

## Exposure to fine particulate matter (PM<sub>2.5</sub>) hampers myelin repair in a mouse model of white matter demyelination

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### ABSTRACT

Epidemiological studies show a strong association between exposure to air pollution – and particularly to particulate matter (PM) –, increased prevalence of Multiple Sclerosis (MS) and higher rates of hospital admissions for MS and MS relapses. Besides having immunomodulatory effects and sustaining a systemic oxidative-inflammatory response, PM may participate in MS pathogenesis by targeting also Central Nervous System (CNS)-specific processes, such as myelin repair. Here we show that, in a mouse model of lysolecithin-induced demyelination of the subcortical white matter, post-injury exposure to fine PM hampers remyelination, disturbs oligodendroglia differentiation dynamics and promotes astroglia and microglia reactivity. These findings support the view that exposure to fine PM can contribute to demyelinating pathologies by targeting the endogenous regenerative capability of the CNS tissue.

### 1. Introduction

Multiple sclerosis (MS) is a chronic immune-mediated disease of the Central Nervous System (CNS), characterized by neuroinflammation, multifocal demyelination and eventually neurodegeneration. Epidemiological studies have shown a strong association between exposure to air pollution and the development and exacerbation of autoimmune diseases, including MS. Short term exposure to ambient airborne particulate matter (PM) has been associated with a higher rate of hospital admissions for MS and MS relapses (Angelici et al., 2016; Roux et al., 2017; Oikonen et al., 2003; Jeanjean et al., 2018). An association between long-term PM exposure and increased prevalence of the disease was also recently shown (Tateo et al., 2019; Lemprière, 2020), suggesting that exposure to airborne PM may operate as an aetiological factor in MS.

PM is a mixture of particles released into the atmosphere during the combustion of fuels, from industrial activity and natural sources,

comprising inorganic compounds, hydrocarbons, metals and microbial components. PM particles are defined according to their size, with coarse, fine and ultrafine particles having an aerodynamic diameter smaller than 10  $\mu\text{m}$  (PM<sub>10</sub>), 2.5  $\mu\text{m}$  (PM<sub>2.5</sub>) and 0.1  $\mu\text{m}$  (PM<sub>0.1</sub>), respectively. When inhaled, fine PM percolates through the upper respiratory tract and deposits in the alveolar area. Here, PM triggers the response of a first set of defensive mechanisms, including components of both innate and acquired immunity. This pulmonary local oxidative-inflammatory reaction in turns leads to systemic inflammation and to modifications of the homeostasis of other organs/systems, including the CNS (Peeples, 2020).

PM is thought to contribute to MS pathogenesis by promoting autoimmunity and immune cell infiltration, disrupting the blood brain barrier (BBB) integrity and exacerbating local neuroinflammation (Bergamaschi et al., 2017; Calderón Garcidueñas et al., 2008; Cortese et al., 2020). Whether, beside these effects, PM exposure can also affect myelin repair in the CNS remains to be investigated. Studies have shown that the normal response to acute demyelination events, including acute

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### Abbreviations

+	positive
BrdU	5-bromo-2-deoxyuridine
CNS	Central Nervous System
CC1	adenomatous polyposis coli (APC) clone CC1
cCASP3	cleaved caspase 3
DAPI	4,6-diamidino-2-phenylindole
dpi	day post-injury
EVs	extracellular vesicles
GFAP	glial fibrillary astrocytic protein
GSTpi	Glutathione S-transferase pi
Iba1	ionized calcium-binding adapter molecule 1
iNOS	inducible Nitric Oxide Synthase
LPC	lysophosphatidylcholine (lysolecithin)
MBP	myelin basic protein
miRNA	micro ribonucleic acid
NG2	neural/glial antigen 2
OPCs	oligodendrocyte progenitor cells
PM	particulate matter
SE	standard error

MS lesions, is spontaneous remyelination (Franklin and Ffrench-Constant, 2008). Oligodendrocyte progenitor cells (OPCs) serve as a major source of remyelinating cells. After migrating to the demyelinated area and expanding by active proliferation, OPCs differentiate into mature oligodendrocytes, engage naked axons and form new myelin sheaths. Each step of this process is tightly regulated by both cell-intrinsic and environmental factors, including signals provided by neurons, astrocytes and microglia (Traiffort et al., 2020). Remyelination re-enables the axonal saltatory conduction, a property that is lost upon demyelination, and contributes to maintain axon integrity and neuronal survival. When remyelination fails, this leaves axons and neurons prone to degeneration. Experimental data indicate that differentiating oligodendroglia are highly vulnerable to insults and their maturation is the most critical phase of the remyelination process (Franklin and Ffrench-Constant, 2008).

Here we show that, in a mouse model of lysolecithin (LPC)-induced demyelination of the subcortical white matter, exposure to fine PM during post-injury oligodendroglia differentiation hampers myelin repair and promotes astroglia and microglia reactivity. These findings support the view that exposure to fine PM can contribute to demyelinating pathologies by targeting the endogenous regenerative capability of the CNS tissue.

## 2. Materials and methods

### 2.1. Animal care and experimental design

Perfusions of mice were carried out under deep general anesthesia obtained by intraperitoneal administration of Zoletil (zolazepam/tiletamine 100 mg/kg; Virbac, Milan, Italy) supplemented by xylazine (5 mg/kg; Rompun; Bayer; Leverkusen, Germany). To fate map cells generated during the post-injury phase, we employed the thymidine analog 5-bromo-2-deoxyuridine (BrdU, Sigma Aldrich) that is incorporated in the DNA during the S-phase of the cell cycle. Two subsequent BrdU injections (100 mg/kg body weight, i. p.) were performed at a 2 hours (h) distance at 8 days post-injury (dpi).

The experimental plan was designed according to the guidelines of the NIH, the European Communities Council (2010/63/EU) and the Italian Law for Care and Use of Experimental Animals (DL26/2014). It was also approved by the Italian Ministry of Health (authorization 510/2020-PR to EB) and the Bioethical Committee of the University of Turin.

The study was conducted according to the ARRIVE guidelines. Mice were housed in groups of 4, under a 12-hr light/12-hr dark cycle at 21 °C, with food and water ad libitum. Experiments were carried out on 3- to 4-month-old male C57BL/6 mice (Charles River, Calco, Italy).

### 3. Acute LPC-mediated demyelination

Acute demyelination was obtained by a unilateral stereotaxic microinjection of 1 µl of 1% lysolecithin (lysophosphatidylcholine, LPC, Sigma-Aldrich) in 0.9% NaCl (saline) solution into the subcortical white matter at coordinates: 1 mm mediolateral, 0.1 mm rostral to bregma and at 1.5 from the cortical surface.

### 4. PM<sub>2.5</sub> collection, extraction and chemical characterization

Ambient aerosol samples were collected at an outdoor urban site in the historical center of Milan, Northern Italy, by means of a high volume air sampler (Air Flow PM2.5-HVS, Model 600/AFPM2501K, AMS Analytica) equipped with a size-selective inlet for the collection of PM<sub>2.5</sub>. Particles to be used for biological experiments were extracted and characterized for elements (major elements and trace metals) and inorganic ions, as described in Suppl. methods and [Suppl Table 1](#).

### 5. PM<sub>2.5</sub> administration

Prior to the treatment, PM<sub>2.5</sub> was diluted in sterile saline and the obtained suspension was thoroughly mixed under sonication. Mice were randomly divided into control and treatment groups. The treatment group (n = 4) was treated by a single intratracheal instillation of PM<sub>2.5</sub> suspension (10 µg in 50 µl of saline), and the control group (n = 4) was treated with saline (50 µl). For adult mice, the respiratory volume is 0.04 m<sup>3</sup>/day. For seriously polluted cities, the reported daily PM<sub>2.5</sub> concentrations were frequently above 250 µg/m<sup>3</sup>. Therefore, in order to use a PM<sub>2.5</sub> dose relevant for human exposition, the dose used in this study was set as 10 µg (0.04 m<sup>3</sup>/day × 250 µg/m<sup>3</sup>).

### 6. Histological procedures

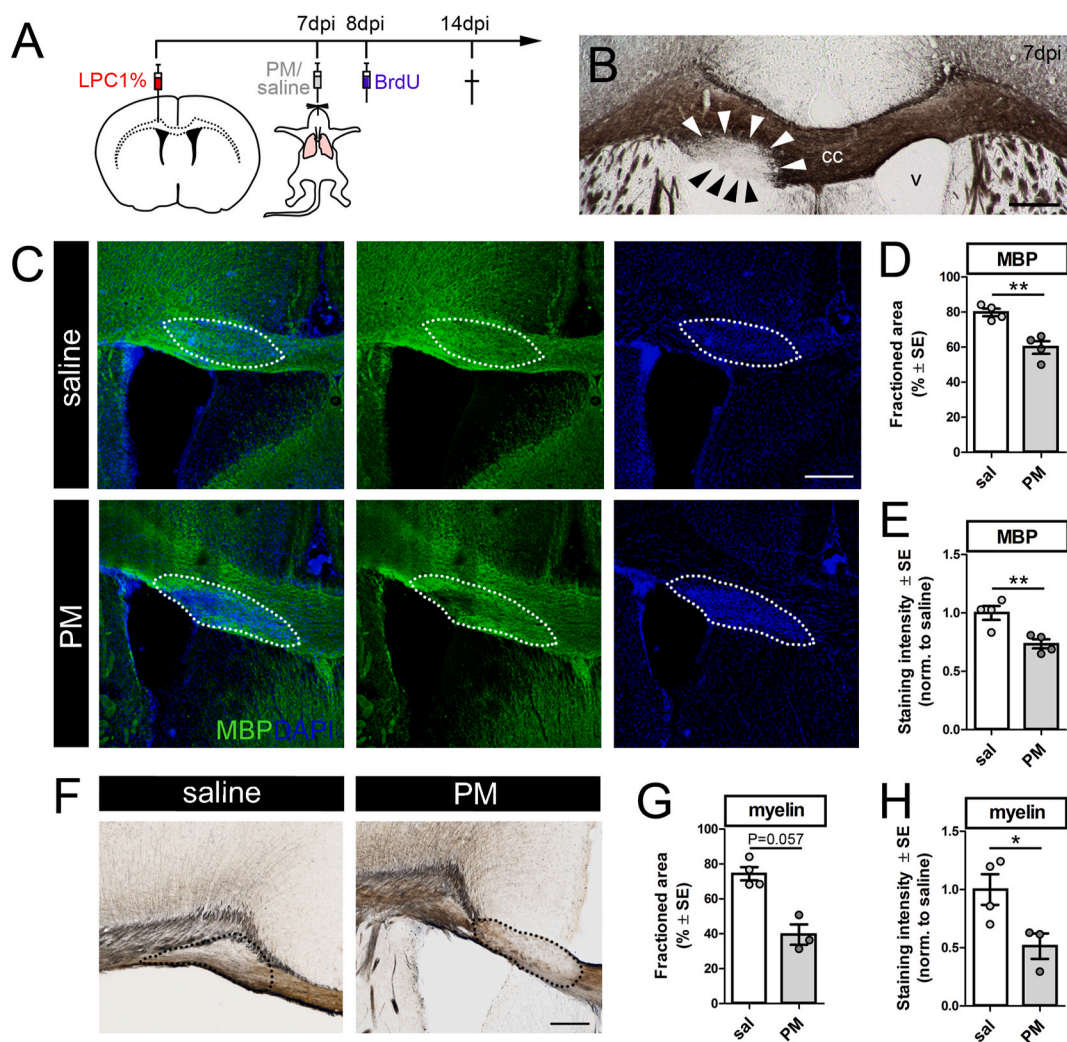
At 14 dpi animals were anesthetized (as described above) and transcardially perfused with 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer (PB). Brains were postfixed overnight, cryoprotected, and processed according to standard immunohistological procedures. Brains were cut in 30 µm thick coronal sections collected in PBS and then stained to detect the expression of different antigens: NG2 (1:200, Millipore, Burlington, MA, USA); GST-pi (1:500, Eppendorf); CC1 (1:1500; Millipore, Burlington, MA, USA); GFAP (1:1000, Dakopatts, Agilent, Santa Clara, CA); Olig2 (1:500, Millipore, Billerica, MA, USA); Iba1 (1:1000; Wako Chemicals, Richmond, VA); MBP (Smi-99 clone, 1:1000 Sternberger); BrdU (1:500; Abcam, Cambridge, UK); iNOS (1:100; Biorbyt, Cambridge, UK); cleaved-caspase 3 (cCASP3; 1:150, Cell Signaling Technology, Danvers, MA, USA). Incubation with primary antibodies was made overnight at 4 °C in PBS with 0.5% Triton-X 100. To allow BrdU recognition, slices were treated with 2N HCl for 20 min at 37 °C, followed by 10 min in borate buffer before adding primary antibodies. The sections were then exposed for 2 h at room temperature (RT) to secondary Cy3, Cy5 (Jackson ImmunoResearch Laboratories, West Grove, PA) or Alexa Fluor 488, Alexa Fluor 555 (Molecular Probes Inc, Eugene, Oregon) -conjugated antibodies. 4,6-diamidino-2-phenylindole (DAPI, Fluka, Saint Louis, USA) was used to counterstain cell nuclei. After processing, sections were mounted on microscope slides with Tris-glycerol supplemented with 10% Mowiol (Calbiochem, LaJolla, CA). Silver nitrate Gallyas staining was performed to detect myelin (Pistorio et al., 2006).

## 7. Image processing and data analysis

Histological specimens were examined using an E–800 Nikon microscope (Nikon, Melville, NY) connected to a color CCD Camera and a Leica TCS SP5 (Leica Microsystems, Wetzlar, Germany) confocal microscope. Adobe Photoshop 6.0 (Adobe Systems, San Jose, CA) was used to assemble the final plates. Quantitative evaluations were performed on confocal images followed by ImageJ (Research Service Branch, National Institutes of Health, Bethesda, MD; available at <http://rsb.info.nih.gov/ij/>) analyses. Density of oligodendroglial (NG2<sup>+</sup>, CC1<sup>+</sup>, GSTpi<sup>+</sup>) cells in the lesion was calculated as number of cells per mm<sup>2</sup>. To analyze the expression level of MBP and myelin (Gallyas staining) in the lesion area, the positive fractioned area (i.e. the percentage of positive pixels throughout the entire lesioned area) and mean staining intensity were quantified. To analyze GFAP and Iba1 immunostainings, where the abundance of positive cell ramifications did not allow a precise quantification of the cell numbers, we quantified the positive fractioned area.

To analyze the percentages of coexpression of CC1/BrdU, BrdU/Olig2 and cCASP3/CC1 at least 100 cells were inspected per individual.

iNOS/Iba1 colocalization index was obtained by using the ImageJ Colocalization plugin. Lesion boundaries were assessed using DAPI staining (of the same slice for immunofluorescence analyses or of the adjacent slice for the analysis of myelin Gallyas staining). To scan the entire rostro-caudal lesion extension, all sections including the lesion were considered for the quantifications. At least three animals and at least three sections per animal were analyzed for each experimental condition. Statistical analyses were carried out with GraphPad Prism 5 (GraphPad software, Inc). The Kolmogorov-Smirnov test was first applied to test for a normal distribution of the data. When normally distributed, two-tailed unpaired Student's *t*-test was used. Alternatively, when data were not normally distributed, Mann-Whitney *U* test was used. Statistics also included Chi-square test (to compare frequencies). In all instances,  $P < 0.05$  was considered as statistically significant. Histograms represent mean  $\pm$  standard error (SE). Statistical differences were indicated with \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ . The list of the applied tests and number of animals in each case are included in [Suppl. Table 2](#).

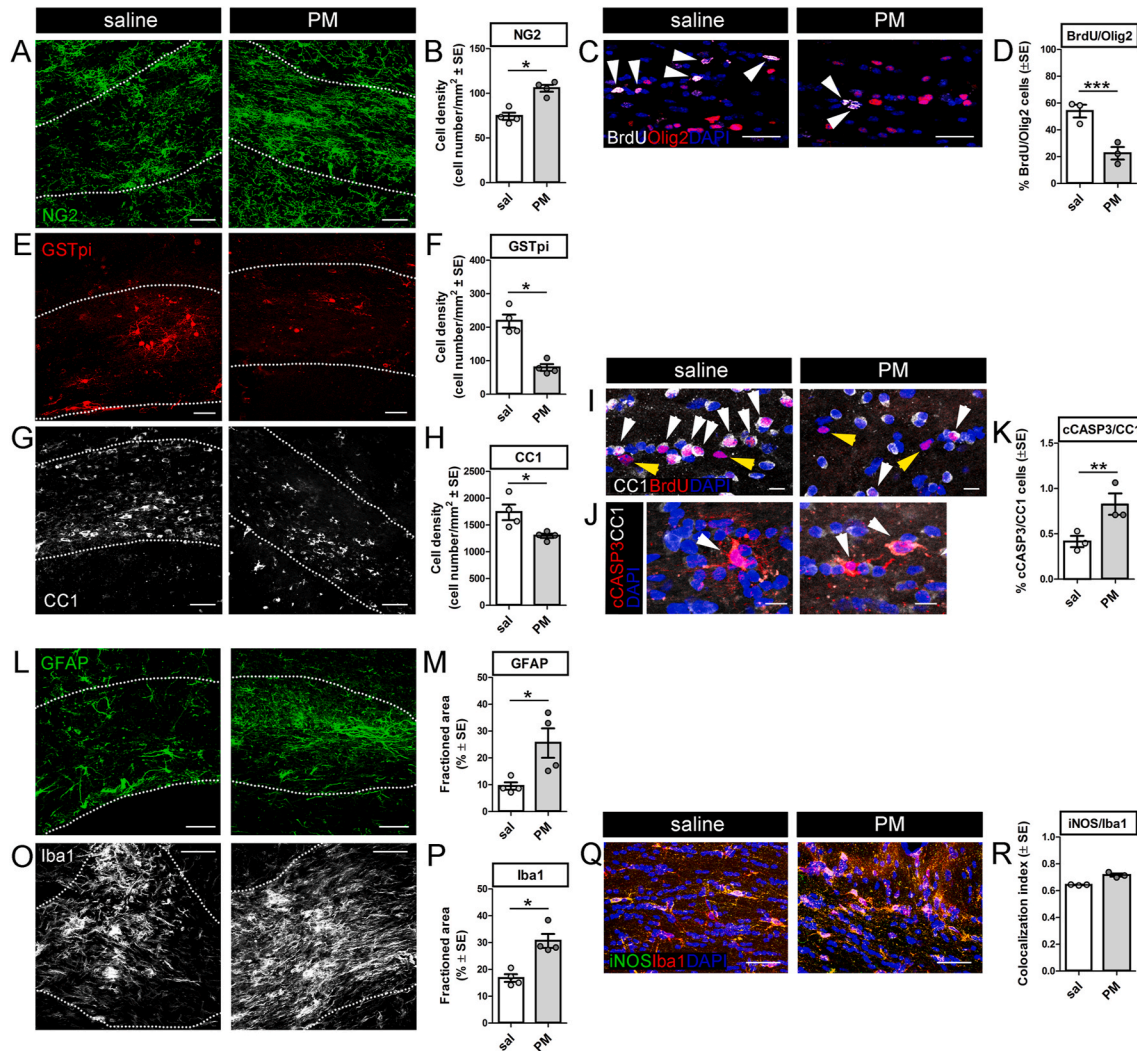


**Fig. 1.** Exposure to fine PM inhibits myelin repair in a model of LPC-induced white matter demyelination. (A) Schematic representation of the experimental plan: PM<sub>2.5</sub> or saline was administered to mice at 7dpi. (B) Absence of Gallyas myelin staining (brown) reveals the area of demyelination (indicated by arrowheads) in the subcortical white matter at 7 dpi. (C) MBP (green) protein expression in the subcortical white matter of mice exposed to saline or PM<sub>2.5</sub>. DAPI (blue) counterstains cell nuclei. (D,E) Quantification of MBP<sup>+</sup> fractioned area (D) and MBP staining intensity at lesion in ctrl (saline) vs. PM mice. (F) Gallyas myelin staining in the subcortical white matter of mice exposed to saline or PM. Dotted lines in (C,F) indicate the edges of the lesions. (G,H) Quantification of Gallyas myelin fractioned area (G) and staining intensity (H) at lesion sites. Data are mean  $\pm$  SE. Differences between groups were assessed using Unpaired *t*-test and Mann-Whitney *U* test, as detailed in [Supplementary Table 1](#). \*\*,  $P < 0.01$ , \*,  $P < 0.05$ . Scale bars: 500  $\mu$ m. Abbreviations: cc, corpus callosum; dpi, days post-injury; LPC, lysolecithin; MBP, myelin basic protein; PM, particulate matter; v, lateral ventricle; sal, saline.

## 8. Results and discussion

After assessing that the an acute exposure to PM<sub>2.5</sub> was not able to induce an overt demyelination *per se* (Suppl. Fig. 1A–D), to investigate whether PM<sub>2.5</sub> exposure can affect myelin repair in the CNS, we exploited a well-established mouse model of white matter lesion, i.e. LPC-induced injury, where acute demyelination is followed by spontaneous myelin repair sustained by resident OPCs. Mice were acutely exposed to PM<sub>2.5</sub> at 7dpi, a time window corresponding to the start of OPC differentiation. To increase the probability to detect even fine differences in lesion repair, the effects of this manipulation were studied at 14 dpi, when OPC maturation is still ongoing and remyelination has not

reached a plateau (Fig. 1A; Woodruff and Franklin, 1999). As expected, at 7dpi, Gallyas myelin staining revealed a focal area of demyelination in the white matter under the cingulate cortex in the LPC-injected hemisphere (indicated by arrowheads in Fig. 1B). At 14 dpi, a 25% decrease of MBP immunolabeling (Fig. 1C–E) and an even more pronounced reduction (about 50%) of Gallyas myelin staining (Fig. 1F–H) were observed at lesion sites in PM<sub>2.5</sub>-exposed mice, compared to controls (saline). These data indicate that PM exposure during post-injury oligodendroglia differentiation phase negatively impacts on myelin repair. Failure of a prompt remyelination leaves axons prone to damage and degeneration in MS, resulting in cognitive, sensory and motor deficits (Franklin and Ffrench-Constant, 2008). Thus, our findings suggest



**Fig. 2.** Exposure to PM<sub>2.5</sub> alters the post-injury oligodendroglia response and sustains astroglia and microglia reactivity. (A) Representative images of the abundance of NG2<sup>+</sup> (green) OPCs at lesion in the subcortical white matter of mice exposed to saline or PM<sub>2.5</sub>. (B) Quantification of NG2<sup>+</sup> OPC density at lesion in ctrl (saline) vs. PM mice. (C) Representative images of BrdU incorporation in Olig2<sup>+</sup> cells at lesion (single confocal slice). Arrowheads indicate double-positive cells. (D) Quantification of the percentage of BrdU<sup>+</sup> cells among all Olig2<sup>+</sup> oligodendroglial cells at lesion. (E) Representative images of the abundance of GSTpi<sup>+</sup> (red) mature oligodendrocytes at lesion. (F) Quantification of GSTpi<sup>+</sup> cell density at lesion. Note that GSTpi expression identifies only a subset of mature oligodendrocytes. (G) Representative images of the abundance of CC1<sup>+</sup> (white) mature oligodendrocytes at lesion. (H) Quantification of CC1<sup>+</sup> cell density at lesion. (I) Representative images of BrdU/CC1 coexpression at lesion. White arrowheads indicate double-positive cells. Yellow arrowheads point to BrdU<sup>+</sup>/CC1-negative cells (J) Representative images of cCASP3 coexpression in CC1<sup>+</sup> oligodendrocytes at lesion. Arrowheads indicate double-positive cells. (K) Quantification of the percentage of apoptotic cCASP3<sup>+</sup> cells among CC1<sup>+</sup> oligodendrocytes at lesion. (L) Representative images of the abundance and aspect of GFAP<sup>+</sup> (green) astrocytes at lesion. (M) Quantification of GFAP<sup>+</sup> fractionated area at lesion. (O) Representative images of the abundance and aspect of Iba1<sup>+</sup> (white) microglia at lesion. (P) Quantification of Iba1<sup>+</sup> fractionated area at lesion. (Q) Representative images of iNOS coexpression in Iba1<sup>+</sup> microglia at lesion (single confocal slice). (R) Quantification of the iNOS/Iba1 colocalization index at lesion. Data are mean ± SE. Differences between groups were assessed using the Mann-Whitney U test and Chi-square test (D,K), as detailed in Supplementary Table 1. \*, P < 0.05. Scale bars: 50 μm in A,C,E,G,L,O,Q; 10 μm in I,J. Abbreviations: BrdU, bromo-2-deoxyuridine; CC1, adenomatous polyposis coli (APC) clone CC1; cCASP3, cleaved caspase 3; GFAP, glial fibrillary astrocytic protein; GSTpi, Glutathione S-transferase pi; Iba1; ionized calcium-binding adapter molecule 1; NG2, neural/glial antigen 2; OPCs, oligodendrocyte progenitor cells; PM, particulate matter; sal, saline.

that, by hampering myelin reconstitution, exposure to high levels of PM<sub>2.5</sub> could contribute to MS progression and accumulation of disabilities.

To assess whether the observed myelin repair defect resulted from altered dynamics of OPC maturation or from oligodendroglia loss during the course of remyelination, we analyzed the densities of cells expressing typical markers of immature OPCs (i.e. NG2), premyelinating (i.e. GSTpi with both nuclear and cytoplasmic localization) and mature (i.e. cytoplasmic GSTpi and CC1; *Suppl. Fig. 2*) oligodendrocytes at lesion sites.

NG2-positive (+) OPC density showed a 30% increase in PM<sub>2.5</sub>-exposed mice, compared to controls (*Fig. 2A and B*), that could be the result of an increased OPC proliferation or of the accumulation of cells at immature stage at lesion. Yet, in PM<sub>2.5</sub>-exposed mice the fraction of newborn cells (i.e. that had incorporated BrdU at 8 dpi) among all oligodendroglial cells was significantly lower than in control mice (*Fig. 2C and D*), suggesting a negative effect of PM on the post-injury oligodendrocyte expansion and/or on the survival of newly-generated oligodendrocytes (see also below).

In line with the idea that exposure to PM<sub>2.5</sub> could impair/delay oligodendroglia maturation during white matter repair, in a complementary way, both GSTpi<sup>+</sup> and CC1<sup>+</sup> cells appeared significantly reduced (*Fig. 2E–H*) and the fraction of newly-generated cells expressing the mature marker CC1 was significantly decreased in PM-exposed mice compared to controls ( $37.63 \pm 5.76\%$  PM vs.  $71.06 \pm 6.24\%$  ctrl;  $P < 0.0001$  Chi-square test; *Fig. 2I*). These data clash with the accelerated maturation rate of OPCs observed in mice exposed to PM in utero (*Klocke et al., 2018*). Although remyelination is frequently interpreted as a recapitulation of the developmental myelin deposition, oligodendroglia as well as their cellular environment show important differences in the perinatal vs. juvenile/adult CNS, including a diverse responsiveness to inflammatory cues, that may subserve different outcomes of PM exposure at distinct stages (*Christensen et al., 2014*).

Nevertheless, because the decrease of GSTpi<sup>+</sup>/CC1<sup>+</sup> oligodendroglia largely outnumbered the increase in NG2<sup>+</sup> cells in PM-exposed mice, oligodendrocyte loss during the post-injury response could also participate in the reduction of mature oligodendrocytes observed in conditions of PM exposure. Consistent with this, we found a higher percentage of cCASP3<sup>+</sup>/CC1<sup>+</sup> cells at lesion site in PM-exposed mice compared to controls (*Fig. 2J and K*), indicating a higher fraction of oligodendrocytes undergoing apoptosis during the reparative phase. This finding is consistent with the known high vulnerability to insults of premyelinating cells and mature oligodendrocytes actively engaged in myelination, due to the high metabolic demand of myelin production (*Butts et al., 2008*).

Microglia and astroglia reactivity is a cardinal feature of the CNS tissue response to injury (*Traiffort et al., 2020*) and can be exacerbated by PM exposure (*Gomez-Budia et al., 2020*). Consistently, in mice exposed to PM<sub>2.5</sub>, both cells types appeared highly activated at lesion site, as assessed by cell hypertrophy and by the upregulation of the expression of GFAP (astrocytes; *Fig. 2L,M*) and Iba1 (microglia; *Fig. 2O, P*). Notably, upon demyelination, astrocytes and microglia can exert both detrimental or beneficial effects and importantly impact on oligodendroglia maturation/survival and remyelination rate (*Traiffort et al., 2020*). After being initially engaged in myelin debris phagocytosis, they control the lesion microenvironment by secreting pro- or anti-inflammatory cues, trophic factors and extracellular matrix components, depending on the modulation of their reactive state. Specifically, microglial activation is heterogeneous and spans a continuum of phenotypes ranging from the pro-inflammatory and cytotoxic M1 phenotype, prevailing in the first post-lesion phases, to the pro-regenerative and neurosupportive M2 microglia, appearing at the initiation of remyelination (*Traiffort et al., 2020*). Since chronic PM exposure was previously associated with the acquisition of a pro-inflammatory reactive phenotype in microglia (*Gomez-Budia et al., 2020*), we investigated the coexpression of the M1 marker iNOS in

Iba1+ microglia at lesion. Notably, although showing increased density and/or hypertrophy (*Fig. 2O,P*), in our experimental setting, microglia reactive phenotype did not appeared different in PM-exposed compared to control mice (*Fig. 2Q,R*). Nevertheless, it is conceivable that the sustained astroglia and microglia reactivity induced by PM<sub>2.5</sub> exposure can negatively impact on oligodendroglia survival and maturation and eventually on white matter repair (*Traiffort et al., 2020*). Further experiments exploiting cell-specific depletion approaches or the manipulation of glial cell reactivity (*Fumagalli et al., 2018*) would possibly clarify the role of microglia and astrocytes in the observed effects of PM on remyelination.

As regards the possible mediators of the inter-organ communications between the lungs and the CNS, alongside the release of pro-inflammatory cytokines, a newly-recognized mechanism is the secretion of pulmonary cell derived extracellular vesicles (EVs). Interestingly, in humans, plasma EVs released following PM exposure carry a miRNA cargo with a signature relevant for the modulation of remyelination (e.g. miR-7, miR-9, miR-17, miR-21, miR-200, miR-302c, limiting oligodendroglia differentiation or enriched in immature OPCs) and of glial cell reactivity (e.g. miR-9, involved in microglia activation; *Boda et al., 2020*). Further studies will be required to understand whether EVs and their miRNA cargo mediate the effects of fine PM exposure on white matter repair and glial cell reactivity.

## 9. Conclusions

Our findings show that an acute exposure to high levels of fine PM during post-injury oligodendroglia differentiation hampers myelin repair and sustains astroglia and microglia reactivity in a mouse model of LPC-induced demyelination. These results support the view that, besides operating as an immunomodulatory factor and altering BBB integrity, exposure to PM can contribute to demyelinating pathologies by targeting the endogenous reparative capability of the CNS tissue.

Assessing whether and how exposure to PM may operate as a risk/aetiological factor for neurological disorders such as MS is a very relevant issue. Epidemiological studies have provided strong evidence that environmental factors act at a population level to influence the temporal distribution of the hospital admissions for MS and the geographical distribution of MS patients. In presence of a predisposing genetic background, environmental factors have been proposed to “set the disease threshold” (*Ebers, 2008*). Thus, the recognition of modifiable environmental factors and of the mechanisms mediating their actions may provide important information to prevent or revert the emergence/exacerbation of the pathology, in terms of environmental policies, actions on people’s lifestyle and, possibly, design of novel preventive/therapeutic interventions.

## Author contributions

RP, FM: Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing –review & editing.

AP, SR, AC: Methodology, Writing –review & editing.

AB, AB: Conceptualization, Writing –review & editing.

VB: Conceptualization, Methodology, Writing –review & editing, Funding acquisition, Project administration, Supervision.

EB: Conceptualization, Data curation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing, Funding acquisition, Project administration, Supervision.

## Data availability

All data are available in the main text or in the supplementary materials.

## Declaration of competing interest

The authors declare no conflict of interest. The funding sponsors had no role in the interpretation of data or in the writing of the manuscript.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.neuint.2021.104991>.

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