatue@uchceu.es

1 Department of Animal Medicine and Surgery, School of Veterinary Medicine, CEU-Cardenal Herrera University, Valencia, Spain

2 Department of Animal Medicine and Surgery, Faculty of Veterinary and Experimental Sciences, Catholic University of Valencia "San Vicente Mártir", Valencia, Spain

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Section 2

Dopamine in Biomedical Research

Chapter 6

Oxidative Polymerization of Dopamine: A High-Definition Multifunctional Coatings for Electrospun Nanofibers - An Overview

Rajamani Lakshminarayanan,
Srinivasan Madhavi and Christina Poh Choo Sim

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.81036

Abstract

The invention that catecholamines undergo oxidative polymerization under alkaline conditions and form adhesive nanocoatings on wide variety of substrates has ushered their potential utility in engineering and biomedical applications. The oxidative polymerization of catecholamines can be triggered by light, chemical and physical methods, thus representing one of the widely explored surface coating methods. The overall objectives of this chapter are to compile the various methods of accomplishing surface coatings and compare the structural diversity of catecholamines. The progress achieved so far on polydopamine (pDA) coatings on electrospun polymers will be discussed. Finally, we will summarize the research efforts on catecholamine coatings for biomedical applications as well as their potential as a high definition coating method.

Keywords: surface coatings, polydopamine, electrospinning, functional coatings, tissue engineering

1. Introduction

There has been a great demand on modification of material surfaces with functional coatings that will present superior translation of desirable features in both biomedical and industrial settings. In particular, the coating methods with wide substrate applicability, ease of processing and subsequent modification and optimum durability are highly desired. One of the key aims of coatings is transform surface functions instead of altering the bulk composition

of the substrate materials. Among the various surface coating methods, the water-resistant wet adhesive bonding by marine mussels has become a leading model for biomimetic modification of surfaces. Ever since the first report that oxidative polymerization of dopamine under alkaline conditions generates material-independent nanocoatings on wide variety of substrates, the topic has become one of the most widely explored area in material science [1]. In their pioneering work by Lee et al., the substrates were immersed in 2 g/L dopamine solution in 10 mM Tris-buffer (pH 8.5) overnight with constant stirring to generate 45 ± 5 nm thick polydopamine (pDA) coating. Organic substrates coated by the above method were more stable to combined acid and ultrasonication than the coated inorganic substrates. The versatility of pDA coating is attributed to the wide variety of chemical interactions conferred by the catecholamine chemistry [2].

The mechanism involves slow oxidation of dopamine (DA) to dopamine quinone (DQ) via dopamine semiquinone (DSQ), which rapidly undergoes Michael-type intramolecular cycloaddition reaction forming leucodopaminechrome (DAL). Oxidation of DAL and subsequent rearrangement results in the formation of heteroaromatic 5,6-dihydroxyindole (DHI) and its oxidized product 5,6-indolequinone (**Figure 1**). The latter two

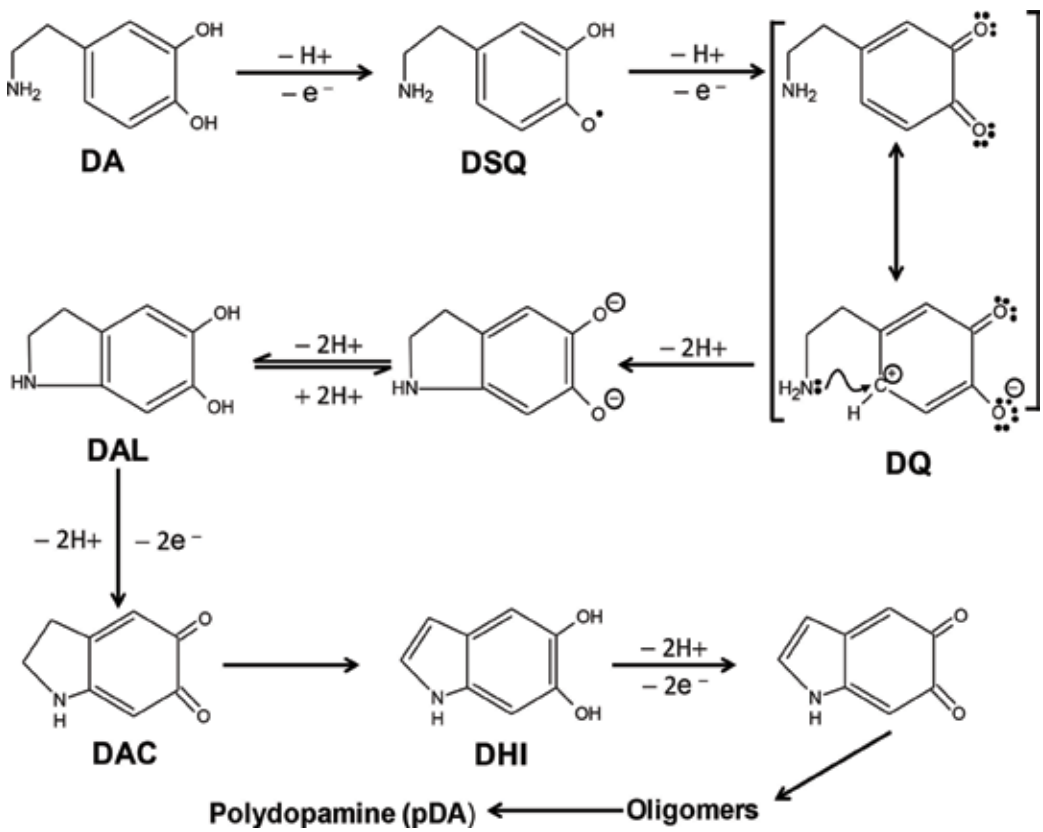

Figure 1. Initial steps in the autoxidation of dopamine to form polydopamine nanocoating in alkaline pH.

http://dx.doi.org/10.5772/intechopen.81036

molecules undergo branching reactions at positions 2, 3, 4 and 7 leading to variety of isomeric dimers or higher order oligomers, which self-assemble to form thin film coating of substrates [3].

Thus, pDA coating generates supramolecular assembly of diverse structures which are resistant to common organic solvents but can be detached under high basic conditions (pH > 10.5). The key step in the formation of polydopamine coating is the oxidation of dopamine to dopamine quinone. Several factors can trigger the conversion of dopamine to dopamine quinone, thus pDA coating can be accomplished by a number of experimental conditions. We will detail these methods in the following section.

2. Factors controlling pDA nanocoatings and its stability

2.1. Dissolved oxygen, dopamine concentration, pH, buffers and temperature

The pDA coating process by Tris-HCl route is slow (1.2–2.1 nm/h) and the thickness of the coating leveled off (45 ± 5 nm) in 24 h. When the coating was carried out at the air-water interface, the thickness increased linearly over entire period and coating speed increased to 3.2 nm/h [4]. These results together with the observations that no detectable coating was observed when the coating process was carried out in nitrogen confirm that the presence of dissolved oxygen was critical for the pDA nanocoatings by Tris-HCl route [5]. When compared to aerial oxidation, exogenous addition of oxygen in solution accelerates the rate of pDA nanocoatings (8 nm/h). It has been shown that the dopamine consumption rate constant was doubled when the coating was carried out in pure oxygen compared to aerial oxidation [5]. The pDA coating in an oxygen atmosphere displayed homogenous thickness distribution compared with coating performed in air.

The aerial deposition of pDA films in Tris-HCl follows a two-step model in which a rapid increase in thickness during the early stages (stage I, <2 h) is accompanied by a slower deposition with increasing time (stage II, 2–10 h), indicating depletion of dopamine concentration with increasing time which resulted in lower rate of deposition [6]. Thus, the coating speed can be accelerated by increasing the concentration of dopamine. Indeed, increasing the concentration of dopamine resulted in an increase in coating speed of 4 nm/h with a maximum thickness of ~80 nm could be achieved with no further increase observed above 8 g/L [7, 8]. Alternatively, the thickness of the coating can be improved by multiple immersion of the substrates in freshly prepared dopamine solution for 15 minutes [9]. A maximum coating speed of 4 nm/h could be achieved by the multiple immersion process while decreasing the immersion cycle to 5 minutes increased the coating speed to 7 nm/h.

The presence of surfactants such as sodium dodecyl sulfate or hexadecyltrimethylammonium bromide and polyvinylpyrrolidone or boric acid, which interact strongly with dopamine, completely prevented or decreased the formation of pDA coating on quartz [10–12]. These results further suggest that the presence of free unoxidized dopamine in solution is essential for the formation of pDA nanocoating as well as to increase the coating thickness.

The buffer pH plays an important role in achieving optimum coating thickness by Tris-HCl method. At a given dopamine concentration, the coating speed increased in a step-wise manner between pH 7 and 10.2 and maximum speed could be achieved between pH 9 and 10.2 [8]. The coating speed approached 10.8 nm/h at pH 8.5 and increased 15.6 nm/h between pH 9.0 and 10.2. The choice of buffers (i.e., phosphate, carbonate or Tris) also determines the thickness of the coating [13]. Dynamic light scattering and small angle neutron scattering studies showed that the aggregates formed in inorganic buffers (phosphate or bicarbonate) contained slow diffusing particles (hence higher molecular weight) than aggregates present in Tris buffers. Higher film deposition rates achieved in inorganic buffers than in Tris was attributed to the covalent interaction of Tris with dopamine oligomers, thus modulating the nanocoating thickness [14]. Zangmeister et al. reported a pDA coating thickness of 8–10 nm in 1 h by using carbonate/bicarbonate buffer (pH = 8.5, Ref. 15). These authors further showed that an immersion time of at least 10 minutes was required to form continuous pDA nanocoatings.

For a given substrate, the rate of pDA coating could also be accelerated by increasing the temperature of coating. Increasing the temperature of coating from 25 to 35°C increased the coating speed from 1.8 to 2.2 nm/h [6]. However, more than 10-fold increase in film thickness was achieved within 8 h by increasing the temperature to 60°C than pDA coating carried out under ambient conditions for 24 h. The high temperature deposited coatings displayed increased surface roughness and greater relative friction coefficient with heterogeneous distribution of pDA nanoparticles [16].

2.2. Accelerating the coating speed of pDA coatings

In the absence of any external additives, the formation of pDA coating takes place slowly but can be accelerated by metal ions, enzymes or organic amines. A number of redox active metal ions and salts have been shown to catalyze the oxidative polymerization of dopamine under neutral or weakly acidic conditions, thus expanding the repertoire pH of pDA deposition. Bernsmann et al. have shown that the presence of stoichiometric excess of Cu^{2+} ions in Tris buffer at pH 4.5 resulted in an increase in coating thickness of >70 nm which was difficult to achieve by conventional Tris-HCl route wherein the thickness did not increase beyond 45 ± 5 nm [17]. When compared to copper ions, the presence of other transition metal ions such as Fe^{3+} and Ce^{4+} has also been shown to accelerate the pDA coating under weakly acidic conditions [18]. Park et al. reported the pDA coating under neutral pH by adding fourfold stoichiometric excess of vanadyl (VO^{2+}) ions to the dopamine solution. Addition of vanadium accelerated the pDA coating speed by about 7 times when compared to conventional pDA coating [19].

Alternatively, the combined use of metal ion and hydrogen peroxide has been shown to greatly accelerate the pDA coating speed on variety of substrates under alkaline pH [20]. The reactive oxygen species produced by Cu^{2+}/H_2O_2 increased the coating speed to 43 nm/h and produced defect-free pDA coating with inherent antioxidant and antimicrobial properties. The pDA nanocoatings prepared by this method displayed remarkable resistance to solvents and acid/alkali treatment in comparison to the pDA coating prepared by Tris-HCl

method. Similarly, Zhu et al. reported solvent-resistant and rapid pDA deposition on ultrafiltration membrane by using Fe^{3+}/H_2O_2 under acidic (pH = 3.5) conditions [21].

In addition to metal ions, oxidizing agents such as ammonium persulfate and sodium periodate catalyze the pDA formation. The presence of ammonium persulfate (pH 7.0) could accelerate the pDA formation with coating speed as high as 35 nm/h [22]. In a systematic study, Ponzio et al. showed that pDA coating with superhydrophilic/superoleophobic properties could be accomplished by the addition of stoichiometric excess of sodium periodate under weakly acidic conditions in acetate buffer [23]. The coating speed can be controlled by appropriate oxidant-dopamine ratio. These authors further showed that increasing the temperature of sodium periodate containing dopamine solution to 70°C accelerated the coating speed to 90 ± 5 nm/h. These results suggest that the combined effect of oxidant and temperature could enhance the coating speed of pDA nanocoatings. Interestingly, Hong et al. demonstrated that more than 200-fold increase in coating speed when compared to Tris-HCL route could be achieved by controlling the molar ratio of dopamine concentration, sodium periodate:dopamine ratio and pH [8]. These authors further demonstrated the utility of such approach in preparing ultrafast coating of substrates by spraying the dopamine solution containing the oxidant. The use of oxidant-induced pDA formation is advantageous since the process can be carried out under deoxygenated conditions at acidic pH values, thus useful for substrates that are sensitive to alkali pH. However, the presence of stoichiometric excess of oxidants (dopamine:oxidant = 2–4) or metal ions is necessary to achieve a higher coating speed. The process may leave impurities in the resultant films and modify the surface properties. In addition to the metal ions or oxidants, multicopper oxidase enzyme, laccase, could catalyze the pDA coating, and the coating speed was doubled in the presence of enzyme (2.7 nm/h) compared to Tris-HCl route [24]. The enzymatic process can also be accomplished in neutral pH [25, 26]. A smooth coating of pDA could also be achieved by the enzyme, tyrosinase, which catalyzes the oxidation of dopamine with a coating speed of ~2.3 nm/h [27].

Organic bases such as hexamethylenediamine (HD), polyethylenimine (PEI), aminopropyl triethoxy silane (APTES) and dihydroxy indazole have been shown to catalyze the pDA coating. In a systematic study, Yang et al. reported the biocompatible coating of stainless steel by HD along with dopamine hydrochloride (4:1 molar ratio) in Tris-HCl buffer (pH 8.5) [28]. The methodology produced fourfold higher coating thickness (140 nm) that was difficult to achieve by traditional Tris-HCl route with a coating speed of 6 nm/h. In an another approach, a free-standing pDA-PEI composite film can be prepared at the air-water interface in Tris-HCL buffer (pH = 8.5) [29]. Using this method, a coating speed of 50 nm/h can be achieved at dopamine:PEI ratio of 4:1. A coating thickness of ~1 μm was possible to achieve by this method by varying dopamine:PEI ratio and reaction time. Similarly, Knorr et al. reported the use of APTES as organic base for the preparation of pDA-silicate composite films in both neutral and basic pH conditions [30]. In both pH, the coating thickness and coating speed depend on APTES:dopamine ratio. A maximum coating speed of 19.6 nm/h in both pH and a thickness of 140 nm can be achieved at APTES:dopamine ratios 3.5 and 5. Interestingly, the composite films facilitated the subsequent functionalization such as metallization, mineralization and covalent immobilization of hyaluronic acid [28, 29]. Similarly,

Fan et al. used a DHI:dopamine molar ratio of 1:3 to obtain a coating speed of 7 nm/h and threefold higher coating thickness than pDA coating prepared by Tris-HCl route [31].

The presence of oxidizing agents, enzymes or organic bases may interfere with the intrinsic properties of pDA; however, a number of physical approaches have been reported that can generate pDA coatings without any chemical interfering agents with higher coating speed. For example, Wang et al. showed that pH-independent (in the range pH 4–8) pDA coating with a coating speed as high as 53 nm/h could be achieved by the use of argon microplasma [32]. Since the pDA coating occurred at the plasma-liquid interface, the method can be extended for the preparation of direct pDA patterning of substrates. Chen et al. used plasma-activated water for pDA coating under acidic conditions (pH 2.5–5.4, Ref. [33]). The pDA particles formed under these conditions remained stable for 3 months, whereas those prepared by conventional method precipitated in 24 h. The use of microwave-assisted radical initiation also accelerates the pDA coating speed. Lee et al. reported a coating thickness of 72 nm/h in Tris buffer (pH ~ 8.5) by using high-power microwave radiation [34]. Recently, Coskun et al. reported pDA coating on glass substrates by chemical vapor deposition of dopamine in the presence of sulfuric acid/sodium sulfate as the oxidizing agents at 300°C in a nitrogen atmosphere [35]. The methodology produced homogenous and highly conductive coating with a coating speed of 339 nm/h, the highest pDA deposition speed achieved so far. The changes in electrical properties are attributed to the homogenous structure of the pDA films that was different from pDA formed by alkaline route.

pDA coating of electrically conductive substrates can also be accomplished by electrochemical methods. pDA coating of metallic implants or gold-coated non-metallic substrates can be accomplished by cyclic voltammetry methods under neutral pH [35, 36]. Unlike solution-based routes, the electrochemical deposition relies on the conductivity of the substrate and the coating is confined to the substrate surface. As a result, the method produced a higher coating speed (6–8 nm/h) and smoother coating than coating prepared by Tris-HCl method. The method has the advantage of direct pDA coating of cardiovascular stents or metallic implants and simultaneous/subsequent functionalization with biomolecules or metal ions [36–38].

pDA nanocoatings can also be triggered photochemically which has the advantage of controlling the onset and the termination of the process [39]. The reactive oxygen species triggered by UV irradiation encouraged the pDA nanocoatings in acidic, neutral and basic pH values. Though fourfold increase in the coating speed was observed by UV irradiation, the method has the advantage of making 2D surface patterns using photomasks or surface grafting of polymers [40]. **Figures 2** and **3** summarize the working pH range for various pDA coating methods and coating speed reported so far.

2.3. pDA nanocoatings in organic solvents

All the above methods utilize the aqueous buffers/conditions for the generation of pDA nanocoatings. To broaden the scope of the coating, You et al. reported the use of organic solvents with relative polarity ≥0.386 and organic bases such as piperidine, trimethylamine and 2-methoxyethyl amine (dopamine:organic ratio 1:2) for the controlled coating of pDA [41].

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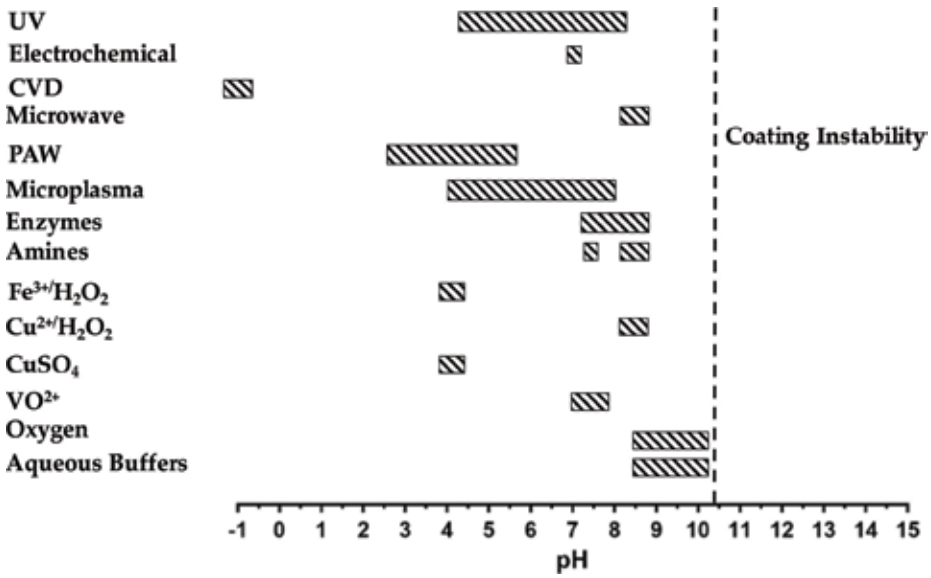

Figure 2. pH map of the various pDA coating methods reported in the literature so far. PAW is plasma-assisted water.

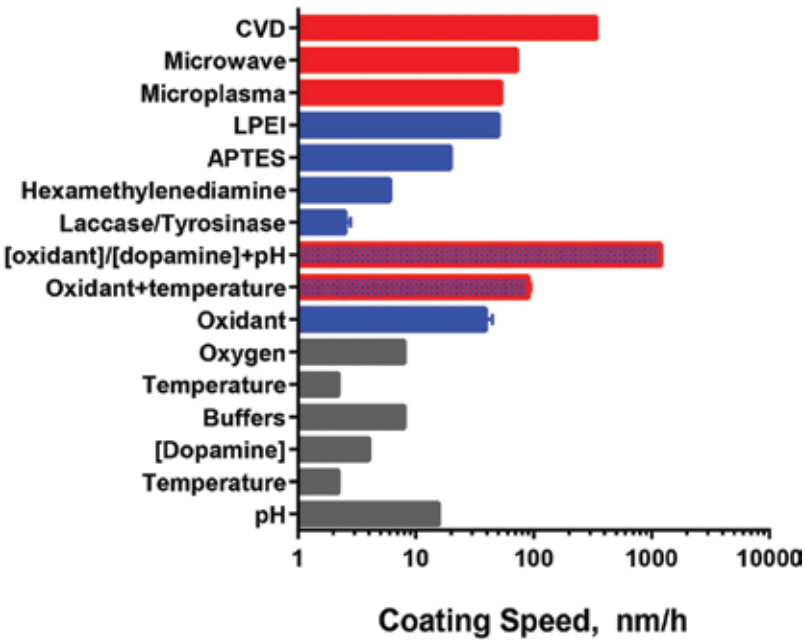

Figure 3. Coating speed achieved with various pDA coating methods reported in the literature. APTES – 3-aminopropyl triethoxy silane; LPEI – linear polyethylenimines; CVD-chemical vapor deposition.

The presence of an organic base is important for the deprotonation of dopamine and subsequent oxidative polymerization. Among the three organic bases, piperidine showed rapid pDA coating within 12 h whereas in the presence of 2-methoxy ethylamine as high as 60 h

was required. The coating speed and coating thickness can be controlled by the polarity of the solvent. At dopamine:piperidine ratio 1:2, a coating speed of 27 nm/h could be achieved in methanol which was decreased to 11 nm/h in ethanol. The authors demonstrated the utility of this approach in establishing pDA coating of water-labile electrospun nanofibers, controlled release of hydrophobic drug, paclitaxel and immobilization of organothiols. Liu et al. reported the pDA coating in ethanol and in the presence of large excess of tetramethylethylenediamine (TEMED) [42]. Despite the large excess of (TEMED: dopamine = 26), a maximum coating thickness of 28 nm was achieved in 48 h. When compared to other organic bases, piperidine showed higher coating thickness and coating speed and attributed to the strong basic properties (low pKb) and higher nucleophilicity of piperidine.

Thus, pDA coating of various thicknesses and surface smoothness can be accomplished by altering the variables such as concentration, temperature and oxidizing agents. The combined effects of oxidizing agents and temperature could maximize the coating thickness in short time. The non-chemical approach broadens the scope of the coating, thus achieving high coating speed in extreme pH conditions.

2.4. Stability of pDA nanocoating

The versatile material-independent pDA nanocoating has the potential in repertoire of applications including industrial and biomedical applications. However, the stability of nanocoatings to harsh conditions realized in the end-use industrial and biomedical applications would impact the lifespan and performance characteristics of the pDA-modified materials. Here, we detail the stability of pDA-coated substrates reported so far in the literature. Ou et al. reported that pDA coating of ATPS-modified silicon was resistant to electrochemical oxidation, thus conferring corrosion-resistant properties [6]. In an another study, Chen et al. showed that the corrosion resistance of dodecanethiol functionalized pDA nanocoating of copper can be improved by 1000-fold in comparison to pristine copper films [43]. The alkane thiol-modified film remained intact even after immersion in simulated sea water for 20 days. In a systematic study, Singer et al. investigated the effect of pH, dipping angle, immersion time and dopamine concentration on the stability of pDA-coated magnesium [44]. The results suggested that pDA-coated magnesium prepared by using 1 mg/mL of dopamine in 50 mM Tris buffer for 2 h (pH 10 and a dipping angle 0°) produced corrosion-resistant coating. The stability of pDA coating is dependent on the pH of the solution and substrates as well. Wei et al. compared the pH stability of pDA coatings on three different polymer films. Among them, pDA coating of polypropylene was the lowest, whereas maximum for nylon films [45]. These authors further showed that the presence of unreacted dopamine was responsible for the poor alkali stability of pDA coating and could be improved by the addition of oxidants. Zhang et al. compared the chemical and pH stability of the pDA coating obtained by two different methods [20]. Their results suggest that the oxidant ($CuSO_4/H_2O_2$) catalyzed pDA coating displayed remarkable chemical resistant to organic solvents etching and superior acid/alkali stability than air oxidized films. Kang et al. investigated the stability of pDA coating on gold substrates in phosphate buffered saline (pH 7.4, 5% CO_2 at 37°C). Only a marginal decrease in coating thickness (4–15.8%) was observed for the pDA coatings after 26 days, confirming excellent

aqueous durability of the coatings under physiological conditions [46]. Similarly, Yang et al. showed that pDA-coated 316 stainless steel immersed in PBS displayed increased swelling of the nanocoatings after 30 days, corroborating the above results [28]. An elegant demonstration on the stability of pDA nanocoatings to pH, chemical and ultrasonication was reported using surface plasmon resonance recently by Yang et al. [47]. The method has the advantage of monitoring the formation and detachment of the coatings directly and in real times. The results suggested that the pDA coating on gold chips was poor in extreme pH values, that is, pH 1–3 and 11–14, as well as in polar organic solvents such as dimethyl sulfoxide and dimethylformamide. The coating was also stable to ultrasonication in water for 1 h and the stability could be enhanced by increasing the ionic strength of the buffer. Alternatively, the alkali pH stability could also be enhanced by the addition of metal ions [48, 49]. In our work on pDA coating, we have shown that pDA-coated titanium implant covalently linked with an antimicrobial peptide prevented the *Staphylococcus aureus* colonization in rabbit cornea and was superior to prophylactic antibiotic treatment, thus confirming the stability of the coating in a biological milieu [50]. In addition to chemical and biostability, the pDA coatings were stable to UV radiation and protect UV-sensitive compounds from rapid degradation [51].

2.5. Structure: activity relationship on pDA coating

Few reports discussed the structure-activity relationship of modified dopamine derivatives. The presence of catechol group and aminoethyl group is essential for the oxidative polymerization of dopamine and the concomitant material-independent adhesive properties. The aromatic ring of dopamine has been modified to generate structures in order to control the formation of pDA nanocoatings. Cui et al. reported the effects of electron withdrawing groups in the aromatic ring on the coating thickness [52]. Substitution of Cl- or $–NO_2$ groups at the 6th position in the aromatic ring retarded the pDA formation. Chlorodopamine formed 7-nm thick pDA-like coating after 48 h, a threefold decrease in coating thickness observed for dopamine under the same conditions. No pDA-like structure was observed for the nitrodopamine even after incubation in alkaline pH for 48 h. The presence of 2 mM sodium periodate, however, catalyzed the oxidative process, and a film thickness of 2.8 nm was achieved after 48 h. Taken together, these results suggest that the presence of an electron with drawing group in the aromatic ring conferred greater oxidative stability, decreased the pDA nanocoating formation and increased metal ion chelation and interfacial adhesion [53, 54]. When compared to dopamine, the presence of electron donating group has been shown to accelerate the pDA formation. Zhang et al. have shown that the presence of 5-methoxy group in the aromatic ring of dopamine accelerated the pDA formation with concomitant twofold increase in coating thickness in comparison to dopamine [55]. However, the methoxy derivative weakens the thermal stability of nanocoating.

Substituents at the 2-amino ethyl group of dopamine have also been shown to affect the polymerization kinetics and surface characteristics of the coating. Norepinephrine, the natural analogue of dopamine with hydroxyl group at the benzylic position of DA displayed similar coating potential as dopamine by alkaline pH or in the presence of laccase [56–58]. When compared to pDA coating, polynorepinephrine (pNE) coating appeared smoother and the

benzylic –OH group facilitated the ring opening polymerization of ε-caprolactone, whereas the secondary amine readily formed diazonium diolates with nitric oxide, thus providing a source for the controlled release of NO [56, 57].

In our work, we compared the changes in mechanical properties of polyvinyl alcohol (PVA) films reinforced with various catecholamines. Among them, pDA-reinforced films displayed the highest mechanical strength and toughness in comparison to other catecholamines; an indication that any functionalization in the amino ethane weakened the interfacial adhesion [26]. Interestingly, the polyepinephrine-reinforced PVA films inhibited the growth of various Gram-positive strains [26]. The results highlight the material-independent coating with inherent antimicrobial properties of a naturally occurring dopamine derivative.

In a seminal work, Hu et al. investigated the effect of increasing the chain length of 2-amino ethyl group on properties of various catecholamines [58]. Their results suggest that dopamine and 3-aminopropyl catechol readily formed material-independent coating with similar mechanism. However, catecholamines containing 4, 5 and 12 methylene groups do not form heteroaromatic products. The adhesion strength of the polycatecholamine coating was not affected by increasing the chain length from 2 (dopamine) to 5, whereas substantial decrease was observed for catecholamine containing 12 methylene groups.

In summary, the polydopamine nanocoating offers a convenient way of transforming an inert surface into one with multifunctional features. The ease of achieving appropriate coating thickness, availability of methods with higher coating speed and the structural diversity of dopamine or catecholamine present ample opportunities to develop surfaces with specific surface features. Subsequent derivatizations of pDA layers expand the robustness of the approach.

3. Applications of pDA coating for electrospun polymers

Besides the premise that nanoscale structures of the extracellular matrix play an important role in tissue regeneration, numerous methods have been introduced for producing ultrathin nanofibers. Electrospinning, the oldest among them, has become a very attractive technique due to its versatility in spinning wide range of polymeric fibers [59]. The method is capable of producing polymer fibers with diameters ranging from 10 nm to 10 μm using both synthetic and biosynthetic polymers by controlling the intrinsic and extrinsic parameters [60]. The inherent hydrophobicity of synthetic polymers such as polycaprolactone (PCL), poly(lactide-coglycolide) and poly(lactide-co-caprolactone) and the absence of cell recognition sites render them unsuitable for biomedical applications. Conventional surface modification methods of electrospun polymers, however, require tedious preparation steps, rigorous reaction conditions and limited choice of substrate materials [61, 62]. Taking into consideration the simplicity, hydrophilicity, aqueous durability under physiological conditions, biocompatibility and ease of functionalization with cell recognition molecules, pDA nanocoatings have been reported on electrospun polymers for various tissue engineering.

Ku and Park were the first to demonstrate the utility of pDA coating for possible vascular tissue engineering applications [63]. These authors compared the growth of human umbilical

vein endothelial cells on pDA- or gelatin-coated PCL nanofiber mats. The results showed that pDA-coated PCL mats displayed threefold to sevenfold higher cell viability, cell attachment and spreading with well-stretched cytoskeletal components than gelatin-coated PCL nanofibers. In addition, the cells grown on pDA-coated mats displayed increased expression of endothelial cell markers highlighting the healthy status of the cells. Similar to the above work, pDA coating of poly(L-lactic acid) (PLLA) conferred higher human mesenchymal stem cell adhesion, penetration, proliferation and osteogenic differentiation than pristine fibers [64]. These authors showed that 1 h immersion of the as-electrospun nanofibers in dopamine in Tris-HCl (pH = 8.5) was enough to achieve optimum biological properties. Similarly, pDA nanocoating of PCL/gelatin nanofibrous membrane was shown to enhance the mouse adipose-derived stem cell (mASC) adhesion, penetration and spreading compared to PCL/gelatin nanofiber mats. The layer-by-layer assembly of pDA-coated PCL/gelatin showed higher expression of key osteogenic marker proteins and calcium deposition than PCL/gelatin [65]. In an interesting approach, Roy et al. reported the utility of pDA coating for macroporous 3D electrospun PVA for hard tissue engineering [66]. These authors performed pDA coating on glutaraldehyde crosslinked electrospun polymers. The pDA-coated PVA was shown to have excellent shape recovery properties and higher cell adhesion, spreading, penetration and PVA scaffolds.

In a systematic study, Sun et al. reported the utility of pDA coating of poly(lactide-co-glacolic acid) (PLGA) nanofibers and subsequent covalent functionalization of basic fibroblast growth factor (bFGF) on pDA-coated PLGA nanofibers [67]. The pDA coating and subsequent bFGF functionalization enhanced primary human dermal fibroblast adhesion and proliferation. In a rabbit model of wound healing, pDA coating followed by bFGF functionalization increased the wound closure and higher re-epithelialization than pristine and pDA-coated PLGA. Wounds treated with pDA-coated PLGA also showed higher wound closure and re-epithelialization than pristine PLGA, highlighting biocompatibility of pDA coating. In a subsequent work, these authors further showed the feasibility of pDA coating followed by bFGF immobilization in drug-loaded PLGA fiber mats [68]. The pDA nanocoating by Tris-HCl route of a drug-loaded PLGA could be achieved with minimum drug efflux, by optimizing dopamine concentration and pH.

Shin et al. reported the utility of pDA coating of electrospun nanofibers poly-L-lactide-co-ε-caprolactone (PLCL) followed by functionalization with gelatin for cardiac tissue engineering [69]. These authors compared the biological properties of gelatin immobilization on PLCL scaffolds by two different methods. The results suggested that pDA nanocoating followed by subsequent immobilization of gelatin resulted in higher rat myoblast adhesion and spreading, superior cytoskeletal organization and cell proliferation than gelatin immobilized by 1-ethyl-3-(3-dimethylaminopropyl)-1-carbodiimide hydrochloride/N-hydroxysuccinimide (EDC/NHS) coupling. Interestingly, the pDA-coated PLCL (without gelatin immobilization) showed superior biological properties than gelatin immobilized with EDC/NHS method, possibly due to immobilization of serum protein on pDA-coated nanofibers. In an extension, these authors investigated the ability of RGD peptide immobilized onto pDA coating with PLCL [70]. In serum-free conditions, the peptide-immobilized pDA-coated PLCL scaffolds displayed higher mouse myoblast adhesion and spreading while enhancing the cell proliferation synergistically with serum proteins. These observations suggest possible inherent cell supportive nature of pDA coatings. Extending the approach further, Ku and Park have demonstrated that pDA-coated uniaxially oriented (aligned) electrospun nanofibers enhanced mouse myoblast adhesion, increased expression of myosin

heavy chain and maturation than mats without pDA coating [71]. To enhance the preferential migration of cells, Shin et al. reported the use of pDA-coated radially aligned PCL nanofibers [72]. The surface modification and radial alignment of the fibers enhanced the human mesenchymal stem cell adhesion, proliferation and spreading. In addition, cells displayed an elongated morphology along the fiber axis. These results highlight the importance of surface chemistry and topographical cues for possible skeletal tissue engineering. It has been suggested that serum proteins such as fibronectin and vitronectin react readily to the pDA surfaces and the presence of integrin-binding sites in the immobilized proteins promotes focal adhesion and spreading. Davoudi et al. reported the dual functionalization of pDA-coated electrospun polyurethane nanofibers with heparin and vascular endothelial growth factor (VEGF) [73]. The biomolecules immobilized nanofiber mats displayed higher endothelial cell adhesion and spreading and poor platelet adhesion, demonstrating the potential utility of pDA nanocoatings for cardiovascular tissue engineering. Recently, pDA coating of PCL nanofibers followed by immobilization of basement membrane fragments (laminin-111 fragments) was demonstrated by Horejs et al. [74]. In vitro assays demonstrated that the laminin-111 fragment immobilized nanofiber mats prevented the TGFβ1-induced epithelial mesenchymal transition of mouse mammary gland epithelial cells and downregulated the expression of matrix metalloprotease 2 (MMP2). In a mice model of TGFβ1-induced peritoneal fibrosis, the laminin or laminin fragment immobilized nanofiber mats decreased the MMP2 expression and controlled the tissue fibrosis without causing any inflammatory response at the site of implant. The immobilization strategy is advantageous owing to the restricted access of the ligand to the target receptors and to overcome any off-target effects.

All the above approaches report pDA coating under alkaline conditions for biodegradable and water-insoluble polymers. The aqueous alkaline condition used for the polymers is not suitable for hydrogel polymers such as gelatin. To overcome this, we electrospun dopamine and gelatin and expose the resultant as-electrospun nanofibers to ammonium carbonate. The methodology takes advantage of the sublimation of $(NH_4)_2CO_3$ (referred hereafter as ammonium carbonate diffusion method, ADM) to generate ammonia and carbon dioxide. The ammoniacal conditions raised the pH ≥ 9.5 and triggered the oxidative polymerization of catecholamines in situ [75]. As a result, the pDA-coated electrospun gelatin displayed better aqueous durability and mechanical properties than pristine gelatin nanofibers. We further demonstrated that alkaline exposure did not alter the antimicrobial properties of cationic polymers, antibacterials or antifungal compounds [76, 77]. Interestingly, the combined effect of pDA coating and antibacterials/antifungals which interact with the gelatin nanofibers resulted in long-term antimicrobial activity and excellent durability of gelatin nanofibers. In porcine model of partial thickness burn injury, gelatin nanofibers coated with pDA did not interfere with the wound closure, whereas the antibiotics-loaded mats display higher wound healing than untreated wounds. Taken together, these results highlight that pDA coating of gelatin did not interfere with the wound healing while the addition of vancomycin accelerated the process when compared to untreated burns.

In an another work, we showed that electrospinning of a collagen dope solution containing dopamine and Ca^{2+} permits the partial oxidation of dopamine [78]. Subsequent ammonium carbonate exposure of the Ca^{2+}-pDA mats would result in the complete formation of pDA and mineralized nanofibers. The mineralized nanofibers displayed superior mechanical properties than collagen or collagen mats crosslinked with pDA. The mechanically robust scaffold displayed superior cell adhesion and spreading than electrospun collagen scaffold.

The ammonium carbonate diffusion method has numerous advantages over conventional Tris-HCl route. Electrospun water labile polymers such as PVA and gelatin could not be pDA coated under aqueous alkaline methods, owing to their poor stability in aqueous media. However, such polymers can be readily coated by ADM. The pDA coating by ADM produced homogenous products, contrary to heterogeneous mixture of products formed by Tris-HCl. No ammonia or amine group is incorporated into the product, whereas Tris-base is covalently linked to oxidative products of dopamine, adding further complexity to the final products. As a result, Tris-HCl route produced aggregates of pDA nanoparticles on fiber surface, whereas the vapor phase alkaline exposure triggered smooth pDA coating both along the surface and at the nanofiber contact points, forming "soldered" junctions. Avoiding the use of aqueous or organic solvents could minimize any morphological defects caused by the interference from solvents. The method did not interfere with the biological properties of additives. Simultaneous in situ mineralization and crosslinking of electrospun nanofibers produced mats with excellent mechanical properties and aqueous durability.

4. Conclusion

pDA nanocoating of organic/inorganic substrates is an effective way to modify surface properties of the materials. The availability of myriads of protocol to achieve the nanocoatings in both neutral and extreme pH conditions would expand the application landscape of the method. The development of high speed coating methods together with the diversity of catecholamines will have wide impact on the design and fabrication of polycatecholamine interfaces. The development of high speed spray coating method may overcome the difficulties posed by solution-based methods for industrial applications. One step co-deposition of biologically relevant macromolecules with high speed coating will be useful for the preparation of biointerfaces. The ammonium carbonate diffusion method would allow facile formation of smooth pDA coating on water labile electrospun polymers in solid state, thus potentially avoiding the use of hazardous solvents/organic bases. The protocol would overcome the difficulties in using water-soluble substrates as well as hydrophobic compounds for the preparation of functionalized surfaces.

Acknowledgements

RL thanks the funding support from the Centre Grant Programme-Optimization of core platform Technologies for Ocular Research (INCEPTOR)-NMRC/CG/M010/2017_SERI and SNEC Ophthalmic Technologies Incubator Program grant (Project no. R1181/83/2014)). CPCS is a recipient of National Medical Research Council Clinician Scientist-New Investigator Grant (Project no. NMRC/CNIG/1169/2017). This work was financially supported by NTU-HUJ Create Phase II which is a joint research programme between the Hebrew university of Jerusalem (HUJ, Israel) and Nanyang Technological University (NTU, Singapore) with CREATE (Campus for Research Excellence and Technological Enterprise) funding from National Research Foundation of Singapore (NRF, Singapore).

Conflict of interest

N/A.

Author details

Rajamani Lakshminarayanan[1,2]*, Srinivasan Madhavi[3] and Christina Poh Choo Sim[4,5]

*Address all correspondence to: lakshminarayanan.rajamani@seri.com.sg

1 Anti-Infectives Research Group, Singapore Eye Research Institute, The Academia, Singapore

2 Ophthalmology and Visual Sciences Academic Clinical Program, Duke-NUS Graduate Medical School, Singapore

3 School of Materials Science and Engineering, Nanyang Technological University, Singapore

4 Department of Restorative Dentistry, National Dental Centre Singapore, Singapore

5 Oral Health Academic Clinical Program, Duke-NUS Graduate Medical School, Singapore

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Edited by Sarat Chandra Yenisetti

The chemical basis of human emotions has been an exciting aspect in biology. The "feel-good chemical" dopamine (DA) is a hormone and also a neurotransmitter, which performs a critical role in reward and movement control in the brain. DA also performs multiple other functions outside the brain. Regulating unrelated critical biological functions makes this chemical a vital factor for sustaining life in both health and disease. *Dopamine - Health and Disease* is an endeavour with an objective to understand and appreciate the biological functions of DA in human wellbeing and its potential utility in biomedical research. This effort will supplement scientific and non-scientific communities in stimulating a critical understanding of the biological purpose of "ticklish" DA, which eventually supports the human relentless effort to reduce the burden of disease. As the most exciting molecule,dopamine directly impacts day-to-day life. Anyone who has an eye for health and disease-related concepts will find this book a good read.

Published in London, UK

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ISBN 978-1-83881-792-3
9 781838 817923