Understanding the effects of air pollution on neurogenesis and gliogenesis in the growing and adult brain

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Abstract

Exposure to air pollution – and particularly to particulate matter (PM) – is strongly associated with higher risk of neurodevelopmental disorders, poor mental health and cognitive defects. In animal models, disruption of CNS development and disturbances of adult neurogenesis contribute to PM neurotoxicity. Recent studies show that gestational PM exposure not only affects embryonic neurodevelopment, but also disturbs postnatal brain growth and maturation, by interfering with neurogenic/gliogenic events, myelination and synaptogenesis. Similarly, adult neurogenesis is affected at many levels, from neural stem cell amplification up to the maturation and integration of novel neurons in the adult brain parenchyma. The underlying mechanisms are still by and large unknown. Beyond microglia activation and neuroinflammation, recent studies propose a role for novel epigenetic mechanisms, including DNA methylation and extracellular vesicles-associated microRNAs.

Exposure to air pollution is increasingly acknowledged as one of the main contributors to the global disease burden [1]. It has been estimated that in 2016 91% of the world population was living in places where the WHO air quality guidelines levels were not met (https://www.who.int/news-room/fact-sheets/detail/ambient-(outdoor)-air-quality-and-health). Among the key air pollutants that pose health risks, particulate matter (PM) is one of the most widespread. PM is a heterogeneous mixture of small solid or liquid particles released into the atmosphere during combustion processes or emitted by industrial activities and natural sources. PM generally comprises water soluble and insoluble components, including inorganic compounds, polycyclic aromatic hydrocarbons, heavy metals and other toxic substances, and microbial components, such as bacteria and their products of degradation (e.g. lipopolysaccharide) and viruses [2]. PM is defined according to its aerodynamic diameter, with coarse PM smaller than 10 μm (PM_{10}) and fine and ultrafine PM smaller than 2.5 ($PM_{2.5}$) or 0.1 ($PM_{0.1}$) μm, respectively. Thanks to their small size, when inhaled, PM particles have the capability to percolate through the respiratory tract. While PM_{10} is trapped in the upper airways, $PM_{2.5}$ reaches the lungs and deposits in the alveolar area. Ultrafine particles could even penetrate into the blood circulation and overcome the blood-brain-barrier (BBB) [3,4], or pass through the nasal mucosa and directly enter the brain [5,6]. Of note, inhaled nanoparticles have been shown to cross the placental barrier and to deposit in the fetal tissues in animal models [7], suggesting a possible mother-to-fetus transfer of airborne ultrafine PM.

Chronic exposure to air pollution has been consistently associated with risk of cardiovascular and respiratory diseases, and different types of cancer [1]. Increasing evidence also indicates that the central nervous system (CNS) is a target for air pollution. In utero and early child exposure to high levels of air pollution, and in particular to PM, is associated with higher risk of neurodevelopmental disorders, long-lasting behavioral alterations and cognitive defects [8,9]. Moreover, during adulthood, chronic PM exposure

has been associated with poor mental health, increased risk of onset and worsening of depression [9], while both short and long term exposure has been associated with cognitive/memory deterioration [10,11].

Most studies in animal models that aimed at establishing a causative link between air pollution and anatomical/functional CNS alterations, and at unveiling the underlying mechanisms, are focused on the effects of PM. In rodents, PM exposure results in neurodevelopmental, cognitive and behavioral alterations reminiscent of those observed in humans, whose extent and duration depend on PM size, doses and timing of exposure [12–17]. Mechanistically, disruption of CNS development and of adult neurogenesis were found to contribute to PM detrimental effects, suggesting the occurrence of similar events in humans.

In this review, we summarize recent advancements toward the understanding of the cellular and molecular mechanisms mediating PM effects on the developmental and adult neurogenesis and gliogenesis, discuss limitations of the available studies and highlight persisting open issues.

In utero and neonatal exposure to PM induces neurodevelopmental alterations in animal models

In mice, chronic prenatal exposure to high levels of fine and ultrafine PM was reportedly associated with reduced brain weight and ventriculomegaly at birth and during the first postnatal period [13,18]. This is the outcome of the disruption of specific and diverse neurodevelopmental events. Exposure to diesel exhaust particles (DEP) in mouse pregnant dams throughout gestation resulted, in the offspring, in increased cortical (i.e. prefrontal cortex) and hippocampal (i.e. dentate gyrus, DG) volumes at embryonic day (E)18, which switched to decreased cortical volume and normalized hippocampal size in postnatal day (P)30 males (but not in females), compared to untreated animals [19].

Similarly, maternal inhalation of carbon black nanoparticles (produced by the incomplete combustion of petroleum products) resulted in an initial increase of parvalbumin-positive (+) neurons in the uppermost layers of the motor cortex, followed by a large reduction at later time points [20]. These results suggest that gestational PM exposure may differentially affect distinct phases of brain development and cause an initial tissue overgrowth – possibly due to neural stem cell (NSC)/progenitor over-expansion - followed by postnatal regressive events. Thus, the effects on CNS development of in utero PM exposure can be persistent and extend beyond the embryonic period. In line with this interpretation, two recent studies [12,21] have shown that chronic prenatal exposure to high dosages of $PM_{2.5}$ resulted in increased neuronal and astrocyte apoptosis in the cortex and distinct hippocampal subregions, including the DG, of the offspring at P14-P30. Postnatal hippocampal neurogenesis and astrogliogenesis appeared also dramatically reduced, due to the suppression of NSC proliferation in the subgranular zone (SGZ). Similarly, parenchymal astro- and oligo-dendroglia amplification was affected, as indirectly assessed by the large decrease of the proliferation marker PCNA in the cortex of P1-P30 offspring [21]. In agreement with this finding, gestational chronic exposure to fine and ultrafine particles has been associated with precocious myelination and premature oligodendroglia proliferation/differentiation switch in the corpus callosum of the adolescent offspring [13,22]. Dendritic complexity [15] and number of asymmetric excitatory synapses impinging on hippocampal neurons were also significantly reduced in adolescent (P14) mice prenatally exposed to $PM_{2.5}$. The remaining synapses showed altered -and possibly compensatory- features, including increased number of presynaptic vesicles, thickened postsynaptic density and decreased synaptic space [12].

Thus, gestational PM exposure not only affects embryonic neurodevelopment, but also disturbs postnatal brain growth and maturation, by interfering with neurogenic/gliogenic events, myelination and synaptogenesis. Pregnancy appears to be a particularly

vulnerable time window, since neonatal exposure had milder effects, and mostly affected myelination [23,24] and expression of synaptic proteins [14].

PM exposure disturbs adult neurogenesis in animal models

In the adult mouse brain, generation of new neurons continues in the subventricular zone (SVZ) of the lateral ventricles and in the SGZ of the hippocampus [25]. Adult neurogenesis in the SVZ cannot be detected in humans, whereas controversial evidence has been provided about the generation of new neurons in the adult human hippocampus [26–28]. Thus, while adult hippocampal neurogenesis is implicated in cognitive processes and mood regulation in rodents [29], whether this occurs also in adult humans is highly debated. Nevertheless, adult neurogenesis in rodents recapitulates many aspects of the developmental neurogenic/gliogenic events. Therefore, the study of the mechanisms mediating PM-induced perturbations of the adult neurogenic niches is still of interest, as it can unveil critical toxicity processes operating in both developing and mature CNS.

In a recent study, acute exposure to fine DEP caused an impairment of adult neurogenesis in mice. This effect was gender-specific, with males showing fewer newly-generated neurons in SGZ, SVZ and olfactory bulb (OB), compared to control animals, and females displaying fewer new neurons only in the OB [30]. Reduced neurogenesis was a consequence of decreased proliferation of NSCs/progenitors, reduced survival of immature neurons, and altered specification/differentiation of newborn elements (i.e. reduced fraction of newborn cells expressing the mature neuronal marker NeuN 3 weeks after their generation [30]). Moreover, life-long exposure to concentrated water-soluble subfraction of $PM_{0.2}$ dramatically reduced the number of SGZ newborn neurons -but not of newborn astrocytes- in adult male rats, which also showed contextual memory defects and depressive behaviors [16]. Thus, PM appears to negatively modulate the neurogenic events at many levels, from NSCs division up to the maturation and integration of novel

neurons in the adult brain parenchyma. In line with this view, chronic inhalation of ammonium sulfate, the major inorganic component in $PM_{2.5}$ (as resulting from the reaction of ammonia, mostly originating from animal farming and synthetic fertilizers, with sulfur dioxide emitted by the burning of fossil fuels [31]), diminished the dendritic complexity of immature neurons in the DG of aged rats [32]. However, in this latter study, no alteration of SGZ/SVZ NSC/progenitor proliferation and of the specification of their derivatives could be detected, highlighting a specific neurotoxicity of the distinct components of PM.

Proposed mechanisms underlying the effects of PM on neurogenesis and gliogenesis

In rodents, neuroinflammation accompanied by microglia and astrocyte activation were cardinal effects of PM exposure, whenever it occurs [12–16,19,20,23,24,30]. Pharmacological treatments aimed at blocking microglia polarization – such as the peroxisome proliferator-activated receptor γ (PPARγ) agonist pioglitazone - protected against PM-induced suppression of SGZ proliferation and rescued the number of newborn neurons, indicating a major role of microglia reactivity in the negative modulation of adult hippocampal neurogenesis [30]. Nevertheless, mechanistically, which activated microglia phenotype (i.e. proregenerative M2 vs. neurotoxic M1 vs. “dark microglia” [33]) is favored upon/after PM exposure and how microglia activation inhibits the neurogenic events remain obscure. Beyond the release of high levels of pro-inflammatory cytokines or reactive oxygen species, that can inhibit NSC/progenitor proliferation and alter the specification and survival of their derivatives [34], an interesting hypothesis is that PM-induced microglia activation could result in increased phagoptosis (i.e. the engulfment of immature viable neurons [35]). In line with this hypothesis, Bolton and colleagues [19] reported increased microglia-neuron physical interactions in the cortex of the offspring of PM-exposed dams.

Notably, upon prenatal and neonatal PM exposure, microglia activation and astrogliosis occurred predominantly in males [19,23,24,36]. Consistently, neuroinflammation was more pronounced in males than in females upon exposure to DEP during adulthood [37], in line with a more marked reduction of adult neurogenesis [30]. This suggests that sex-dependent factors, including the hormonal background, may influence the individual's vulnerability to PM effects. Interestingly, microglia activation and neuroinflammation extended well beyond PM-exposure, when it occurred in utero, in line with a priming action of air pollution.

Moreover, what is the trigger for microglia and astrocyte activation remains elusive. Fine and ultrafine particles could enter the CNS and directly stimulate glial reactivity. Given the relatively small extension of the olfactory mucosa, it is likely that in humans – at difference with rodents - the main entrance route for PM is the blood. In line with this view, astroglia reactivity was observed predominantly around blood vessels [38]. Nevertheless, glial cells and NSCs/progenitors may be reached by a plethora of other factors – and even cells- from the periphery, thanks to the disruption of BBB integrity and increased leakage induced by PM exposure [13,16]. Among these elements, pulmonary cell-derived extracellular vesicles (EVs) may represent important lung-to-brain mediators of PM effects [39,40]. EVs are lipid bilayer-delimited particles, actively released from cells in response to stress. After internalization within target cells, EVs deliver their content, including proteins, lipids and miRNAs, and profoundly influence the recipient cell molecular state and function [41]. Interestingly, recent studies [39,40] showed that, in humans, the miRNA cargo of plasma EVs released following PM exposure has a signature relevant for the modulation of glial cell reactivity (e.g. miR-9, involved in microglia activation and neuroinflammation [42]) and NSC/progenitor functions (e.g. miR-128, miR-302, let-7 and miR-9, regulating neural precursor proliferation and neurogenesis [43]; miR-21, miR-9, miR-200, miR-17, miR-7, miR-302c, limiting oligodendroglia differentiation or enriched in immature oligodendrocyte

186 precursors [44]). Finally, a novel epigenetic mechanism possibly mediating PM effects on
187 developmental and adult neurogenesis may be the regulation of DNA methylation in NSCs
188 and their derivatives, that has been shown to be responsive to extrinsic signals and to
189 influence multiple aspects of neurogenesis from stem cell maintenance up to
190 synaptogenesis [45]. This hypothesis is corroborated by the observation of increased DNA
191 methyltransferase DNMT1 in the brains of male mice perinatally exposed to DEP [46].
192 Notably, in human placenta, PM exposure was associated with altered methylation level of
193 DNA repair and clock genes [47,48], which are also essential for adult and developmental
194 neurogenesis [49–51].
195
196 **Concluding remarks and open issues**
197 Convincing evidence, obtained in animal models, shows that CNS development and adult
198 neurogenesis are profoundly impacted by PM exposure throughout life, with significant
199 behavioral and cognitive alterations. This field of research is still in its infancy and
200 strenuous efforts are still needed to clarify the precise mechanisms by which PM affects
201 neurodevelopmental events and adult neurogenesis, and the molecular substrates of
202 gender and time window -specific differences in PM sensitivity. Available mechanistic
203 studies have frequently exploited heterogeneous PM dosages, composition, administration
204 modalities and timing. This scenario has so far impeded a complete understanding of the
205 processes subserving PM effects. Nevertheless, research on the effects of PM on other
206 systems has greatly advanced in the last years and identified interesting candidate
207 mechanisms that could be also at the basis of PM neurotoxicity.

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Declaration of interests

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Figure legend

Figure 1. PM-induced alterations detected in the adult mouse brain following in-utero or adult exposure. Orange boxes (above) include the proposed underlying mechanisms. BBB, blood-brain barrier; CC, corpus callosum; DG/SGZ, hippocampal dentate gyrus/subgranular zone; EV, extracellular vesicles; NSCs, neural stem cells; OPC, oligodendrocyte precursor cell; PM, particulate matter; PV, parvalbumin.

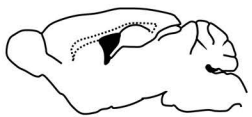
Microglia & astrocyte reactivity

BBB breakdown

EV-associated miRNAs

DNA methylation

In-utero PM exposure



Effects in young adults

CORTEX

- -volume
- ↓ -PV+ neurons
- -proliferation
- ↑ apoptosis of neurons and astrocytes

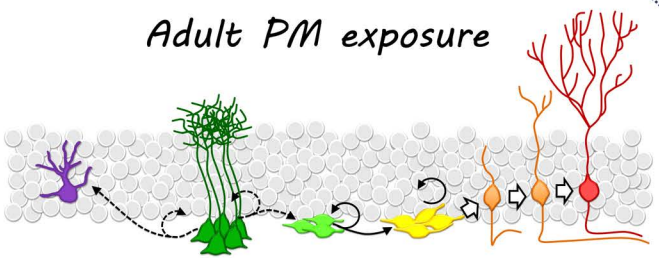
CC

- ↓ OPC expansion
- ↑ OPC maturation & myelination

DG/SGZ

- ↓ -proliferation
- ↓ -dendritic complexity
- ↑ apoptosis of neurons and astrocytes

Adult PM exposure



NSCs/intermediate progenitors:

↓ proliferation

Unaltered astrogliogenesis

Newborn neurons:

- -survival
- ↓ -acquisition of mature markers
- -dendritic complexity