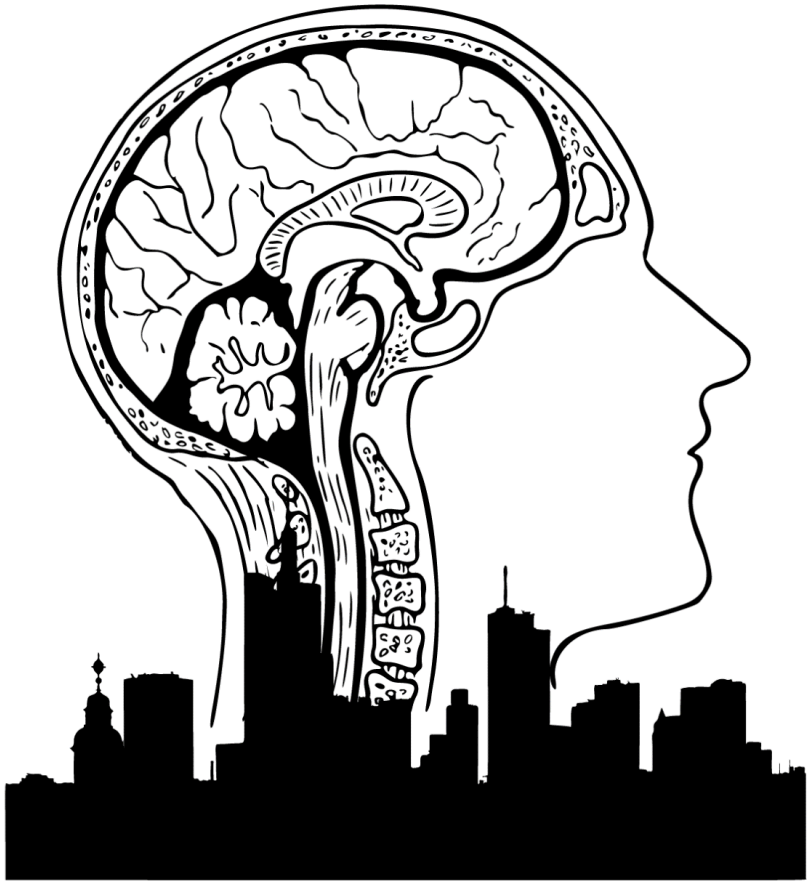
Could air pollution cause brain pollution? How ultra-fine particle air pollution is associated with Alzheimer's disease.



Author: Alicia Barten
Student number: 3114791
Field of study: Master Biomedical Sciences (Neuroscience)
Research group: Neurobiology
First examiner:
Ulrich Eisel
Date (completion): 19-12-2022

Abstract

Around 91% of the world's population lives in areas where the air quality levels exceed the limits set by the World Health Organization (WHO) air quality guidelines. Previous studies have demonstrated that fine particle pollution (PM2.5) can be associated with cardiovascular disease, respiratory disease, and lung cancer. However, more recent studies indicated PM2.5 can be associated with neurodegenerative disease as well. To further elucidate the role of PM2.5 in the development of neurodegenerative disease and specifically Alzheimer's Disease (AD) this essay analyzed a broad range of literature. This literary analysis demonstrated that PM2.5 exposure affects the CNS through different mechanisms that include: peripheral systemic inflammation, disruption of the blood-brain barrier, pathological abnormalities in of olfactory neurons and gut-microbiota brain axis alterations. Consequently, this leads to neuroinflammation, mitochondrial dysfunction, oxidative stress, and cell death. All of which have shown to play a crucial role in the pathophysiological process of AD as well. Due to the complexity and diversity of PM components, the development of AD is likely to result from multiple pathways and pathological interactions.

Table of contents

Introduction

Around 91% of the world's population lives in areas where the air quality levels exceed the limits set by the World Health Organization (WHO) air quality guidelines. Approximately 4.2 million premature deaths are caused by ambient air pollution (Tsai et al.,2019). Air pollution is generated by a mixture of various substances including gases, particles, and biological components from the earth's atmosphere. The toxicity of particulate matter is dependent on its size, superficial area, and chemical composition (Thangravel et al.,2022). Urban air pollution consists of two elements: gaseous components and particulate matter (PM) (Glencross et al.,2022). PM consists of solid and liquid particles that result from diesel usage, road and agricultural dust, and industrialization (Thangravel et al.,2022). PM stays suspended in the air because of the following properties: size, density, thermal conditions, and wind speed. PM can be subdivided into fractions based on particle size (Thangravel et al.,2022). Only particles smaller than 10 μm such as PM2.5, PM10 and UFPM can reach the lower airways (Glencross et al.,2022). Since particulate matter that is smaller in size (PM2,5) has the capability to carry various toxic chemicals and can penetrate from the alveolar space into the circulatory system, it has a greater potential to impact human health than coarse particulate matter (PM10) (Tsai et al., 2019). PM2.5 is predominantly composed of both organic and inorganic compounds consisting of sulfates, nitrates, carbon ammonium, hydrogen ions, lipopolysaccharides (LPS), metals, and water (Madrigano et al.,2011). The major source of this pollutant is the combustion of fossil fuels (Tsai et al.,2019). In past studies, it has been shown that there is robust evidence to support an apparent association between long-term exposure to PM and pulmonary and cardiovascular diseases (Thiankhaw et al.,2022).

However, that is not the only pathological manifestation due to PM, there is growing evidence that indicates that PM2.5 exposure is associated with neurodegenerative diseases including Alzheimer's Disease (AD) (Thiankhaw., 2022). AD can be defined as a slowly progressive condition that is characterized by the presence of neuritic plaques and neurofibrillary tangles that occur as a result of the accumulation of amyloid-beta peptide's (Aβ) in predominantly the medial temporal lobe and neocortical structures (Breijyeh et al., 2020).

In vitro studies suggest that exposure to acute and high doses of PM2.5 can promote AD pathologies in the brain which may be caused by several factors including induction of neuroinflammation, increased ROS activity, and an increase in the pro-amyloidogenic processing of the amyloid precursor protein (APP) (Thiankhaw et al.,2022).

With regard to in vivo experiments, animal models have demonstrated that long-term and high-dose exposure to PM2.5 is associated with neuronal synaptic changes and the release of several inflammatory cytokines (Thiankhaw et al.,2022). Additionally, Ku et al. established that PM2.5 exposure induces the release of inflammatory cytokines in the hippocampus of C57BL/6 mice in a dose-dependent manner (Ku et al.,2017). AD pathology has equivalently been found in animal models that were subjected to long-term PM2.5 exposure. After a 9-month period of exposure to PM2.5, a C57BL/6 mice model exhibited a significant increase in Aβ and immunoreactivity in the temporal cortex. Additionally, PM2.5 exposure caused an upregulation of APP processing, Aβ 1-40 levels, and beta-site amyloid precursor protein cleaving enzyme 1 (BACE1), which promoted the processing of APP in an amyloidogenic pathway (Bhatt et al.,2015). Animal models have correspondingly found neuronal morphological changes following PM2.5 exposure, aged Sprague-Dawley exhibited a reduction in doublecortin cells, and dendritic

complexity of mature neurons in the hippocampal subgranular zone subsequent to 28 days of exposure (Cheng et al.,2017).

There are also supplementary clinical outcomes indicating that long-term exposure to above-standard levels of PM2.5 is associated with the presence of pathological AD hallmarks (Thiankhaw et al.,2022). This has been found in young adults as well as the elderly. In two prospective pilot studies conducted by Calderón-Garcidueñas et al., children and young adults who lived in an area with an annual average PM2.5 concentration above the United States environmental protection agency (US EPA) standards level were studied. It was reported that these individuals experienced a decrease in CSF Aβ and brain-derived neurotrophic factor (BDNF). Additionally, an elevation of CSF tau, in both the total and phosphorylated form was registered (Calderon-Garciduenas et al., 2008; Calderon-Garciduenas et al., 2015). Furthermore, a population-based cohort study conducted in Taiwan on adults has shown that an increase of 4.34 g/m3 in PM2.5 exposure led to a 138% increased risk of newly diagnosed AD during the 10-year follow-up period (Jung et al.,2015).

In summary, there is considerable evidence from in vitro, in vivo, and clinical studies that long-term high-dose exposure to PM2.5 is associated with AD biomarkers. However, the exact pathways by which PM2.5 mediates neurotoxicity and cognitive dysfunction still remain unclear. The current essay has collected literature to analyze the association between PM2.5 exposure and the neurodegenerative disease Alzheimer's to answer to the following question: What pathways are involved that could link the inhalation of PM2.5 to neurodegenerative diseases, specifically Alzheimer's Disease?

Chapter 1: Entry of PM2.5 from the external to internal environment

Since PM2.5 is in the air we breathe the initial sites of PM2.5 entry and deposition are the airways and lungs. Considering this characteristic, the airway and lungs are the primary targets of PM2.5 toxicity (Thangravel et al.,2022). Once PM2.5 is inhaled the particles deposit on the surface of the airways, pulmonary bronchi and alveoli. Thereafter PM2.5 is internalized in the lung cells including epithelial cells and alveolar macrophages (Thangravel et al.,2022). Following, PM2.5 induces oxidative stress and impairs the normal function of the cells and can ultimately lead to apoptosis. Additionally, PM2.5 induces an inflammatory response which plays an important factor in the respiratory damage observed. These proceedings occur through different mechanisms by which PM2.5 can affect the respiratory system. Most recently the following mechanisms have been uncovered: injury from free radical peroxidation, imbalanced intracellular homeostasis, and inflammatory injury (Xing et al.,2016) (Figure 2).

1.1 Injury from radical peroxidation

The PM2.5 surface is rich in copper, iron, zinc, manganese, and other transition elements. These elements increase the free radical output in the lungs, consume antioxidant ingredients and cause oxidative stress (Xing et al., 2016). Furthermore, these soluble metals including transition metals are capable of activating redox cycling (Kelly., 2003). Consequently, PM2.5 can induce free radical production that can oxidize lung cells (Dellinger et al.,2001). In accordance, it has been demonstrated that the surface of the particle itself can produce free radicals (Donaldson et al.,1997). Subsequent studies have confirmed that PM-induced radicals are able to reduce oxygen to superoxide, which can then form hydrogen peroxide and eventually hydroxyl radicals (Dellinger et al.,2001)). Hydroxyl radicals (• OH) are the primary factor causative to oxidative damage of DNA (Xing et al.,2016). Hydroxyl radicals can react with the elements of DNA near or at diffusion-controlled rates, which can then cause damage to the heterocyclic DNA bases and the sugar moiety (Dizdaroglu et al.,2012).

Furthermore, it has been demonstrated that PM cannot only damage DNA and suppress repair but also promote the replication of damaged DNA fragments and therefore prompt carcinogenesis (Xing et al.,2016). These effects are caused by the property of PM to inhibit nucleotide excision repair (NER) and to enhance both spontaneous and DNA damage-induced mutagenesis. It has been suggested that this results from the following occurrences: components in PM including heavy metals and aldehyde directly modify repair proteins and DNA, ROS and secondary products resulting from ROS modify repair proteins and DNA, direct modification of DNA replication proteins by heavy metals and aldehydes reduces the fidelity of DNA replication (Mehta et al.,2008).

1.2 Imbalanced intracellular calcium homeostasis

One of the most important second messengers constitutes that mediates and regulates cell function both physiologically and pathologically is calcium. It has been reported that PM2.5 induces ROS production and decreases the antioxidant capacity of cells. Not only does this cause oxidation of lung cells and induction of oxidative damage to DNA it can also result in the peroxidation of lipids on the cell membrane. This peroxidation can result in the destruction of the ultrastructure of mitochondria (Xing et al.,2016; Wei et al.,2021). Subsequently, the release of Ca^{2+} is induced from mitochondria into the cytoplasm which leads to alveolar macrophage (AM) apoptosis. Apoptosis is induced after mitochondria have released Cytochrome C into the cytoplasm as a result of the Ca^{2+} overload-dependent activation of phosphatase. After which, Cytochrome C and caspase-9 form apoptotic bodies which activate caspase-3 and thereby lead to cellular apoptosis (Wei et al.,2021). In addition, when there is an abnormally increased concentration of calcium this activates a series of inflammatory reactions which consequently leads to inflammation and cell damage (Xing et al.,2016). Furthermore, the increase in intracellular Ca^{2+} concentrations can lead to further elevation of radical or ROS production (Xing et al.,2016). Moreover, Ca^{2+} can activate endonuclease and degrade nuclear DNA (Wei et al.,2021).

1.3 Inflammatory injury

It has been demonstrated that PM2.5 is related to the release of inflammatory cytokines which leads to the overexpression of various transcription factor genes and inflammation-related cytokine genes that induce inflammatory injury (Xing et al.,2016). Similar to the above-stated determinants for respiratory damage resulting from PM2.5 oxidative stress and the generation of ROS is of importance in the PM-induced inflammatory response (Wang et al.,2017). It has been established that PM2.5-induced inflammation leads to an increase in the amount of neutrophils (Sigaud et al.,2008). Furthermore, PM2.5 and its microenvironment affect the function of the following polarized alveolar macrophages: M1 and M2. M1 polarized macrophages are mainly induced by Thl-type cytokines (IL-12, IFN-γ) and pathogens and promote inflammation. M2-polarized macrophages are related to Th2-type cytokines (IL-4 and IL-13) and the anti-inflammatory cytokine IL-10 (Xing et al.,2016). Studies have reputed that when human alveolar macrophages are treated with PM2.5 they express high levels of M1- associated cytokines including IL-et 12 and IFN-Y and low levels of M2-associated cytokines including IL-4, IL-10 and IL-13 (Xing et al.,2016;Wang et al.,2017). Zhao et al., established that this inflammatory response could be partly generated by a ROS-dependent pathway resulting from mitochondrial dysfunction. Furthermore, the inhibition of anti-inflammatory cytokines could be induced by the PM2.5-mediated activation of mTOR which inhibits M2 macrophage polarization (Zhao et al.,2016) (Figure 1).

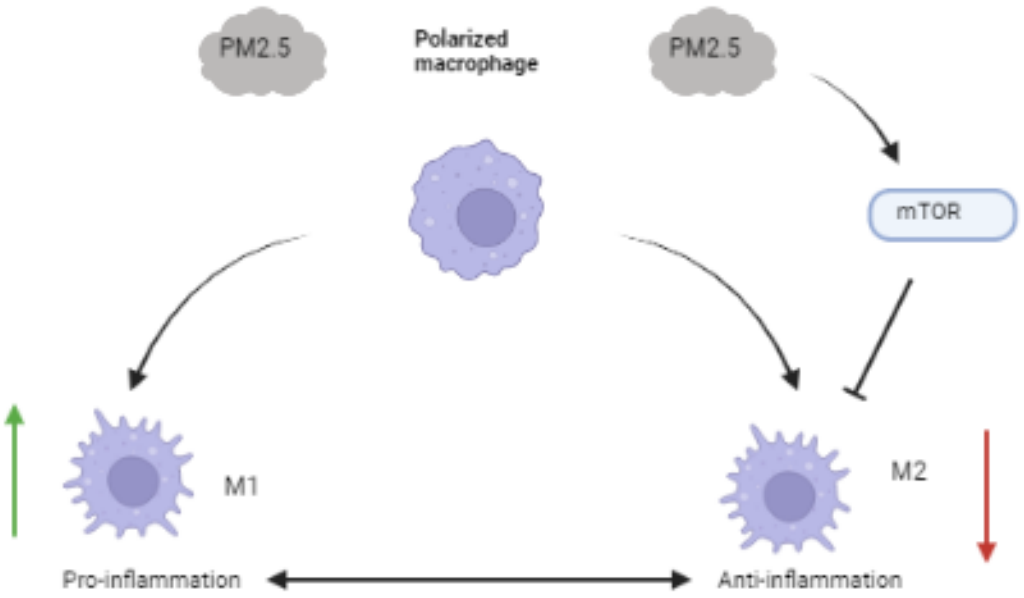


Figure 1: PM2.5 induced upregulation of M1 polarized macrophages. PM2.5 is associated with a higher level of cytokines expressed by the proinflammatory M1 macrophages and a lower level of anti-inflammatory M2 expressed cytokines. This is regulated by the activation of mTOR which inhibits M2 activation.

In addition, urban PM could be causative for the release of L-1β, IL-6, and IL-8, and increase the expression of MMP-9. MMP-9 is associated with tissue remodeling in several pulmonary diseases and partakes in the breakdown of extracellular matrix. In addition, urban PM can induce COX-2 expression in human bronchial epithelial cells (HBECs). COX-2 is a pro-inflammatory enzyme that is demonstrated to be associated with the inflammatory response and cytotoxicity perceived from PM exposure (Wang et al.,2017).

Moreover, numerous studies have reported that PM can induce multiple different signalling pathways to regulate the PM-induced inflammatory response these include the Mitogen-activated protein kinase (MAPK) and Nuclear factor-κB (NF-κB) pathways (Wang et al.,2017).

The MAPK pathways play an essential role in the transduction of extracellular signals into cellular reactions. MAPK cascades relay, amplify and integrate impulses from a wide scope of stimuli and consequently elicit an adequate physiological response. These elicited responses include cellular proliferation, differentiation, inflammation, and apoptosis (Wang et al.,2017; Zhang et al.,2002). The MAPK pathway is generally activated by a triple kinase cascade. This cascade consists of: MAPK, MAPK kinase (MAPKK), and MAPKK kinase (MAPKKK). ERK, JNK and P38 are three common members of the MAPK family and participate in distinct biological processes, however, there is some crosstalk between these pathways (Wang et al.,2017). The NF-κB pathways are involved in various cellular responses including cell proliferation and apoptosis, neural development, reaction toward infection and

inflammation (Biswas et al.,2016). NF-κB can mediate the induction of an array of pro-inflammatory genes in innate immune cells. Furthermore, NF-κB is able to regulate activation, differentiation and the function of inflammatory T cells (Lui et al.,2017).

Furthermore, subsequent to the PM2.5 induced a dose- and time-dependent manner increase of intracellular ROS generation the upregulation of JNK, ERK1/2, p38 MAPK, and AKT phosphorylation, and nuclear translocation of NF-κB has been demonstrated in a human umbilical vein cell line (Rui et al.,2016). In addition, Wang et al., reported that urban PM is capable of generation of the phosphorylation of ERK, JNK, p38 MAPK and NF-κB. ERK, JNK and p38 MAPK which further activates the NF-κB pathway and causes the expression of the following pro-inflammatory proteins: IL-1β, IL-6, IL-8, MMP-9 and COX-2. It is suggested that the PM-induced MAPK signaling pathway is able to activate the NF-κB pathway downstream to promote further inflammatory responses (Wang et al.,2017).

Chapter 2: How PM2.5 affects the Central Nervous System (CNS)

There are four proposed mechanisms by which air pollutants including PM2.5 can affect the CNS: peripheral systemic inflammation, direct transport or signaling through receptors at the BBB, direct transportation through the olfactory tract, and signaling via the sensory afferents in the gastrointestinal tract (brain-gut axis) (Shou et al.,2019; Oppenheim et al.,2013) (Figure 2).

2.1 Peripheral systemic inflammation

As previously mentioned, when PM2.5 is inhaled in the lungs it can result in an acute pulmonary inflammatory response and release various inflammatory factors (Jia et al.,2021). Initial inflammation in the lungs can be followed by inflammation in distant tissues (Xu et al.,2013). In vivo and in vitro experiments demonstrated that PM2.5 increases the expression of inflammatory cytokines including TNFα, ICAM-, IL-6, and the release of chemokines (ICAM-1 and MCP-1) which facilitate the infiltration of inflammatory cells (Li et al.,2021). Additionally, PM2.5 induced systemic chronic inflammation as signaled through leukocyte recruitment in the microcirculation after 3 weeks of exposure. Markers for macrophage migration were detectable as short as 5 days after exposure (Xu et al.,2013). Furthermore, there is a strongly increased risk for the development of dementia after systemic infections indicating that the peripheral immune system has a strong effect on the brain (Hoogland et al.,2015).

Microglia are the most prominent immune cells of the central nervous system and are the first responders to irregularities in the brain (Augusto-Oliveira et al., 2019). Microglia exist in two states: a resting state and an activated state. When in the resting state microglia survey the micro-environment for damage, to be prepared to support endangered neurons or interfere with any potential threats to tissue integrity (Hoogland et al.,2015). When a threat does arise microglia transform to their activated states which are also referred to as the M1 and M2 phenotypes. M1-activated microglia produce pro-inflammatory factors and are suggested to function as neurotoxic cells. M2-activated microglia have a role in remodeling and repair and are induced by signals from apoptotic cells (Hoogland et al.,2015). Experimental studies have demonstrated that systemic challenge through LPS in gram-negative bacteria leads to microglial activation. Even though the exact mechanism connecting systemic inflammatory

challenge to microglial activation remains unclear. Several mechanisms have been shown to play a role in this occurrence. The microglia could be activated by primary autonomic afferents, in particular, the vagal nerve and active transport through the blood-brain barrier of pro-inflammatory chemo and/or cytokines. Or through the passive transport of pro-inflammatory factors through the circumventricular organs (Hoogland et al.,2015).

An important factor influencing the level of microglial activation after the systemic inflammatory challenge is age (Hoogland et al.,2015). It has also been known that the greatest risk factor for Alzheimer's disease is advanced age (Guerreiro et al.,2015). When we age this process induces changes in the microglial phenotype which is also defined as "priming". When aged mice were given a systemic LPS challenge it caused a hyperactive microglial response in the brain, which is associated with higher induction of inflammatory IL-1β and anti-inflammatory IL-10 cytokines. Additionally, a peripheral CFA injection, which is a component used to induce inflammation, induced hippocampal activation in middle-aged rats. In comparison, there was only a moderate activation in young rats. The microglial activation that was observed in middle-aged rats was associated with neurocognitive deficits (Hoogland et al.,2015).

In conjunction, several studies have shown that there is an association between systemic LPS challenge, microglial activation, and cognitive deficits in mice. The possible underlying mechanism is caused by the fact that LPS challenge causes cyclooxygenase-1 (COX-1), COX-2, and inducible nitric oxide synthase (iNOS) expression in the brain which is a factor hypothesized to make mice susceptible to cognitive deficits (Hoogland et al.,2015).

Furthermore, systemic inflammation which leads to an increase in the circulating levels of pro-inflammatory cytokines could cause disruption of the blood-brain barrier (BBB) (Koyama et al.,2022). When there is a disruption of this barrier particles could pass through the BBB into the brain and subsequently trigger neuroinflammation and neurodegeneration (Shou et al.,2019).

2.2 Disruption of the blood-brain barrier

The BBB creates a highly stable internal environment for the brain and prevents any foreign objects such as toxins to invade brain tissue (Cai et al.,2018). It is a highly selective semipermeable structure and a chemical barrier that creates a division between the circulating blood from the brain and the extracellular fluid. The BBB is comprised of endothelial cells of the capillary walls, pericytes, astrocytes, and tight junctions (TJ) (Koyama et al.,2022; Cai et al.,2018). Dysfunction of the BBB is associated with a wide range of neurological disorders including AD (Cai et al.,2018).

PM exposure studies have demonstrated that 30-day inhalation of PM resulted in an increased BBB permeability and altered BBB function in both in vivo and in vitro models. The exposure resulted in increased levels of ROS and matrix metalloproteinase 2 (MMP2) as well as MMP9 activity in the cerebral microvasculature and parenchyma. These increased levels were associated with a decreased expression of TJ proteins which included occluding and claudin in Apo E−/ mice (Oppenheim et al.,2013).

MMPs disrupt the BBB via the degradation of TJ and basal lamina proteins which subsequently leads to leakage of the BBB. Both MMP2 and MMP9 target type IV collagen, laminin, and fibronectin which are all significant components of the basal lamina of cerebral blood vessels (Lakhan et al.,2013).

Additionally, oxidative stress can damage a variety of cell types in the BBB including pericytes, astrocytes, and brain microvascular endothelial cells (BMVEC) (Song et al.,2020).

Furthermore, there are various different mechanisms by which oxidative stress can affect the integrity of the BBB. Oxidative stress can lead to an increase in oxidative damage to biomolecules including proteins and lipids. The BBB is largely compromised of membrane lipids, these constituents provide a large surface area over which lipid-soluble molecules can diffuse through the transcellular pathway. With an increase of oxidative stress, membrane lipids could be oxidized and form cytotoxic lipid peroxidation products such as malondialdehyde and 4-hydroxynonenal which are elements that may adversely affect the integrity of the BBB (Pun et al.,2009). ROS can also affect the integrity of the BBB through DNA modifications. Elevated ROS levels can cause the downregulation of E-cadherin through hypermethylation of the promoter region (Pun et al.,2009).

Moreover, ROS can affect the expression of TJ proteins including occludin and claudin-5 which could influence the permeability of the BBB. Oxidative stress can also cause the phosphorylation of these proteins thus compromising the barrier function (Pun et al.,2009). Additionally, increased ROS levels could alter the BBB's integrity by causing alterations to the cytoskeleton. One of the underlying factors is an increased expression of chemokine receptors. When there is an increase in signaling through these receptors this contributes to the myosin light chain (MLC) phosphorylation by the activation of myosin light chain kinase (MLCK) which consequently modulates the actin structure. In addition, MLCK can phosphorylate TJ proteins which then further disturbs the organization of the cytoskeleton and thus increase BBB permeability (Pun et al.,2009). Furthermore, in conditions of oxidative stress protein tyrosine kinases (PTK) are activated. PTK's are suggested to play a role in MMP activation (Pun et al.,2009).

Since the transcription factor NF-κB is activated in a redox-dependent manner it can become activated by ROS. NF-κB can then stimulate the expression of the adhesion molecules ICAM-1 and VCAM-1. ICAM-1 can compromise the BBB by cross-linking and activating Ca2+- signaling pathways that lead to cytoskeletal alterations. The expression of ICAM-1 and VCAM-1 can also promote the recruitment of activated neutrophils and leukocytes. These cells then lead to BBB breakdown by releasing inflammatory mediators including TNF-α and IL-1β. The disruption of BBB integrity can get exacerbated since recruited leukocytes such as neutrophils and macrophages can cause an increase in ROS production (Pun et al.,2009).

2.2 Pathological abnormalities olfactory neurons

PM2.5 does not only gain entry to the brain from the bloodstream, but it has also been demonstrated that translocation of the particulate matter to the brain occurs through the olfactory system. The olfactory system is responsible for the transmission of smells to the olfactory bulb through the olfactory nerve. It has been shown that an impaired olfactory function can be a premature sign of neurodegenerative disease. Once particles are inhaled the olfactory mucosa (hOM) acts as the first line of defense. The hOM is located on the upper part of the nasal cavity and lies closest to the cribriform plate. The hOM is a tissue that contains sensory neurons, horizontal basal cells, a small number of multipotent stem cells that give rise to globose basal cells, and sustentacular cells (Chew et al.,2020).

It has been demonstrated through animal studies that both nasal and olfactory epithelial barriers are disrupted after concentrated urban PM exposure (Zhao et al.,2018). PM exposure caused an increased permeability of the nasal epithelial barrier and disruption of the structure of TJ between neighboring epithelial cells. As is the underlying origin for many of the detrimental effects exerted by PM2.5, intracellular ROS production is responsible for this barrier disruption in nasal epithelial cells. Furthermore, downregulation of zone occludens-1 (ZO-1) was reported after PM2.5 exposure. ZO-1 plays a critical role in the formation and regulation of TJs (Zhao et al.,2018). Additionally, PM2.5 exposure has been demonstrated to induce pathological olfactory bulb (OB) alterations, including decreased cells and disorganization in a mouse model with depressive-like responses (Ji et al.,2022). Moreover, when exposed to PM2.5 shrunken neurons with condensed chromatin were observed in the OB of mice.

PM2.5 can reach the brain tissue from the olfactory system by entering the olfactory receptor neurons through the following methods: pinocytosis, simple diffusion, or receptor-mediated endocytosis. Subsequently, the PM2.5 components get transported further along the axons to the olfactory bulbs and the olfactory cortex. Moreover, when PM2.5 is deeply inhaled it can penetrate the alveolar blood barrier and the BBB and thus reach the brain regions (Song et al.,2022).

As microglia are the resident immune cells of the CNS and an accumulation of them can lead to neuronal damage it has been reported to play a critical role in the association between CNS impairment and PM exposure. Ji et al. subsequently observed that the area proportion of IBA-1-positive microglia was significantly increased after PM2.5 exposure. Furthermore, PM2.5 changed the morphology of microglia in the following manner: larger cell body decreased branches along the granule cell layer and the glomerular layer of the OB. The activated microglia have been reported to release excessive amounts of TNF-α which resulted in neuronal death. Neuronal death is suggested to be conducted by the following mechanism: PM2.5 activates microglia which is followed by the release of TNF-α that can bind to TNF receptor 1 (TNFR1). TNFR1 can then trigger caspase-3-mediated signaling resulting in neuronal death (Ji et al.,2022).

2.3 Gut microbiota- brain axis alterations

The gut microbiota consists of a complex network of microorganisms that reside in the digestive tract (Gomaa, 2020). The bacterial populations in the microbiome are constantly changing and are susceptible to variations in the host environment and internal bodily conditions (Niu et al.,2018). When dysbiosis occurs, inflammation and disruption of the gut's permeability can consequently impact the host's health. Gut dysbiosis can occur from environmental pollutants and lead to modifications in the gut-brain axis (GBA). The gut-brain axis can be defined as the communication between the enteric nervous system (ENS) and the central nervous system. This system links peripheral digestive processes to the emotional, behavioral and cognitive centers in the brain (Singh et al.,2022). Changes in the composition and amount of gut microbes that could be caused by different environmental contaminants, diet and host-derived- metabolites can affect the CNS as well as the ENS.

It has been proposed that PM2.5 is able to affect the gut microbiota through the gastrointestinal tract since approximately 5% of the particulate matter that is inhaled enters the gastrointestinal tract and consequently increases the diversity of the gut microbiota (Li et al.,2022). This is conducted through the following pathway: PM2.5 is inhaled from the ambient air after which it can get transported to the lower

airways such as the trachea and larger bronchi. Due to the small diameter of PM2.5, it can further reach the bronchioles and alveolar spaces after which the particles are phagocytosed by alveolar macrophages (Mutlu et al.,2018). Moreover, PM can reach the GI tract through the ingestion of food and water contaminated by PM.

After PM infiltrates the GI tract it can affect the host's health through multiple mechanisms. One suggested mechanism is that PM may have direct effects on the GI epithelial cells. Considering the intestinal epithelial cells are the primary physical barrier of the gut they can regulate its permeability. Important for this permeability is the tight junctions between epithelial cells that are strengthened by intracellular cytoskeletal proteins (Mutlu et al.,2018). Exposure to PM has been shown to cause a reduction in tight junction proteins and thus enhance the paracellular permeability in alveolar epithelial cells. This is partially caused by the reduction of occludin at the plasma membrane and ZO-1 dissociation in alveolar epithelial cells (Caraballo et al.,2011). Furthermore, Mutlu et al., demonstrated that PM induces mitochondrial ROS production in colonic epithelial and increases permeability through apoptosis in colonic epithelial cells. Second, PM may influence permeability by inflammatory response via ROS production and NF-κB activation in the GI tract (Mutlu et al.,2018).

In accordance with this, Li et al., have demonstrated the effect of environmental contaminants including PM2.5 on the composition of the gut microbiota. Significant changes were found in 20 members of the gut microbiota after PM2.5 exposure (Li et al.,2022). A trend was reported toward a statistically significant increase in alpha diversity in the GI tract, except in the cecum. Moreover, PM exposure altered microbiota composition in which some bacterial taxa are favored. One taxon that has been reported to be differentially existent in a copious amount is the Bacteriodales order (Sheng et al.,2022). Additionally, a decrease was reported in Firmicutes and an increase in Bacteroidetes after PM2.5 inhalation (Minter et al.,2016). It has been suggested that alterations in the gut microbiota could be associated with neurological conditions including multiple sclerosis and Parkinsons' disease. In accordance, Minter et al., demonstrated that long-term treatment with a high dose of antibiotics (ABX) can induce a distinct disruption in microbiota diversity and can additionally alter the peripheral circulating cyto and chemokine composition (Minter et al.,2016). Furthermore, these disruptions influenced the neuro-inflammatory responses by inducing reduced plaque-localized gliosis and altered microglial morphology (Minter et al.,2016).

The connection between the microbiota and the CNS is primarily mediated by neurological, hormonal (HPA-axis), and immunological pathways (cyto and chemokines), all of which are linked to each other (Singh et al.,2022). Niu et al., have reported that short-term PM2.5 exposure is associated with an increase in serum levels of three stress hormones that can conduce HPA axis stimulation (Niu et al.,2018). Specifically, water-soluble inorganic ions (especially NO3-) are potentially capable of having stronger influences in comparison to carbonaceous and elemental components of PM2.5 (Niu et al., 2018). The exact underlying mechanism by which PM2.5 exposure can activate the HPA axis is to be further investigated but there are some biologically possible mechanisms that are suggested. First, exposure to PM2.5 can induce the production of ROS and inflammatory responses in the hypothalamus. Subsequently, this process may activate the HPA axis by diminishing the glucocorticoid negative feedback system. Another possible mechanism works by the upregulation of glucocorticosteroid-sensitive gene expression. Thereupon increasing the synthesis of stress hormones from the HPA axis (Niu et al.,2018). In accordance with this theory Li et al., have found that inhalation of PM2.5 promotes the release of a corticotropin-releasing hormone (CRH) and ACTH from the hypothalamus and subsequently stimulates the synthesis and release of cortisol which is a marker for the HPA stress axis (Li

et al., 2022). Last, specific chemical constituents may be able to transit from the respiratory tract to the brain through the cranial nerve axons or a more permeable BBB (Niu et al.,2018).

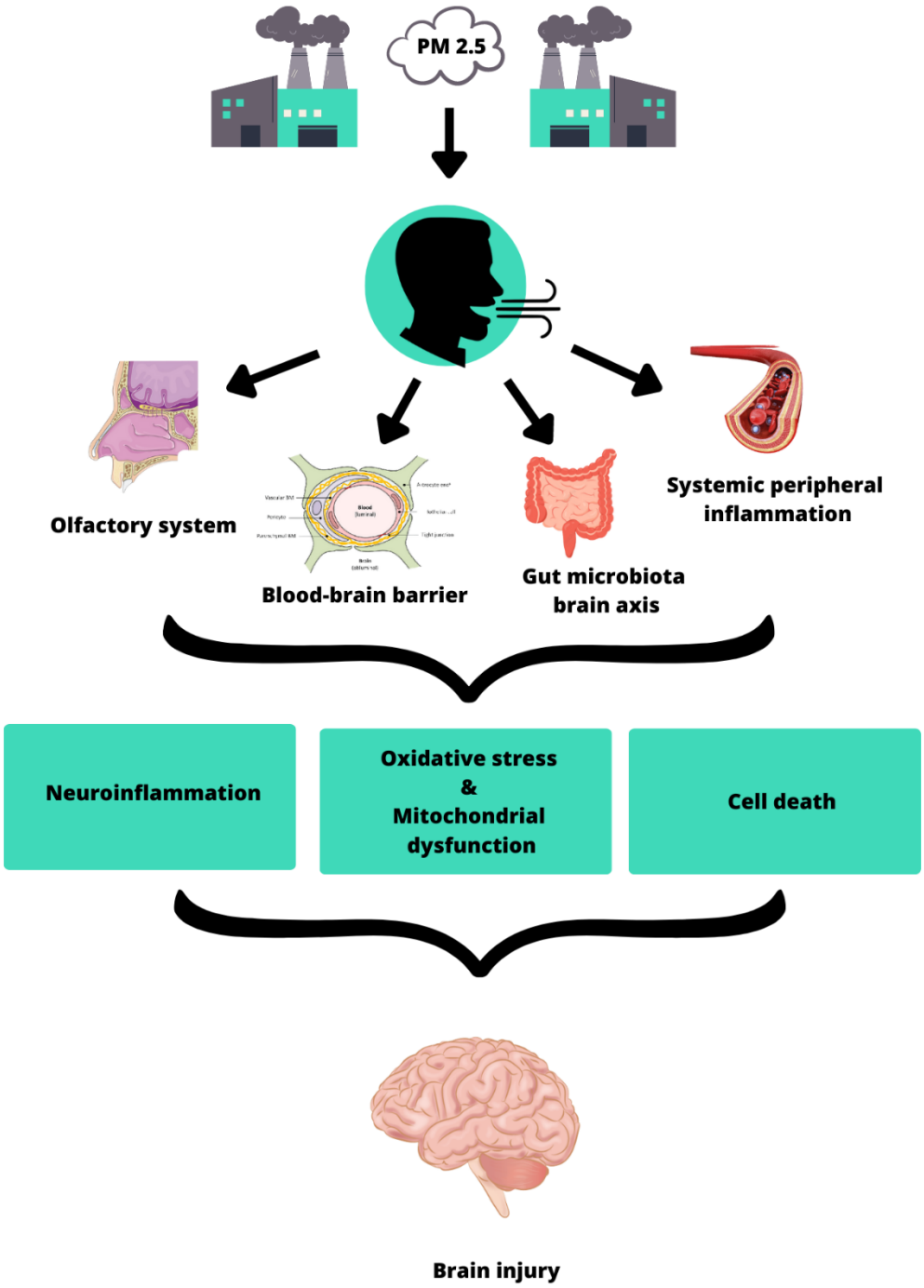


Figure 2: The pathways involved in PM2.5-induced brain injury.

Chapter 3: How PM2.5 causes neurotoxicity

Once PM2.5 has achieved access to the CNS through various different entry routes it can induce neurotoxicity. This is supported by the decrease of cell viability and loss of neuronal antigens observed after PM2.5 exposure. Furthermore, it has been reported that supernatants from PM2.5 treated macrophages and microglia are neurotoxic (Lui et al.,2015). This neurotoxic effect elicited by PM2.5 can occur through a variety of different mechanisms which include: neuroinflammation, oxidative stress, mitochondrial dysfunction, and ultimately neuronal cell death (Zhu et al.,2020).

3.1. Neuroinflammation

Brain matter exposed to PM2.5 has been demonstrated to have a prominent activation of innate immune cells including astrocytes and microglia, which is preceded by an increase of neuroinflammation. While the exact mechanisms between environmental PM2.5 exposure and adverse neuroinflammation are yet to be clarified there are some suggestions disclosed.

A suggestion described by Kang et al., specifies the importance of astrocytes and microglia on the neuroinflammatory process. Astrocytes and microglia are glial cells that are crucial for maintaining the homeostasis and function of neurons (Garland et al.,2022). Furthermore, it has been demonstrated that the activation of glial cells plays an important role in eliciting the inflammatory pathway that is involved in neurodegeneration (Fakhoury, 2018). The chronic stimulation of microglia and astrocytes activates an immunomodulatory response which can subsequently trigger an increase in Aβ deposits, tau hyper-phosphorylation, and cerebral amyloid angiopathy (Al-Ghraiybah et al.,2022).

When exposed to PM2.5 astrocytes acquire a hyper-reactive state and show elevated markers of proinflammation and oxidative stress (Kang et al.,2021). Specifically, glial TNF-α is suggested to play a role in the microglia-macrophage activation that occurs after PM inhalation. This is supported by the observation that microglia contributed 60% of the TNF-α in the conditioned media from mixed glia cultures. These results were consistent with the inhibition of neurite outgrowth by the cultures from microglia. Additionally, microglial cultures showed more inhibition of neurite outgrowth and neurite density than astrocyte cultures (Cheng et al,.2016). In accordance, it was reported that astrocytes cocultured with neurons activated by PM2.5 increased the expression levels of glial fibrillary acidic protein (GFAP) (Kang et al,.2021). GFAP is an intermediate filament-III protein that is found in the following structures: astrocytes in the CNS, non-myelinating Schwann cells in the PNS, and enteric glial cells. GFAP expression is regulated by various nuclear-receptor hormones, growth factors, and lipopolysaccharides (Yang et al.,2015). In addition, cocultured PM2.5 models with reactive astrocytes formed significant levels of proinflammatory chemokines including CCL1 and CCL2, and cytokines including IL-1β and IFN-γ (Kang et al.,2021).

Once peripheral IFN-γ enters the CNS this can induce activation of M1 microglia. The activated microglia can then further induce a neuro-immunoregulatory response through the initiation of the JAK/STAT1 signaling pathway that can upregulate pro-inflammatory gene expression (Zhang et al.,2020). Moreover, IL-1β is able to increase the expression levels of IFN-γ receptors (IFN-γR1) on a variety of innate immune cells which further contributes to the promotion of proinflammation. It is suggested that IFN-γ and IL-1β have priming effects on one another, IFN-γ could increase the sensitivity of microglia

toward IL-1β and vice versa, which consequently can lead to the polarization of M1-microglia (Kang et al.,2021).

The combined effect reported from IL-1β and IFN-γ from cocultured neurons and astrocytes in PM2.5 conditions can lead to the generation of NO and proinflammatory cytokines by polarized M1 microglia (Kang et al.,2021; Kim et al.,2020). Furthermore, it has been demonstrated that this enhanced induction of the proinflammatory M1 and disease-associated microglia phenotype (DAM) is a result of the activation of receptors expressed on myeloid cells 2 (TREM2) and Toll-Like-Receptor 2 and 4 (TLR2/4) both of which inhibit the development of the anti-inflammatory phenotype (Kim et al.,2020).

Potential regulatory mediators of the PM2.5-induced M1 polarization are inducible nitric oxide synthase (iNOS) and Heme oxygenase-1 (HO-1). This is based on the finding that both iNOS and HO-1 expression significantly increased in BV-2 microglial cells after PM2.5 exposure (Kim et al.,2020). iNOS is a key mediator of immune activation and inflammation and produces nitric oxide (NO) from l-arginine. When iNOS is dysregulated or overexpressed it is associated with numerous pathologies including neurodegeneration (Cinelli et al.,2020). HO-1 is the main protein that appears in diseases that arise as a result of oxidative and inflammatory insults (Waza et al.,2018). The interaction between NO and HO-1 plays a crucial role in the modulation of the adaptive response of activated microglia (Kim et al.,2020). Both of these factors are known to be mediators of the inflammatory response in microglia and neuronal cells. NO exerts this effect by increasing oxidative stress by various stimuli and consequently generates more toxic products that include peroxynitrite (ONOO-), which is the product of the reaction of NO with a superoxide radical that ultimately leads to neurotoxicity and brain damage (Kim et al.,2020; Szabó et al., 2007).

Additionally, factors that increase NO expression can also increase HO-1 expression and the production of ONOO- which is important in the NO-mediated modulation of HO-1. Therefore, the PM2.5-induced increase in HO-1 expression can augment inflammation and reinforce feedback loops which trap cells in a hyperinflammatory state through a vicious cycle (Kim et al.,2020). This is reported to be a result of expression via the Nrf2 transcription factor. Nrf2 is a transcription factor that plays a crucial role in protection from oxidative stress through the increase of the production of antioxidant enzymes including HO-1. Nfr2 is known to be involved in inflammatory diseases by the regulation of the activity of the NLR family pyrin domain containing 3 (NLRP3) inflammasome. Furthermore, Nrf2 activation is associated with pro-inflammatory signaling pathways and the inhibition of the NF-κB cascade. In accord, Kohandel et al., reported that treatment with ATX stimulated Nrf2 expression in BV2- microglial cells after PM2.5 exposure. ATX is a transcription factor that maintains redox status, and plays a role in inflammatory modulation, protein degeneration and biotransformation that are regulated by transcription factors of various genes subsequent to Nfr2 binding to a DNA sequence also referred to as the antioxidant-responsive element (ARE) (Kohandel et al.,2021). The inhibitory effects of this treatment are suggested to be mediated by the inhibition of inflammatory mediators such as HO-1 and iNOS through the adjustments in the expression of Nfr2 and d NF-κB. (Kim et al.,2020).

3.2. Oxidative stress and mitochondrial dysfunction

ROS are crucial elements of the proinflammatory signalling pathway in microglia. In the microglia ROS derived from NADPH-oxidase (NOX) and the mitochondria has the ability to act as second messengers to transit immune activation, excessive inflammation and oxidative stress (Song et al.,2022). When the balance of oxidation and the antioxidant system is disturbed, this could have effects on cell survival and the state of dynamic equilibrium. In AD, oxidative stress influences microglia morphology modifications and enhances microglial activation. This occurs by stimulating neurotoxic oligomerization of Aβ peptide and Tau tangles (Peters et al.,2018).

It has been demonstrated that the enzymatic activity of the following antioxidants: SOD and GSH-Px show decreased patterns in rats after PM2.5 exposure (Zhang et al.,2018). In addition, an increase in malondialdehyde (MDA) levels was reported, which is a biomarker that is widely used for monitoring oxidative stress (Zhang et al.,2018; Jadoon et al.,2017). The reduction of GSH-Px activity that was observed is mostly caused by the overproduction of hydrogen peroxide and a decrease of GSH after xenobiotic intoxication. Once SOD is overexpressed it can reduce oxidative stress and thus provide protection against brain damage while also alleviating mitochondrial dysfunction and cell apoptosis. Consequently, the reduction in SOD observed after PM2.5 exposure could be one of the mechanisms behind the mitochondrial structural damage and cell death that were reported (Zhang et al.,2018).

Environmental toxicants including PM2.5 can cause mitochondrial dysfunction and induce ROS accumulation which consequently can further deplete antioxidant defenses and mediate oxidoreduction reactions. Considering this PM2.5 could lead to mitochondrial damage in brain cells by inducing ROS generation. This can then further affect many cellular functions since mitochondria play an important role in ATP generation and metabolism which in turn affect cell survival and apoptosis (Zhang et al., 2018). Furthermore, pollutant-triggered mitochondrial imbalance can not only result in neuronal energy metabolism disorder, increase of ROS, and apoptosis but is also one of the key events in the early pathological process of AD (Jadoon et al.,2017).

Wang et al., reported PM2.5 mitochondrial-induced damage in SHSY-5Y human neuroblastoma cells. This was expressed by the detection of a dominant morphology of mitochondrial swelling in SHSY-5Y cells exposed to PM2.5 (Wang et al.,2019). There are several factors that are measured to indicate mitochondrial function these include mPTP, ATP levels, MMP, mtDNA copy number, and Ca^{2+} levels. mPTP plays a crucial role in the regulation of solute exchange between the mitochondrial matrix and the cytoplasm. Under toxic circumstances, mPTP opens and consequently increases the permeability of inner mitochondrial membranes. This leads to small molecules entering the mitochondrial matrix which induces mitochondrial swell and influences cell survival. Important factors that regulate mPTP opening are high levels of CypD, and excessive ROS production. When mPTP opens this leads to MMP decline, cytochrome C release, mitochondrial swelling, impairment ATP synthesis, reduction of mitochondrial respiration function, and eventually cell apoptosis or death (Wang et al.,2019).

Furthermore, a calcium overload can influence the physiological function of the mitochondria. CypD, which is an important component of mPTP has binding sites for Ca^{2+} and Adenine nucleotide translocase (ANT). ANT is a mitochondrial protein that aids in the exchange of ADP and ATP across the inner mitochondrial membrane and plays a crucial role in the cellular energy metabolism (Chevrollier et al.,2011). When there is an increase in Ca^{2+} uptake CypD binds to Ca^{2+} and ANT, which further changes the ANT confirmation and promotes opening of mPTP (Figure 3). Furthermore, the mitochondria are an important site for intracellular ROS production through the electron transport chain (Chevrollier et

al.,2011). ROS can cause mPTP opening, when there is excessive ROS, it is coupled with the transient mPTP opening. When mPTP opens this can cause superoxide radical (O2•–) release into the cytoplasm which consequently leads to the accumulation of intracellular ROS and oxidative stress. Once there is oxidative stress this can damage the function of the respiratory chain, which in turn produces more ROS and thus leads to the destruction of mitochondria through a vicious cycle of oxidative stress.

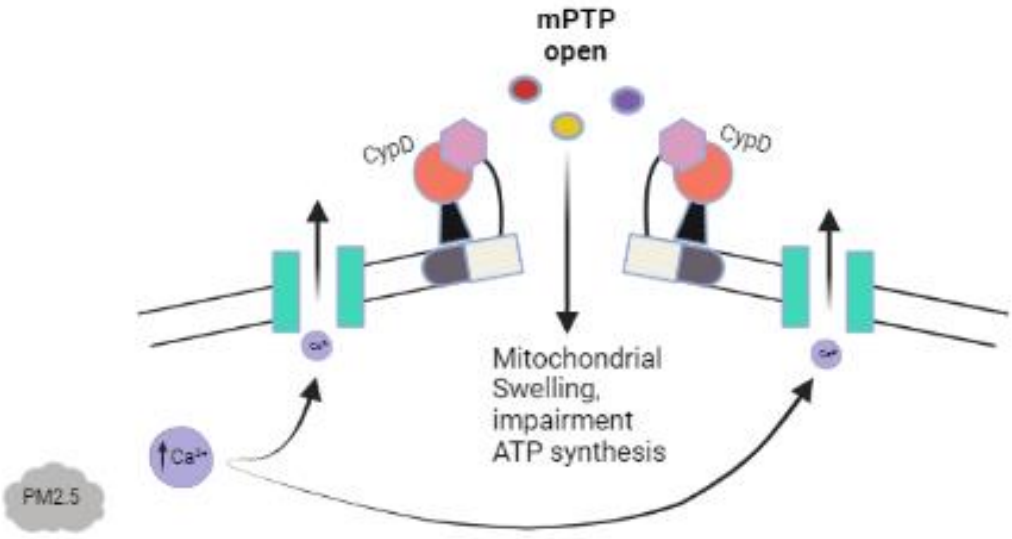


Figure 3: Ca^{2+} induced opening of mPTP channels. CypD binds to Ca^{2+} and ANT, which changes the ANT confirmation and promotes opening of mPTP. mPTP opening leads to various consequences such as mitochondrial swelling and the impairment of ATP synthesis.

An important mediator for mtDNA copy number variation is oxidative stress, mtDNA replication and transcription-related autophagy (Wang et al.,2019). During of oxidative stress, the copy number might increase or decrease depending on the circumstance. While mild oxidative stress may increase the mtDNA molecules to compensate for the weakened respiratory function, severe oxidative stress and excess ROS can exacerbate mitochondrial damage by decreasing mtDNA copy numbers. This occurs as a result of mitochondrial dysfunction and a diminished replication and transcription rate. In conformity, a decrease in mtDNA has been proven to be a characteristic of early events of AD pathology (Delbarba et al.,2016).

Moreover, COX IV is a crucial enzyme of the mitochondrial inner membrane and a vital enzyme in the electron transport chain. It has a central role in the maintenance of the inner mitochondrial proton gradient and ATP production. When there is a suppression of the COX IV gene this could lead to a decrease in ATP synthesis and MMP collapse (Wang et al.,2019). It is suggested that PM2.5-stimulated mPTP opening can induce mitochondrial calcium homeostasis imbalance and cellular oxidative stress. Consequently, this leads to an increased mitochondrial membrane permeability that can supress ATP

synthesis and induce mitochondrial swelling. Furthermore, these occurrences can subsequently elicit mitochondrial dysfunction of the nerve cell. In addition, it was demonstrated that the upregulation of CypD and downregulation of COX IV could have an important regulatory role in the mPTP opening process which is triggered by PM2.5 exposure (Wang et al.,2019).

Mitochondrial fusion and fission genes are crucial for the regulation of mitochondrial dynamics. OPA1 is a gene that is mainly involved in the process of mitochondrial inner membrane fusion and Drp1 is principally involved in mitochondrial fission (Wang et al.,2019). When exposed to PM2.5 protein expression levels of OPA1 and Drp1 have shown to be increased, however, OPA1 was only significant relative to the control. Since OPA1 can regulate and promote excessive mitochondrial fusion these results suggest that OPA1 could be critical for PM2.5-induced impairment (Wang et al.,2019).

3.3. Neuronal cell death

The above-mentioned processes: neuroinflammation, oxidative stress, and mitochondrial dysfunction driven by PM2.5 exposure can lead to cell death including autophagy and apoptosis and ultimately lead to brain damage (Wang et al.,2021). Autophagy can be described as a physiological subcellular degradation process that includes the decomposition of folded proteins, protein complexes, and dysfunctional organelles. These components are subsequently sequestered into autophagosomes which are fused with lysosomes to form autolysosomes and get further degraded into micro molecules by lysosomal hydrolases (Wang et al.,2021). In neurons, autophagy is an essential pathway for homeostasis. Once we age, neuronal cells will accumulate intracellular toxicants or damaged organelles. To remain appropriate intracellular homeostasis these must be cleared appropriately by autophagy. Unlike other cell types, neurons are post-mitotic cells that can not dilute toxic substances through mitosis. This aspect makes autophagy-dependent clearance of proteins/organelles crucial for neurons (Li et al.,2017).

Wei et al., have observed that consequent to PM2.5 exposure, LC3B-II protein expression increased together with the appearance of autophagosomes, both of these factors indicate autophagy. Further investigation revealed that PM2.5 blocked autophagic flux, leading to a dysregulation of autophagy and the accumulation of the autophagy substrate known as p62 (Wei et al.,2022). Autophagy is a lysosome-dependent degradation cascade. Once impairment of lysosomes occurs it can affect the degradation of autophagic capacity and lead to blockage of autophagic flux. In various neurodegenerative diseases, it has been reported that lysosomal dysfunction and especially the increase of Lysosomal membrane permeabilization (LMP) occurs (Wei et al.,2022). When there is an increase in LMP this is a marker for lysosomal membrane instability or integrity. LMP is induced by various different stimuli including ROS (Boya et al.,2008). In accordance, Wei et al., further demonstrated that PM2.5 exposure causes lysosomal pH elevation and an increase in LMP. Furthermore, leakage of cathepsin B from lysosomes was observed. Cathepsin B is considered a lysosomal proteolytic enzyme, once a limited amount is released into the cytoplasm this can activate apoptosis. An excessive release of cathepsin B can promote necrosis (Wei et al.,2022).

Another PM2.5-induced form of cell death is apoptosis. Apoptosis is crucial for development and function establishment this includes non-functional cell elimination and mammary gland involution. Apoptosis generally involves two different mechanisms: cell death through caspase-3 or 7 and the

recruitment of macrophages for cell engulfment (Wang et al.,2021). It was previously demonstrated that long-term PM2.5 exposure can potentially lead to neuronal apoptosis in the brain tissue of mice via impaired depolarization of the mitochondrial membrane potential and reduced mitochondrial-related proteins (Zhu et al.,2021). In a more recent study by Ji et al., it was reported that PM2.5 exposure led to an increase in the expression levels of cleavage of caspase-3 in the hippocampus and PC12 cells in mice. Furthermore, subsequent to PM exposure apoptotic cells in the hippocampal CA1 area was observed (Ji et al.,2022). However, it has been suggested that MeCbl plays a crucial role in the normal functioning of the nervous system and brain. Methylcobalamin (MeCbl) could have a neuronal protective effect by promoting the regeneration of injured nerves and antagonizing glutamate-induced neurotoxicity (McCaddon et al.,2010). Furthermore, in animal models, it has been demonstrated that MeCbl acts as a neuroprotective agent against neuronal apoptosis after peripheral nerve injury (Xu et al.,2016). Ji et al., reported that treatment with MeCbl could effectively reduce the neuronal apoptosis that is induced after PM2.5 exposure in both in vivo as in vitro experiments. An important pathway of apoptosis is the mitochondrial pathway. Once an apoptotic signal is received Bax is relocated to the mitochondrial surface after which cross-mitochondrial membrane pores are formed. These occurrences lead to the reduction of MMP and an increased membrane permeability which thereby can release apoptotic factors. MeCbl shows to have a protective function for mitochondria and thereby could inhibit apoptosis. This is confirmed by Ji et al., based on the following, MeCbl treatment significantly reduced the expression of mitochondrial apoptotic proteins generated after PM2.5 exposure in primary hippocampal neurons and PC12 cells (Ji et al.,2022). The mitochondrial apoptotic proteins which showed a decrease included: mitochondrial apoptosis-related proteins, cleaved-caspase 9, Cyt C, and Bax/Bcl2. In addition, treatment with MeCbl recovered the diminished MMP levels following PM2.5 exposure (Ji et al.,2022).

Chapter 5: Discussion

Approximately 4.2 million premature deaths are caused by ambient air pollution (Tsai et al.,2019). Thus, increasing amount of environmental particulate matter as a result from ambient air pollution should not be neglected. Previous studies have demonstrated that PM2.5 can be associated with cardiovascular disease, respiratory disease, and lung cancer. However, more recent studies indicated PM2.5 can be associated with neurodegenerative disease as well. Epidemiological studies have reported an increased risk of newly diagnosed AD in areas with an average PM2.5 concentration above WHO air quality guidelines. Moreover, AD-associated biomarkers have been measured in this population (Calderon-Garciduenas et al., 2008; Calderon-Garciduenas et al., 2015). In addition, in vitro, in vivo, and clinical studies show that high doses of PM2.5 can promote AD-like pathologies in the brain (Thiankhaw et al.,2022; Cheng et al.,2017). Thus, there is considerable evidence stating that there is an interconnection between PM2.5 exposure and AD-like pathology and biomarkers. However, the exact pathways by which PM2.5 mediates neurotoxicity and cognitive dysfunction still remain unclear. This essay has elucidated potential mechanisms by which PM2.5 can go from an external pollutant to an internal one and presumably induce AD pathological hallmarks.

For PM2.5 to exert effects on the CNS it has to gain access from the external to the internal environment first. PM2.5 enters through the airways and can consequently lead to respiratory damage in various

manners. In all PM2.5 induced oxidative stress and the generation of ROS is of importance. First, PM2.5 can lead to radical peroxidation in the respiratory system, in which transition metals derived from PM2.5 can induce free radical production that can oxide lung cells. Furthermore, the ROS and secondary products resulting from ROS can lead to DNA damage. Second, PM2.5 can generate imbalanced intracellular calcium homeostasis. This is caused by the peroxidation of lipids which leads to the destruction of the ultrastructure of mitochondria after which Ca2+ is released which can ultimately lead to cellular apoptosis. Third, PM2.5 can induce inflammatory injury through the release of pro-inflammatory cytokines (IL-1β, IL-6, and IL-8) and modification to polarized alveolar macrophage morphology that promotes inflammation. Furthermore, PM2.5 can induce various signaling pathways to regulate this inflammatory response these include the MAPK and the NF-κB pathway. It is suggested that the PM-induced MAPK signaling pathway is able to activate the NF-κB pathway downstream to promote further inflammatory responses.

However, to affect the central nervous system PM2.5 has to gain entry it first. This can occur through several different routes. One route is peripheral systemic inflammation. There is a strongly increased risk for the development of dementia after systemic infections indicating that the peripheral immune system has a strong effect on the brain. Once PM2.5 is inhaled in the lungs the initial inflammation can be followed by inflammation in distant tissues. The systemic challenge can consequently lead to microglial activation. Furthermore, the increase in circulating levels of pro-inflammatory cytokines from systemic inflammation can cause disruption of the BBB. Which is subsequently the alternative entry route for PM2.5. Dysfunction of the BBB is associated with a wide range of neurological disorders including AD The integrity of the BBB can be influenced by PM2.5 through increased levels of ROS, MMP2 and MMP9 in the cerebral microvasculature and parenchyma. Following MMPs can disrupt the BBB by degradation of TJ and basal lamina proteins. Additionally, Oxidative stress derived from the increased ROS level can affect the expression of TJ proteins including occluding and claudin-5 both of which influence the permeability of the BBB. Oxidative stress can also affect the BBB permeability through the following: alterations of the cytoskeleton through activation MLCK and NfkB and PTK activation. NfkB activation subsequently leads to the release of inflammatory mediators that can increase the ROS production. An alternative entry route is through the olfactory system. It has been shown that an impaired olfactory function can be a premature sign of neurodegenerative disease. Exposure to PM can increase the permeability of the nasal epithelial barrier. This is mediated by ROS production and the downregulation of ZO-1 which play crucial roles in the regulation of TJs. Consecutively, PM2.5 can reach brain tissue by the following methods: pinocytosis, simple diffusion, or receptor-mediated endocytosis. Last, PM2.5 can reach the GI tract through the ingestion of food and water contaminated by PM. PM2.5 has been suggested to influence the Gut microbiota- brain axis through the following mechanisms. First, exposure to PM2.5 can induce the production of ROS and inflammatory responses in the hypothalamus. Subsequently, this process may activate the HPA axis by diminishing the glucocorticoid negative feedback system. Another possible mechanism works by the upregulation of glucocorticosteroid-sensitive gene expression. Last, specific chemical constituents may be able to transit from the respiratory tract to the brain through the cranial nerve axons or a more permeable BBB.

Once PM2.5 has achieved access to the CNS through various different entry routes it can induce neurotoxicity. This neurotoxic effect elicited by PM2.5 can occur through a variety of different mechanisms. First, this occurrence can result from neuroinflammation. PM2.5 exposure can change

microglia into a hyperreactive state caused by elevated proinflammatory chemokines (CCL1, CCL2) , cytokines (IL-1β, IFN-γ), and oxidative stress. The chronic stimulation of microglia and astrocytes activates an immunomodulatory response which can subsequently trigger an increase in Aβ deposits, tau hyper-phosphorylation, and cerebral amyloid angiopathy. Activated microglia can induce a neuro-immunoregulatory response through initiation of the JAK/STAT1signalingg pathway that can upregulate pro-inflammatory gene expression. Furthermore, IL-1b and IFN-γ can lead to generation of NO and proinflammatory cytokine by polarized M1 microglia. This is regulated by iNOS and HO-1. When iNOS is dysregulated or overexpressed it is associated with numerous pathologies including neurodegeneration. The PM2.5-induced increase of HO-1 expression can augment inflammation and reinforce feedback loops which trap cells in a hyperinflammatory state through a vicious cycle (Kim et al.,2020). This a result of expression via the Nrf2 transcription factor that plays a crucial role in protection from oxidative stress through the increase of the production of antioxidant enzymes including HO-1 and is associated with pro-inflammatory signaling pathways and the inhibition of the NF-κB cascade. Second, oxidative stress and ROS are crucial to PM2.5-induced neurotoxicity as well. After PM2.5 exposure SOD is diminished which can lead to mitochondrial structural damage and cell death. PM-triggered mitochondrial imbalance can not only result in neuronal energy metabolism disorder, increase of ROS, and apoptosis but is also one of the key events in the early pathological process of AD. Once there is oxidative stress this can damage the function of the respiratory chain, which in turn produces more ROS and thus leads to the destruction of mitochondria through a vicious cycle of oxidative stress. A proposed underlying mechanism of this PM2.5-induced mitochondrial dysfunction is mPTP opening which can induce mitochondrial calcium homeostasis imbalance and cellular oxidative stress. Consequently, this leads to an increased mitochondrial membrane permeability that can suppress ATP synthesis and induce mitochondrial swelling. The above-mentioned neurotoxic processes can lead to cell death which includes autophagy and apoptosis. This can be a result of lysosomal membrane instability and the leakage of cathepsin B from lysosomes. Furthermore, it is opposed that PM2.5 exposure leads to an increase in the expression levels of cleavage of caspase 3 in the hippocampus and PC12 cells in mice thus inducing apoptosis.

Thus, there is epidemiological, in vivo, and in vitro evidence that PM2.5 can increase biomarkers for AD and elicit physiological responses which are known to be involved in the pathophysiology of AD. Furthermore, the articles analysed in this essay demonstrate that PM2.5 exposure affects the CNS through different mechanisms that lead to neuroinflammation, mitochondrial dysfunction, oxidative stress, and cell death. All of which have shown to play a crucial role in the pathophysiological process of AD as well. Furthermore, PM2.5 is not the only environmental pollutant that can be associated with the risk of developing AD. Other pollutants such as pesticides have been shown to cause genotoxicity and neurotoxicity. In addition, antimicrobials that are commonly used as preservatives have neurotoxic, immunotoxic, and brain-impairing effects that could be crucial to AD etiology. Such pollutants often show a similar toxicity mechanism in which oxidative stress production plays a crucial role just as with PM2.5 exposure and AD pathology (Mir et al.,2020).

However, there are some limitations to be stated from the available studies that elucidate the role of PM2.5 in neurodegenerative diseases. First, PM2.5 has a size definition but can be very different in nature depending on the measured location. Second, while short-term exposure to PM can elicit a stress response in the brain and long-term exposure can result in AD-like cognitive impairment and neurological changes the exact time course of the AD-like pathology induced by PM is still to be elucidated. In accord, the long-term studies analyzed for this essay have exposure time frames that range from 30 days of exposure (Oppenheim et al.,2013) to 12 months of exposure (Zhang et al.,2018).

Third, confounding factors such as the aging process, and comorbidities that are associated with AD should be further elucidated before a definite causal relationship can be confirmed.

To conclude, considering the available evidence it is highly possible for PM2.5 exposure to be associated with neurodegenerative diseases such as AD. This is supported by studies that not only show how PM2.5 can gain entry into the well-protected CNS but also that PM2.5 exposure stimulates activation of pathways and pathophysiological processes which occur in AD as well. Due to the complexity and diversity of PM components, the development of AD is likely to result from multiple pathways and pathological interactions. Although there seems to be an association further epidemiological and pathological studies are required to further clarify these mechanisms. However, it is of absolute importance to take better care of the planet we all reside in and take action to reduce the trend of increasing air pollution rates because healthy air can aid in a healthy brain.

Reference list

1. Al-Ghraiybah, N. F., Wang, J., Alkhalifa, A. E., Roberts, A. B., Raj, R., Yang, E., & Kaddoumi, A. (2022). Glial Cell-Mediated Neuroinflammation in Alzheimer's Disease. *International Journal of Molecular Sciences*, *23*(18), 10572.
2. Anthony, D. C., ... & Crespo-Lopez, M. E. (2019). What do microglia really do in healthy adult brain?. *Cells*, *8*(10), 1293.
3. Bhatt, D. P., Puig, K. L., Gorr, M. W., Wold, L. E., & Combs, C. K. (2015). A pilot study to assess effects of long-term inhalation of airborne particulate matter on early Alzheimer-like changes in the mouse brain. *PLoS One*, *10*(5), e0127102.
4. Biswas, R., & Bagchi, A. (2016). NFkB pathway and inhibition: an overview. *Computational Molecular Biology*, *6*.
5. Boya, P., & Kroemer, G. (2008). Lysosomal membrane permeabilization in cell death. *Oncogene*, *27*(50), 6434-6451.
6. Breijyeh, Z., & Karaman, R. (2020). Comprehensive review on Alzheimer's disease: causes and treatment. *Molecules*, *25*(24), 5789.
7. Cai, Z., Qiao, P. F., Wan, C. Q., Cai, M., Zhou, N. K., & Li, Q. (2018). Role of blood-brain barrier in Alzheimer's disease. *Journal of Alzheimer's Disease*, 63(4), 1223-1234.
8. Calderon-Garciduenas, L., Mora-Tiscareno, A., Melo-Sanchez, G., Rodriguez-Diaz, J., Torres-Jardon, R., Styner, M., ... & Jewells, V. (2015). A critical proton MR spectroscopy marker of Alzheimer's disease early neurodegenerative change: low hippocampal NAA/Cr ratio impacts APOE ε4 Mexico City children and their parents. *Journal of Alzheimer's Disease*, *48*(4), 1065-1075.
9. Calderón-Garcidueñas, L., Solt, A. C., Henríquez-Roldán, C., Torres-Jardón, R., Nuse, B., Herritt, L., ... & Reed, W. (2008). Long-term air pollution exposure is associated with neuroinflammation, an altered innate immune response, disruption of the blood-brain barrier, ultrafine particulate deposition, and accumulation of amyloid β-42 and α-synuclein in children and young adults. *Toxicologic pathology*, *36*(2), 289-310.
10. Caraballo, J. C., Yshii, C., Westphal, W., Moninger, T., & Comellas, A. P. (2011). Ambient particulate matter affects occludin distribution and increases alveolar transepithelial electrical conductance. *Respirology*, *16*(2), 340-349.
11. Cheng, H., Davis, D. A., Hasheminassab, S., Sioutas, C., Morgan, T. E., & Finch, C. E. (2016). Urban traffic-derived nanoparticulate matter reduces neurite outgrowth via TNFα in vitro. *Journal of neuroinflammation*, *13*(1), 1-11.
12. Cheng, L., Lau, W. K., Fung, T. K., Lau, B. W., Chau, B. K., Liang, Y., ... & Lee, T. (2017). PM2. 5 exposure suppresses dendritic maturation in subgranular zone in aged rats. *Neurotoxicity Research*, *32*(1), 50-57.
13. Chevrollier, A., Loiseau, D., Reynier, P., & Stepien, G. (2011). Adenine nucleotide translocase 2 is a key mitochondrial protein in cancer metabolism. *Biochimica et Biophysica Acta (BBA)-Bioenergetics*, *1807*(6), 562-567.
14. Chew, S., Lampinen, R., Saveleva, L., Korhonen, P., Mikhailov, N., Grubman, A., ... & Kanninen, K. M. (2020). Urban air particulate matter induces mitochondrial dysfunction in human olfactory mucosal cells. *Particle and fibre toxicology*, *17*(1), 1-15.
15. Cinelli, M. A., Do, H. T., Miley, G. P., & Silverman, R. B. (2020). Inducible nitric oxide synthase: Regulation, structure, and inhibition. *Medicinal research reviews*, *40*(1), 158-189.
16. Delbarba, A., Abate, G., Prandelli, C., Marziano, M., Buizza, L., Arce Varas, N., ... & Uberti, D. (2016). Mitochondrial alterations in peripheral mononuclear blood cells from Alzheimer's disease and mild cognitive impairment patients. *Oxidative Medicine and Cellular Longevity*, *2016*.
17. Dellinger, B., Pryor, W. A., Cueto, R., Squadrito, G. L., Hegde, V., & Deutsch, W. A. (2001). Role of free radicals in the toxicity of airborne fine particulate matter. *Chemical research in toxicology*, *14*(10), 1371-1377.

18. Dizdaroglu, M., & Jaruga, P. (2012). Mechanisms of free radical-induced damage to DNA. *Free radical research*, *46*(4), 382-419.
19. Fakhoury, M. (2018). Microglia and astrocytes in Alzheimer's disease: implications for therapy. *Current neuropharmacology*, *16*(5), 508-518.
20. Gao, M., Monian, P., Pan, Q., Zhang, W., Xiang, J., & Jiang, X. (2016). Ferroptosis is an autophagic cell death process. *Cell research*, *26*(9), 1021-1032.
21. Garland, E. F., Hartnell, I. J., & Boche, D. (2022). Microglia and Astrocyte Function and Communication: What Do We Know in Humans?. *Frontiers in Neuroscience*, *16*.
22. Glencross, D. A., Ho, T. R., Camina, N., Hawrylowicz, C. M., & Pfeffer, P. E. (2020). Air pollution and its effects on the immune system. *Free Radical Biology and Medicine*, *151*, 56-68.
23. Gomaa, E. Z. (2020). Human gut microbiota/microbiome in health and diseases: a review. *Antonie Van Leeuwenhoek*, *113*(12), 2019-2040.
24. Guerreiro, R., & Bras, J. (2015). The age factor in Alzheimer's disease. *Genome medicine*, *7*(1), 1-3.
25. Hoogland, I., Houbolt, C., van Westerloo, D. J., van Gool, W. A., & van de Beek, D. (2015). Systemic inflammation and microglial activation: systematic review of animal experiments. *Journal of neuroinflammation*, *12*(1), 1-13.
26. Jadoon, S., & Malik, A. (2017). A review article on the formation, mechanism and biochemistry of MDA and MDA as a biomarker of oxidative stress. *Int. J. Adv. Res*, *5*, 811-818.
27. Ji, X., Li, C., Zhu, X., Yu, W., Cai, Y., Zhu, X., ... & Wang, H. (2022). Methylcobalamin Alleviates Neuronal Apoptosis and Cognitive Decline Induced by PM 2.5 Exposure in Mice. *Journal of Alzheimer's Disease*, (Preprint), 1-14.
28. Ji, X., Liu, R., Guo, J., Li, Y., Cheng, W., Pang, Y., ... & Tang, J. (2022). Olfactory bulb microglia activation mediated neuronal death in real-ambient particulate matter exposure mice with depression-like behaviors. *Science of The Total Environment*, *821*, 153456.
29. Jia, H., Liu, Y., Guo, D., He, W., Zhao, L., & Xia, S. (2021). PM2. 5-induced pulmonary inflammation via activating of the NLRP3/caspase-1 signaling pathway. *Environmental Toxicology*, *36*(3), 298-307.
30. Jung, C. R., Lin, Y. T., & Hwang, B. F. (2015). Ozone, particulate matter, and newly diagnosed Alzheimer's disease: a population-based cohort study in Taiwan. *Journal of Alzheimer's Disease*, *44*(2), 573-584.
31. Kang, Y. J., Tan, H. Y., Lee, C. Y., & Cho, H. (2021). An air particulate pollutant induces neuroinflammation and neurodegeneration in human brain models. *Advanced Science*, *8*(21), 2101251.
32. Kelly, F. J. (2003). Oxidative stress: its role in air pollution and adverse health effects. *Occupational and environmental medicine*, *60*(8), 612-616.
33. Kim, R. E., Shin, C. Y., Han, S. H., & Kwon, K. J. (2020). Astaxanthin suppresses PM2. 5-induced neuroinflammation by regulating Akt phosphorylation in BV-2 microglial cells. *International Journal of Molecular Sciences*, *21*(19), 7227.
34. Kohandel, Z., Farkhondeh, T., Aschner, M., & Samarghandian, S. (2021). Nrf2 a molecular therapeutic target for Astaxanthin. *Biomedicine & Pharmacotherapy*, *137*, 111374.
35. Koyama, Y., Sun, Y., & Shimada, S. (2022). Inflammation from peripheral organs to the brain: How does systemic inflammation cause neuroinflammation?. *Frontiers in Aging Neuroscience*, 601.
36. Ku, T., Li, B., Gao, R., Zhang, Y., Yan, W., Ji, X., ... & Sang, N. (2017). NF-κB-regulated microRNA-574-5p underlies synaptic and cognitive impairment in response to atmospheric PM2. 5 aspiration. *Particle and fibre toxicology*, *14*(1), 1-19.
37. Lakhan, S. E., Kirchgessner, A., Tepper, D., & Leonard, A. (2013). Matrix metalloproteinases and blood-brain barrier disruption in acute ischemic stroke. *Frontiers in neurology*, *4*, 32.
38. Li, Q., Liu, Y., & Sun, M. (2017). Autophagy and Alzheimer's disease. *Cellular and molecular neurobiology*, *37*(3), 377-388.

39. Li, T., Fang, J., Tang, S., Du, H., Zhao, L., Wang, Y., ... & Shi, X. (2022). PM2. 5 exposure associated with microbiota gut-brain axis: Multi-omics mechanistic implications from the BAPE study. *The Innovation*, *3*(2), 100213.
40. Li, Y., Batibawa, J. W., Du, Z., Liang, S., Duan, J., & Sun, Z. (2021). Acute exposure to PM2. 5 triggers lung inflammatory response and apoptosis in rat. *Ecotoxicology and Environmental Safety*, *222*, 112526.
41. Liu, F., Huang, Y., Zhang, F., Chen, Q., Wu, B., Rui, W., ... & Ding, W. (2015). Macrophages treated with particulate matter PM 2.5 induce selective neurotoxicity through glutaminase-mediated glutamate generation. *Journal of neurochemistry*, *134*(2), 315-326.
42. Liu, T., Zhang, L., Joo, D., & Sun, S. C. (2017). NF-κB signaling in inflammation. *Signal transduction and targeted therapy*, *2*(1), 1-9.
43. Madrigano, J., Baccarelli, A., Mittleman, M. A., Wright, R. O., Sparrow, D., Vokonas, P. S., ... & Schwartz, J. (2011). Prolonged exposure to particulate pollution, genes associated with glutathione pathways, and DNA methylation in a cohort of older men. *Environmental health perspectives*, *119*(7), 977-982.
44. McCaddon, A., & Hudson, P. R. (2010). L-methylfolate, methylcobalamin, and N-acetylcysteine in the treatment of Alzheimer's disease-related cognitive decline. *CNS spectrums*, *15*(S1), 2-5.
45. Mehta, M., Chen, L. C., Gordon, T., Rom, W., & Tang, M. S. (2008). Particulate matter inhibits DNA repair and enhances mutagenesis. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, *657*(2), 116-121.
46. Minter, M. R., Zhang, C., Leone, V., Ringus, D. L., Zhang, X., Oyler-Castrillo, P., ... & Sisodia, S. S. (2016). Antibiotic-induced perturbations in gut microbial diversity influences neuro-inflammation and amyloidosis in a murine model of Alzheimer's disease. *Scientific reports*, *6*(1), 1-12.
47. Mir, R. H., Sawhney, G., Pottoo, F. H., Mohi-Ud-Din, R., Madishetti, S., Jachak, S. M., ... & Masoodi, M. H. (2020). Role of environmental pollutants in Alzheimer's disease: a review. *Environmental Science and Pollution Research*, *27*(36), 44724-44742.
48. Mutlu, E. A., Comba, I. Y., Cho, T., Engen, P. A., Yazıcı, C., Soberanes, S., ... & Mutlu, G. M. (2018). Inhalational exposure to particulate matter air pollution alters the composition of the gut microbiome. *Environmental Pollution*, *240*, 817-830.
49. Niu, Y., Chen, R., Xia, Y., Cai, J., Ying, Z., Lin, Z., ... & Kan, H. (2018). Fine particulate matter constituents and stress hormones in the hypothalamus–pituitary–adrenal axis. *Environment international*, *119*, 186-192.
50. Oppenheim, H. A., Lucero, J., Guyot, A. C., Herbert, L. M., McDonald, J. D., Mabondzo, A., & Lund, A. K. (2013). Exposure to vehicle emissions results in altered blood brain barrier permeability and expression of matrix metalloproteinases and tight junction proteins in mice. *Particle and Fibre Toxicology*, *10*(1), 1-14.
51. Osama, A., Zhang, J., Yao, J., Yao, X., & Fang, J. (2020). Nrf2: a dark horse in Alzheimer's disease treatment. *Ageing Research Reviews*, *64*, 101206.
52. Peters, D. G., Pollack, A. N., Cheng, K. C., Sun, D., Saido, T., Haaf, M. P., ... & Meadowcroft, M. D. (2018). Dietary lipophilic iron alters amyloidogenesis and microglial morphology in Alzheimer's disease knock-in APP mice. *Metallomics*, *10*(3), 426-443.
53. Pun, P. B., Lu, J. I. A., & Moochhala, S. (2009). Involvement of ROS in BBB dysfunction. *Free radical research*, *43*(4), 348-364.
54. Rui, W., Guan, L., Zhang, F., Zhang, W., & Ding, W. (2016). PM2. 5-induced oxidative stress increases adhesion molecules expression in human endothelial cells through the ERK/AKT/NF-κB-dependent pathway. *Journal of Applied Toxicology*, *36*(1), 48-59.
55. Sheng, C., Yang, K., He, B., Du, W., Cai, Y., & Han, Y. (2022). Combination of gut microbiota and plasma amyloid-β as a potential index for identifying preclinical Alzheimer's disease: a cross-sectional analysis from the SILCODE study. *Alzheimer's research & therapy*, *14*(1), 1-15.

56. Shou, Y., Huang, Y., Zhu, X., Liu, C., Hu, Y., & Wang, H. (2019). A review of the possible associations between ambient PM2. 5 exposures and the development of Alzheimer's disease. *Ecotoxicology and Environmental Safety*, *174*, 344-352.
57. Sigaud, S., Goldsmith, C. A. W., Zhou, H., Yang, Z., Fedulov, A., Imrich, A., & Kobzik, L. (2007). Air pollution particles diminish bacterial clearance in the primed lungs of mice. *Toxicology and applied pharmacology*, *223*(1), 1-9.
58. Singh, S., Sharma, P., Pal, N., Kumawat, M., Shubham, S., Sarma, D. K., ... & Nagpal, R. (2022). Impact of Environmental Pollutants on Gut Microbiome and Mental Health via the Gut–Brain Axis. *Microorganisms*, *10*(7), 1457.
59. Song, J., Han, K., Wang, Y., Qu, R., Liu, Y., Wang, S., ... & Wu, W. (2022). Microglial Activation and Oxidative Stress in PM2. 5-Induced Neurodegenerative Disorders. *Antioxidants*, *11*(8), 1482.
60. Song, J., Han, K., Wang, Y., Qu, R., Liu, Y., Wang, S., ... & Wu, W. (2022). Microglial Activation and Oxidative Stress in PM2. 5-Induced Neurodegenerative Disorders. Antioxidants, 11(8), 1482.
61. Song, K., Li, Y., Zhang, H., An, N., Wei, Y., Wang, L., ... & Gao, Y. (2020). Oxidative stress-mediated blood-brain barrier (BBB) disruption in neurological diseases. *Oxidative Medicine and Cellular Longevity*, *2020*.
62. Szabó, C., Ischiropoulos, H., & Radi, R. (2007). Peroxynitrite: biochemistry, pathophysiology and development of therapeutics. *Nature reviews Drug discovery*, *6*(8), 662-680.
63. Thangavel, P., Park, D., & Lee, Y. C. (2022). Recent insights into particulate matter (PM2. 5)-mediated toxicity in humans: an overview. *International Journal of Environmental Research and Public Health*, *19*(12), 7511.
64. Thiankhaw, K., Chattipakorn, N., & Chattipakorn, S. C. (2022). PM2. 5 exposure in association with AD-related neuropathology and cognitive outcomes. *Environmental Pollution*, *292*, 118320.
65. Tsai, T. L., Lin, Y. T., Hwang, B. F., Nakayama, S. F., Tsai, C. H., Sun, X. L., ... & Jung, C. R. (2019). Fine particulate matter is a potential determinant of Alzheimer's disease: A systemic review and meta-analysis. *Environmental research*, *177*, 108638.
66. Ulland, T. K., & Colonna, M. (2018). TREM2—a key player in microglial biology and Alzheimer disease. *Nature Reviews Neurology*, *14*(11), 667-675.
67. Wang, B. R., Shi, J. Q., Ge, N. N., Ou, Z., Tian, Y. Y., Jiang, T., ... & Zhang, Y. D. (2018). PM2. 5 exposure aggravates oligomeric amyloid beta-induced neuronal injury and promotes NLRP3 inflammasome activation in an in vitro model of Alzheimer's disease. *Journal of neuroinflammation*, *15*(1), 1-12.
68. Wang, J., Huang, J., Wang, L., Chen, C., Yang, D., Jin, M., ... & Song, Y. (2017). Urban particulate matter triggers lung inflammation via the ROS-MAPK-NF-κB signaling pathway. *Journal of thoracic disease*, *9*(11), 4398.
69. Wang, Y., Zhang, M., Li, Z., Yue, J., Xu, M., Zhang, Y., ... & Li, R. (2019). Fine particulate matter induces mitochondrial dysfunction and oxidative stress in human SH-SY5Y cells. *Chemosphere*, *218*, 577-588.
70. Wang, Y., Zhong, Y., Liao, J., & Wang, G. (2021). PM2. 5-related cell death patterns. *International Journal of Medical Sciences*, *18*(4), 1024.
71. Waza, A. A., Hamid, Z., Ali, S., Bhat, S. A., & Bhat, M. A. (2018). A review on heme oxygenase-1 induction: is it a necessary evil. *Inflammation Research*, *67*(7), 579-588.
72. Wei, H., Yuan, W., Yu, H., & Geng, H. (2021). Cytotoxicity induced by fine particulate matter (PM2. 5) via mitochondria-mediated apoptosis pathway in rat alveolar macrophages. *Environmental Science and Pollution Research*, *28*(20), 25819-25829.
73. Wei, M., Bao, G., Li, S., Yang, Z., Cheng, C., & Le, W. (2022). PM2. 5 exposure triggers cell death through lysosomal membrane permeabilization and leads to ferroptosis insensitivity via the autophagy dysfunction/p62-KEAP1-NRF2 activation in neuronal cells. *Ecotoxicology and Environmental Safety*, *248*, 114333.

74. Xing, Y. F., Xu, Y. H., Shi, M. H., & Lian, Y. X. (2016). The impact of PM2. 5 on the human respiratory system. *Journal of thoracic disease*, *8*(1), E69.
75. Xu, J., Wang, W., Zhong, X. X., Feng, Y. W., Wei, X. H., & Liu, X. G. (2016). Methylcobalamin ameliorates neuropathic pain induced by vincristine in rats: effect on loss of peripheral nerve fibers and imbalance of cytokines in the spinal dorsal horn. *Molecular pain*, *12*, 1744806916657089.
76. Xu, X., Jiang, S. Y., Wang, T. Y., Bai, Y., Zhong, M., Wang, A., ... & Sun, Q. (2013). Inflammatory response to fine particulate air pollution exposure: neutrophil versus monocyte. *PLoS One*, *8*(8), e71414.
77. Yang, Z., & Wang, K. K. (2015). Glial fibrillary acidic protein: from intermediate filament assembly and gliosis to neurobiomarker. *Trends in neurosciences*, *38*(6), 364-374.
78. Zhang, J., He, H., Qiao, Y., Zhou, T., He, H., Yi, S., ... & You, Z. (2020). Priming of microglia with IFN-γ impairs adult hippocampal neurogenesis and leads to depression-like behaviors and cognitive defects. *Glia*, *68*(12), 2674-2692.
79. Zhang, Q., Li, Q., Ma, J., & Zhao, Y. (2018). PM2. 5 impairs neurobehavior by oxidative stress and myelin sheaths injury of brain in the rat. *Environmental Pollution*, *242*, 994-1001.
80. Zhang, W., & Liu, H. T. (2002). MAPK signal pathways in the regulation of cell proliferation in mammalian cells. *Cell research*, *12*(1), 9-18.
81. Zhao, Q., Chen, H., Yang, T., Rui, W., Liu, F., Zhang, F., ... & Ding, W. (2016). Direct effects of airborne PM2. 5 exposure on macrophage polarizations. *Biochimica et Biophysica Acta (BBA)-General Subjects*, *1860*(12), 2835-2843.
82. Zhao, R., Guo, Z., Zhang, R., Deng, C., Xu, J., Dong, W., ... & Zhuang, G. (2018). Nasal epithelial barrier disruption by particulate matter≤ 2.5 μm via tight junction protein degradation. *Journal of Applied Toxicology*, *38*(5), 678-687.
83. Zhu, X., Ji, X., Shou, Y., Huang, Y., Hu, Y., & Wang, H. (2020). Recent advances in understanding the mechanisms of PM2. 5-mediated neurodegenerative diseases. *Toxicology Letters*, *329*, 31-37.
84. Zhu, X., Shou, Y., Ji, X., Hu, Y., & Wang, H. (2021). S-adenosylmethionine decarboxylase 1 and its related spermidine synthesis mediate PM2. 5 exposure-induced neuronal apoptosis. *Ecotoxicology and Environmental Safety*, *224*, 112678.