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The Role of Olfactory-Nigral Circuitry in Neurodegeneration and Hyposmia in Rodent Model of Parkinson’s Disease

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February 4, 2019
Abstract
Parkinson’s Disease (PD) is a chronic and progressive neurodegenerative disorder characterized by both motor and non-motor symptoms. The motor symptoms include bradykinesia and resting tremor while the non-motor symptoms associate with sleeping disorders and olfactory dysfunctions. Besides that, patients may experience hyposmia prior being diagnosed with PD. Thus, the present project’s objective is to confirm a new dopaminergic pathway, which contributes to hyposmia link in pre-exposed PD patients. In order to validate the nigro-olfactory pathway, the MitoPark model was set up in the combination of several techniques including cryosectioning, mounting, and fluorescent microscopy. After collecting data, there were significant fluorescent of FG tracers emitted at Striatum. In order to validate the hypothesis, more fluorescent data need collecting specifically for Dil and CTB at the olfactory bulb.
Introduction

According to National Institute of Environmental Health Sciences, neurodegenerative diseases affect millions of people worldwide. Alzheimer’s disease and Parkinson’s disease are the most common types, with more than five million Americans living with Alzheimer’s, and at least 500,000 Americans living with Parkinson’s (Hollander & Lawler, 2019). Thus, understanding the pathogenesis, which underlies these chronic diseases, is essential to identify and explore new cures or treatments. In particular, Parkinson’s disease (PD) is a chronic pathology characterized by massive degeneration of dopaminergic neurons in the substantia nigra, the loss of striatal dopaminergic fibers and a dramatic reduction of the striatal dopamine levels (Schober, 2004).

Many PD-diagnosed patients may experience both motor and non-motor symptoms, which were associated with the loss of striatal dopaminergic neurons. In PD, the motor symptoms include resting tremor, stiffness, slow walking, bradykinesia, and muscular rigidity while the non-motor pathophysiological changes associated with sleeping disorders, depression, olfactory dysfunction, and cognitive changes (DeMaagd & Philip, 2015).

In order to study PD and generate symptom-like on rodent model, there were several toxins utilized in the project. Several studies from Dr. Schober showed that exposing to dopamine (DA)-toxic compounds/chemicals via olfactory bulb such as 6-hydroxyl-dopamine (6-OHDA) and 1-methyl-4-phenyl-1, 2,3,6-tetrahydropyridine (MPTP) was able to destroy catecholaminergic neurons (Schober, 2004) in PD model. According to Dr. Schober, 6-ODHA is an analogue of neurotransmitter dopamine, which induces the efficient and long lasting noradrenaline depletion in central nervous system to the heart. The accumulation of cystolic 6-OHDA initiates apoptosis via mitochondrial pathway. However, his model does not mimic all pathological and clinical features of Parkinsonism. It induces the dopaminergic neuron death but does not show the formation of Lewy bodies. Thus, it does not affect the anterior olfactory structures.

In addition, MPTP is an analogue of narcotic meperidine, which is lipid soluble and able to cross blood-brain barrier. MPTP is metabolized to MPDP via Monoamine oxidase B, which in turn accumulates as MPP+ as the final product. MPP+ uptake depends on active carriers such as VMAT and MPP since they transport vesicle-containing DA inside the cytoplasm. MPTP fails to show the formation of Lewy bodies in patients and monkey model. Summarizing Dr. Schober’s studies, there are two essential take-home messages. First, the presence of Lewis bodies at cellular level indicates the progression of PD. Second, MPP+ and 6-ODHA are two common toxins used in rodent model to initiate the apoptosis of dopaminergic neurons (Schober, 2004).

According to Dr. Alexander from Emory University School of Medicine, the neurons in substantia nigra are selectively vulnerable to the motor deficits in PD. They produce the neurotransmitter dopamine, which are responsible to relay messages that plan and control body movement. Progression of motor dysfunction
in PD is correlated with reductions of nigrostriatal dopamine terminals within the same striatal territories. Besides that, a complex chain of decisions involving interconnected ganglia controls the body movement. Information comes to a central area of the brain called striatum, which works with the substantia nigra to send impulses back and forth from the spinal cord to the brain (Alexander, 2004). Therefore, in PD patients, there is a significant decrease in the concentration of dopamine neural modulators from substantia nigra to striatum. This is known as a classic pathway observed in PD patients.

On a different study performed by Dr. Doty of University of Pennsylvania School of Medicine, the xenobiotics (prions, notably viruses) are able to enter the brain via the olfactory mucosa. The loss of smell occurs during preclinical phase of PD. 90% patients with early stage PD exhibit olfactory dysfunction via psychological and electrophysiological test (Doty, 2008). The neuropathy begins with Lewy bodies localized within the olfactory bulb, anterior olfactory nucleus, and dorsal motor nucleus of the vagus nerve and then advances through regions of medulla oblongata, mid brain, and basal forebrain. The finding confirmed the presence of Lewis bodies in olfactory bulb and anterior olfactory nucleus (Doty, 2008). This suggested a different dopaminergic pathway, which starts from substantia nigra to olfactory bulb. If nigral neurons project tracts to the olfactory bulb in an addition to the striatum, then this pathway may provide a direct link between hyposmia and Parkinson’s disease.

In order to support the existence of new finding dopaminergic pathway, Dr. Bohnen from University of Michigan School of Medicine and his laboratory performed University of Pennsylvania Smell Identification Test (UPSIT) on 29 subjects. The result showed that the correlation coefficients between total UPSIT scores and regional brain dopamine transporter binding potentials were highest for the hippocampus and lowest for dorsal striatum. Under selective hyposmia condition, the study suggested that, besides striatum, neurotransmitter dopamine might innervate other brain regions including hippocampus, which is in charged of olfactory cognitive and memory processing (Bohnen, Gedela, Herath, Constantine, & Moore, 2008).

In addition, olfactory system and olfactory signal transduction play crucial roles in hyposmia condition. The olfactory system detects airborne agents in the human respiratory system, which dissolve in the mucus that covering the surface of olfactory epithelium in the nasal cavity. In addition, olfactory epithelium contains around 6 million of bipolar receptor cells (cell bodies and dendrites) that contain odorant receptors. Together with taste sense, the sense of the smell is critical for food selection and has a strong affective component in the sexual and other behaviors. Moreover, olfactory bulb contains mitral cells that receive information from sensory neurons and in turn send projections to central pathways. It also contains other types of neurons and performs the first stage of information processing (Doty, 2009).
According to Drs. Ronnett and Moon from Johns Hopkins Institute for Basic Biomedical Sciences, olfactory signal transduction is an interface of the environment and nervous system. The membranes of olfactory cilia contain odorant and G-protein coupled receptors. When odorants bound, the receptors are activated by adenyl cyclase, which catalyzes formation of cyclic AMP (second messenger). Increased levels of cAMP open cAMP-gated Na+ channels, which depolarize cell receptor and generate an action potential leading to releasing glutamate neurotransmitter. Neurotransmitters then activate mitral cells in the olfactory bulb. Therefore, olfactory bulb plays vital role in olfactory signal transduction (Ronnett & Moon, 2002).

Hyposmia is a condition where the patients may lose partial of smell sensation. In a study published in January of 2004, Dr. Uylings showed that the total number of tyrosine hydroxylase-immunoreactive neurons in the olfactory bulb was twice as high in Parkinson patients compared to age and gender-matched controls. Because dopamine was known to inhibit olfactory transmission in the olfactory glomeruli, his team suggested that the increase of dopaminergic neurons in the olfactory bulb was responsible for the hyposmia in Parkinson patients (Huisman, Uylings, & Hoogland, 2004). He also found that increasing dopamine in olfactory bulb explained why olfaction did not improve with levodopa therapy, which was the current treatment for PD (Schober, 2004). Under stress condition, dopaminergic neurons secreted high levels of chemokine-like signaling protein called Prokineticin-2 (PK2) as the compensatory protective response. In Dr. Kanthasamy’s study, a PK2 agonist IS20 was used to mimic the effect of PK2 in the primary culture. In addition, IS20 blocked MPTP-induced reductions in astrocytes (Neal, 2018).

Methodology

Mouse Model

The rodent model was utilized in order to trace the pathway of dopamine neurotransmitter from substantial nigra to olfactory bulb. The model consisted of 12 mice, which was divided into two groups: littermate control and MitoPark. In the MitoPark group, the mice were further divided into two subgroups: one subgroup was treated with IS20, and the other subgroup was labeled vehicle (non-ingested IS20). The littermate control group underwent the same division as the MitoPark group (Langleley et al., 2017). The MitoPark model is a newer model recently popularized for PD study. It is a model knockout of tfam genes in dopaminergic neurons, which caused mitochondrial deficits (reduction of ATP) and death of dopaminergic neurons. In summary, the animal model recapitulates man PD-symptoms seen in human patients including olfactory deficits.

Neuronal Tracers

There were three neuronal tracers injected in the model including 1,1’-dioctadecyl-3, 3, 3,3’-tetramethylindocarbocyanine perchlorate – Dil (Bartheld, Cunningham, & Rubel, 1990), cholera toxin unit B – CTB (Conte, Kamishina, &
Reep, 2009), and Fluoro-Gold (Schmued & Fallon, 1986). 1μl of Dil and 1μl of CTB were injected into the olfactory bulb site simultaneously from the same syringe at a rate of 0.4 μl/min for total of 5 minutes. Flouro-Gold was injected to the striatum site at a rate of 0.4 μl/min for total of 2.5 minutes. Later, the model brains were collected and stored at -20 degree Celsius with optimal cutting temperature (OCT) compound.

**Cryosectioning Technique**

The technique was incorporated to separate brain cross-sectional images at coronal and sagittal planes. The frozen brain was mounted with OCT compound into the sample stubs. In the cryostat chamber, the stub was placed securely into the chuck with horizontal and vertical locks. The sectioning was performed at a thickness of 35 ηm. Each section was collected with angled brush and stored in sucrose and 1XPBS solution for cryoprotection (Beedle, 2016).

**Florescent Microscopy Technique**

In order to visualize the dopaminergic pathway, the laboratory used fluorescent microscopy to trace the neurotransmitter. Generally, a fluorescence microscope contains an excitation filter, a dichroic beam splitter, and an emission filter. First, the excitation filter selects the interested wavelengths to excite a particular dye. Then, the emission filter serves as a kind of quality control since it only let the interested wavelengths pass through. After that, the dichroic mirror reflects the light in the excitation band and transmits the light in emission band for light illumination (Sanderson & Bootman, 2016). In the experiment, there were three stains emitted in the application. Dil stain emitted red color while CTB stain emitted green color at olfactory bulb injecting site. The Flouro-Gold stain emitted gold at striatum injecting site.

**Immunohistochemistry Technique**

Immunohistochemistry (IHC) was utilized for additional section visualization. It combines anatomical, immunological, and biochemical techniques to image discrete components in tissues by using appropriately labeled antibodies to bind specifically to their target antigens in situ (Matos, Trufelli, De Matos & Da Silva Pinhal, 2010). Specifically, IHC is a 3-day long process. In first day, the interested sections are washed with phosphate buffer solution (PBS), and then incubated with methanol containing 3% H₂O₂. Next, blocking took place where the sections are incubated in blocking buffer for 60 minutes. To wrap up day 1, the sections are incubated in primary antibodies for 24 hours at 4°C.

In the second day, the process begins with washing the sections with PBS and incubating in second antibodies for 60 minutes at room temperature. Next, the sections are washed with PBS and incubated in ABC solution for 1 hour. After washing with PBS, the color development took place with the combination of 50μl of 30% H₂O₂ in 15 ml dH₂O, 25 mg DAB in 50 ml PBS, and 250 diluted H₂O₂. The sections are incubated in DAB solution for 60 seconds and washed with PBS. Then, the sections are dipped in cresyl violet dye for 4-6 minutes. Washing in deionized water stops the reaction moving forward. Next, washing in 70% ethanol
for 3 minutes washes off cresyl violet deionized H₂O. In day 3, the washing continues taking place with the following order: 50% Ethanol, 70% Ethanol, 95% Ethanol, 100% Ethanol, Xylene 1, and Xylene 2 (Matos, Trufelli, De Matos & Da Silva Pinhal, 2010).

**Results**

Table 1. Identification of MitoPark Mice Table

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<th>Mouse ID</th>
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<tr>
<td>03B</td>
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Table 2. Identification of Littermate Control Mice Table

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Figure 1. FlouroGold Tracer in Striatum of 02K-MPV

Figure 2. CTB Tracer is Olfactory Bulb of 02L-MPV
Discussion

In order to validate the hypothesis, the MitoPark model was utilized with conditional TFAM knockout. Parkinson’s Disease tends to disrupt mitochondrial activity. One of the essential genes is tfam gene, which regulates the mitochondria DNA replication and transcription. Moreover, the gene also got involved in dopaminergic transporter (DAT) transcription. Therefore, inactivating tfam gene in
MitoPark model would result in dopamine deficits in these mice. Moreover, the role of dopamine in olfactory bulb is not well understood (Hollinger et al., 2015). 6-OHDA and MPTP were neurotoxins used in the experiment to generate the loss of dopaminergic neurons.

In Table 1 and Table 2, each mouse was identified and recorded with its date of birth for both littermate control and MitoPark model. Unfortunately, 02X in littermate control was found dead before injection day. However, its brain was collected for further experiment. According to Figure 1, the FlouroGold tracer emitted blue fluorescent in the striatum of 02K (MitoPark-Vehicle), where it was initially injected. FlouroGold was a retrograde tracer starting from the terminal ends toward the cell bodies, which were located at Substantia Nigra par compacta. In Figure 3, there was not significant fluorescent of FlouroGold in Substantia Nigra due to the retrograde effect of the tracer. In Figure 2, the CTB tracer emitted green fluorescent in the olfactory bulb of 02L (MitoPark-Vehicle). However, in the experiment, there was not significant fluorescent of CTB in olfactory bulb. Figure 5 and Figure 6 gave a better visualization of CTB and Dil fluorescents. For future studies, we may collect more images having CTB and Dil tracers to validate the hypothesis.

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References


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