Neurological effects of ambient air pollution

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i. Abstract

Ambient air pollution is a global health problem and it is an important factor associated with morbidity and mortality worldwide. In high-income countries air pollution was associated with 2.5% of all deaths, making it the eighth leading risk factor for mortality(Narayan, Ali et al. 2010). Cardio respiratory effects and mechanisms have been extensively investigated. There is also mounting evidence that exposure to air pollution can cause stroke-related sickness and death. However, little is known regarding neurological effects. Air pollutants are substances in the air that can cause harm to humans and the environment. They arise in different forms like gasses, solid particles and liquid droplets. They are also divided in pollutants from natural sources in the environment, like volcanic activity and forest fires (when started by lightning) and pollutants that are man-made or anthropogenic. The most investigated air pollutants that have an adverse health effect are particulate matter (PM), ground level ozone (O_3), black carbon (BC) and nitrogen dioxide (NO_2). These pollutants can enter the brain via different pathways, including diffusion trough the lung epithelial and vascular endothelial layers, via the neuronal olfactory nerve and through disrupting the blood brain barrier by inducing oxidative stress and the expression of pro-inflammatory molecules. Several groups have found associations between exposure to ambient air pollutants and neuro-degenerative effects and cognitive function, both in toxicology studies and epidemiology studies. The aim of this review is to give some information about the exposure to ambient air pollutant and the adverse effects on the function of the brain. It will include studies on the effect on development due to prenatal or early childhood exposure as well as studies that investigated the effect of air pollution exposures on the adult cognition function.

Chapter overview

1. Introduction

Ambient air pollution is a global health problem and it is an important factor associated with morbidity and mortality worldwide (Narayan, Ali et al. 2010). In high-income countries air pollution was associated with 2.5% of all deaths, making it the eighth leading risk factor for mortality (Narayan, Ali et al. 2010). Pollutants do not stay within the national borders. They are carried by winds, contaminating water and soil far from their origin. The great London smog of 1952 focussed the world's attention on the problem of air pollution (LOGAN 1953). Since then there has been an improvement in air quality in developed countries, but a in developing countries air pollution continues to be a big problem. The air quality in large cities in developing countries is remarkably poor and large numbers of people living in these cities are exposed to levels of air pollutants well above the World Health Organization guidelines for air quality (Kim, 2004).
Cardiorespiratory effects and mechanisms have been extensively investigated. There is also mounting evidence that exposure to air pollution can cause stroke-related sickness and death (Hong, Lee et al. 2002) (Mateen, Brook 2011). However, little is known regarding neurological effects. Especially children are a population at risk, because childhood and adolescence are crucial periods of brain development. Environmental exposures during early life may permanently change the body's structure and cause problems during childhood but also later in life. The central nervous system (CNS) has mainly unprotected barriers and the long period of conformations can lead to prolonged vulnerability of developmental processes. Research conducted among a limited series of pollutants showed that early life exposure to chemicals at current environmental levels can be neurotoxic years or even decades after exposure (Grandjean, Landrigan 2006). The possibility of a link between chemicals and neurobehavioural changes was first raised by research showing that lead was toxic to the developing brain (Landrigan, Whitworth et al. 1975). The US National Research Council concluded that 3% of developmental disabilities are the direct result of environmental exposure to pollutants and 25% is induced by the interaction between environmental factors and genetic susceptibility. (rapport NRC)
Although urban air pollution crosses geographical and socioeconomical boundaries, urban minority populations are likely to be more exposed to indoor and outdoor air pollution than other populations (Breysse, Buckley et al. 2005). Also, children may have increased exposures to air pollutants compared to adults, because of higher minute ventilation and higher levels of physical activity. They are also more exposed to ambient air pollution, because they usually spend much more time outdoors than adults (Kim, American Academy of Pediatrics Committee on Environmental Health 2004).

2. Air pollution

Pollutants are substances in the air that can cause harm to humans and the environment. They arise in different forms like gasses, solid particles and liquid droplets. They can also divided in pollutants from natural sources in the environment, like volcanism and forest fires (when started by lightning) and pollutants that are man-made or anthropogenic, like fossil fuel combustion emitted by traffic, power generation and industry. Both the natural and anthropogenic pollutants can also be classified as either primary or secondary pollutants. Primary pollutants are directly formed and emitted, whereas secondary pollutants are formed in the atmosphere, when primary pollutants react or interact. Since a lot of air pollutants are emitted by natural sources, industry and traffic, it is logical that people are mainly focussed on the outdoor air pollution. However, it is important to realise that indoor air pollution (IAP) might also play a role in the origin of neurological effects. Especially in developing countries, most people still rely on solid fuels for cooking and heating, which generates high levels of pollutants like particulate matters and carbon monoxide. Women and young children may be significantly more exposed to this form of air pollution because of their traditional role in the house hold. But also in developing countries IAP plays a role, since people tend to spend 80-90% of their time indoors and good ventilation is often lacking.
In this review we will mainly focus on the neurological effects of ambient air pollution. Toxicological studies will give an indication of the possible effects the air pollutants can have on the neurological development, whereas epidemiology studies will give inside in the extension of these effects. Although children are thought to be more sensitive to adverse neurological effects, we will also focus on the adverse neurological effects in adults.

2.1 Pollutants of interest

2.1.1 Particulate matter

One of the most important groups of primary pollutants is the group of fine particles, also known as particulate matter (PM). This PM contains particles from different sizes, sources and chemical composition. Based on size, they are divided in three categories. Particles indicated by PM_{10} have a aerodynamic diameter of less than 10 µm. $PM_{2.5}$ are particles smaller than 2.5 µm and $PM_{0.1}$ are the particles smaller than 0.1 which are also called ultra-fine particles (Miyata, van Eeden 2011). Because of their size, the largest particles (2.5-10 µm) are not able to penetrate the respiratory tract very deeply. They are mainly deposited in the nasal cavity, larynx and trachea(Heyder 2004). The particles that are between 0.1 and 2.5 µm penetrate the bronchi and bronchioles. However, the larger particles in this group will still deposit mostly in the upper airway, whereas the particles between 0.1 and 1 µm hardly deposit at all(NRPB, 2004). The ultrafine particles (<0.1 µm) are able to penetrate the alveoli and have a deposition rate of more than 80% (NRPB). The origin of particulate matter can be natural (volcanoes, dust storms and forest fires), but mostly they are emitted as a result of fuel combustion from traffic and industry or agriculture.

2.1.2 Nitrogen dioxide & Ozone

Nitrogen dioxide (NO_2) is formed in most combustion processes that use oxygen as an oxidant. NO_2 is a highly reactive and nitrogen-centered free radical that can induce airway inflammation, like asthma and acute bronchitis (Braun-Fahrlander,

Vuille et al. 1997)(Shima, Adachi 2000). NO_2 is also a major precursor for a number of harmful secondary pollutants. The most important form of a secondary pollutant is ozone (O_3). Ozone is formed by photochemical reactions of sunlight on air containing hydrocarbons and nitrogen oxides. This newly formed ozone reacts with UV light again, resulting in the production of hydroxyl radicals. These hydroxyl radicals are the first step in the creation of smog components, including peroxyacyl nitrates that can be powerful eye irritants. But also neuronal deficits have been shown after O3 exposure. Upon O3 inhalation, Gackiere *et al*. showed a time- and dose-dependant neuronal activation pattern similar to that induced by systemic stress (Gackiere, Saliba et al. 2011). This chronic stress is known to disrupt sleeping patterns, anxiety, depression and social isolation (Chrousos 2009).

2.2 Air pollution levels

Air pollution is usually concentrated in densely populated areas, especially in developing countries where environmental regulations are relatively lax or non existent, but also areas in the developed world can be highly polluted.
In many developing countries the absence of surface-based air pollution monitors makes it difficult and sometimes impossible to get even a rough estimate of the abundance of a subcategory of airborne particles that epidemiologists suspect contributes to millions of premature deaths each year. Therefore, van Donkelaar *et al*. created a map (see figure 1) by blending total-column aerosol measurements with information about the vertical distribution of aerosols from a computer model (van Donkelaar, Martin et al. 2010). Figure 2 clearly shows that the areas with the highest concentration of $PM_{2.5}$ are concentrated around the equator and especially in Northern Africa and the rapidly developing countries in Asia.

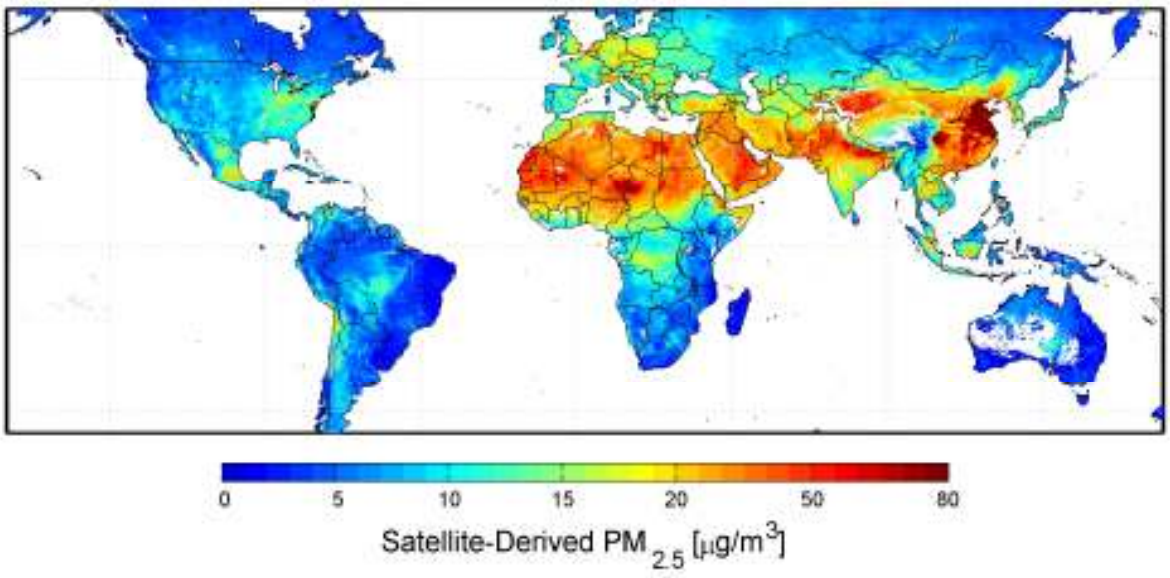


Figure 1; Worldwide PM $_{2.5}$ concentration
The picture shows the worldwide concentration of fine particles. The highest concentrations are found in the developing countries of Africa and Asia (Source: van Donkelaar, 2010).

Figure 2 shows the worldwide concentrations of O_3. The spatial variability of O_3 is less pronounced than that of $PM_{2.5}$ and levels are not systematically higher in the rapidly developing countries (Brauer, Amann et al. 2011). The observed patterns

follow the increase of emissions of ozone precursors in Asia and decrease because of less biomass burning in South America (HEI, 2010)

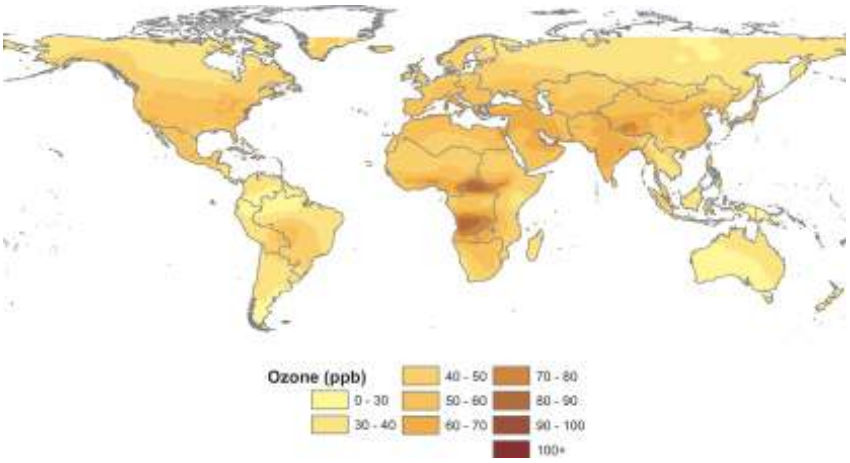


Figure 2; Estimated seasonal (3 month) hourly maximum ozone concentrations (ppb). *Highest levels are evident in North America, Europe, Asia and some specifically high concentrations are seen in regions of Africa (Source: Brauer 2011).*

2.4 Guidelines and legislation

Due to the large impacts of air pollution on health and mortality, many governments, non-governmental organisations and international organisations came up with legislations and strategies for reducing air pollution and enhancing air quality.

2.4.1 WHO guidelines

Air quality guidelines have been published by the World Health Organisation in 1987 and were revised in 1997 and 2005 (WHO 2006)

Pollutant	Guidelines	Timepoint	Basis for selected level
PM_{10}	50 µg/m^3	24-hour mean	Based on relationship between 24-hour and annual PM levels.
	25 µg/m^3	Annual mean	These are the lowest levels at which total, cardiopulmonary and lung cancer mortality have been shown to increase with more than 95% confidence in response to long-term exposure to PM2.5.
$PM_{2,5}$	25 µg/m^3	24-hour mean	Based on relationship between 24-hour and annual PM levels.
	10 µg/m^3	Annual mean	These are the lowest levels at which total, cardiopulmonary and lung cancer mortality have been shown to increase with more than 95% confidence in response to long-term exposure to PM2.5.
Ozone (O_3)	100 µg/m^3	8-hour mean	Provides adequate protection of public health, though some health effects may occur below this level.
NO_2	200 µg/m^3	1-hour mean	

	40 µg/m^3	Annual mean	
SO_2	500 µg/m^3	10-minute mean	
	20 µg/m^3	24-hour mean	

Table 1; WHO guidelines for maximum exposure to air pollutants

2.4.2 EU air quality legislation

In 1996 the EU adopted the directive on ambient air quality assessment and management. This directive was supplemented with four daughter directives that set limit values for maximum allowed concentrations of air pollutants in ambient air. In 2008 a new air quality directive entered into force (EP, 2008). This new directive did not change the previous set of standards but allowed the member state more time to meet the already existing standards. The directive already stated that In 2015 the standard for $PM_{2,5}$ will be changed to 25 µg/m^3. However, this is still much higher than the 10 µg/m^3 guideline that was set by the WHO. In 2013 the air quality directive will be reviewed and revised.
The maximum exposure concentrations stated by the European commission are shown in table 2.

Pollutant	**Guidelines**	**Timepoint**
PM10	50 µg/m^3	24-hour mean
	40 µg/m^3	Annual mean
PM2.5	25 µg/m^3	Annual mean
Ozone	120 µg/m^3	Max. daily 8-hour mean
NO2	200 µg/m^3	1-hour mean
	40 µg/m^3	Annual mean
SO2	350 µg/m^3	1-hour mean
	125 µg/m^3	24-hour mean

Table 2; EC guidelines for maximum exposure to air pollutants

2.4.3 The US Clean Air Act

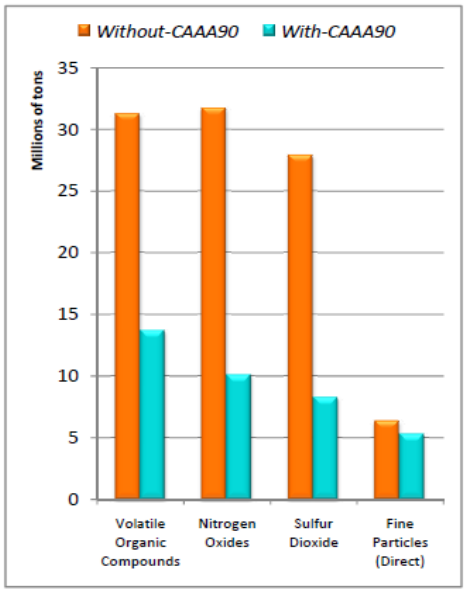
In 1970 president Nixon signed the Clean Air Act (CAA). This CAA is the law that defines the responsibilities of the Environmental Protection Agency (EPA) for protecting and improving the nation's air quality and stratospheric ozone layer. In 1990 the last major changes were taken up in the law with the CAA Amendments (*The Clean Air Act.* 1970)
The law focuses on:

- Reducing ambient concentrations of air pollutants that cause smog, haze, acid rain, and other problems.
- Reducing emissions of toxic air pollutants that are known to, or are suspected of, causing cancer or other serious health effects.
- Phasing out production and use of chemicals that destroy stratospheric ozone

In 2010 the EPA performed a study to examine the effects of the CAA (with the 1990 amendments) on the ambient pollutant concentration and health. Figure 3A shows the emission of volatile organic compounds (VOC), Nitrogen oxide (NO), Sulfur dioxide (SO_2) and fine particles. For the first 3 compounds the emission decreased

around one third compared to the estimated emissions without the CAAA. Only for particulate matter this effect was rather small. Figure 3B shows the effects on health outcomes for particulate matter and ozone. It describes the reduction in numbers in 2010 and the estimated reduction in 2020 compared to the estimated numbers without the establishment of the CAAA (EPA report 2010).

A



B

Health Effect Reductions (PM2.5 & Ozone Only)	Pollutant(s)	Year 2010	Year 2020
PM2.5 Adult Mortality	PM	160,000	230,000
PM2.5 Infant Mortality	PM	230	280
Ozone Mortality	Ozone	4,300	7,100
Chronic Bronchitis	PM	54,000	75,000
Acute Bronchitis	PM	130,000	180,000
Acute Myocardial Infarction	PM	130,000	200,000
Asthma Exacerbation	PM	1,700,000	2,400,000
Hospital Admissions	PM, Ozone	86,000	135,000
Emergency Room Visits	PM, Ozone	86,000	120,000
Restricted Activity Days	PM, Ozone	84,000,000	110,000,000
School Loss Days	Ozone	3,200,000	5,400,000
Lost Work Days	PM	13,000,000	17,000,000

Figure 3: The effects of the Clean Air Act on air pollutant emission and health. *A) Emission of pollutants in millions of tons, with or without the clean air act. B) Numbers of health effect reductions for different pollutants as a result of the clean air act (Source: EPA report on costs and benefits of the CAAA)*

3. The brain

The brain, or encephalon (derived from Greek), is the centre of the nervous system. It is the organ that is responsible for sensing, controlling and processing signals and is therefore found close to primary sensory apparatus like vision, hearing, balance, taste and smell. The brain consists of billions of neurons each connected via synapses. Neurons communicate via these synapses with chemical signals called neurotransmitters. Electrical signals are transmitted through the axons that carry action potentials to distant parts of the brain or body to target specific recipient cell. Every function will involve multiple brain regions and every brain region may be involved in several other functions. Therefore, understanding the brain is not simple and straightforward (Silverthorn 2004).

3.1 Anatomy

In the early embryo, the cells that will form the nervous system are positioned in the neural plate. During the development of the embryo, neural crest cells migrate to the middle and thereby creating a neural tube. The anterior portion of the neural tube will specialize into the regions of the brain, being the forebrain, midbrain and hindbrain. The posterior part of the neural tube will form the spinal cord. Finally the six main parts of the brain are formed: (1) the cerebrum, (2) the diencephalon, (3) the mesencephalon, (4) the cerebellum, (5) the pons and (6) the medulla oblongata (see figure 4). The cerebrum is the largest and most distinctive part of the human brain and fills most of the cranial cavity (silverthorn, 2004).

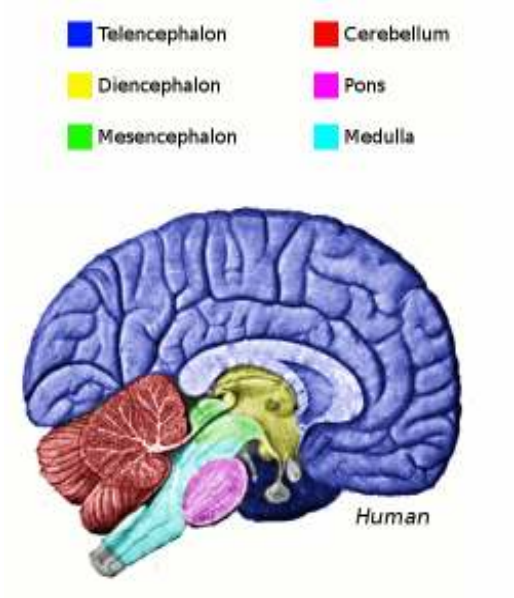


Figure 4; The anatomy of the human brain. *The different parts of the brain are indicated with the colour legend. The cerebrum is by far the largest part of the brain. (Source: Ranson S.W, 1920)*

3.1.1 Gray and white matter

The cerebrum has distinct regions of gray and white matter. The outer layer of the cerebrum, which is only a few millimetres thick, forms the gray matter of the cerebral cortex. Gray matter consists of unmyelinated nerve cell bodies, dendrites and axon terminals. The cell bodies form layers in some parts of the brain or cluster into groups of neurons with a similar function. White matter in the cerebrum is found primarily in the interior. White matter is made up mostly of myelinated axons and contains very

few cell bodies. Its pale (white) colour comes from the myelin sheaths that surround the axons. Bundles of fibres allow different regions of the cortex to communicate with each other and transfer information from one hemisphere to the other primarily through the corpus callosum (Purves 2008).

3.1.2 Blood brain barrier

The blood brain barrier is a separation of circulation blood and the brains extracellular fluid and prevents potentially harmful particles from being delivered into the brain. It is formed by the brains capillary endothelium. These endothelial cells form tight junctions that are composed of transmembrane proteins that are anchored in the endothelial cell (see figure 5). The capillary endothelium uses selected membrane carriers and channels to move nutrients and other useful material from the blood to the brains interstitial fluid. Other transporters move wastes from the interstitial fluid into the plasma. If a water soluble molecule is not transported on one of these carriers, it cannot cross the blood brain barrier (Liddelow 2011). Although the blood-brain barrier excludes many water-soluble substances, smaller lipid-soluble molecules can simply diffuse through the cell membranes. Because of the high demand of oxygen of the brain, oxygen can pass the blood-brain barrier freely. Neurons consume oxygen at such high rates that interruption of the blood flow to the brain can have devastating effects within only a few minutes (Pritchard).

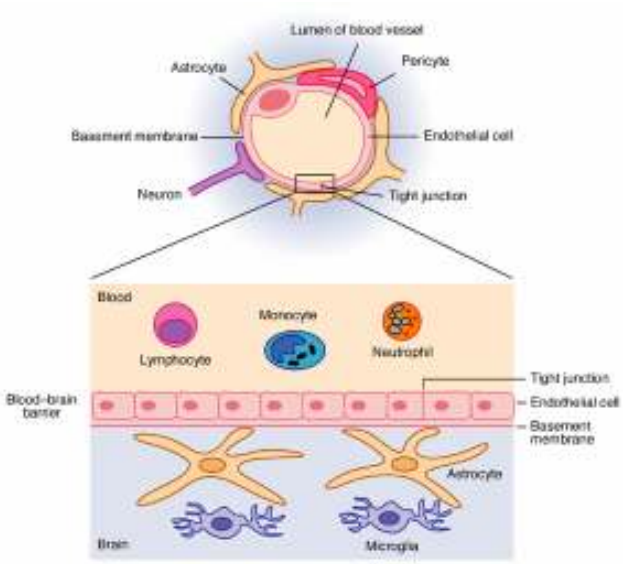


Figure 5; The blood brain barrier.
The blood brain barrier consists of tightly bound endothelial cells that prevent particles and immune cells from entering the brains interstitial fluid.
Source: Expert Reviews in molecular medicine@2003 Cambridge University Press

3.2 Function

The brain receives sensory input from the internal and external environments. After processing these incoming signals it creates a response. However, the brain is also able to generate information and act without the external input of signals. There are three different systems that influence the output signals, being (1) the sensory system that monitors the internal and external environment and initiates reflex responses, (2) the cognitive system that is able to initiate voluntary responses and (3) a behavioural state system, which governs sleep-wake cycles and other behaviours. Cognition refers to mental processes, which include: attention, memory, understanding a language, solving problems and making decisions. It can be natural or artificial and conscious or unconscious (Silverthorn 2004).

3.2.1 Learning

Learning can be classified into two broad types. The first is associative learning, which occurs when two stimuli are associated with each other. The second form of learning is non-associative learning. This includes imitative behaviours such as learning a language. This form of learning can be divided in habituation and sensitization. Habituation is when a decreased response is shown to an irrelevant stimulus that is repeated over and over (Rankin, Abrams et al. 2009). For example, the brain of people who live close to a railway will ignore the sound of passing trains after a while. This allows people to filter out signals that we know are insignificant. Sensitization is the opposite of habituation. In sensitization learning, exposure to an intense stimulus causes an increased response upon repeated exposure. People who become ill while eating certain foods, may loose their desire to that kind of food. Therefore, sensitization helps us to avoid potentially harmful stimuli (Dayan, Kakade et al. 2000)

3.2.2 Memory

Memory is the ability to retain and recall information. There are several different types of memory, like short-term and long-term, automatic and explicit memory. The learning of a single task may involve parallel routes. Processing via these parallel routes helps to provide a backup in case of damage somewhere in the brain. It is also believed that this parallel processing allows new information to be matched to already stored information. For example, someone who has never seen a volleyball, will recognize it as a ball because it has the same characteristics as all other balls that person has seen (Silverthorn 2004). Memory has multiple levels of storage and our memory is constantly changing. When a stimulus reaches the central nervous system, it first goes into the short-term memory. This is a limited store of information that can hold only about 7 to 12 pieces of information at the time. These memories will disappear from the short term memory, if you do not take the effort to store them in a more permanent form, for example by repeating. The working memory is a special form of short-term memory that stores information long enough to use it for tasks that takes place after the information about that task has been processed (Baddeley 2003). Reflexive (implicit) memory is automatic and does not require conscious processes for its creation or recall. Information that is stored in this kind of memory is acquired slowly by repetition (Graf, Schacter 1985). Declaritive (explicit) memory, on the other hand, required conscious attention for its recall. The creation of these kinds of memories depends on the use of higher-level cognitive skills such as interference, comparison and evaluation (Eichenbaum 1997). Sometimes information can shift from the declarative to the reflexive memory. This usually happens when specific movements become a reflex and is therefore often referred to as motor memory (Krakauer, Shadmehr 2006).

3.3 Determinants of cognition

Different psychosocial and bio-physiological factors play a role in the development, functioning and maintenance cognitive skills. A well examined determinant of cognitive development in children is the environment. Different environments can both facilitate or hinder child development. Research in this area has mainly focussed on social environment. The impact of social environment is divided in distal (social,

historical and cultural context) and proximal (daily interactions with family, teachers, etc.), which includes verbal stimulation, childcare and family organisation. The existence of play materials and games and the attendance at preschool were found to be the most important determinants in cognitive development (Marques dos Santos, Neves dos Santos et al. 2008). Santos *et al*. showed that cognitive function at the age of five was negatively associated with poor socioeconomic conditions, poor maternal education, paternal absence and poor sanitary conditions both at home and in the neighbourhood (Santos, Assis et al. 2008). Figure 6 gives an overview of all the factors that were found to influence the cognitive score. Early health indicators like birth weight and linear growth were also factors that influenced the cognitive score, but they were found to be mostly primary factors instead of mediators of the socioeconomic status.

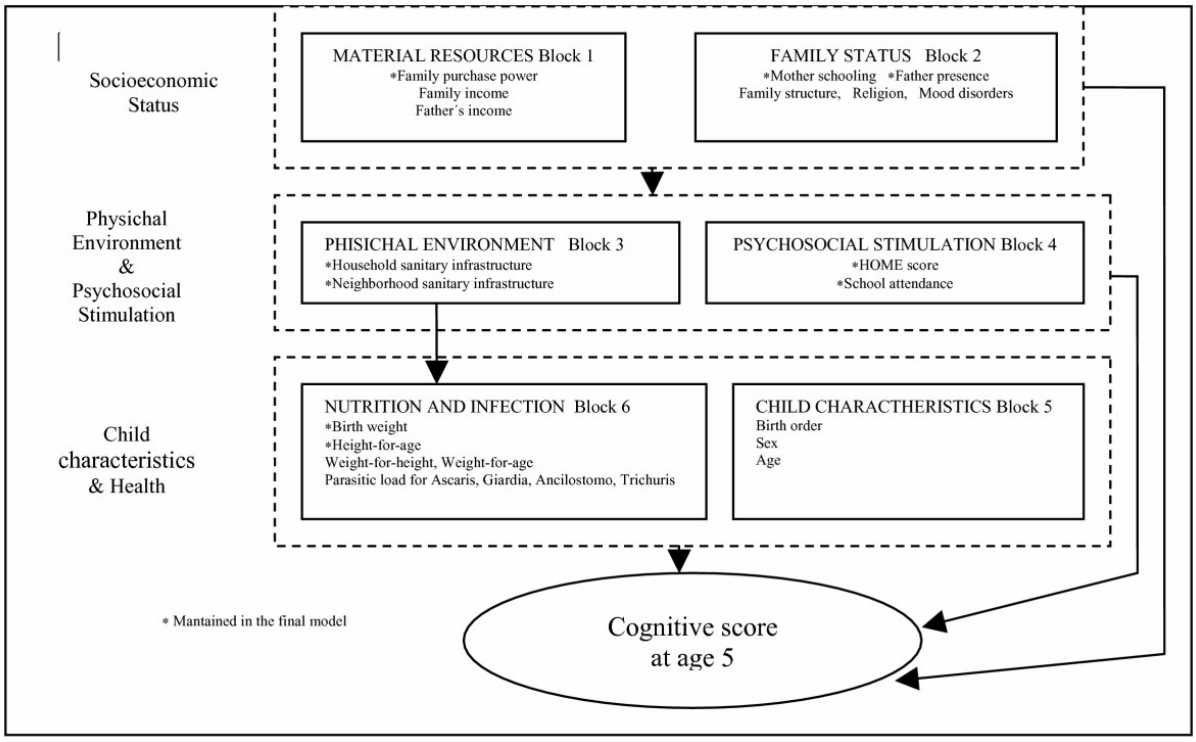


Figure 6; Overview of different factors that determine cognitive function
The schedule shows the factors of socioeconomic status, physical environment and child characteristics that influence cognitive function either in positive or negative ways. These factors can influence cognitive function directly or act as mediators for other factors. (Source: Santos, D.N. 2008)

Except for impaired development, cognition can also be negatively influenced later in life. One of the best known determinants for later life cognition is age. Using the Mini-Mental State Examination (MMSE), Peters *et al*. showed that increasing age is associated with poorer cognitive function (Peters, Beckett et al. 2009). Interestingly, this association was still present in the very elderly, even within the 80-105 year group. Finally, exposure to psychoactive compounds can also influence cognitive skills in adults. People with a prolonged history of alcohol or cannabis use had a greater deficit in working memory (Coullaut-Valera, Arbaiza-Diaz Del Rio et al. 2011). The cannabis users also showed impaired alternating attention and needed more time to execute tasks that required logical and sequential thinking.

3.4 Cognition testing

Choosing a specific measure of cognition in epidemiology studies that investigate cognitive function and skill is a challenging task. A powerfull measure must be able to detect small cognitive changes in all the range of cognition observed in the target population. And there are a lot of different tests that scale cognitive functions and skills to choose from. Two often used tests for cognition measurements are the Bayley Scales of Infant Development (BSID) and the Wechsler intelligence scales. The BSID is a standard series of measurements to assess the motor, language and cognitive development of infants from 0-3 years. It consists of a series of developmental play tasks. The raw scores of successfully completed items are converted to scale scores. These scores are than used to determine the child's performance compared to the standardized scores of typically developing children from the same age (Black 1999). The Wechsler intelligence scales were developed by Dr. David Wechsler. This test is not based on the quantity of intelligence, but how well you use your intelligence. The three most important tests are the Wechsler Adult Intelligence Scale (WAIS), the Wechsler Infant Intelligence test (WIIS) and the Wechsler Preschool and Primary Scale of Intelligence (WPPSI) (Kaufman, 2006). However, there are three considerations that are of great practical importance in epidemiology studies on cognative function. First, the performance on cognitive tests is affected by many factors other than the one under investigation. For instance: prior testing experience, emotional state and measurement error. Also in contrast to studies of specialized clinic populations, it is often necessary to consider a wide range of cognition, which imposes demands on cognitive tests so that ceiling and floor effects in the measurements often occur. Finally, epidemiologic studies are limited to briefer tests. Therefore, the tests usually summarize multiple facets of cognition, which again increased the likely hood of ceiling and floor effects (Morris, Evans et al. 1999).

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4. Neurological effects of air pollution

4.1 Toxicology studies

4.1.1 Translocation of particles to the brain

Translocation to extrapulmonary sites after respiratory tract deposition is an important mechanism for particles to cause effects in secondary organs. Whether this process occurs and to which extent, depends on several factors including particle size, solubility, site of deposition and the integrity of the epithelial lining. Elder *et al.* showed that ultra fine and fine particles can translocate from the lungs by penetrating pulmonary tissue and enter the capillaries, reaching other organs, including the brain by circulation (Elder, Gelein et al. 2006). As mentioned before, the blood brain barrier is supposed to inhibit harmful particles from entering the brain. However, some particles are still able to cross the blood brain barrier either because they are small enough to leak through the endothelial tight-junctions, or because they disrupt the blood brain barrier by inflammatory responses. Pollutants can also enter the brain through direct translocation. Animal studies have shown that inhaled particles can be translocated to the brain via the olfactory nerve that connects the nose and the brain directly (Elder, Gelein et al. 2006, Oberdorster, Sharp et al. 2004).

4.1.2 Neuro-inflammation and degeneration

The first and main form of active immune defence in the central nervous system is formed by the action of microglia. These microglia are a type of glial cells that reside in the brain and spinal cord. They respond to tissue insult with a complex array of inflammatory cytokines and actions. They are recognized as the prime components of an intrinsic brain immune system. Before neuro-inflammation became a commonly used term, scientists used the term 'reactive gliosis' (Streit, Mrak et al. 2004). This term specifically referred to the accumulation of enlarged glial cells, notably microglia and astrocytes, immediately after CNS injury had occurred. Activation of immune cells in the periphery leads to leukocyte infiltration of tissues, but this leukocyte infiltration is absent in the brain, unless there has been destruction of the blood brain barrier. Without breakdown of the blood brain barrier, leukocytes are not able to cross this barrier and there is a much subtler response of the brains own immune system. Although these specific responses might be included in the term neuro-inflammation, this term generally applies to a more chronic, sustained cycles of injury and response. This chronic microglial activation likely contributes to injury, loss of neurons and neuronal dysfunction (Bellucci, Westwood et al. 2004). Neuro-degeneration is the overall term for progressive loss of structures or function of neurons and axons in the central nervous system. Immune activation in the CNS is a classical feature of neuro-degeneration

4.1.3 IL-1 and COX-2

Among the pro-inflammatory molecules, cytokines are thought to play a central role in the self-propagation of neuro-inflammation, with a prominent function for interleukin-1 (IL-1). IL-1 is family of three related proteins, being Il-1a, Il-1b and Il-1ra. Normally, Il-1 is expressed at low levels, but is upregulated rapidly in response to local or peripheral insults. The specific cellular source of these proteins is unclear, but microglia cells appear to be the early primary source. Astrocytes and neurons have also been reported to express Il-1. (Pearson, Rothwell et al. 1999) It remains

uncertain whether IL-1 plays a major role in the normal, healthy brain, because the expression is barely detectable. However, IL-1 has been shown to act by increasing fever, hypophagia, slow-wave sleep, sickness behaviour and neuro-endocrine changes. It's expression is also increased in human degenerative conditions and inhibition of IL-1 in rodents reduced neuronal loss dramatically. (Rothwell, Luheshi 2000)
Cyclooxygenase (COX) generates reactive oxygen species (ROS) as a by product of the conversion of prostaglandin G2 to prostaglandin H2 in the synthetic pathway of prostaglandins and thromboxanes. There are two different isoforms of COX. COX-1 is expressed and predominant in peripheral tissues, while COX-2 has been shown to be expressed at high levels in the CNS and is induced by a variety of stimuli (Yamagata, Andreasson et al. 1993). It is rapidly upregulated at sites of inflammation and it is primarily expressed by neurons, whereas microglia and astrocytes are almost unlabelled. Given the fact that oxidative stress is involved in neuro-degeneration, it is likely that the induction of COX-2 and the generation of free radicals by this protein are related to the underlying mechanism (Oka, Takashima 1997).

4.1.4 White matter lesions

White matter lesions are small area's of disrupted neurons in the white matter, commonly seen in older people, since it is a normal result of aging (Sierra 2001). However, aging is not the only factor that induces these lesions. They also appear in the brains of people who have suffered stroke or have progressive neurological diseases and they can be induced by exposure to toxicants. While it is not clear that white matter lesions directly cause brain dysfunction, they are seen as good indicators. Namely there is a clear connection between lesions and decreases in brain volume, loss of memory and vision, and cognitive impairment (De Groot, De Leeuw et al. 2002).

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4.2 Epidemiology studies

4.2.1 Air pollution exposure and cognitive deficits in Mexico City Children

In 2002, the group of Calderon compared the nasal mucosae, olfactory bulb, and cortical and subcortical structures of dogs from the SouthWest Mexico City (SWMC), which is a highly polluted area, with those of dogs living in Tlaxcala, a less polluted control city (Calderon-Garciduenas, Azzarelli et al. 2002). In the next stage of their research, they found an acceleration of Alzheimer's like pathology in the dogs that were living in the polluted area, compared to the control dogs, suggesting that the brain is adversely affected by pollutants (Calderon-Garciduenas, Maronpot et al. 2003). They found that dogs living in the polluted area had an disrupted blood-brain-barrier, degenerated coritial neurons and apoptotic glial white matter cells, compared to the control dogs. There is an important knowledge gap regarding the impact that chronic exposure to air pollution has on cognitive performance, neuro-inflammation and neuro-degeneration in healthy children. Therefore, in 2008 Calderon *et al*. evaluated the neuro-physiological functioning and the structural brain alterations as detected by MRI of clinically healthy children that live in two significantly different urban environments (Calderon-Garciduenas, Mora-Tiscareno et al. 2008). One group lived in Mexico City (MC), which is known for high concentrations of air pollutants.

This group consisted of 6 males and 7 females with an average age of 10.69. The other group of children lived in the region of Polotitlan, with air pollution levels within the current USA National Ambient Air Quality Standards. This group consisted of 11 males and 12 females with an average age of 10.73. The Wechsler Intelligence Scale for Children-Revised was used as the standardized neuro-physiological instrument to measure cognitive impairment, like attention, working-memory and executive functions. Brain MRI was done to look at the occurrence of white matter lesions. Their conclusions were that the MC clinically healthy children showed significant differences for a number of WISC variables, like in areas of fluid cognition, memory and executive functions, compared to the children in the low polluted area. In parallel, 56.5% of MC children showed prefrontal white matter lesions (WML), compared to 7.6% of Polotitlan children. This result suggest that exposure to air pollution is a key factor of brain damage in these MC children(Calderon-Garciduenas, Solt et al. 2008). Their most recent work includes data from 20 children from Mexico city (mean age 7.1 years) and 10 children from the region of Polotitlan (mean age 6.8 years) (Calderon-Garciduenas, Engle et al. 2011). The children had a negative smoking history and ETS exposure, life long residency in the chosen area, full term birth, living within 5 miles of a monitoring station and no remarkable clinical history. And again the children were matched by age and socioeconomical status. Baseline and 1-year follow-up measurements of global and regional brain MRI volumes, WISC scores and serum inflammatory mediators were collected. Significant differences in white matter volume (WMV) were observed in the MC children and they showed progressive deficits on the vocabulary and digit span subtests of the WISC. These cognitive deficits match the localization of the volumetric changes detected over the 1-year follow-up. These findings suggest that exposure to air pollution may disrupt the cerebral development.

4.2.2 Exposure to traffic-related air pollution and neurobehavioural functions

For this study, children from two different primary schools were selected to explore the association between traffic-related air pollution and neurobehavioral function in children (Wang, Zhang et al. 2009). The first school was located 3.5km away from primary traffic roads in the north of Quanzhou and had low traffic density. The second was located on a three-way-intersection in the centre of the city. Levels of ambient air PM10 and NO2 were measured for two days at five different school sites. The children were 8-10 years old and selected based on a questionnaire about their socioeconomic status and neurobehaviour was tested based on nine standardized tests. The NO2 levels in the polluted area where significantly higher than in the clear area. However, levels of PM10 did not show any differences. Children that were going to school in a polluted area had significantly lower scores on all nine tests, compared to the children that were going to school in de clean area. Therefore, Wang et al. state suggests that there is a significant relationship between traffic-related air pollution and neurobehavioral functions in children.

4.2.3 PAH exposure and developmental delay

Another prospective cohort study was done by Perera *et al*. and evaluated the role of prenatal exposure to urban pollutants in the pathogenesis of neuro-behavioural disorders (Perera, Rauh et al. 2006). The urban pollutants included polycyclic aromatic hydrocarbons (PAHs), environmental tobacco smoke (ETS) and pesticides.

The woman who participated in this cohort were Dominican or African-American, non-smokers, free of diabetes, hypertension and known HIV; did not use drugs and had resided in the area for at least one year. During the third trimester of pregnancy, personal PAH exposure was monitored for 2 consecutive days and measures of child behaviour and neurodevelopment were done with the use of the Bayley Scales of Infant Development-Revised. Infants who had been exposed to the highest concentration of PAH, scored significantly lower on the mental development index (MDI) at three years of age, compared to infants that were lower exposed (see figure 7). The odds of having low MDI scores were 2.89 times higher in the high exposed children, suggesting that more exposed children are potentially at risk for performance deficits.

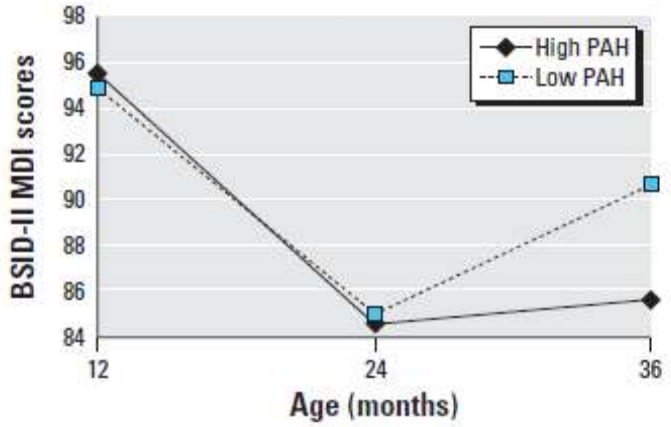

Figure 7; The effect of prenatal PAH exposure on the BSID-II MDI scores*. The BSID-II MDI score is used as a measure of developmental delay. (Source: Perera et al. 2006)*

4.2.4 Black carbon exposure and cognition

The relation between black carbon and cognition among 202 Boston children was examined by Suglia *et al*. in a prospective birth cohort study (1989-2001) (Suglia, Gryparis et al. 2008). Pregnant woman over 18 years, receiving prenatal care at an urban community health center in Boston were fitted for enrolment. At each clinic visit during their pregnancy the women were asked about their smoking status and the smoking habits of other members of the household. Also a urine sample was taken to determine the cotinine level. The postnatal exposure of the child to secondhand smoke was obtained by a questionnaire every month for the first 26 months, twice a year for the period between 26 months to 4 years and annually for the remaining years. To estimate the residential black carbon levels, data was used from pollution measurements at more than 80 sites performed at more than 2000 different days. For the follow-up study, children were selected based on their birth weight, blood-lead-level and ETS exposure. When the children were aged 8-11 years, cognitive tests were administered, including the Kaufman Brief Intelligence Test (K-BIT) for verbal and non verbal intelligence and the Wide Range Assessment of Memory and Learning (WRAML) for verbal and visual memory and learning (Putzke, Williams et al. 2001). In this study, long-term concentration of black carbon particles from mobile sources was associated with decreases in cognitive test scores, both on verbal and non-verbal intelligence and on memory. Socioeconomic status could have been a confounder, since it can be a determinant of cognitive abilities during childhood. Also it can determine whether a family lives close to traffic areas. However, since all families were recruited from one neighbourhood, the variability in socioeconomic

status was restricted and therefore the potential of confounding was reduced. (Suglia, Gryparis et al. 2008).

4.2.5 Traffic-related air pollution and cognitive function of older men

Since children are not the only group of individuals that are exposed to traffic-related air pollution, Power *et al*. recently performed a study that looked at the effects of traffic-related air pollution on the cognitive function of older men (Power, Weisskopf et al. 2011). A total of 680 participants (mean age 71 years) of an ongoing longitudinal cohort study about ageing were selected for this study. They were selected on medical history, lifestyle and demographic factors and they were asked to complete different cognitive tests including the MMSE. During the 12 years of this study, the participants were invited to undergo an examination every 3 years, including physical examination and laboratory test. Estimates of BC exposure were at the residence of each participants were used as a surrogate for individual exposure to traffic-related air pollution. Daily averages from 83 monitoring sites, were used to develop a BC prediction model. To decrease the confounding effect of lead, which is also an environmental pollutant, the lead levels in the blood were also measured during the examination of the study subjects. They found that BC exposure was significantly associated with the risk of a low MMSE score (odds ratio 1.3). They also found some effect modification, since their was a higher adverse BC effect level in overweight or obese people and heavy smokers.

4.2.6 PM and cognitive function in elderly women

To investigate whether long term exposure to traffic-related PM is linked to the development of cognitive impairment, neurophysiological test were done in a cohort of elderly women (Ranft, Schikowski et al. 2009). The study subjects were chosen from the SALIA cohort, which was a Study on the influence of Air pollution on Lung function, Inflammation and Ageing. The Ruhr district and the rural counties to the north of the Ruhr district were chosen as study area's, because of the range of exposure to PM in ambient air due to traffic, coal and steel industries. PM_{10} levels were provided by monitoring stations that were equally distributed over the Ruhr district. The individual exposure to background PM_{10} was estimated by using data of a monitoring station close to the participants' residencies and was averaged over the 5 years before baseline investigation. To ensure the reliability, only women (average age of 74.1 years) that had not moved over the last 20 years were included in the study. Since decreased olfactory function could be an important confounder, the odor identification ability was tested with the use of sniffing sticks. Also, some psycological tests were included, since depression could also influence the cognitive test outcomes. Finally, based on a questionnaire, information about gas cooking, chronic diseases, smoking habits and educational levels was obtained. Regression analysis revealed that for women younger than or equal to 74 years of age, distance to traffic was a consistent and significant risk factor for mild cognative impairment (MCI), because the test scores of participants living within a 50 meter range to a busy road were significantly lower, compared to the standardized scores. They found an indication that chronic exposure to traffic related PM may be involved in the development of mild cognative impairment (MCI). However, it only provides indirect

evidence for an effect, since air pollution near streets is a complex mixture and PM is only one of many toxic compounds.

4.2.7 Ozone reduces neural behaviour in adults

Although children may be at higher risk for neurological effects caused by air pollution, exposure to air pollutants also effects the neurological behaviour in adults. Chen *et al*. used data on central nervous system function from the Third National Health and Nutrition Examination Survey (NHANES III) conducted by the National Centre for Health Statistics (NCHS) between 1988 and 1994 (Chen, Schwartz 2009). In this survey CNS function was measured by three neurobehavioral tests. (1) a simple reaction time test (SRTT) which is a basic measure for visuomotor speed. (2) a symbol digit substitution test (SDST) which tests the ability of coding. And (3) a serial digital learning test (SDLT) which measured the attention and short-term memory. For the follow-up tests, the study population was restricted to people that participated between 1989 and 1991, completed at least one measurement of CNS function and who had estimable air pollution data. They also adjusted for individual-level confounders like age, sex, race and socio-economic status, but also lifestyle factors (smoking, alcohol consumption and exercise) were considered confounders. Since it was impossible to measure real cumulative exposure, the annual average exposure was used for the approximate long-term exposure. Their analysis demonstrated that reduced performance in neurobehavioral tests was associated with estimated increasing levels of annual exposure to ambient ozone.
The estimated annual ozone levels were consistently associated with reduced performance in both SDST (attention) and SDLT (short-term memory), but not in SRTT (reaction test). High levels of estimated PM_{10} were also correlated with reduced performance in SDST and SDLT in sex- and age-corrected models. However, these effects disappeared after adjustment for race/ethnicity and socio-economic status, suggesting the presence of confounding by the residential separation of ethnic minorities and poor people in areas with high levels of PM_{10} exposure. Though, it is possible that the lack of consistent association may result from the limited spatial resolution of estimated PM_{10} levels. Also, the inconsistent association between PM10 and cognitive function should not preclude the possible adverse effects of other or smaller ambient particles on the CNS.

4.2.8 Schematic overview

Study	Age (years	Exposure data	Findings
Calderon *et* al. 2008	10-11	Lifelong exposure	Increase in WML and decrease in fluid cognition, memory and executive fundtions
Calderon *et al*. 2011	6-7	Baseline + 1 year follow-up	Decreased WMV and deficits on vocabulary and digit span
Wang *et al*. 2009	8-10	Exposure during school years	Decreased scores on 9 different cognition tests
Perera *et al*. 2006	3	Prenatal	Lower MDI
Suglia *et al*. 2008	8-11	Prenatal + first 8 childhood	Decreased verbal and non-verbal intelligence and

		years	decreased memory
Power *et al*. 2011	64-78	Previous 10 years	Risk of low MMSE score
Ranft *et al*. 2009	68-79	Previous 20 years	Development of MCI
Chen *et al*. 2009	27-47	Long-term annual exposure	Reduced attention and short term memory

Table 3; Schematic overview of the epidemiology studies

5. Discussion

For neurological effects to occur it is important to know whether air pollutants are able to translocate from the respiratory system to the central nervous system. Several research groups have shown that the air pollutants are indeed able to translocate to the CNS via different pathways. Dependent on their size, particles are can diffuse through the epithelial cells from the lung into the blood stream (Oberdorster, Sharp et al. 2002). The ultra fine particles are so small they can also cross the blood brain barrier, without disrupting the BBB. Once translocated to the brain, they activate microglia (Minghetti 2005), induce oxidative stress (Peters, Veronesi et al. 2006) and they induce the expression of pro-inflammatory molecules, including IL-1 and COX-2 (Pinteaux, Trotter et al. 2009) (Hoozemans, Veerhuis et al. 2006) All of the studies that were described in this review have their strengths and limitations. Calderon *et al* recruited children from middle class families, based on parental occupation and income criteria, to avoid socioeconomic confounders. Also the selection of the control city was not only based on the low air pollution levels, but also because it was on the same altitude and relatively close to Mexico City, to make sure that the environmental influences were mostly the same for both groups of children. Finally, given that Apolipoprotein E (APOE) plays a crucial role in the maintenance and repair of neurons and since it is associated with neuro-pathological processes (Gozal, Capdevila et al. 2007), the cohorts were also genotyped for the APOE alleles to determine if subjects had a known risk factor for Alzheimer's disease. The study that was performed by Wang *et al*. looked at the effects of attending a school in a highly polluted area. The levels of NO_2 were significantly higher at the school that was located in the polluted area, but they found similar levels at PM_{10}. Therefore, they argued that traffic related air pollutants would probably be smaller than 2.5μm and that these small particles would not contribute to the levels of PM_{10}, because otherwise the levels in the polluted school would definitely by higher. But without measuring the real pollution levels, it is hard to make assumptions about the actual air pollution. Besides, they were only able to measure pollution at both schools twice in the same month and therefore they could not estimate seasonal differences. Also, the found that lead levels, which is also a contributor to cognitive impairment, were similar in both school. But they did not include the possibility of lead poisoning at home, since the questionnaire only included socioeconomic factors and information about fuel burning and heating at home. The observed association between cognitive performance and air pollution found by Chen *et al*. cannot be interpreted as definitively causal, because it is statistically arguable that residents from clean air communities might happen to have better baseline cognitive function and thus having better test performances during the survey. Also the use of one time residential information to represent the geographical location associated with long-term exposure to ambient air pollution, does not allow characterization of life-course cumulative exposure. Therefore, exposure during vulnerable periods was not captured in cross-sectional data. To avoid this problem, cohort studies with longitudinal residential information should be done. Another limitation in this survey is the fact that there was no personal air pollution exposure data available, which means the exposure assessment can result in an under- or overestimation of individual exposure to ozone. Since the exposure also depends on occupational exposure and the daily indoor and outdoor activities. Finally, in this analysis it is impossible to rule out the possibility that the effects of ozone represent the adverse effects of other photoreactive pollutants correlated with ozone, because

other products of photochemical processes are likely correlated with ozone, even though they are not monitored.

The advantage of the PAHs exposure cohort study is that the researchers had an extensive medical record and questionnaire data to work with. On the other hand, the individual exposure data is very limited, because exposure levels were based on a measurement of only two days during the third trimester. The fact that the exposure was high or low at that time point, does not mean that the average exposure during the entire pregnancy was the same. And there was no data about the postnatal exposure to PAH although that might be equally important, since brain development continues during complete childhood. Also, it is possible that high levels of PAHs may be associated with living near an exposure source such as a bus route or garage leading to some uncontrolled confounding by socioeconomic status even within the low-income population. Finally, the mean developmental scores in this cohort population were lower than the normed population, which might mean that the low income nature of this cohort population and the low levels of mother-child interaction effect the developmental scores more than the prenatal PAH exposure.

The most important limitations of studies performed by Power *et al*. and Ranft *et al*. are the mean age of the participants. Although the subjects were carefully selected to avoid as many confounders as possible, age is still the main confounder in these studies. Even though they found associations between air pollution exposure and decreased cognitive function, these effects can still be due to naturally ageing, because ageing happens differently in every person and can not be ignored, just by matching people based on their socioeconomic and educational status.

There is however one major confounder that probably applies to all of the above studies, which is noise. There is evidence that noise exposure can not only induce hearing impairment, hypertention and sleeping disorders, but also decreased school performance. That last effect is not only due to the lack of attention, but also to cognitive impairment, which was already shown in two old epidemiologic studies. But also more recently, Stansfeld *et al*. showed that the noise of airplanes during the night was significantly associated with impaired reading and recognition memory (Stansfeld, Hygge et al. 2010). Since, outdoor air pollution is often associated with noise (especially traffic, but also building cites, power plants, etc.), this confounder could influence the outcome of every study that investigates the effects of outdoor air pollution and cognitive function. But on the other hand, evidence from indoor air pollution studies might ground the previous associations between air pollution and cognitive function. A small birth cohort study in spain was the first to report a negative association between exposure to gas appliances in indoor NO2 with general cognition and inattention symptoms in four year old children (Morales, Julvez et al. 2009). However, these effects were modified by a polymorphism in a detoxification gene. A more recent prospective birth cohort study that was set up to investigate associations between NO2 pollution (indoor gas cooking) and neurodevelopment, also found some adverse effects (Vrijheid, Martinez et al. 2012). In this study, decreases in BSID scores were related to gas cooking, independent of social class, maternal education and other measured potential confounders. Although all of these studies have their limitations, it does not mean that the observed effects on neurodevelopment and neuro-behaviour are not reliable. The results just require more conformation and therefore, the issue of air pollution causing cognitive deficits or structural brain changes, with all their potentially consequences should still be of major public importance. Especially, because there are still living areas, where monitored air fails to meet the Air Quality Standard for critical air pollutants.

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