



Review

# Molecular Regulation in Dopaminergic Neuron Development. Cues to Unveil Molecular Pathogenesis and Pharmacological Targets of Neurodegeneration

Floriana Volpicelli <sup>1</sup>, Carla Perrone-Capano <sup>1,2</sup>, Gian Carlo Bellenchi <sup>2,3</sup>,  
Luca Colucci-D'Amato <sup>4,\*</sup> and Umberto di Porzio <sup>2</sup>

<sup>1</sup> Department of Pharmacy, University of Naples Federico II, 80131 Naples, Italy; floriana.volpicelli@unina.it (F.V.); perrone@unina.it (C.P.C.)

<sup>2</sup> Institute of Genetics and Biophysics "Adriano Buzzati Traverso", CNR, 80131 Rome, Italy; bellenchi@igb.cnr.it (G.C.B.); diporzioumberto@gmail.com (U.d.P.)

<sup>3</sup> Department of Systems Medicine, University of Rome Tor Vergata, 00133 Rome, Italy

<sup>4</sup> Department of Environmental, Biological and Pharmaceutical Sciences and Technologies, University of Campania "Luigi Vanvitelli", 81100 Caserta, Italy

\* Correspondence: luca.colucci@unicampania.it; Tel.: +39-0823-274577

Received: 28 April 2020; Accepted: 1 June 2020; Published: 3 June 2020



**Abstract:** The relatively few dopaminergic neurons in the mammalian brain are mostly located in the midbrain and regulate many important neural functions, including motor integration, cognition, emotive behaviors and reward. Therefore, alteration of their function or degeneration leads to severe neurological and neuropsychiatric diseases. Unraveling the mechanisms of midbrain dopaminergic (mDA) phenotype induction and maturation and elucidating the role of the gene network involved in the development and maintenance of these neurons is of pivotal importance to rescue or substitute these cells in order to restore dopaminergic functions. Recently, in addition to morphogens and transcription factors, microRNAs have been identified as critical players to confer mDA identity. The elucidation of the gene network involved in mDA neuron development and function will be crucial to identify early changes of mDA neurons that occur in pre-symptomatic pathological conditions, such as Parkinson's disease. In addition, it can help to identify targets for new therapies and for cell reprogramming into mDA neurons. In this essay, we review the cascade of transcriptional and posttranscriptional regulation that confers mDA identity and regulates their functions. Additionally, we highlight certain mechanisms that offer important clues to unveil molecular pathogenesis of mDA neuron dysfunction and potential pharmacological targets for the treatment of mDA neuron dysfunction.

**Keywords:** dopamine; microRNAs; midbrain; morphogens; Parkinson's disease; transcription factors

## 1. Overview of the Midbrain Dopaminergic System

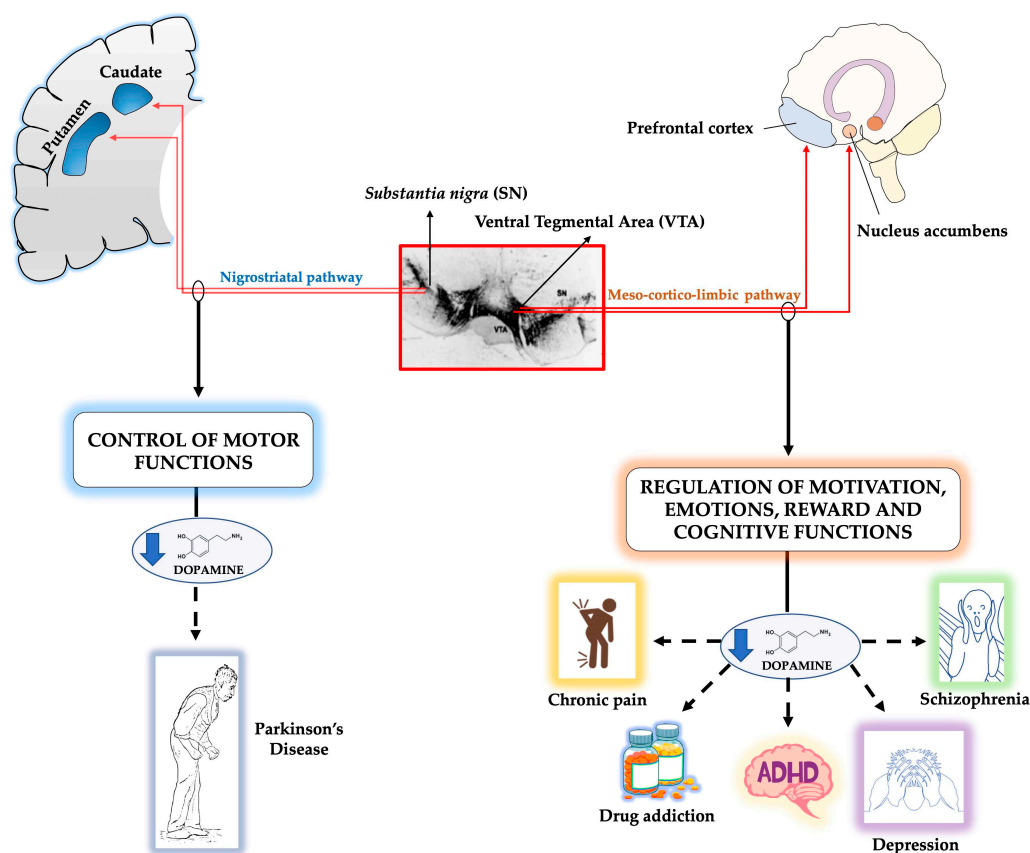
In mammals, the dopaminergic neurons are predominantly located in the midbrain. They play an important role in several neural functions including regulation of voluntary movement, attention, motivational and cognitive processing, emotive behavior and reward and responses to unusual or unpleasant experiences. mDA neurons also play a crucial role in decision-making and in reward prediction error signaling. These higher cognitive functions guide complex learning mechanisms subserved by basal ganglia and frontal cortex.

mDA neurons originate in the midbrain floor plate [1,2] and migrate in a tangential direction towards their final location [3,4]. They give rise to the DA neurons in the substantia nigra (SN, A9), which projects to the dorsal striatum via the nigrostriatal pathway, and controls voluntary movement. The floor plate precursors also give rise to two other mDA neuron groups, in the ventral tegmental area

(A10, VTA) and the retrorubral field (A8), involved in the regulation of emotion and reward through the meso-cortico-limbic system, which innervates the ventral striatum (or nucleus accumbens) and the prefrontal cortex [5].

The SN, besides including the DA cell bodies in the pars compacta (SNc), encompasses a second half called pars reticulata where DA dendrites are laid out, interspersed among GABAergic neurons, which exert an inhibitory activity on mDA neurons in SNc and, indirectly, on their target area (such as thalamus and superior colliculus). The VTA also comprises a heterogeneous mixture of DA (about 65%), GABAergic (30%) and glutamatergic (5%) neurons.

Degeneration of the nigro-striatal mDA pathway is the cause of Parkinson's disease (PD) [6], while dysfunction of the meso-cortico-limbic pathway is associated with schizophrenia, drug addiction, attention deficit hyperactive disorder (ADHD), depression and chronic pain [7–10], see Figure 1.



**Figure 1.** Schematic representation of the midbrain dopaminergic pathways. The figure illustrates in a schematic representation the two dopaminergic pathways originating from the midbrain (delimited by a red rectangle). The ventral tegmental area (VTA) and the substantia nigra (SN) in the adult ventral midbrain are visualized by anti-tyrosine hydroxylase antibodies. On the left: the nigrostriatal pathway connects the SN pars compacta in the midbrain with the dorsal striatum (i.e., caudate nucleus and putamen) in the forebrain. The main function of the nigrostriatal pathway is to control voluntary movement through basal ganglia motor loops. Degeneration of SN dopaminergic neurons leads to diminished concentrations of dopamine in the caudate-putamen. Degeneration of this pathway causes Parkinson's disease (see text). On the right: the meso-cortico-limbic system pathway connects the VTA in the midbrain to the ventral striatum (i.e., nucleus accumbens) and to the prefrontal cortex. It regulates motivation, emotions, reward and cognitive functions. Dysfunction of this pathway is associated to schizophrenia, drug addiction, attention deficit hyperactive disorder (ADHD), depression and chronic pain (see text).

## 2. Features of Midbrain Dopaminergic Neurons

### 2.1. Molecular Characteristics of Dopaminergic Neurons

A dopaminergic neuron is a typical cell that releases the neurotransmitter dopamine (DA). As mentioned above, DA is involved in important homeostatic responses and motor control in humans. This wide range of adaptive behavioral responses is attained by a relatively small number of mDA neurons (about 400,000–600,000 in human) [11], which develop a very extended axonal arborization, sending their terminals for long-distance in many brain areas, with extensive branching and connections, including en passant synapses. The mDA neurons show a remarkable diversity, recognized only recently [12–14]. Molecularly, a dopaminergic neuron expresses: i) a set of genes common to all neurons; ii) a set of genes necessary for DA synthesis and neurotransmission. The DA synthesis and neurotransmission require genes encoding tyrosine hydroxylase (TH) and dopa decarboxylase (Ddc). They are necessary for the production of DA from its precursor L-tyrosine. The vesicular monoamine transporter 2 (VMAT2), also known as Slc18a2, is necessary for DA packaging into vesicles [15]. Since these genes are expressed in other catecholaminergic neurons, such as noradrenergic neurons, which requires dopamine  $\beta$ -hydroxylase (Dbh) to synthesize noradrenaline from dopamine, the presence of TH and absence of Dbh characterizes DA neurons. Besides the Central Nervous System (CNS) noradrenergic neurons, the DA synthesis pathway is present also in peripheral blood lymphocytes, in catecholaminergic neurons of the Enteric Nervous System (ENS) and in the gut microbiota [16].

DA released at the dopaminergic nerve terminals activates postsynaptic dopamine receptor D1- or D2-type G-coupled receptors, and presynaptic autoreceptors of the D2-type. The latter are involved in the regulation of DA synthesis, metabolism, and release. The dopamine transporter plasma membrane glycoprotein (DAT), also known as Slc6a3, a member of the sodium/chloride-dependent neurotransmitter transporter family, terminates DA neurotransmission by high-affinity uptake into the presynaptic DA fibers [17]. DAT expression, together with TH, identifies bona fide DA neurons [18].

An additional feature of mDA neurons is the expression of developmental genes such as forkhead box A 1/2 (Foxa1/2), LIM homeobox transcription factor 1 alpha/beta (Lmx1a/b) and Nurr1, also known as Nuclear Receptor Subfamily 4 Group A Member 2 (NR4A2) [19,20]. These genes are not concertedly expressed in DA neurons located in hypothalamus and olfactory bulbs, which do not originate in the floor plate. A number of the above mentioned genes are also expressed in non-DA neurons located in rostral linear nucleus, subthalamic nucleus and ventral premammillary nucleus; therefore, they are not sufficient to identify an mDA phenotype [21,22]. Interestingly, mDA neurons exhibit a pacemaker activity, characterized by spontaneous rhythmic spike activity at low-frequency [23], which persists in vitro in brain slices and in dissociated cultures, representing a hallmark of dopaminergic differentiation and maturation from embryonic or transdifferentiated fibroblasts [24,25].

### 2.2. The Metabolic Rate and Vulnerability of Dopaminergic Neurons

Oxidative stress is a key player in the loss of mDA neurons. mDA neurons have an intrinsic vulnerability, mainly due to their high metabolic demands. In addition, the DA metabolism generates intermediate metabolites that cause oxidative stress [26]; DA degradation generates reactive oxygen species (ROS) and DA oxidation generates many o-quinones, including DA o-quinones, aminochrome and 5,6-indolequinone. Therefore, DA metabolism is important for neuronal redox-homeostasis and viability. Furthermore, the pacemaker activity of DA neurons is accompanied by large oscillations in intracellular  $\text{Ca}^{2+}$  concentration. The  $\text{Ca}^{2+}$  in the cytoplasm is relatively free and high, while the levels of  $\text{Ca}^{2+}$ -buffering proteins, such as calbindin, are low. Thus, the  $\text{Ca}^{2+}$  overload results in mitochondrial oxidant stress, which largely contributes to the degeneration of mDA neurons and the pathogenesis of PD [27]. Recent data have demonstrated that dysfunction in  $\text{Ca}^{2+}$  signaling may cause the increased nuclear translocation of the transcriptional repressor histone deacetylase 4 (HDAC4) in PD iPSC-derived dopamine neurons and repression of genes that promote neuronal survival [28]. In addition, the misfolding of  $\alpha$ -synuclein ( $\alpha$ -syn, encoded by the SNCA gene) causes widespread

aggregation of the  $\alpha$ -syn protein in the form of Lewy bodies, leading to Lewy pathology, a hallmark of PD [29,30].  $\alpha$ -syn oligomers are highly toxic and their extracellular release activate astrocytes and microglia, generating local inflammation and neuroimmune reaction [31].

Unbiased “omic” approaches have unveiled intracellular and secreted molecular targets involved in the survival, development and cell death of neurons including dopaminergic [32–35]. In particular, mortalin, also known as Glucose Regulated Protein 75 (GRP75) or mitochondrial heat shock protein 70 (mtHsp70), a protein discovered by means of this methodological approach, has proven to play an important role in the regulation of survival and death of dopaminergic neurons [36]. Mortalin has a multifaceted role since it can interact with a variety of partners within a number of sub-cellular compartments (i.e., mitochondria, cytosol, endoplasmic reticulum and vesicles); nevertheless, in DA neurons, it can regulate their vulnerability. Indeed, mortalin has been found downregulated in the midbrain of PD patients and in preclinical animal models its loss of function leads to mitochondrial proteolytic stress and neuronal death. Interestingly, Parkin and PINK1, two proteins related to PD, are able to rescue the deleterious effects of its loss [37].

Recent evidence has shown that in most PD patients, Lewy pathology is also present in ENS and in the vagus nerve, which express high levels of  $\alpha$ -syn. It has been suggested that the vagus nerve could represent a route by which  $\alpha$ -syn pathology can bidirectionally spread between the gut and the CNS, thus constituting an important etiological factor in PD [38]. Interestingly, gut dysfunction and gut microbiota modifications occur in most PD patients, supporting that the alteration of the complex cross-talk between brain and gut might play a crucial role in this neurodegeneration [39,40].

### 2.3. ERK Signaling in the Pathophysiology of Dopaminergic Neurons

The aforementioned factors involved in the death of mDA neurons (i.e., ROS, 6-hydroxydopamine (6-OHDA),  $\text{Ca}^{2+}$  and mitochondrial dysfunction) can regulate, or be regulated by, the extracellular signal-regulated kinases 1/2 (ERK) also known as p42/p44 extracellular mitogen-activated protein kinases (MAPK). ERK is part of a highly conserved signaling module including its direct upstream activator, MEK1/2, a serine-threonine kinase, and RAF-1 or B-RAF, the MEK activators, the latter being highly expressed in the brain. ERK is present within the cytoplasm and translocates into the nucleus upon activation following a variety of stimuli such as growth factors, depolarizing signals and neurotransmitters, including DA, and glutamate (Glu). ERK is also present in the mitochondria of neurons as well as of non-neuronal cells. Alterations of ERK signaling has been involved in the pathophysiology of several neurodegenerative diseases [41,42]. An increase of phosphorylated ERK is found in the cytoplasm and mitochondria of mDA neurons of patients with PD and dementia with Lewy bodies [43]. Moreover, chemical and genetic models of PD and/or neurotoxicity have shown that ERK can exert an essential role in mediating the noxious effects of various pro-cell death stimuli, including the well-known selective dopaminergic toxic compounds such as 6-OHDA, 1-metil 4-fenil 1,2,3,6-tetraido-piridine (MPTP)/MPP<sup>+</sup>, rotenone and high doses of dopamine. In particular, the study of 6-OHDA has shed light on the role of ERK in DA neurons cell death. Although widely used as an exogenous neurotoxin to generate cellular and animal models of DA neurons cell death, 6-OHDA can be produced in vivo from dopamine and it has been found in the urine of PD patients treated with levodopa [44]. 6-OHDA is capable to generate ROS including  $\text{H}_2\text{O}_2$  and superoxide that are highly toxic in mDA neurons. Interestingly, experimental findings show that the mechanisms of cell death, at least partly, are mediated by the activation of ERK induced by ROS; thus, ERK blockade using MEK inhibitors is able to protect cells from death [45]. Surprisingly, ERK is also a pivotal mediator of many pro-survival as well as differentiation factors such as neurotrophins and growth factors [46]. It has been proposed that the reason underlying such a paradox may lie upon a different kinetic of ERK activation. Indeed, in PC12 cells, as well as in neurons, transient or sustained ERK kinetics determine its noxious or beneficial effects [41,47–50]. In turn, the different kinetics of activation is shown to mediate, at least partly, the cellular compartmentalization of ERK. Sustained activation of ERK leads to its consistent translocation into the nucleus and cell death, whereas a transient activation, localizing

ERK mainly in the cytoplasm, has a protective effect [51]. Cell type and the nature of the stimulus are likely to play an important role in determining the temporal and spatial pattern of ERK signaling. Thus, in order to plan any pharmacological intervention, it appears to be of utmost importance to unveil the cellular and molecular mechanisms underlying pro cell death or survival effects of ERK signaling in mDA neurons. Besides being involved in the pathogenesis of neurodegeneration, ERK signaling also plays an important role in the physiological processes of neurogenesis as well as the maturation of mDA neural progenitors [52]. Neurogenic stem cell niches remain active in the adult brain, giving rise to neurons in the hippocampus and the olfactory bulb, the latter being composed, among the others, of dopaminergic neurons [53]. Notably, in the adult subventricular zone (SVZ), one of the two neurogenic areas, IL-10 is essential to foster neurogenesis by regulating the activation of ERK and STAT 3 in nestin<sup>+</sup> neural progenitors. Indeed, ERK blockade inhibits neurogenesis in SVZ [54]. Moreover, elegant experiments using D2R<sup>-/-</sup> mice clearly showed that in midbrain neuronal cultures, Wnt5a controls the number of TH neurons as well as their morphogenic features such as neurite length through ERK activation [52].

### 3. Pathways Involved in the Priming Process of the Ventral Midbrain and mDA Precursor Neurogenesis

Given the role of mDA neurons in PD and neuropsychiatric diseases, intense research activity in the last decades has been directed to unravel the mechanisms of DA phenotype induction and maturation and to elucidate the role of factors involved in the specification, development and maintenance of these neurons and their functions [55]. This remarkable research activity is also based on the assumption that the identification of molecules and cellular interactions involved in the development of the mDA circuits may be the key to generate mDA neurons *in vitro* for use in regenerative medicine for PD patients.

The development of mDA neurons is a highly coordinated process, which requires the activity of numerous morphogens and transcription factors. The genes encoding this complex network are characterized by time- and cell-specific gene expression patterns that regulate their complex interaction. This articulated differentiation process begins with the establishment of the midbrain-hindbrain boundary by the mutually repressive activities of orthodenticle homeobox 2 (Otx2) in the midbrain and gastrulation brain homeobox 2 (Gbx2) in the hindbrain. In addition, Otx2 suppresses the expression of the transcription factor NK2 Homeobox 2 (Nkx2.2), thus preventing the formation of serotonergic neurons [56]. Therefore, this transcriptional complex is also necessary to suppress non-dopaminergic neural fates. Otx2 also regulates the expression of “proneural” genes (that is, they promote neural differentiation) in the proliferating mDA progenitors, such as Mash1, also known as Ascl1 (Achaete-Scute Family BHLH Transcription Factor 1) and Neurogenin 2 (Ngn2). Transcription factor Foxa2 (also known as HNF3 $\beta$ ) is amongst the first expressed in the ventral midbrain. Gain- and loss-of-function experiments indicate that Foxa2 is necessary to produce mDA neurons during development, as well as from embryonic stem cells and fibroblasts *in vitro*, and to maintain Nurr1 expression in engineered pluripotent stem cells [57]. Foxa2 is also expressed in adult DA neurons and seems to regulate their survival. Mice with one copy of this gene display loss of mDA neurons [58].

#### 3.1. The Role of Morphogens and Their Effectors

The commitment of neuroblasts to the mDA phenotype is promoted by the secretion by the floor plate of sonic hedgehog (SHH) [59] and its synergy with fibroblast growth factor 8 (FGF8), secreted *in vivo* by the isthmus [60]. These two morphogens establish an epigenetic cartesian grid that defines positional information necessary for mDA induction [61]. SHH activates two transmembrane receptors, Patched (PTC) and Smoothened, which elicit an intracellular response with the activation of the zinc-finger transcription factor Gli1 (glioma-associated oncogene 1). The latter therefore represents an early marker of the mDA precursors. Both the floor plate and the isthmus express the basic helix-loop-helix (bHLH) transcription factor Hes1, which suppresses proneural gene expression

and induces cell cycle exit, at about day 9 during mouse embryogenesis (E9) [62]. The loss of Hes1 modifies the inductive and repulsive activities of the isthmic organizer. Hes1 regulates the localization and density of mDA neurons [63]. Several genes are required for the correct positioning of the isthmus and the secretion of FGF8. These include the paired box gene 2 (Pax2), Lmx1b, Wingless-type MMTV integration site family member 1 (Wnt1) and Engrailed-1 (En1) (for review, see [64]). It is now established that mDA precursors are floor plate radial glial-like cells [65]. In these precursor neuroblasts, SHH regulates the expression of the Lmx1a, which is necessary to commit midbrain neuroblasts as well as human embryonic stem cells to mDA fate [66,67]. Lmx1a, in turn, activates the expression of Msh homeobox 1 (Msx1), which induces the bHLH proneural gene Ngn2 expression. Lmx1a and Ngn2 gene ablation or silencing result in absence or reduction in the number of mDA neurons. Direct involvement of the morphogen SHH in mDA differentiation has been recently questioned [68]. This conclusion is drawn from the lack of PTC or Gli1 receptors in more mature mDA developing neurons. It has been suggested that other receptors (such as Cdon, Boc and Gas1), in addition to PTC, could modulate SHH signaling activity [69], or that SHH is only indirectly involved in mDA development through early patterning of the midbrain. However, a plethora of in vitro experiments point to a direct role of SHH in mDA differentiation. Thus, in the recent view, SHH is necessary for midbrain progenitors to acquire a dopaminergic cell fate in vitro and in vivo. Furthermore, a direct role of SHH in mDA development is indicated by its induction of the Lmx1a and its downstream gene Msx expression. While Lmx1a expression is maintained in post-mitotic mDA neurons in mice, Msx1 expression is restricted to the mDA neuroblasts [70]. The important role of Msx1 in mDA neurogenesis is revealed by its induction of Ngn2 expression. The latter is also indirectly controlled by Otx2, through the regulation of Lmx1a expression. A well-established role in mDA differentiation in early (mouse E8-9) and more mature (mouse E13) developing mDA neurons has been attributed to the canonical Wnt signaling pathway [71,72]. Indeed, Wnt1 induces the proliferation of mDA precursors by expanding their pool, while Wnt family member 5A (Wnt5a) increases mDA differentiation. These two proteins work by activating the G protein-coupled receptor frizzled, which in turn activates the cytosolic Dishevelled phosphoprotein; the latter inhibits glucose synthetase kinase 3 and blocks phosphorylation of  $\beta$ -catenin and its degradation.  $\beta$ -catenin can thus enter the nucleus, activating a transcriptional response, or causing an increase in intracellular  $\text{Ca}^{2+}$  through the frizzled receptors and the activation of intracellular protein Dishevelled.  $\beta$ -catenin is a subunit of the cadherin protein complex and the major intracellular signal transducer in the Wnt signaling pathway [73]. Finally, together with Foxa1 and Foxa2, the transcription factors En1 and 2 are also implicated in the determination, differentiation and maintenance of mature mDA neurons and prevent their death from apoptosis [74,75]. Wnt1 and En1 are expressed in graded fashion along the rostrocaudal axis and are more expressed near the mid-hindbrain boundary [76]. Lmx1A, Foxa2 and Otx2 are commonly used to identify mDA progenitors during development [77] and in stem cell cultures [78–80]. However, a recent study has revealed that these potential ventral midbrain markers, as well as several other mDA genes, are not sufficient to predict yield or functionality of these cells in vivo since they are also co-expressed in the subthalamic nucleus [22]. Interestingly, current data show that markers expressed by midbrain cells close to the midbrain-hindbrain boundary (i.e., En1, ETS Variant Transcription Factor 5, Canopy FGF Signaling Regulator 1, PAX8 and Sprouty RTK Signaling Antagonist 1) correlate with a successful graft outcome [81].

### 3.2. Genes Involved in the Acquisition and Stabilization of the Functional mDA Phenotype

A number of transcription factors necessary for the development of mDA neurons and their occurrence during development have been described and extensively investigated [82].

Up to E13.5 of mouse embryogenesis, SN and VTA mDA precursors appear indistinguishable, although these two subpopulations of mDA neurons have different markers already at the neural progenitor cell stage. These are the transcription factor Sox6 (or SRY-box 2, sex-determining region Y box 6), known to be involved in establishing pluripotency in stem cells, which is expressed mainly in the

SNc neurons, and both Otx2 and the zinc-finger transcription factor Nolz1, which are expressed in VTA precursors [83]. It is worth mentioning that mDA neurons co-express and often co-release GABA/or Glu neurotransmitters. VGLUT2 is more broadly expressed in mDA neurons during development [84–86], but its expression pattern is restricted to smaller subsets in adult mouse, in particular in the medial regions of VTA [87–91]. Recent evidence indicates that VGLUT2 co-expression in SNc DA neurons re-emerges in adult mice after DA neuron insult. Interestingly, conditional deletion of VGLUT2 makes DA neurons more vulnerable to neurotoxins, suggesting that the physiological balance of VGLUT2 expression is crucial to DA neuron survival [92].

As previously described, a sequential gene-cohort activation is required to promote the expression of genes, including other transcription factors, that are essential to complete the dopaminergic program in already determined mDA precursors. These genes include Nurr1, which is an “orphan” nuclear receptor belonging to the steroid-thyroid receptor family, the homeogene Pitx3 (Paired-like homeodomain 3), En1 and 2 and Lmx1b. It must be noted that none of these transcription factors alone is sufficient to mature all aspects of the mDA phenotype, indicating that they must act in a coordinated and combinatorial fashion.

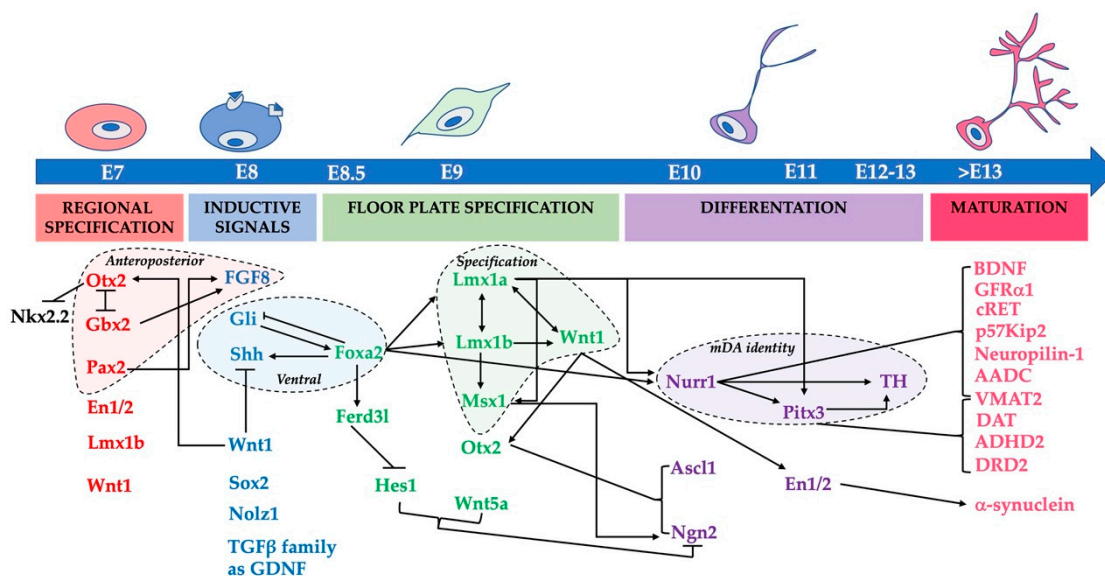
Nurr1 (NR4A2) exerts a fundamental role [93]; its expression is under a complex regulation, which includes the activity of a battery of genes as well as depolarization [94]. Nurr1 null mice show loss of mDA neurons, which initially express the dopaminergic transcription factor Pitx3 (see below), but subsequently degenerate and die [95]. Thus, Nurr1 is necessary for the development as well as for the maintenance of mDA neurons. Indeed, Nurr1 regulates the expression of various genes governing DA function, including TH, the rate-limiting enzyme in the biosynthesis of catecholamines, which synthesizes L-DOPA from tyrosine; the aromatic L-amino acid decarboxylase (AADC), which transforms L-DOPA in DA (although its dependence from Nurr1 is not ascertained, as shown in Smits et al. [96]. In addition, Nurr1 regulates the expression of the VMAT2 [97], which concentrates the catecholamines from the cytoplasm into the synaptic vesicles; the cytoplasmic dopamine transporter DAT [98]; the cyclin kinase inhibitor p57/Kip2 [99], which is a negative regulator of cell proliferation; neuropilin-1, a receptor protein of the Vascular Endothelial Growth Factor (VEGF) cytokine family, involved in axon guidance and angiogenesis [97]. Moreover, Nurr1 also regulates the response to neurotrophic factors such as glial derived neurotrophic factor (GDNF) by controlling the expression of its receptors Ret and GDNF Family Receptor alpha (GFR $\alpha$ ) [100,101]. Nurr1 also controls the expression of the brain derived neurotrophic factor (BDNF), which in this case could act as an autocrine signal, being involved in a neuroprotective loop for mDA neurons [102]. Nurr1 and Foxa2 act synergistically in microglia decreasing the production and release of proinflammatory cytokines and enhancing the synthesis and secretion of many trophic/pleiotropic molecules (GDNF, BDNF, Neurotrophin 3, SHH, erythropoietin, thioredoxin, transforming growth factor family  $\beta$  and Insulin like Growth Factor 1) [103].

Although olfactory bulb DA neurons express Nurr1, no change in TH in these neurons is observed in Nurr1 null mutant mice, whereas in these mutants there is the loss of mDA. Apparently, Nurr1 plays a role in retina DA neurons differentiation, but has no role in hypothalamic DA neurons [104]. Nevertheless olfactory dysfunction is present in PD patients, and often smell alterations represent a first symptom of the disease [105].

Nurr1 binds to DNA as a monomer, homodimer or heterodimer with retinoid X receptor (RXR)  $\alpha$  or RXR $\gamma$ . In midbrain DA neurons, Nurr1 heterodimerizes with RXR $\alpha$ , and heterodimer activation in mouse and cellular models of PD has a protective effect on mDA neurons, increases TH and other DA functional proteins, augments DA in the striatum and ameliorates and improves symptoms in genetic PD animal models [106]. Nurr1 also has a role in the induction of Pitx3 expression, unveiling a strict relationship between these two transcription factors [107]. Although Pitx3 is expressed in both SN and VTA neurons, the Pitx3-deficient aphakia mouse mutant shows a selective loss of DA neurons only in the SN [108]. Indeed, DA cell physiology does differ between SN and VTA mDA neurons, although these midbrain areas are subject to many common regulatory pathways.

The maturation and survival of mDA neurons *in vitro* and *in vivo* is regulated by several families of transcription factors, including BDNF [109,110] and GDNF [111,112]. It has been demonstrated that conditional deletion of GDNF in adult mice causes mDA neuronal loss [113] indicating that GDNF is necessary for mDA neuron survival. Similarly, silencing of gene encoding BDNF in mice results in loss of DA neurons. Moreover, GDNF has been suggested to regulate BDNF expression via a GDNF-Pitx3-BDNF trophic loop [114]. BDNF treatment in animal models before the induction of PD prevented the loss of SN DA neurons and their projections to the striatum. BDNF delivery after induction of PD did not alter the number of DA neurons; however, it elevated the DA level in striatum, increased synaptic plasticity and induced DA axon regrowth [115]. It remains unclear whether the delivery of neurotrophic factors by gene therapy in humans reduces the progression of PD, and the results of the initial trials have been disappointing [115,116]. To date, the delivery of neurotrophic factors to the dorsal striatum of animal models by gene therapy has proved very effective for neurorestoration of dopaminergic neurons [117,118].

Thus, multiple lines of evidence indicate that the network formed by morphogens, transcriptional factors and neurotrophic factors promotes mDA differentiation and survival. In summary, the large number of genes involved in mDA neuron development allows the distinction of five sequential stages schematized in Figure 2.



**Figure 2.** Genetic networks controlling midbrain dopaminergic neuron development in the mouse brain. The diagram summarizes the sequential stages and the molecules involved in mouse midbrain DA neuron embryonic development. The expression of transcription factors (TF) and secreted molecules involved in midbrain regional specification, induction, floor plate specification, differentiation and maturation of the mDA phenotype are indicated, at various embryonic (E) ages. Lines depict possible interactions among these molecules. Arrows indicate stimulatory effects, while perpendicular lines denote inhibitory effects. The orange area, delimited by a dotted line, clusters the TFs and molecules involved in anteroposterior patterning (see text). The light blue area, delimited by a dotted line, clusters the TFs involved in ventral patterning (see text). The green area, delimited by a dotted line, groups the TFs involved in midbrain floor plate specification (see text). The violet area, delimited by a dotted line, collects the TFs involved in mDA identity (see text).

Recent advances in the field of single-cell transcriptomics and in bioinformatics studies have enabled detection of coordinated gene expression profiles within individual cells [119]. In the last years at least six independent groups have identified molecularly distinct populations of mDA neurons, but their location and molecular signature are only partially overlapping (reviewed by Poulin et al. [14]). For instance, La Manno et al. [120], identified three types of embryonic DA neurons during E11.5



-E18.5 mouse development: i) immature cells expressing TH and other makers of DA neurons, ii) mature neurons expressing TH and DAT and iii) mature neurons expressing—in addition to TH and DAT—also Aldehyde Dehydrogenase 1 family member A1 (Aldh1a1) and LIM Domain Only 3 (Lmo3). Conversely, Hook et al. [121], identified only two embryonic populations at E15 mouse development: a neuroblast population and more mature neurons expressing high levels of TH and DAT. Finally, Tiklova et al. [122] demonstrated that different subgroups of mDA neurons begin to diversify as early as E13 and this process continues during development. These results strongly suggest that after DA neuron maturation and integration into defined neuronal circuits, neurons continue to refine their molecular signature. Additional studies conducted during postnatal development demonstrated that combinatorial expression of Gastrin releasing peptide (Grp) and Neuronal differentiation 6 (Neurod6) defines a dopaminergic subpopulation that resides in the ventromedial VTA and sends projections to the medial shell of the nucleus accumbens [123]. In summary, single-cell gene expression profiling studies, although partially discrepant, highlight the great molecular heterogeneity of the midbrain DA system, challenging the traditional anatomically-based classifications.

How and when DA neuron diversity is generated during development remains unknown, and at the moment, it is not possible to control this subtype diversity during stem cell differentiation. An accurate and molecular definition of mDA subtypes will be crucial for understanding molecular cascades underlying the selective vulnerability in PD, and for enabling an accurate differentiation of iPSC in different DA subtypes.

#### **4. Interplay between miRNAs, Dopaminergic Neurons Differentiation, Maintenance and Dysfunction**

In recent years, posttranscriptional regulation of gene expression has emerged as an additional important regulatory mechanism in neuronal differentiation, in addition to well defined transcriptional programs. microRNAs (miRNAs) appear crucial in regulating neuronal differentiation and neural circuit development. miRNAs are a class of evolutionary conserved small non-coding single-strand RNA molecules, 18–25 nucleotide long. They are transcribed as long pre-miRNAs, which are processed by subsequent RNase III activities (Drosha in the nucleus and Dicer in the cytoplasm) forming a duplex, one strand of which is then loaded in a silencing complex. miRNAs regulate gene expression by binding to mRNAs containing a miRNA recognition sequence [124]. miRNAs are expressed abundantly within the nervous system in a tissue-specific manner [125] and are crucial players in several biological processes including neurogenesis, neuronal maturation, synapses formation, axon guidance, neurite outgrowth and neuronal plasticity [126–128]. Alterations in miRNAs contribute to the pathogenic mechanisms in neurodegenerative diseases, including PD [129]. The essential role of miRNAs in neurodegenerative disorders has been highlighted using Dicer knock-out mice [130,131]. Dicer is the cytoplasmatic ribonuclease III and is essential to generate mature miRNA [132]. Several studies have demonstrated that cell type-specific deletion of miRNAs expressed in mDA neurons causes progressive loss of these cells [133], suggesting a pivotal role for miRNAs in mDA neuron formation, survival and function. The relationship between miRNAs and mDA neuron differentiation, as well as miRNAs dysregulation and mDA system dysfunction, such as PD, has been intensively studied in recent years [133,134]. Thus, the detection of miRNAs expressed in mDA neurons could bring about promising information for the early diagnosis and prognosis of PD and offer new potential pharmacological tools.

Overexpression of miRNAs has emerged as a potential additional strategy to improve DA survival in the attempt to counteract the progressive neuron loss in PD and related disorders. In addition, this approach represents an attractive alternative to increase in vitro dopaminergic differentiation starting from undifferentiated ES cells or through the reprogramming of human fibroblasts. In this context, two microRNAs, miR-34b/c and miR-218, have been very promising, since once expressed in combination with specific DA-related transcription factors are able to increase the overall yield of functional DA neurons. The overexpression of miR-34b/c together with the transcription factors *Ascl1*

and Nurr1 reduces Wnt1 expression and promotes maturation into functional DA neurons that show the typical electrophysiological properties of DA neurons, consisting in a burst of action potential firing pattern [24].

In the same way, miR-218 combined with Neurod1, Ascl1 and Lmx1a is able to promote in vivo the transdifferentiation of astrocytes into fully functional DA neurons and rescues, at least partially, the progressive loss of DA neurons [67]. In both cases, miRNAs, rather than promoting by themselves the differentiation of DA neurons, act to potentiate the effect of specific transcription factors. These results make miRNAs particularly interesting, from a pharmacological point of view, and highlight their potential for therapeutic purposes.

Interestingly, miR-34b/c expression is significantly downregulated in brain areas of PD patients with different degrees of disease, suggesting their role in the early and late phases of the pathology. Here, the miR-34b/c cluster exerts a protective role by regulating Parkin and DJ-1, two proteins associated with familial forms of PD whose expression is affected in PD brain [135]. Similarly, the downregulation of miR-34b/c in differentiated SH-SY5Y neuroblastoma causes cell vitality reduction, affecting mitochondrial function and oxidative stress [135,136].

Among the various miRNAs expressed abundantly in the brain, miR-7a seem to be important to control the site-specific generation of DA neurons in the mouse forebrain [137]. miR-7a inhibits the expression of Pax6, a key transcription factor that controls DA neuron differentiation, along the entire ventricle wall except for the most dorsal part. There, the absence of miR-7a expression allows Pax6 expression, and hence, the occurrence of DA neuron differentiation [137].

In addition, miR-132 has been shown able to regulate the differentiation of DA neurons by directly targeting Nurr1 [134], suggesting its importance for DA neuronal maturation and maintenance. It is now well established that miR-132-3p targets important pathways involved in neuronal plasticity such as long term potentiation, DNA methylation and neuronal cAMP response element-binding protein, N-methyl-D-aspartate receptor and BDNF signaling [138]. On the other hand, the role of miR-132 in PD is not yet totally established. Contrasting evidence has been reported, showing downregulation of miR-132 in the brain of an  $\alpha$ -syn transgenic mouse [139], or upregulation of miR-132 in a rat mouse model of PD [140]. In addition, the expression of miR-132-3p was three times higher in the peripheral blood lymphocytes of treated PD patients compared to untreated PD patients [141]. miRNAs also act as protectors of neurons through inhibition of neuroinflammation, apoptosis or autophagy [142]. miR-132 regulates the inflammatory response, while miR-124 plays a protective role in cellular models MPTP-intoxicated SH-SY5Y through the involvement of cell apoptosis and autophagy [143]. At the same time, in MPTP-induced mouse models of PD, miR-124 overexpression promotes DA receptor expression and neuronal proliferation and suppresses neuronal apoptosis by downregulating endothelin 2 through activation of the SHH signaling pathway [144]. Similarly, miR-410 exerts neuroprotective effects in a 6-OHDA cellular model of PD by inhibiting the PTEN/AKT/mTOR signaling pathway [145].

Basically, it can be said that miRNAs play an important role in neurogenesis and survival, by conferring identity and specificity to subtypes of neural cells and modulating the spatial and temporal expression profiles of some fundamental genes. In this context, they represent an important class of molecules useful to better understand PD pathology that could be used as biomarkers in diagnosis and prognosis of this neurodegenerative disorder, as well as new promising pharmacological tools. Knowledge of miRNA-based regulation of the chosen neural phenotype could help develop new strategies for obtaining different types of animal and human neuronal cells to replace damaged mDA neurons.

## 5. Conclusions

The elucidation of the gene network involved in mDA neuron development and function could open new avenues and will be crucial to identify early changes of DA neurons that occur in the pre-symptomatic stages of PD. It can help identify targets for effective new therapies and for

regenerative medicine through lineage reprogramming of other cell types (iPSC cells, neurons, glial cells and fibroblasts) into dopaminergic neurons. In addition, understanding the role of genetic and epigenetic factors involved in mDA neurons ontogeny and survival may provide new information for pharmacological manipulation of DA neurons in DA-related disorders, such as schizophrenia, drug addiction, ADHD, depression and chronic pain.

**Author Contributions:** Writing and editing, F.V.; original draft preparation and editing, C.P.-C., G.C.B. and L.C.-D.; original draft preparation, writing and review, U.d.P. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by MIUR-PRIN 2015R9ASHT\_003 and MIUR-PRIN 2017T9JNLT\_004 to G.C.B.; VALERE: VANviteLli pEr la RicErca to L.C.D.A

**Conflicts of Interest:** The authors declare no conflict of interest.

## Abbreviations

AADC	Dopa Decarboxylase (Aromatic L-amino acid decarboxylase)
ADHD	Attention Deficit Hyperactive Disorder
ALDH1A1	Aldehyde Dehydrogenase 1 family member A1
Ascl1	Achaete-scute family bHLH transcription factor 1
BDNF	Brain Derived Neurotrophic Factor
$\alpha$ -syn	$\alpha$ -synuclein
bHLH	basic Helix–Loop–Helix
CNS	Central Nervous System
DA	Dopamine
DAT	Dopamine transporter (SLC6A3)
Dbh	$\beta$ -hydroxylase
Ddc	Gene encoding AADC
En1	Engrailed-1
ENS	Enteric Nervous System
ERK	Extracellular-signal regulated kinases 1/2
FGF8	Fibroblast Growth Factor 8
Foxa1/2	Forkhead box A1/2
Gbx2	Gastrulation brain homeobox 2
GDNF	Glial Derived Neurotrophic Factor
GFAP	Glial Fibrillary Acid Protein
GFRa	GDNF Family Receptor alpha
Gli1	Glioma associated oncogene 1
Glu	Glutamate
GRP75	Glucose Regulated Protein 75
HDAC4	Histone deacetylase 4
Grp	Gastrin releasing peptide
LMX1a/b	LIM homeobox transcription factor 1 alpha/beta
Lmo3	LIM Domain Only 3
MAPK	Mitogen-activated protein kinases
mDA	Midbrain dopaminergic
mES	Mouse Embryonic Stem cells
miRNAs	microRNAs
MPTP	1-Methyl-4-phenyl-1,2,3,6 tetrahydropyridine
Msx1	Msh homeobox 1
mtHsp70	Mitochondrial heat shock protein 70
mTOR	Mechanistic target of rapamycin (serine/threonine kinase)
Neurod6	Neuronal differentiation 6
Ngn2	Neurogenin 2
Nkx2.2	NK2 Homeobox 2

Nurr1	Nuclear Receptor Subfamily 4 Group A Member 2 (NR4A2)
NRLP3	Nod-like Receptor Protein 3 inflammasome
6-OHDA	6-hydroxydopamine
Otx2	Orthodenticle homeobox 2
Pax2	Paired box gene 2
PD	Parkinson's Disease
Pitx3	Paired-like homeodomain 3
PTC	Patched
Ret	Ret protooncogene
ROS	Reactive oxygen species
RXR	Retinoid X Receptor
SHH	Sonic Hedgehog
SN	Substantia nigra
SNc	Substantia nigra pars compacta
SNG1	Small nucleolar RNA host gene 1
Sox2	SRY-2 (Sex determining region Y) Box 2
Sox10	SRY-10 (Sex determining region Y) Box 10
SVZ	Subventricular zone
TH	Tyrosine Hydroxylase
VEGF	Vascular Endothelial Growth Factor
VGLUT2	Vesicular Glutamate Transporter 2
VMAT2	Vesicular Monoamine Transporter 2
VTA	Ventral Tegmental Area
Wnt1	Wingless-type MMTV integration site family member 1

## References

1. Bayer, S.A.; Wills, K.V.; Triarhou, L.C.; Ghetti, B. Time of neuron origin and gradients of neurogenesis in midbrain dopaminergic neurons in the mouse. *Exp. Brain Res.* **1995**, *105*, 191–199. [[CrossRef](#)]
2. Bonilla, S.; Hall, A.C.; Pinto, L.; Attardo, A.; Götz, M.; Huttner, W.B.; Arenas, E. Identification of midbrain floor plate radial glia-like cells as dopaminergic progenitors. *Glia* **2008**, *56*, 809–820. [[CrossRef](#)] [[PubMed](#)]
3. Di Porzio, U.; Zuddas, A.; Cosenza-Murphy, D.B.; Barker, J.L. Early appearance of tyrosine hydroxylase immunoreactive cells in the mesencephalon of mouse embryos. *Int. J. Dev. Neurosci.* **1990**, *8*, 523–532. [[CrossRef](#)]
4. Kawano, H.; Ohyama, K.; Kawamura, K.; Nagatsu, I. Migration of dopaminergic neurons in the embryonic mesencephalon of mice. *Brain Res. Dev. Brain Res.* **1995**, *86*, 101–113. [[CrossRef](#)]
5. Schultz, W.; Dayan, P.; Montague, P.R. A neural substrate of prediction and reward. *Science* **1997**, *275*, 1593–1599. [[CrossRef](#)] [[PubMed](#)]
6. Poewe, W.; Seppi, K.; Tanner, C.M.; Halliday, G.M.; Brundin, P.; Volkmann, J.; Schrag, A.E.; Lang, A.E. Parkinson disease. *Nat. Rev. Dis. Primers* **2017**, *3*, 17013. [[CrossRef](#)] [[PubMed](#)]
7. Leo, D.; Sorrentino, E.; Volpicelli, F.; Eyman, M.; Greco, D.; Viggiano, D.; di Porzio, U.; Perrone-Capano, C. Altered midbrain dopaminergic neurotransmission during development in an animal model of ADHD. *Neurosci. Biobehav. Rev.* **2003**, *27*, 661–669. [[CrossRef](#)]
8. Purves-Tyson, T.D.; Owens, S.J.; Rothmond, D.A.; Halliday, G.M.; Double, K.L.; Stevens, J.; McCrossin, T.; Shannon Weickert, C. Putative presynaptic dopamine dysregulation in schizophrenia is supported by molecular evidence from post-mortem human midbrain. *Transl. Psychiatry* **2017**, *7*, e1003. [[CrossRef](#)]
9. Li, A.; Zalesky, A.; Yue, W.; Howes, O.; Yan, H.; Liu, Y.; Fan, L.; Whitaker, K.J.; Xu, K.; Rao, G.; et al. A neuroimaging biomarker for striatal dysfunction in schizophrenia. *Nat. Med.* **2020**, *26*, 558–565. [[CrossRef](#)]
10. Serafini, R.A.; Pryce, K.D.; Zachariou, V. The Mesolimbic Dopamine System in Chronic Pain and Associated Affective Comorbidities. *Biol. Psychiatry* **2020**, *87*, 64–73. [[CrossRef](#)]
11. Pakkenberg, B.; Møller, A.; Gundersen, H.J.; Mouritzen Dam, A.; Pakkenberg, H. The absolute number of nerve cells in substantia nigra in normal subjects and in patients with Parkinson's disease estimated with an unbiased stereological method. *J. Neurol. Neurosurg. Psychiatry* **1991**, *54*, 30–33. [[CrossRef](#)] [[PubMed](#)]

12. Morello, F.; Partanen, J. Diversity and development of local inhibitory and excitatory neurons associated with dopaminergic nuclei. *FEBS Lett.* **2015**, *589*, 3693–3701. [[CrossRef](#)] [[PubMed](#)]
13. Anderegg, A.; Poulin, J.F.; Awatramani, R. Molecular heterogeneity of midbrain dopaminergic neurons—Moving toward single cell resolution. *FEBS Lett.* **2015**, *589*, 3714–3726. [[CrossRef](#)] [[PubMed](#)]
14. Poulin, J.F.; Gaertner, Z.; Moreno-Ramos, O.A.; Awatramani, R. Classification of Midbrain Dopamine Neurons Using Single-Cell Gene Expression Profiling Approaches. *Trends Neurosci.* **2020**, *43*, 155–169. [[CrossRef](#)]
15. Björklund, A.; Dunnett, S.B. Dopamine neuron systems in the brain: An update. *Trends Neurosci.* **2007**, *30*, 194–202. [[CrossRef](#)]
16. Buttarelli, F.R.; Fanciulli, A.; Pellicano, C.; Pontieri, F.E. The dopaminergic system in peripheral blood lymphocytes: From physiology to pharmacology and potential applications to neuropsychiatric disorders. *Curr. Neuropharmacol.* **2011**, *9*, 278–288. [[CrossRef](#)]
17. Perrone-Capano, C.; Tino, A.; di Porzio, U. Target cells modulate dopamine transporter gene expression during brain development. *Neuroreport* **1994**, *5*, 1145–1148. [[CrossRef](#)]
18. Lammel, S.; Steinberg, E.E.; Földy, C.; Wall, N.R.; Beier, K.; Luo, L.; Malenka, R.C. Diversity of transgenic mouse models for selective targeting of midbrain dopamine neurons. *Neuron* **2015**, *85*, 429–438. [[CrossRef](#)]
19. Pristerà, A.; Lin, W.; Kaufmann, A.K.; Brimblecombe, K.R.; Threlfell, S.; Dodson, P.D.; Magill, P.J.; Fernandes, C.; Cragg, S.J.; Ang, S.L. Transcription factors FOXA1 and FOXA2 maintain dopaminergic neuronal properties and control feeding behavior in adult mice. *Proc. Natl. Acad. Sci. USA* **2015**, *112*, E4929–E4938. [[CrossRef](#)]
20. Yan, C.H.; Levesque, M.; Claxton, S.; Johnson, R.L.; Ang, S.L. Lmx1a and lmx1b function cooperatively to regulate proliferation, specification, and differentiation of midbrain dopaminergic progenitors. *J. Neurosci.* **2011**, *31*, 12413–12425. [[CrossRef](#)]
21. Nouri, N.; Awatramani, R. A novel floor plate boundary defined by adjacent En1 and Dbx1 microdomains distinguishes midbrain dopamine and hypothalamic neurons. *Development* **2017**, *144*, 916–927. [[CrossRef](#)] [[PubMed](#)]
22. Kee, N.; Volakakis, N.; Kirkeby, A.; Dahl, L.; Störvall, H.; Nolbrant, S.; Lahti, L.; Björklund, Å.; Gillberg, L.; Joodmardi, E.; et al. Single-Cell Analysis Reveals a Close Relationship between Differentiating Dopamine and Subthalamic Nucleus Neuronal Lineages. *Cell Stem Cell* **2017**, *20*, 29–40. [[CrossRef](#)] [[PubMed](#)]
23. Kang, Y.; Kitai, S.T. Calcium spike underlying rhythmic firing in dopaminergic neurons of the rat substantia nigra. *Neurosci. Res.* **1993**, *18*, 195–207. [[CrossRef](#)]
24. Caiazzo, M.; Dell’Anno, M.T.; Dvoretzskova, E.; Lazarevic, D.; Taverna, S.; Leo, D.; Sotnikova, T.D.; Menegon, A.; Roncaglia, P.; Colciago, G.; et al. Direct generation of functional dopaminergic neurons from mouse and human fibroblasts. *Nature* **2011**, *476*, 224–227. [[CrossRef](#)] [[PubMed](#)]
25. De Gregorio, R.; Pulcrano, S.; De Sanctis, C.; Volpicelli, F.; Guatteo, E.; von Oerthel, L.; Latagliata, E.C.; Esposito, R.; Piscitelli, R.M.; Perrone-Capano, C.; et al. miR-34b/c Regulates Wnt1 and Enhances Mesencephalic Dopaminergic Neuron Differentiation. *Stem Cell Reports* **2018**, *10*, 1237–1250. [[CrossRef](#)] [[PubMed](#)]
26. Meiser, J.; Weindl, D.; Hiller, K. Complexity of dopamine metabolism. *Cell Commun. Signal.* **2013**, *11*, 34. [[CrossRef](#)]
27. Surmeier, D.J.; Halliday, G.M.; Simuni, T. Calcium, mitochondrial dysfunction and slowing the progression of Parkinson’s disease. *Exp. Neurol.* **2017**, *298*, 202–209. [[CrossRef](#)]
28. Lang, C.; Campbell, K.R.; Ryan, B.J.; Carling, P.; Attar, M.; Vowles, J.; Perstenko, O.V.; Bowden, R.; Baig, F.; Kasten, M.; et al. Single-Cell Sequencing of iPSC-Dopamine Neurons Reconstructs Disease Progression and Identifies HDAC4 as a Regulator of Parkinson Cell Phenotypes. *Cell Stem Cell* **2019**, *24*, 93–106. [[CrossRef](#)]
29. Tran, H.T.; Chung, C.H.; Iba, M.; Zhang, B.; Trojanowski, J.Q.; Luk, K.C.; Lee, V.M. A-synuclein immunotherapy blocks uptake and templated propagation of misfolded  $\alpha$ -synuclein and neurodegeneration. *Cell Rep.* **2014**, *7*, 2054–2065. [[CrossRef](#)]
30. Mehra, S.; Sahay, S.; Maji, S.K.  $\alpha$ -Synuclein misfolding and aggregation: Implications in Parkinson’s disease pathogenesis. *Biochim. Biophys. Acta Proteins Proteom.* **2019**, *1867*, 890–908. [[CrossRef](#)]
31. Rocha, E.M.; De Miranda, B.; Sanders, L.H. Alpha-synuclein: Pathology, mitochondrial dysfunction and neuroinflammation in Parkinson’s disease. *Neurobiol. Dis.* **2018**, *109*, 249–257. [[CrossRef](#)]

32. Colucci-D'Amato, L.; Farina, A.; Vissers, J.P.; Chambery, A. Quantitative neuroproteomics: Classical and novel tools for studying neural differentiation and function. *Stem Cell Rev. Rep.* **2011**, *7*, 77–93. [[CrossRef](#)] [[PubMed](#)]
33. Colucci-D'Amato, L.; Cicatiello, A.E.; Reccia, M.G.; Volpicelli, F.; Severino, V.; Russo, R.; Sandomenico, A.; Doti, N.; D'Esposito, V.; Formisano, P.; et al. A targeted secretome profiling by multiplexed immunoassay revealed that secreted chemokine ligand 2 (MCP-1/CCL2) affects neural differentiation in mesencephalic neural progenitor cells. *Proteomics* **2015**, *15*, 714–724. [[CrossRef](#)] [[PubMed](#)]
34. Severino, V.; Farina, A.; Colucci-D'Amato, L.; Reccia, M.G.; Volpicelli, F.; Parente, A.; Chambery, A. Secretome profiling of differentiated neural mes-c-myc A1 cell line endowed with stem cell properties. *Biochim. Biophys. Acta* **2013**, *1834*, 2385–2395. [[CrossRef](#)] [[PubMed](#)]
35. Willis, S.D.; Hanley, S.E.; Beishke, T.; Tati, P.D.; Cooper, K.F. Ubiquitin-proteasome-mediated cyclin C degradation promotes cell survival following nitrogen starvation. *Mol. Biol. Cell* **2020**, *31*, 1015–1031. [[CrossRef](#)]
36. Jin, J.; Hulette, C.; Wang, Y.; Zhang, T.; Pan, C.; Wadhwa, R.; Zhang, J. Proteomic identification of a stress protein, mortalin/mthsp70/GRP75: Relevance to Parkinson disease. *Mol. Cell. Proteomics* **2006**, *5*, 1193–1204. [[CrossRef](#)]
37. Burbulla, L.F.; Fitzgerald, J.C.; Stegen, K.; Westermeier, J.; Thost, A.K.; Kato, H.; Mokranjac, D.; Sauerwald, J.; Martins, L.M.; Voitalla, D.; et al. Mitochondrial proteolytic stress induced by loss of mortalin function is rescued by Parkin and PINK1. *Cell Death Dis.* **2014**, *5*, e1180. [[CrossRef](#)]
38. Breen, D.P.; Halliday, G.M.; Lang, A.E. Gut-brain axis and the spread of  $\alpha$ -synuclein pathology: Vagal highway or dead end? *Mov. Disord.* **2019**, *34*, 307–316. [[CrossRef](#)]
39. Scheperjans, F.; Derkinderen, P.; Borghammer, P. The Gut and Parkinson's Disease: Hype or Hope? *J. Parkinsons Dis.* **2018**, *8*, S31–S39. [[CrossRef](#)]
40. Lubomski, M.; Tan, A.H.; Lim, S.Y.; Holmes, A.J.; Davis, R.L.; Sue, C.M. Parkinson's disease and the gastrointestinal microbiome. *J. Neurol.* **2019**. [[CrossRef](#)]
41. Colucci-D'Amato, L.; Perrone-Capano, C.; di Porzio, U. Chronic activation of ERK and neurodegenerative diseases. *Bioessays* **2003**, *25*, 1085–1095. [[CrossRef](#)] [[PubMed](#)]
42. Fusco, F.R.; Anzilotti, S.; Giampà, C.; Dato, C.; Laurenti, D.; Leuti, A.; Colucci D'Amato, L.; Perrone, L.; Bernardi, G.; Melone, M.A. Changes in the expression of extracellular regulated kinase (ERK 1/2) in the R6/2 mouse model of Huntington's disease after phosphodiesterase IV inhibition. *Neurobiol. Dis.* **2012**, *46*, 225–233. [[CrossRef](#)]
43. Zhu, J.H.; Kulich, S.M.; Oury, T.D.; Chu, C.T. Cytoplasmic aggregates of phosphorylated extracellular signal-regulated protein kinases in Lewy body diseases. *Am. J. Pathol.* **2002**, *161*, 2087–2098. [[CrossRef](#)]
44. Andrew, R.; Watson, D.G.; Best, S.A.; Midgley, J.M.; Wenlong, H.; Petty, R.K. The determination of hydroxydopamines and other trace amines in the urine of parkinsonian patients and normal controls. *Neurochem. Res.* **1993**, *18*, 1175–1177. [[CrossRef](#)]
45. Kulich, S.M.; Chu, C.T. Sustained extracellular signal-regulated kinase activation by 6-hydroxydopamine: Implications for Parkinson's disease. *J. Neurochem.* **2001**, *77*, 1058–1066. [[CrossRef](#)]
46. Vaudry, D.; Stork, P.J.; Lazarovici, P.; Eiden, L.E. Signaling pathways for PC12 cell differentiation: Making the right connections. *Science* **2002**, *296*, 1648–1649. [[CrossRef](#)] [[PubMed](#)]
47. Chu, C.T.; Levinthal, D.J.; Kulich, S.M.; Chalovich, E.M.; DeFranco, D.B. Oxidative neuronal injury. The dark side of ERK1/2. *Eur. J. Biochem.* **2004**, *271*, 2060–2066. [[CrossRef](#)]
48. Subramaniam, S.; Unsicker, K. Extracellular signal-regulated kinase as an inducer of non-apoptotic neuronal death. *Neuroscience* **2006**, *138*, 1055–1065. [[CrossRef](#)]
49. Lin, E.; Cavanaugh, J.E.; Leak, R.K.; Perez, R.G.; Zigmond, M.J. Rapid activation of ERK by 6-hydroxydopamine promotes survival of dopaminergic cells. *J. Neurosci. Res.* **2008**, *86*, 108–117. [[CrossRef](#)] [[PubMed](#)]
50. Colucci-D'Amato, G.L.; D'Alessio, A.; Califano, D.; Cali, G.; Rizzo, C.; Nitsch, L.; Santelli, G.; de Franciscis, V. Abrogation of nerve growth factor-induced terminal differentiation by ret oncogene involves perturbation of nuclear translocation of ERK. *J. Biol. Chem.* **2000**, *275*, 19306–19314. [[CrossRef](#)]
51. Wainstein, E.; Seger, R. The dynamic subcellular localization of ERK: Mechanisms of translocation and role in various organelles. *Curr. Opin. Cell Biol.* **2016**, *39*, 15–20. [[CrossRef](#)] [[PubMed](#)]

52. Yoo, D.Y.; Kim, W.; Nam, S.M.; Kim, D.W.; Chung, J.Y.; Choi, S.Y.; Yoon, Y.S.; Won, M.H.; Hwang, I.K. Synergistic effects of sodium butyrate, a histone deacetylase inhibitor, on increase of neurogenesis induced by pyridoxine and increase of neural proliferation in the mouse dentate gyrus. *Neurochem. Res.* **2011**, *36*, 1850–1857. [[CrossRef](#)]
53. Colucci-D'Amato, L.; di Porzio, U. Neurogenesis in adult CNS: From denial to opportunities and challenges for therapy. *Bioessays* **2008**, *30*, 135–145. [[CrossRef](#)] [[PubMed](#)]
54. Pereira, L.; Font-Nieves, M.; Van den Haute, C.; Baekelandt, V.; Planas, A.M.; Pozas, E. IL-10 regulates adult neurogenesis by modulating ERK and STAT3 activity. *Front. Cell. Neurosci.* **2015**, *9*, 57. [[CrossRef](#)] [[PubMed](#)]
55. Perrone-Capano, C.; Di Porzio, U. Genetic and epigenetic control of midbrain dopaminergic neuron development. *Int. J. Dev. Biol.* **2000**, *44*, 679–687. [[PubMed](#)]
56. Puelles, E.; Annino, A.; Tuorto, F.; Usiello, A.; Acampora, D.; Czerny, T.; Brodski, C.; Ang, S.L.; Wurst, W.; Simeone, A. Otx2 regulates the extent, identity and fate of neuronal progenitor domains in the ventral midbrain. *Development* **2004**, *131*, 2037–2048. [[CrossRef](#)]
57. Kim, T.; Song, J.J.; Puspita, L.; Valiulahi, P.; Shim, J.W.; Lee, S.H. In vitro generation of mature midbrain-type dopamine neurons by adjusting exogenous Nurr1 and Foxa2 expressions to their physiologic patterns. *Exp. Mol. Med.* **2017**, *49*, e300. [[CrossRef](#)]
58. Domanskyi, A.; Alter, H.; Vogt, M.A.; Gass, P.; Vinnikov, I.A. Transcription factors Foxa1 and Foxa2 are required for adult dopamine neurons maintenance. *Front. Cell. Neurosci.* **2014**, *8*, 275. [[CrossRef](#)]
59. Hynes, M.; Porter, J.A.; Chiang, C.; Chang, D.; Tessier-Lavigne, M.; Beachy, P.A.; Rosenthal, A. Induction of midbrain dopaminergic neurons by Sonic hedgehog. *Neuron* **1995**, *15*, 35–44. [[CrossRef](#)]
60. Ye, W.; Shimamura, K.; Rubenstein, J.L.; Hynes, M.A.; Rosenthal, A. FGF and Shh signals control dopaminergic and serotonergic cell fate in the anterior neural plate. *Cell* **1998**, *93*, 755–766. [[CrossRef](#)]
61. Volpicelli, F.; Consales, C.; Caiazzo, M.; Colucci-D'Amato, L.; Perrone-Capano, C.; di Porzio, U. Enhancement of dopaminergic differentiation in proliferating midbrain neuroblasts by sonic hedgehog and ascorbic acid. *Neural Plast.* **2004**, *11*, 45–57. [[CrossRef](#)] [[PubMed](#)]
62. Ono, Y.; Nakatani, T.; Minaki, Y.; Kumai, M. The basic helix-loop-helix transcription factor Nato3 controls neurogenic activity in mesencephalic floor plate cells. *Development* **2010**, *137*, 1897–1906. [[CrossRef](#)] [[PubMed](#)]
63. Kameda, Y.; Saitoh, T.; Fujimura, T. Hes1 regulates the number and anterior-posterior patterning of mesencephalic dopaminergic neurons at the mid/hindbrain boundary (isthmus). *Dev. Biol.* **2011**, *358*, 91–101. [[CrossRef](#)] [[PubMed](#)]
64. Hegarty, S.V.; Sullivan, A.M.; O'Keefe, G.W. Midbrain dopaminergic neurons: A review of the molecular circuitry that regulates their development. *Dev. Biol.* **2013**, *379*, 123–138. [[CrossRef](#)] [[PubMed](#)]
65. Mattugini, N.; Bocchi, R.; Scheuss, V.; Russo, G.L.; Torper, O.; Lao, C.L.; Götz, M. Inducing Different Neuronal Subtypes from Astrocytes in the Injured Mouse Cerebral Cortex. *Neuron* **2019**, *103*, 1086–1095. [[CrossRef](#)] [[PubMed](#)]
66. Cai, J.; Donaldson, A.; Yang, M.; German, M.S.; Enikolopov, G.; Iacovitti, L. The role of Lmx1a in the differentiation of human embryonic stem cells into midbrain dopamine neurons in culture and after transplantation into a Parkinson's disease model. *Stem Cells* **2009**, *27*, 220–229. [[CrossRef](#)]
67. Rivetti di Val Cervo, P.; Romanov, R.A.; Spigolon, G.; Masini, D.; Martín-Montañez, E.; Toledo, E.M.; La Manno, G.; Feyder, M.; Pifl, C.; Ng, Y.H.; et al. Induction of functional dopamine neurons from human astrocytes in vitro and mouse astrocytes in a Parkinson's disease model. *Nat. Biotechnol.* **2017**, *35*, 444–452. [[CrossRef](#)] [[PubMed](#)]
68. Mesman, S.; von Oerthel, L.; Smidt, M.P. Mesodiencephalic dopaminergic neuronal differentiation does not involve GLI2A-mediated SHH-signaling and is under the direct influence of canonical WNT signaling. *PLoS ONE* **2014**, *9*, e97926. [[CrossRef](#)]
69. Allen, B.L.; Song, J.Y.; Izzi, L.; Althaus, I.W.; Kang, J.S.; Charron, F.; Krauss, R.S.; McMahon, A.P. Overlapping roles and collective requirement for the coreceptors GAS1, CDO, and BOC in SHH pathway function. *Dev. Cell* **2011**, *20*, 775–787. [[CrossRef](#)] [[PubMed](#)]
70. Andersson, E.; Tryggvason, U.; Deng, Q.; Friling, S.; Alekseenko, Z.; Robert, B.; Perlmann, T.; Ericson, J. Identification of intrinsic determinants of midbrain dopamine neurons. *Cell* **2006**, *124*, 393–405. [[CrossRef](#)]
71. Wurst, W.; Prakash, N. Wnt1-regulated genetic networks in midbrain dopaminergic neuron development. *J. Mol. Cell Biol.* **2014**, *6*, 34–41. [[CrossRef](#)]

72. Marchetti, B. Wnt/ $\beta$ -Catenin Signaling Pathway Governs a Full Program for Dopaminergic Neuron Survival, Neurorescue and Regeneration in the MPTP Mouse Model of Parkinson's Disease. *Int. J. Mol. Sci.* **2018**, *19*, 3743. [[CrossRef](#)] [[PubMed](#)]
73. MacDonald, B.T.; Tamai, K.; He, X. Wnt/beta-catenin signaling: Components, mechanisms, and diseases. *Dev. Cell* **2009**, *17*, 9–26. [[CrossRef](#)] [[PubMed](#)]
74. Ferri, A.L.; Lin, W.; Mavromatakis, Y.E.; Wang, J.C.; Sasaki, H.; Whitsett, J.A.; Ang, S.L. Foxa1 and Foxa2 regulate multiple phases of midbrain dopaminergic neuron development in a dosage-dependent manner. *Development* **2007**, *134*, 2761–2769. [[CrossRef](#)]
75. Alvarez-Fischer, D.; Fuchs, J.; Castagner, F.; Stettler, O.; Massiani-Beaudoin, O.; Moya, K.L.; Bouillot, C.; Oertel, W.H.; Lombès, A.; Faigle, W.; et al. Engrailed protects mouse midbrain dopaminergic neurons against mitochondrial complex I insults. *Nat. Neurosci.* **2011**, *14*, 1260–1266. [[CrossRef](#)]
76. Brown, A.; Machan, J.T.; Hayes, L.; Zervas, M. Molecular organization and timing of Wnt1 expression define cohorts of midbrain dopamine neuron progenitors in vivo. *J. Comp. Neurol.* **2011**, *519*, 2978–3000. [[CrossRef](#)]
77. Arenas, E.; Denham, M.; Villaescusa, J.C. How to make a midbrain dopaminergic neuron. *Development* **2015**, *142*, 1918–1936. [[CrossRef](#)]
78. Doi, D.; Samata, B.; Katsukawa, M.; Kikuchi, T.; Morizane, A.; Ono, Y.; Sekiguchi, K.; Nakagawa, M.; Parmar, M.; Takahashi, J. Isolation of human induced pluripotent stem cell-derived dopaminergic progenitors by cell sorting for successful transplantation. *Stem Cell Reports* **2014**, *2*, 337–350. [[CrossRef](#)] [[PubMed](#)]
79. Kirkeby, A.; Grealish, S.; Wolf, D.A.; Nelander, J.; Wood, J.; Lundblad, M.; Lindvall, O.; Parmar, M. Generation of regionally specified neural progenitors and functional neurons from human embryonic stem cells under defined conditions. *Cell Rep.* **2012**, *1*, 703–714. [[CrossRef](#)] [[PubMed](#)]
80. Kriks, S.; Shim, J.W.; Piao, J.; Ganat, Y.M.; Wakeman, D.R.; Xie, Z.; Carrillo-Reid, L.; Auyeung, G.; Antonacci, C.; Buch, A.; et al. Dopamine neurons derived from human ES cells efficiently engraft in animal models of Parkinson's disease. *Nature* **2011**, *480*, 547–551. [[CrossRef](#)] [[PubMed](#)]
81. Kirkeby, A.; Nolbrant, S.; Tiklova, K.; Heuer, A.; Kee, N.; Cardoso, T.; Ottosson, D.R.; Lelos, M.J.; Rifes, P.; Dunnett, S.B.; et al. Predictive Markers Guide Differentiation to Improve Graft Outcome in Clinical Translation of hESC-Based Therapy for Parkinson's Disease. *Cell Stem Cell* **2017**, *20*, 135–148. [[CrossRef](#)] [[PubMed](#)]
82. Ang, S.L. Transcriptional control of midbrain dopaminergic neuron development. *Development* **2006**, *133*, 3499–3506. [[CrossRef](#)] [[PubMed](#)]
83. Panman, L.; Papanthou, M.; Laguna, A.; Oosterveen, T.; Volakakis, N.; Acampora, D.; Kurtsdotter, I.; Yoshitake, T.; Kehr, J.; Joodmardi, E.; et al. Sox6 and Otx2 control the specification of substantia nigra and ventral tegmental area dopamine neurons. *Cell Rep.* **2014**, *8*, 1018–1025. [[CrossRef](#)] [[PubMed](#)]
84. Mendez, J.A.; Bourque, M.J.; Dal Bo, G.; Bourdeau, M.L.; Danik, M.; Williams, S.; Lacaille, J.C.; Trudeau, L.E. Developmental and target-dependent regulation of vesicular glutamate transporter expression by dopamine neurons. *J. Neurosci.* **2008**, *28*, 6309–6318. [[CrossRef](#)]
85. Descarries, L.; Bérubé-Carrière, N.; Riad, M.; Bo, G.D.; Mendez, J.A.; Trudeau, L.E. Glutamate in dopamine neurons: Synaptic versus diffuse transmission. *Brain Res. Rev.* **2008**, *58*, 290–302. [[CrossRef](#)]
86. Bérubé-Carrière, N.; Riad, M.; Dal Bo, G.; Lévesque, D.; Trudeau, L.E.; Descarries, L. The dual dopamine-glutamate phenotype of growing mesencephalic neurons regresses in mature rat brain. *J. Comp. Neurol.* **2009**, *517*, 873–891. [[CrossRef](#)]
87. Yamaguchi, T.; Qi, J.; Wang, H.L.; Zhang, S.; Morales, M. Glutamatergic and dopaminergic neurons in the mouse ventral tegmental area. *Eur. J. Neurosci.* **2015**, *41*, 760–772. [[CrossRef](#)]
88. Yoo, J.H.; Zell, V.; Gutierrez-Reed, N.; Wu, J.; Ressler, R.; Shenasa, M.A.; Johnson, A.B.; Fife, K.H.; Faget, L.; Hnasko, T.S. Ventral tegmental area glutamate neurons co-release GABA and promote positive reinforcement. *Nat. Commun.* **2016**, *7*, 13697. [[CrossRef](#)]
89. Bodea, G.O.; Blaess, S. Establishing diversity in the dopaminergic system. *FEBS Lett.* **2015**, *589*, 3773–3785. [[CrossRef](#)]
90. Stuber, G.D.; Hnasko, T.S.; Britt, J.P.; Edwards, R.H.; Bonci, A. Dopaminergic terminals in the nucleus accumbens but not the dorsal striatum corelease glutamate. *J. Neurosci.* **2010**, *30*, 8229–8233. [[CrossRef](#)]
91. Hnasko, T.S.; Hjelmstad, G.O.; Fields, H.L.; Edwards, R.H. Ventral tegmental area glutamate neurons: Electrophysiological properties and projections. *J. Neurosci.* **2012**, *32*, 15076–15085. [[CrossRef](#)]



92. Steinkellner, T.; Zell, V.; Farino, Z.J.; Sonders, M.S.; Villeneuve, M.; Freyberg, R.J.; Przedborski, S.; Lu, W.; Freyberg, Z.; Hnasko, T.S. Role for VGLUT2 in selective vulnerability of midbrain dopamine neurons. *J. Clin. Investig.* **2018**, *128*, 774–788. [[CrossRef](#)]
93. Perlmann, T.; Wallén-Mackenzie, A. Nurr1, an orphan nuclear receptor with essential functions in developing dopamine cells. *Cell Tissue Res.* **2004**, *318*, 45–52. [[CrossRef](#)]
94. Volpicelli, F.; Perrone-Capano, C.; Da Pozzo, P.; Colucci-D'Amato, L.; di Porzio, U. Modulation of nurr1 gene expression in mesencephalic dopaminergic neurones. *J. Neurochem.* **2004**, *88*, 1283–1294. [[CrossRef](#)] [[PubMed](#)]
95. Saucedo-Cardenas, O.; Quintana-Hau, J.D.; Le, W.D.; Smidt, M.P.; Cox, J.J.; De Mayo, F.; Burbach, J.P.; Conneely, O.M. Nurr1 is essential for the induction of the dopaminergic phenotype and the survival of ventral mesencephalic late dopaminergic precursor neurons. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 4013–4018. [[CrossRef](#)] [[PubMed](#)]
96. Smits, S.M.; Ponnio, T.; Conneely, O.M.; Burbach, J.P.; Smidt, M.P. Involvement of Nurr1 in specifying the neurotransmitter identity of ventral midbrain dopaminergic neurons. *Eur. J. Neurosci.* **2003**, *18*, 1731–1738. [[CrossRef](#)] [[PubMed](#)]
97. Hermanson, E.; Joseph, B.; Castro, D.; Lindqvist, E.; Aarnisalo, P.; Wallén, A.; Benoit, G.; Hengerer, B.; Olson, L.; Perlmann, T. Nurr1 regulates dopamine synthesis and storage in MN9D dopamine cells. *Exp. Cell Res.* **2003**, *288*, 324–334. [[CrossRef](#)]
98. Sacchetti, P.; Brownschidle, L.A.; Granneman, J.G.; Bannon, M.J. Characterization of the 5'-flanking region of the human dopamine transporter gene. *Brain Res. Mol. Brain Res.* **1999**, *74*, 167–174. [[CrossRef](#)]
99. Joseph, B.; Wallén-Mackenzie, A.; Benoit, G.; Murata, T.; Joodmardi, E.; Okret, S.; Perlmann, T. p57(Kip2) cooperates with Nurr1 in developing dopamine cells. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 15619–15624. [[CrossRef](#)]
100. Wallén, A.; Castro, D.S.; Zetterström, R.H.; Karlén, M.; Olson, L.; Ericson, J.; Perlmann, T. Orphan nuclear receptor Nurr1 is essential for Ret expression in midbrain dopamine neurons and in the brain stem. *Mol. Cell Neurosci.* **2001**, *18*, 649–663. [[CrossRef](#)]
101. Galleguillos, D.; Fuentealba, J.A.; Gómez, L.M.; Saver, M.; Gómez, A.; Nash, K.; Burger, C.; Gysling, K.; Andrés, M.E. Nurr1 regulates RET expression in dopamine neurons of adult rat midbrain. *J. Neurochem.* **2010**, *114*, 1158–1167. [[CrossRef](#)]
102. Volpicelli, F.; Caiazzo, M.; Greco, D.; Consales, C.; Leone, L.; Perrone-Capano, C.; Colucci D'Amato, L.; di Porzio, U. Bdnf gene is a downstream target of Nurr1 transcription factor in rat midbrain neurons in vitro. *J. Neurochem.* **2007**, *102*, 441–453. [[CrossRef](#)]
103. Oh, S.M.; Chang, M.Y.; Song, J.J.; Rhee, Y.H.; Joe, E.H.; Lee, H.S.; Yi, S.H.; Lee, S.H. Combined Nurr1 and Foxa2 roles in the therapy of Parkinson's disease. *EMBO Mol. Med.* **2015**, *7*, 510–525. [[CrossRef](#)] [[PubMed](#)]
104. Jiang, H.; Xiang, M. Subtype specification of GABAergic amacrine cells by the orphan nuclear receptor Nr4a2/Nurr1. *J. Neurosci.* **2009**, *29*, 10449–10459. [[CrossRef](#)] [[PubMed](#)]
105. Tarakad, A.; Jankovic, J. Anosmia and Ageusia in Parkinson's Disease. *Int. Rev. Neurobiol.* **2017**, *133*, 541–556. [[CrossRef](#)] [[PubMed](#)]
106. Spathis, A.D.; Asvos, X.; Ziavra, D.; Karampelas, T.; Topouzis, S.; Courनिया, Z.; Qing, X.; Alexakos, P.; Smits, L.M.; Dalla, C.; et al. Nurr1:RXR $\alpha$  heterodimer activation as monotherapy for Parkinson's disease. *Proc. Natl. Acad. Sci. USA* **2017**, *114*, 3999–4004. [[CrossRef](#)]
107. Volpicelli, F.; De Gregorio, R.; Pulcrano, S.; Perrone-Capano, C.; di Porzio, U.; Belenchi, G.C. Direct regulation of Pitx3 expression by Nurr1 in culture and in developing mouse midbrain. *PLoS ONE* **2012**, *7*, e30661. [[CrossRef](#)]
108. Hwang, D.Y.; Ardayfio, P.; Kang, U.J.; Semina, E.V.; Kim, K.S. Selective loss of dopaminergic neurons in the substantia nigra of Pitx3-deficient aphakia mice. *Brain Res. Mol. Brain Res.* **2003**, *114*, 123–131. [[CrossRef](#)]
109. Frim, D.M.; Uhler, T.A.; Galpern, W.R.; Beal, M.F.; Breakefield, X.O.; Isacson, O. Implanted fibroblasts genetically engineered to produce brain-derived neurotrophic factor prevent 1-methyl-4-phenylpyridinium toxicity to dopaminergic neurons in the rat. *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 5104–5108. [[CrossRef](#)]
110. Hyman, C.; Hofer, M.; Barde, Y.A.; Juhasz, M.; Yancopoulos, G.D.; Squinto, S.P.; Lindsay, R.M. BDNF is a neurotrophic factor for dopaminergic neurons of the substantia nigra. *Nature* **1991**, *350*, 230–232. [[CrossRef](#)]
111. Arenas, E.; Trupp, M.; Akerud, P.; Ibáñez, C.F. GDNF prevents degeneration and promotes the phenotype of brain noradrenergic neurons in vivo. *Neuron* **1995**, *15*, 1465–1473. [[CrossRef](#)]

112. Akerud, P.; Canals, J.M.; Snyder, E.Y.; Arenas, E. Neuroprotection through delivery of glial cell line-derived neurotrophic factor by neural stem cells in a mouse model of Parkinson's disease. *J. Neurosci.* **2001**, *21*, 8108–8118. [[CrossRef](#)] [[PubMed](#)]
113. Pascual, A.; Hidalgo-Figueroa, M.; Piruat, J.I.; Pintado, C.O.; Gómez-Díaz, R.; López-Barneo, J. Absolute requirement of GDNF for adult catecholaminergic neuron survival. *Nat. Neurosci.* **2008**, *11*, 755–761. [[CrossRef](#)] [[PubMed](#)]
114. Peng, C.; Aron, L.; Klein, R.; Li, M.; Wurst, W.; Prakash, N.; Le, W. Pitx3 is a critical mediator of GDNF-induced BDNF expression in nigrostriatal dopaminergic neurons. *J. Neurosci.* **2011**, *31*, 12802–12815. [[CrossRef](#)]
115. Palasz, E.; Wysocka, A.; Gasiorowska, A.; Chalimoniuk, M.; Niewiadomski, W.; Niewiadomska, G. BDNF as a Promising Therapeutic Agent in Parkinson's Disease. *Int. J. Mol. Sci.* **2020**, *21*, 1170. [[CrossRef](#)]
116. Man, J.H.K.; Groenink, L.; Caiazzo, M. Cell reprogramming approaches in gene- and cell-based therapies for Parkinson's disease. *J. Control. Release* **2018**, *286*, 114–124. [[CrossRef](#)]
117. Valdés, P.; Schneider, B.L. Gene Therapy: A Promising Approach for Neuroprotection in Parkinson's Disease? *Front. Neuroanat.* **2016**, *10*, 123. [[CrossRef](#)]
118. Grondin, R.; Littrell, O.M.; Zhang, Z.; Ai, Y.; Huettl, P.; Pomerleau, F.; Quintero, J.E.; Andersen, A.H.; Stenslik, M.J.; Bradley, L.H.; et al. GDNF revisited: A novel mammalian cell-derived variant form of GDNF increases dopamine turnover and improves brain biodistribution. *Neuropharmacology* **2019**, *147*, 28–36. [[CrossRef](#)]
119. Ecker, J.R.; Geschwind, D.H.; Kriegstein, A.R.; Ngai, J.; Osten, P.; Polioudakis, D.; Regev, A.; Sestan, N.; Wickersham, I.R.; Zeng, H. The BRAIN Initiative Cell Census Consortium: Lessons Learned toward Generating a Comprehensive Brain Cell Atlas. *Neuron* **2017**, *96*, 542–557. [[CrossRef](#)]
120. La Manno, G.; Gyllborg, D.; Codeluppi, S.; Nishimura, K.; Salto, C.; Zeisel, A.; Borm, L.E.; Stott, S.R.W.; Toledo, E.M.; Villaescusa, J.C.; et al. Molecular Diversity of Midbrain Development in Mouse, Human, and Stem Cells. *Cell* **2016**, *167*, 566–580.e519. [[CrossRef](#)]
121. Hook, P.W.; McClymont, S.A.; Cannon, G.H.; Law, W.D.; Morton, A.J.; Goff, L.A.; McCallion, A.S. Single-Cell RNA-Seq of Mouse Dopaminergic Neurons Informs Candidate Gene Selection for Sporadic Parkinson Disease. *Am. J. Hum. Genet.* **2018**, *102*, 427–446. [[CrossRef](#)] [[PubMed](#)]
122. Tiklová, K.; Björklund, Å.; Lahti, L.; Fiorenzano, A.; Nolbrant, S.; Gillberg, L.; Volakakis, N.; Yokota, C.; Hilscher, M.M.; Hauling, T.; et al. Single-cell RNA sequencing reveals midbrain dopamine neuron diversity emerging during mouse brain development. *Nat. Commun.* **2019**, *10*, 581. [[CrossRef](#)] [[PubMed](#)]
123. Kramer, D.J.; Risso, D.; Kosillo, P.; Ngai, J.; Bateup, H.S. Combinatorial Expression of Grp and Neurod6 Defines Dopamine Neuron Populations with Distinct Projection Patterns and Disease Vulnerability. *eNeuro* **2018**, *5*. [[CrossRef](#)] [[PubMed](#)]
124. Bartel, D.P.; Chen, C.Z. Micromanagers of gene expression: The potentially widespread influence of metazoan microRNAs. *Nat. Rev. Genet.* **2004**, *5*, 396–400. [[CrossRef](#)] [[PubMed](#)]
125. Lagos-Quintana, M.; Rauhut, R.; Yalcin, A.; Meyer, J.; Lendeckel, W.; Tuschl, T. Identification of tissue-specific microRNAs from mouse. *Curr. Biol.* **2002**, *12*, 735–739. [[CrossRef](#)]
126. Kapsimali, M.; Kloosterman, W.P.; de Bruijn, E.; Rosa, F.; Plasterk, R.H.; Wilson, S.W. MicroRNAs show a wide diversity of expression profiles in the developing and mature central nervous system. *Genome Biol.* **2007**, *8*, R173. [[CrossRef](#)]
127. McNeill, E.; Van Vactor, D. MicroRNAs shape the neuronal landscape. *Neuron* **2012**, *75*, 363–379. [[CrossRef](#)]
128. Volpicelli, F.; Speranza, L.; Pulcrano, S.; De Gregorio, R.; Crispino, M.; De Sanctis, C.; Leopoldo, M.; Lacivita, E.; di Porzio, U.; Bellenchi, G.C.; et al. The microRNA-29a Modulates Serotonin 5-HT7 Receptor Expression and Its Effects on Hippocampal Neuronal Morphology. *Mol. Neurobiol.* **2019**, *56*, 8617–8627. [[CrossRef](#)]
129. Rosas-Hernandez, H.; Chigurupati, S.; Raymick, J.; Robinson, B.; Cuevas, E.; Hanig, J.; Sarkar, S. Identification of altered microRNAs in serum of a mouse model of Parkinson's disease. *Neurosci. Lett.* **2018**, *687*, 1–9. [[CrossRef](#)]
130. Cuellar, T.L.; Davis, T.H.; Nelson, P.T.; Loeb, G.B.; Harfe, B.D.; Ullian, E.; McManus, M.T. Dicer loss in striatal neurons produces behavioral and neuroanatomical phenotypes in the absence of neurodegeneration. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 5614–5619. [[CrossRef](#)]
131. Kawase-Koga, Y.; Otaegi, G.; Sun, T. Different timings of Dicer deletion affect neurogenesis and gliogenesis in the developing mouse central nervous system. *Dev. Dyn.* **2009**, *238*, 2800–2812. [[CrossRef](#)] [[PubMed](#)]

132. Lee, Y.S.; Nakahara, K.; Pham, J.W.; Kim, K.; He, Z.; Sontheimer, E.J.; Carthew, R.W. Distinct roles for *Drosophila* Dicer-1 and Dicer-2 in the siRNA/miRNA silencing pathways. *Cell* **2004**, *117*, 69–81. [[CrossRef](#)]
133. Kim, J.; Inoue, K.; Ishii, J.; Vanti, W.B.; Voronov, S.V.; Murchison, E.; Hannon, G.; Abeliovich, A. A MicroRNA feedback circuit in midbrain dopamine neurons. *Science* **2007**, *317*, 1220–1224. [[CrossRef](#)] [[PubMed](#)]
134. Yang, D.; Li, T.; Wang, Y.; Tang, Y.; Cui, H.; Zhang, X.; Chen, D.; Shen, N.; Le, W. miR-132 regulates the differentiation of dopamine neurons by directly targeting *Nurr1* expression. *J. Cell Sci.* **2012**, *125*, 1673–1682. [[CrossRef](#)]
135. Miñones-Moyano, E.; Porta, S.; Escaramís, G.; Rabionet, R.; Iraola, S.; Kagerbauer, B.; Espinosa-Parrilla, Y.; Ferrer, I.; Estivill, X.; Martí, E. MicroRNA profiling of Parkinson’s disease brains identifies early downregulation of miR-34b/c which modulate mitochondrial function. *Hum. Mol. Genet.* **2011**, *20*, 3067–3078. [[CrossRef](#)]
136. Shanesazzade, Z.; Peymani, M.; Ghaedi, K.; Nasr Esfahani, M.H. miR-34a/BCL-2 signaling axis contributes to apoptosis in MPP. *Mol. Genet. Genomic Med.* **2018**, *6*, 975–981. [[CrossRef](#)]
137. de Chevigny, A.; Coré, N.; Follert, P.; Gaudin, M.; Barbry, P.; Béclin, C.; Cremer, H. miR-7a regulation of *Pax6* controls spatial origin of forebrain dopaminergic neurons. *Nat. Neurosci.* **2012**, *15*, 1120–1126. [[CrossRef](#)]
138. Qian, Y.; Song, J.; Ouyang, Y.; Han, Q.; Chen, W.; Zhao, X.; Xie, Y.; Chen, Y.; Yuan, W.; Fan, C. Advances in Roles of miR-132 in the Nervous System. *Front. Pharmacol.* **2017**, *8*, 770. [[CrossRef](#)]
139. Gillardon, F.; Mack, M.; Rist, W.; Schnack, C.; Lenter, M.; Hildebrandt, T.; Hengerer, B. MicroRNA and proteome expression profiling in early-symptomatic  $\alpha$ -synuclein(A30P)-transgenic mice. *Proteom. Clin. Appl.* **2008**, *2*, 697–705. [[CrossRef](#)]
140. Lungu, G.; Stoica, G.; Ambrus, A. MicroRNA profiling and the role of microRNA-132 in neurodegeneration using a rat model. *Neurosci. Lett.* **2013**, *553*, 153–158. [[CrossRef](#)]
141. Alieva, A.K.h.; Filatova, E.V.; Karabanov, A.V.; Illarioshkin, S.N.; Limborska, S.A.; Shadrina, M.I.; Slominsky, P.A. miRNA expression is highly sensitive to a drug therapy in Parkinson’s disease. *Parkinsonism Relat. Disord.* **2015**, *21*, 72–74. [[CrossRef](#)] [[PubMed](#)]
142. Di Rita, A.; Maiorino, T.; Bruqi, K.; Volpicelli, F.; Bellenchi, G.C.; Strappazzon, F. miR-218 Inhibits Mitochondrial Clearance by Targeting PRKN E3 Ubiquitin Ligase. *Int. J. Mol. Sci.* **2020**, *21*, 355. [[CrossRef](#)] [[PubMed](#)]
143. Gong, X.; Wang, H.; Ye, Y.; Shu, Y.; Deng, Y.; He, X.; Lu, G.; Zhang, S. miR-124 regulates cell apoptosis and autophagy in dopaminergic neurons and protects them by regulating AMPK/mTOR pathway in Parkinson’s disease. *Am. J. Transl. Res.* **2016**, *8*, 2127–2137.
144. Wang, H.; Ye, Y.; Zhu, Z.; Mo, L.; Lin, C.; Wang, Q.; Gong, X.; He, X.; Lu, G.; Lu, F.; et al. MiR-124 Regulates Apoptosis and Autophagy Process in MPTP Model of Parkinson’s Disease by Targeting to Bim. *Brain Pathol.* **2016**, *26*, 167–176. [[CrossRef](#)] [[PubMed](#)]
145. Ge, H.; Yan, Z.; Zhu, H.; Zhao, H. MiR-410 exerts neuroprotective effects in a cellular model of Parkinson’s disease induced by 6-hydroxydopamine via inhibiting the PTEN/AKT/mTOR signaling pathway. *Exp. Mol. Pathol.* **2019**, *109*, 16–24. [[CrossRef](#)]

