Dopaminergic Neurons and Parkinson's Disease: Current Status, Implications and Future Perspectives

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Abstract. Parkinson's disease (PD) is a common neurodegenerative disease that impairs motor functions in the affected individuals, causing increased dependence and even mortality eventually. Therefore, it has become a significant resources and financial burden to the society. Scientists and companies have carried out a myriad of studies to study potentially effective drugs to slow or cure the disease, but they barely pass the Phase III stage of clinical trials. Under these circumstances, establishing the pathogenic mechanisms is really the key to removal of obstacles in the way of mitigating PD. Degeneration of dopaminergic neurons is known as a cause of motor manifestation in PD. Instead of approaching PD from the traditional α-synuclein theory, this review emphasizes on dopaminergic neurons *per se* to discuss their unique features and how they may implicate in PD development and progression. Some latest findings and potential future directions are also summarized and discussed in this review.

Keywords: Dopaminergic Neurons, Parkinson's Disease, Calcium Ion Channels.

1. Introduction

Parkinson's disease (PD) has a morbidity that is the second most frequent in the world, affecting people with an age of 65 years and above predominantly [1]. The prevalence of PD has in According to the World Health Organization, over 8.5 million individuals were diagnosed with PD in 2019 [2]. The prevalence of PD increases at an unbelievable fast speed, such that the number of diagnoses doubled to 6 million from 1990 to 2015. This trend is expected to be maintain and the prevalence is likely to be over 12 million by 2040, as the global population ages (which is the major driving factor) [3].

As a troublesome health problem, PD has become a serious financial burden to the world. For example, the United State is estimated to spend 51.9 billion dollars in 2017 alone, including the combined direct and indirect cost, for PD, while in the United Kingdom the annual cost for PD patients is over 5 thousand pounds per person in 2018 [4,5].

PD displays a variety of motor and non-motor symptoms. Currently, PD has no effective cure or disease modifying treatments. Although numerous studies have been done, the exactly pathogenic mechanism is still unclear. However, two pathological characteristics have been identified for a long time, which are the loss or degeneration of the dopaminergic (DA) neurons and accumulation of Lewy (LB) Bodies inside the neurons. Loss of the DA neurons in the substantia nigra (SN) contributes to the cardinal motor symptoms in PD, while the LBs are abnormal intracellular protein aggregates, containing various proteins (α-synuclein ubiquitin, etc.), that impair normal neuronal functions [1].

Death of the DA neurons, a crucial part of PD pathogenesis, has not been fully understood yet. Several hypotheses and studies have indicated that a number of factors may contribute to this process, including oxidative stress, mitochondrial dysfunction, protein aggregation, etc. Interestingly, DA neurons seem to be particularly susceptible to the Lewy pathology and mitochondrial dysfunction, although the role of Lewy pathology remains controversial. The degeneration of DA neurons occurs systemically in the brain, but different areas are affected to different extent [6]. As a consequence, understanding physiology and the degenerative mechanisms of the DA neurons are critical for explaining PD pathogenesis and developing novel therapies. This review focuses on the DA neurons and which role it plays in the PD researches and treatment.

2. Dopaminergic Neurons

Dopaminergic (DA) neurons are neurons that synthesize and exploit dopamine as neurotransmitter in the central nervous system. Dopamine is of the most important neurotransmitter in physiology, involving in functions of the cardiovascular, renal, hormonal and central nervous systems. It also controls processes, such as movement, rewarding and addiction, etc. In mammals, DA neurons are the major source of dopamine [7].

By definition, DA neurons should possess DA, DA-synthetizing enzymes, DA-degrading enzymes, DA transporters and auto-receptors, while lack dopamine-β-hydroxylase and phenylethyl-N-methyl transferase, which convert DA into noradrenaline and subsequently adrenaline [8]. However, not all DA neurons in the midbrain satisfy the full criteria. Instead, DA neurons are anatomically and functionally different and form ten distinct clusters that distribute in the diencephalon (A11-A15), mesencephalon (A8-A10), olfactory blub (A16) and the retina (A17). (Figure 1) For example, in the non-human primate, the A11 neurons contain the first DA-synthesizing enzymes tyrosine hydroxylase (TH), but lack detectable levels of the second DA-synthesizing enzyme Aromatic l-amino acid decarboxylase (AADC) or DA receptors DAT, indicating that these neurons are probably L-DOPA-ergic, instead of dopaminergic. These DA neurons form a semi-continuous network, projecting from the mesencephalon to the forebrain, including the diencephalon [9].

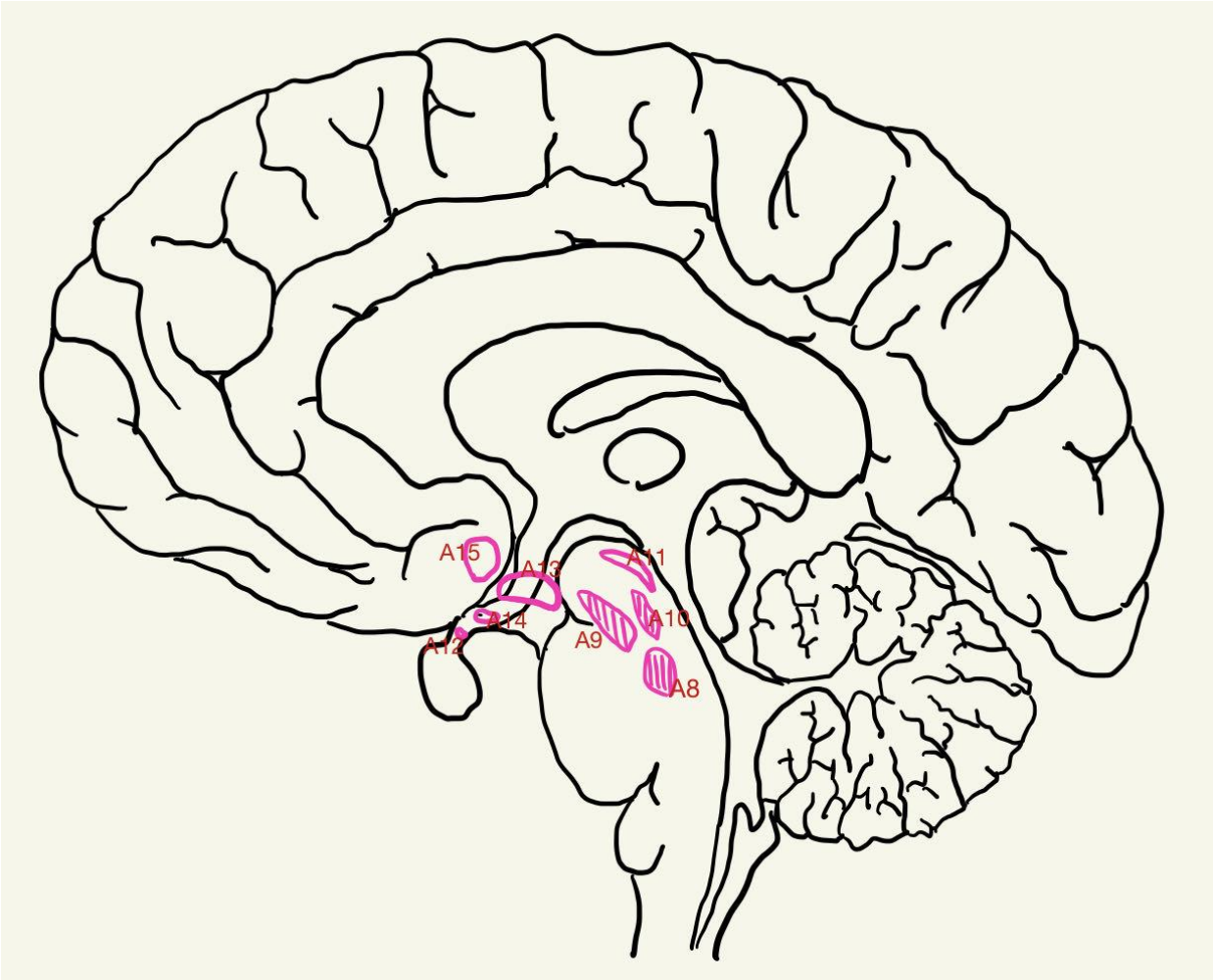

Figure 1. Distribution of different dopaminergic neuron clusters. Shaded magenta: significant degeneration in PD, Blanked magenta: may be degenerated, but no robust evidence. A8-A10: the mesencephalic complex. A8: the retrorubral field. A9: SNpc. A10: the ventral tegmental area. A11-A15: the diencephalic area. In addition, there is also the A16 cluster in the olfactory bulb and the A17 in the retina excluded by the figure.

3. The Relationship between Dopaminergic Neuron and Parkinson's Disease

3.1. Dopaminergic Neuron Degeneration and Parkinson's Disease

PD is a neurodegenerative disease that is associated with DA neuron loss, so that one hallmark of PD pathology is the progressive loss of neuromelanin-containing DA neurons in the SN pars compacta (SNpc), which is the A9 group. Notably, instead of affecting the SNpc alone, loss of DA neurons takes place in the entire dopaminergic system with severity to different levels. Among all ten groups, mesencephalic groups (A8-A10) contain the majority of the DA neurons and are most adversely affected [10]. In contrast, to what extent other neuron groups are affected is still ambiguous, since there is no sufficient information to derive robust conclusions.

In the ventral mesencephalic complex, DA groups A8-A10 are continuous without clear boundaries and neurons within them cannot be distinguished based on morphology because they exhibit the most complete dopaminergic phenotype (which means that they contain almost all the proteins and enzymes required to be a DA neuron by definition). Despite these similarities in traits, these groups are also differentially affected in PD. The A8 group show minor or even no degeneration, while the A10 group exhibit 40%-77% loss of neuromelanin-containing DA neurons. The A9 group is affected the most significantly, with 60%-97% of DA neurons dying. Therefore, there must be other factors that are affecting the viability of the DA neurons in the mesencephalic groups [10,11].

Currently, there are two theories aiming to explain the neurodegeneration in the DA cell nuclei in PD. The first one is the Lewy pathology, since extensive LB accumulation was observed within these areas. The LBs consist of various proteins, including misfolded α-synuclein (aSYN), which are known stimuli that triggers unfolded protein response and subsequent cellular death. Moreover, studies have shown that aSYN has prion-like features, so that it can spread to other brain areas from the injected site and acts as a template to direct misfolding of endogenous aSYN [12]. However, this hypothesis has been controversial, because the pattern of the Lewy pathology is inconsistent both within and between individuals. From pathological analysis, only half of the diagnosed PD brains matching the Braak model and the strength of synaptic connections cannot predict spreading of the misfolded aSYN accurately. In addition, it seems that DA neurons are particularly susceptible to the LB aggregation and LBs can present in many other areas in the brain for years without causing any adverse events. Finally, if the Lewy pathology were the primary reason for PD pathogenesis and progression, disease-modifying therapy should be focusing on mitigating protein misfolding and preventing aggregation. However, current analyses on anti-aSYN therapies predominately focus on evaluating tolerance and toxicity instead of effectiveness and alterations on disease progression [13,14]. Therefore, there is no sufficient evidence to support the causative role of the Lewy pathology. With all these speculations, a second explanation of DA neurodegeneration, which refers to mitochondrial dysfunction, was proposed.

This theory was developed based on the nature of the DA neurons. One notable and long recognized is the long and highly branched axons with multiple neurotransmitter release sites. However, this feature is not confined within the DA neuron type and not all neurons with such properties are susceptible in PD, such as the striatal cholinergic interneurons, indicating that there are other factors underlying the susceptibility. Another feature of the susceptible DA neurons is the slow and broad action potentials. These action potentials maximize Ca2+ ions entry from both extracellular influx and intracellular endoplasmic reticulum and promote autonomous slow rhythmic activities. Different from other neurons that rely on sodium ion channels to evoke action potentials, SNpc DA neurons exploit L-type calcium channels to achieve its pace-making behavior. These L-type calcium channels contain a Cav1.3 subunit that form the central channel pore. These Cav1.3 Ca2+ channels have much lower abundance within the brain and are open at more hyperpolarized membrane potentials compared to those with a Cav1.2 subunit [15].

Entered intracellular Ca2+ ions can then interact with other cellular components, since the level of calcium buffering proteins is low in the SNpc DA neurons. One downstream event is the entry into mitochondria to promote oxidative phosphorylation and ATP synthesis. This is proposed to be an

evolutionary feature to ensure survival, because the intracellular energy source is secured to support the continuous neuronal activity, including mobilization of the sensory and motor systems, of the DA neurons. On the other hand, as a trade-off, SNpc DA neurons are suffering from increased oxidative stress and sustained high levels of intracellular Ca2+ ions. The elevated oxidative stress resulted from reactive oxygen species and reactive nitrogen species can damage cellular components, amplify the detrimental effects of genetic mutations and toxins on mitochondria and promote aggregation of aSYN [15]. The high calcium concentration in the cytosol also increases the probability of the Lewy pathology by promoting aggregation and impairing clearance, such as lysosomal dysfunction. Subsequent to mitochondrial dysfunction, neurodegeneration maybe triggered.

Overall, low and broad action potentials, spontaneous pace generation, low cellular Ca2+ buffer capacity and cytosolic calcium fluctuations characterize together SNpc DA neurons, suggesting that these features may underpin the vulnerability of SNpc DA neurons. In this essence, the plasma membrane calcium channel, Cav1.3, is thought to be an important component. Not only because there is an increasing dependence on Cav1.3 channels to increase the intracellular calcium level associated with aging, but also because of the differential susceptibility among DA neurons with different physiology. For example, in comparison, the A10 DA neurons, which are significantly less vulnerable, utilize Na+ channels to accomplish pace-making and have stronger calcium buffering ability.

3.2. New Discoveries on Degeneration Mechanisms in the Dopaminergic Neurons

Degeneration of DA neurons is a major component in PD progression and scientists have carried out numerous studies, hoping to reach a consensus on the exact mechanism. However, this has never been achieved, because of the complexity of disease. There are a myriad of aspects and interactions between cellular components and among neurons. As a result, various hypotheses were developed.

Although reliance on Cav1.3 channels may be a crucial clue to DA neuron degeneration is not a novel proposal [15], the number of studies on it is relatively limited due to distraction of other hypotheses. The Cav1.3 channels were only begun to be studied as a therapeutic target until recent years. It has been identified decade ago that L-type calcium channel antagonists, such as the dihydropyridine class, can effectively reduce the risk and attenuate the progression of PD. However, the dihydropyridine class has failed to slow the disease progression in a Phase III clinical trial. Isradipine is one best studied drug of the dihydropyridine class. Although it has been justified that isradipine is capable of ameliorate disease progression in MPTP-induced PD model, it fails to perform better than placebo in early PD patients in a randomized trial. Several reasons may be attributable. First, the dose used in the trail may be too low to achieve desirable outcomes. Second, the bioavailability of isradipine is lower than expected. Finally, compared to other members in the dihydropyridine class, isradipine has an affinity for Cav1.3 channels that is approximately 40-fold higher, but this affinity is still 5- to 11-fold lower than that to Cav1.2 channels, depending on the splicing and isoforms of the Cav channels. Moreover, cardiac muscles in the heart also rely on the L-type calcium channels, so that cardiovascular toxicity is a potential side effect [16].

As discussed above, the dihydropyridine class does not distinguish between the Cav1.2 and Cav 1.3 channels. Facing this limitation, PD pathophysiology provides inspiration to break it. In PD, although the expression of Cav1.3 is elevated, DA neurons are not degenerated until later stage is achieved. Therefore, there should be some intrinsic mechanisms to inhibit Cav1.3 activity or promote neuron viability. Sun et al. investigated this problem from the perspective of the calcium oscillation and discovered that TRPC1- STIM1 complex is essential for protecting DA neurons. The store-operated Ca2+ channels (SOCs) were identified to maintain calcium homeostasis, and hence regulate neuronal function and promote cell survival. TRPC1 is a SOC and has been shown to be involved in DA neuronal functioning and protect DA neurons by diminishing ER stress. Stromal interacting molecule-1 (STIM1) regulates SOC-mediated calcium entry. Upon intracellular Ca2+ depletion, STIM1 rearranges and interacts with TRPC1 to activate it. In addition, the STIM1-TRPC1 complex also inhibits Cav1.3 channels. Consistent results were also derived from later study, suggesting that TRPC1 expression is down-regulated in PD brains. This study identified the interaction of Cav1

channels with other cellular components and proved that specific inhibition of Cav1.3 is achievable [16,17].

While there are studies focusing on exploring Cav1.3 channels, Cav1.2 channels are also shown to probably be involved in PD recently. In a 6-hydroxydopamine-induced neurotoxicity PD model, the expression of Cav1.2 channels was proved to be increased in the SNpc and blockade of these channels using nifedipine (another member of the dihydropyridine class) indeed restored DA neuron contents in mice. However, this does not mean Cav1.2 is part of PD pathogenesis, since the level of Cav1.3 was not controlled in such studies and nifedipine also does not bind to Cav1.2 channels selectively. Therefore, further investigations should be carried out to identify causes underlying the increased expression of Cav1.2 and what are the implications [18].

In addition, in a study on interaction between neurons and glial cells, it was demonstrated that Cav1.2 channels are also expressed on non-excitable cells, such as microglia (a type of immune cells residing in the brain). In this case, Cav1.2 inhibition was shown to promote proinflammatory phenotype differentiation and inhibit neuroprotective phenotype transition. As a consequence, when therapies targeting Cav1 channels are being developed, it is crucial to determine the dose carefully, since inhibition of Cav1 channels may have dual effects [19].

In addition to Cav1 channels, mitochondria and oxidative stress per se are also topics subjecting to extensive investigation. The transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2) is a major target to mitigate neuronal injury resulted from oxidative stress. Nrf2 is a key regulator in antioxidative mechanisms and exert anti-inflammatory effects. It can also control mitochondrial functioning and biogenesis. It can direct the expression of several enzymes, and these pathways are neuroprotective and is considered as a novel therapeutic target in PD treatment [19].

In recent studies, wedelolactone was able to tackle PD symptoms via the Nrf2/SKN-1 pathway in a worm model. It was shown that wedelolactone effectively relieve oxidative stress, restore dopamine level and promote mitochondrial health. Wedelolactone is derived from plants, especially from the Asteraceae family. This report raises the probability of combating PD using naturally existing compounds [20].

The Nrf2/Wnt/β-catenin cascade is a crucial pathway involved in DA neuron biogenesis and protection, and downregulation of the cascade is also observed in PD pathology. In the study done by Marchetti, activation of the Nrf2/Wnt/β-catenin pathway rescue SNpc from degeneration and activate cell proliferation and differentiation of the neuronal stem progenitors by rejuvenating astrocytes which are another type of glial cells. Moreover, the Wnt/β-catenin pathway also regulates mitochondrial biogenesis. Study have shown that knockdown of the Wnt/β-catenin inhibitor, Axin-2, protected SNpc DA neurons and improved behavioral functions in a 6-hydrodopamine induced rat PD model [19,21].

Finally, given that DA neurons exhibit a spectrum of phenotypic features and genetic expression, different expression profiles were highly speculated to be related to the differential degeneration pattern. Therefore, some studies were carried out based on this fundamental relationship to establish a clearer pattern of susceptible DA neurons. Kamath et al. used single cell profiling to study the cell population that selectively degenerates in PD. It turned out that a cell population with upregulated expression of AGTR1 that localize at the ventral edge of SNpc, the SOX6_AGTR1 population, was particularly susceptible to degeneration in PD. After further sequencing analysis, they found that this cell population was enriched with all risk genes identified by previous genome-wide association studies and several cell stress pathways were involved, such as those regulated by expression of the genes TP53 and NR2F2. Overall, this analysis provides insights in comparing and contrasting different neurodegenerative diseases, as well as testing potential pharmacological molecules. For example, it has shown that genetic influences act preferentially intrinsically on neurons in PD, while is more extrinsic in Alzheimer's disease. In contrast, the upregulated expression of NR2F2 and TP53 may provide potential linkage to other neurodegenerative diseases. Products of the gene NR2F2 was shown to promote mitochondrial malfunctioning in several models and TP53 was known as a component in motor neuron degeneration [22].

4. Conclusions

Although a large amount of work has been done, there are still many ambiguous points and debates in PD pathogenesis and therapeutic development. There are also many obstacles in the way. The major challenge is the lack of understanding on PD pathogenesis. Although many contributing factors have been identified, the causative role of these factors are not assertive. In addition, in-depth studies on interactions within and between these factors are still needed to clarify the functioning mechanism of neurodegeneration process.

With regard to investigations on DA neurons, further phenotypic profiling is required, so that the question on differential extent of degeneration in PD may be answered by comparing genomics, proteomics or transcriptomics. By identifying potential factors that distinguish DA neurons in different groups, more specific therapeutic targets may be discovered to develop potentially more effect disease-modifying treatment.

Another obstacle that prevents breakthroughs from being made in the field of PD pathogenesis is that current models are not sufficiently representative. Genetic mutation models do exhibit PD features, but the model is not stable enough. Although intra-striatal injections of aSYN can cause parkinsonism, the models are not robust because they do not retain the progressive nature of PD. As in previous discussion, different targets and drugs were only justified in one specific model. Mitochondrial impairment, despite of its importance, is ubiquitous consequence of a large number of cellular stress pathways, making it a rather disappointing indicator in assessing how mitochondrial dysfunction and aSYN aggregation are integrated in PD onset and progression. Without models that can represent PD firmly, it is barely possible to obtain reliable data that can evaluate different hypothesized causative factors, and tests of pharmacological molecules also lack directiveness. Therefore, PD pathogenesis can be studied from both directions, either form causes to treatments or from treatments to causes, and success from either will make great contributions to interpretation of the disease and future studying.

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