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Neurodegenerative effects of air pollutant Particles: Biological mechanisms implicated for Early-Onset Alzheimer's disease

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ABSTRACT

Background: Sporadic Alzheimer's disease (AD) occurs in 99% of all cases and can be influenced by air pollution such as diesel emissions and more recently, an iron oxide particle, magnetite, detected in the brains of AD patients. However, a mechanistic link between air pollutants and AD development remains elusive.
Aim: To study the development of AD-relevant pathological effects induced by air pollutant particle exposures and their mechanistic links, in wild-type and AD-predisposed models.
Methods: C57BL/6 (n = 37) and APP/PS1 transgenic (n = 38) mice (age 13 weeks) were exposed to model pollutant iron-based particle (Fe^0–Fe_3O_4, d_{TEM} = 493 ± 133 nm), hydrocarbon-based diesel combustion particle (43 ± 9 nm) and magnetite (Fe_3O_4, 153 ± 43 nm) particles (66 μg/20 μL/third day) for 4 months, and were assessed for behavioural changes, neuronal cell loss, amyloid-beta (Aβ) plaque, immune response and oxidative stress-biomarkers. Neuroblastoma SHSY5Y (differentiated) cells were exposed to the particles (100 μg/ml) for 24 h, with assessments on immune response biomarkers and reactive oxygen species generation.
Results: Pollutant particle-exposure led to increased anxiety and stress levels in wild-type mice and short-term memory impairment in AD-prone mice. Neuronal cell loss was shown in the hippocampal and somatosensory cortex, with increased detection of Aβ plaque, the latter only in the AD-predisposed mice, with the wild-type not genetically disposed to form the plaque. The particle exposures however, increased AD-relevant immune system responses, including inflammation, in both strains of mice. Exposures also stimulated oxidative stress, although only observed in wild-type mice. The *in vitro* studies complemented the immune response and oxidative stress observations.
Conclusions: This study provides insights into the mechanistic links between inflammation and oxidative stress to pollutant particle-induced AD pathologies, with magnetite apparently inducing the most pathological effects. No exacerbation of the effects was observed in the AD-predisposed model when compared to the wild-type, indicating a particle-induced neurodegeneration that is independent of disease state.

1. Introduction

Alzheimer's disease (AD) is one of the most common forms of dementia affecting approximately 90 % of all dementia cases (Breijyeh and Karaman, 2020). The two characteristic pathologies of the disease are the formations of amyloid-beta (Aβ) plaques and neurofibrillary tangles (NFTs) (Tiwari et al., 2019). The plaques are aggregates of Aβ40 and Aβ42 peptides, which derive from large amyloid precursor protein (APP) *via* proteolytic cleavage, while NFTs are aggregates of hyperphosphorylated tau protein, a microtubule binding protein (DeTure and Dickson, 2019). The plaque formation accumulates around neuron cells, whereas NFTs form intracellularly. Both pathologies have been known to cause neuronal cell death, leading to brain shrinkage which is associated with AD-relevant behavioural changes including increased

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anxiety and stress, memory impairment, as well as mood changes (DeTure and Dickson, 2019). In the present study, we investigated the biological effects of air pollutant particulate matter (PM) on the early onset of AD pathologies, with a focus on sporadic AD. In contrast to familial AD, which is genetically linked, sporadic AD accounts for over 99 % of AD cases and is thought to be associated with lifestyle and environmental factors (Dorszewska et al., 2016). Among other anthropogenic factors, PM has been increasingly implicated in adverse biological activity, such as DNA damage, neuronal cell death, and has been associated with neurodegenerative diseases (Nel, 2005; Wang et al., 2021). Anthropogenic PM varies in composition and sizes, being primarily emitted into the atmosphere through combustion of fossil fuels, including coal, petroleum as well as natural gas, from power plants and vehicle emissions (Anderson et al., 2012). Pyritization (decomposition of materials at elevated temperatures) of unburnt fuel and engine wear can produce iron and iron oxide pollutant particles, including magnetite (Fe_3O_4), as well as the hydrocarbon-based diesel particulates (Resitoglu et al., 2015). Studies have shown that inhaled PM, in particular ultrafine (<100 nm) and sub-micron sized particles, can accumulate in the lower respiratory tract, being further translocated across the alveolar-capillary barrier into the systemic circulation. Inhaled PM can also enter the brain via the nasal olfactory bulb, then travel across the blood brain barrier (BBB). Alternatively, the particles can travel through the paracellular space across the nasal epithelium, then through the perineural space, into the brain (Xie et al., 2019).

Scholarly studies have increasingly shown a correlation between PM air pollution and neuro inflammation responses. In general, studies have deduced an infection-like, 'foreign body invasion' inflammatory response to pollutant exposures. Adivi et al. demonstrated exposure to mixed vehicle PM emissions (200 PM/m^3: 50 μg PM/m^3 from gasoline engine + 150 μg PM/m^3 from diesel engine, for 6 h/day, 7 days/week, for 30 days) had detrimental effects on the integrity of the blood–brain barrier (BBB) that was associated with elevated intracellular adhesion molecule (ICAM-1), TNF and IκB kinase mRNA expressions in mice cerebrum samples (Adivi et al., 2021). Liu et al. also reported upregulations of IL-6 and TNF in the hypothalamic brain region of mice, following exposures to PM (2.5 μm size, 6.3—10.7 μg/m^3 dose for ambient air, 2.3—2.7 μg/m^3 dose for filtered air and 73.6—139.5 μg/m^3 dose for concentrated PM, for 5–8 weeks) (Liu et al., 2014). Another study observed upregulations of TNF, nuclear factor kappa B (NFκB) and other inflammatory mediators following murine exposures to nano-sized (<200 nm) traffic collects (Woodward et al., 2017). Research inquiries have also reported behavioral changes as a result of exposure to particulate matter. Woodward et al., for instance, observed impaired memory, reduced appetite as well as depressive behavior in rats following exposures to traffic collects for 28 weeks (<200 nm size, 340 μg/m^3, 3 days/week) (Woodward et al., 2018). Israel et al. reported increased levels of the AD trademark cellular Aβ42 peptide and phosphorylated tau protein in the cerebral cortex of AD-predisposed mice, upon exposures to different sized PMs (ultrafine < 0.18 μm, fine ≤ 2.5 μm, coarse 2.5 – 10 μm size, 31.4 – 98.4 μg/m^3, 6 months) (Israel et al., 2023). In other study, Herr et al. observed increased levels of phosphorylated tau in the brain tissue of AD-predisposed mice, when exposed to ultrafine PM (<100 nm, 2 weeks) (Herr et al., 2021). In 2016, Maher et al. found the presence of anthropogenically formed magnetite particles in brain samples of AD patients (Maher et al., 2016). The spherical morphology of the sub-micron particles were consistent with those formed as by-products of high temperature combustion. Other studies have also detected pollutant magnetite particles in brain samples of AD patients (<65 y at death and even on younger individuals, <40 y at death), who resided in heavily polluted areas, with more than double the ambient air pollutant concentration limit (10 μg/m^3, (W.H.O. 2016)) (Calderon-Garciduenas et al., 2008; Hammond et al., 2021; Maher, 2019). Iron and iron oxides are naturally present in the brain and are essential for many metabolic processes. An excess of iron however, could be toxic (Justice, 2018). Iron (II) (as Fe^{2+} ions), for instance, can react with hydrogen peroxide (H_2O_2) in biological environments to generate the highly reactive hydroxyl ($OH^{\bullet}$) radical via the Fenton reaction (Justice, 2018), leading to oxidative damage on proteins and lipids (Carrera et al., 2013; Cui et al., 2015; O'Brien and Wong, 2011). While studies have indicated the roles of air pollutant PM on neurodegeneration, the exact pathological features and manifestation remain unclear. Understanding the biological processes that drive the pathologies will provide insights into the role of the PM pollutants in early onset of the disease.

The present work carried out *in vivo* and *in vitro* investigations to study the AD-relevant biological activity of sub-micron sized air pollutant particulate models: (a) iron (Fe^0) – iron oxide (Fe_3O_4) particles (referred herein to as 'IRON'), (b) a hydrocarbon-based diesel particulate emission ('DIESEL') and (c) magnetite ('MAG'; Fe_3O_4). The *in vivo* studies compared the biological effects of the particle exposures on wild-type and double transgenic AD-predisposed mice models over the course of four months to simulate chronic exposures. *In vitro* studies were performed using the human neuroblastoma SH-SY5Y cell line, an already established neuronal cell model in neurodegenerative studies (Agholme et al., 2010; de Medeiros et al., 2019). The findings provide evidence for the ability of the pollutant particles to induce not only behavioural changes and neuronal cell loss, but also a number of interrelated pathological features that link inflammation and oxidative stress responses to the characteristic Aβ plaque and NFT pathologies. Different trends of particle-induced AD-relevant features were observed in the wild-type when compared to those seen in the AD-predisposed mice models. Comparing the wild-type and AD cases however, no exacerbation of the particle effects was seen with the latter.

2. Experimental section

Detailed descriptions of procedures are provided in **SI Material and Methods Section**.

3. Results and discussion

3.1. Exposure to air pollutant particles caused behavioural changes and neuronal cell loss in wild-type and AD-predisposed mice models

Well-known symptoms of early onset AD include anxiety, stress and short-term memory impairment (Justice, 2018; Kelley and Petersen, 2007; Trinchese et al., 2004). To evaluate if exposures to the model air pollutant particles (Fig. 1**A–C**) induced these symptoms, we carried out the EPM (elevated plus maze) and NOR (novel object recognition) tests. The EPM test evaluated anxiety and stress levels, with the mice spending time in open and closed arms of a maze (Walf and Frye, 2007). Results show that the particle-exposed WT mice spent more time in the closed arm ($p = 0.0018$ for IRON, $p = 0.0309$ for DE, $p = 0.0140$ for MAG), relative to the saline-administered WT mice (Fig. 1**E**), which suggests that all the pollutant particles induced anxiety and stress on the WT mice. Studies have reported that excess iron was associated with anxiety-like behaviours and mood swings, seen in rats following daily iron uptake (Kim and Wessling-Resnick, 2014). Analysis between the saline-administered APP/PS1 mice to those of WT mice showed the former spending more time in the closed arm (Figure S2**A;** $p = 0.0225$), indicating anxious and stressed AD-predisposed mice, even prior to exposure to the air pollutant particles. The observations are consistent with previous studies reporting increased anxiety- and stress-like behaviours in APP/PS1 mice when compared to WT (Lok et al., 2013). Exposure of the APP/PS1 mice to the particles did not seem to exacerbate the anxiety effects, with similar time spent in the closed arm, relative to the saline APP/PS1 mice (Fig. 1**F**).

The NOR test evaluated short-term memory impairment, with a recognition index indicating time spent by the mice exploring objects (Lueptow, 2017). We observed different behaviour with the WT and APP/PS1 mice upon particle exposures to those seen with the anxiety

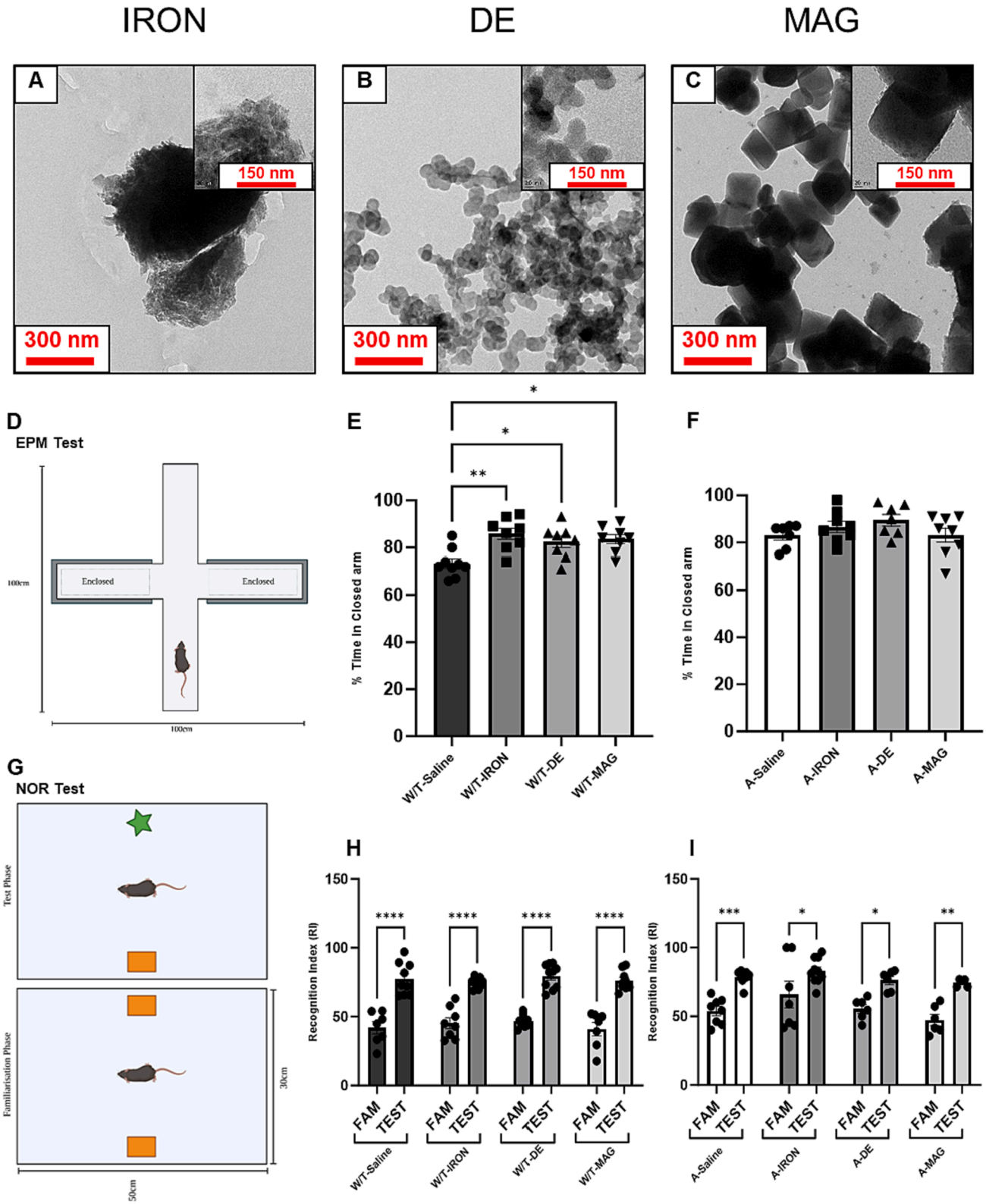

Fig. 1. *In vivo* behavioral studies of the wild-type C57Bl/6 and AD-predisposed APP/PS1 double transgenic mice, following exposures to the air pollutant particulate models 'IRON', 'DE', 'MAG'. Transmission electron microscopy (TEM) images of (**A**) IRON, (**B**) DE and (**C**) MAG. W/T and APP/PS1 mice were exposed to saline, IRON, DE or MAG particles as described in Methods. (**D**) Schematic representation of the elevated plus maze (EPM) test. (**E**) The time spent in the closed arm for the particle-exposed wild-type mice, $^{**}p = 0.0018$ (W/T-IRON), $^{*}p = 0.0309$ (W/T-DE), $^{*}p = 0.0140$ (W/T-MAG) with respect to W/T-Saline, (**F**) time spent in the closed arm for the AD-predisposed mice, note that there is no statistical significance for the particle-exposed groups relative to the A-Saline. (**G**) Schematic representation of novel object recognition (NOR) test, (**H**) showing recognition index for wild-type mice exposed to the different particles, $^{****}p < 0.0001$ FAM vs TEST, (**I**) Recognition index for familiarization and test phase in the AD-predisposed mice exposed to the different particles, $^{***}p = 0.0005$ FAM vs TEST, $^{*}p = 0.0196$ FAM vs TEST, $^{*}p = 0.0147$ FAM vs TEST, $^{**}p = 0.0015$ FAM vs TEST. All error bars indicate mean ± SEM (n = 5–8).

and stress test. The WT mice exhibited almost identical familiarisation-test recognition indexes for all pollutant particle exposures when compared to the saline-administered WT mice, with $p < 0.0001$ for all familiarisation-test recognition index pairings, indicating no short-term memory loss (Fig. 1**H).** The APP/PS1 mice, on the other hand, exhibited discrepancies in the familiarisation-test recognition indexes with the particle exposures ($p = 0.0196$ for IRON, $p = 0.0147$ for DE, $p = 0.0015$ for MAG), relative to the saline APP/PS1 mice ($p = 0.0005$), indicating short-term memory impairment. The different behaviour, at least for the AD-predisposed mice, could stem from the observation that, despite being already anxious and stressed, the APP/PS1 mice did not seem to associate with an 'in-built' memory impairment, showing no difference in their familiarisation-test recognition indexes, relative to the saline WT mice. Earlier studies have observed memory impairment in AD-predisposed mice (AppNL-G-F/+-KI) upon exposures to concentrated ultrafine PM (8-fold ambient concentration, 12 weeks) (Kilian et al., 2023).

Given the known association of anxiety and stress as well as memory impairment to neuronal cell loss in the brain hippocampus region, we next studied the effects of the pollutant particles on cells in this region (Beauquis et al., 2014; Chi et al., 2018). The hippocampus is important for memory storage and retrieval. Memory impairment can lead to increased anxiety and stress and *vice versa*, anxiety and stress have also been shown to disrupt working memory (Lok et al., 2013). The memory storage and retrieval process involves a chain of neuronal signalling, the so-called perforant pathway (Fig. 2**A**), that projects signalling from the parietal (including the somatosensory cortex (SSC), temporal and pre-frontal lobes in the cerebral cortex, via the entorhinal cortex (the primary input structure in hippocampus), which then extends to the dentate gyrus (DG), cornu ammonis (Ca) 3 and Ca1 regions in the hippocampus (Anand and Dhikav, 2012; Setti et al., 2017; Stephen et al., 2010). Results showed less cell count in the hippocampal DG region for the particle-exposed WT mice, particularly for MAG ($p = 0.0102$), compared to the saline-administered group **(**Fig. 2**B–C).** Similar observations were also seen with the Ca3 ($p = 0.0010$ for MAG, Fig. 2**D**) and Ca1 ($p = 0.0005$ for MAG, Fig. 2**E**) regions, as well as with the SSC cells ($p = 0.0394$ for DE, $p = 0.0008$ for MAG, Fig. 2**F**). The neuronal cell loss data are in line with the increased anxiety and stress levels observed with the particle-exposed WT mice (the EPM test), which could also associate with a reduced synaptic density (transmission of electrical impulses between neuron cells) in DG and Ca3, that are known to correlate with increased stress in animal studies (Anand and Dhikav, 2012; Chi et al., 2018).

For the AD-predisposed mice, corresponding to the more anxious and stressed APP/PS1 mice even prior to the particle exposures (the EPM test, Figure S2A), the neuronal cell counts also showed less cells in the DG and Ca3 regions, relative to the WT mice (both saline-administered, Figure S2**B–C**). The (saline) APP/PS1 mice also had less cells in their Ca1 region (relative to the saline WT) (Figure S2D). A decrease in Ca3 and Ca1 neuronal cell density have been reported in APP/PS1, being associated with the known presence of hippocampal lesions in the transgenic mice as a result of the PS1 mutation (Lazarov et al., 2006). The particle exposures cause further cell loss in APP/PS1 mice, in the DG ($p = 0.0369$ for DE, Fig. 2**B and G)**, Ca3 ($p = 0.0428$ for MAG, Fig. 2**H)**, and Ca1 ($p = 0.0048$ for MAG, Fig. 2**I)** regions, as well as the SSC region ($p = 0.0115$ for DE, $p = 0.0057$ for MAG, Fig. 2**J)**, which may correlate with the

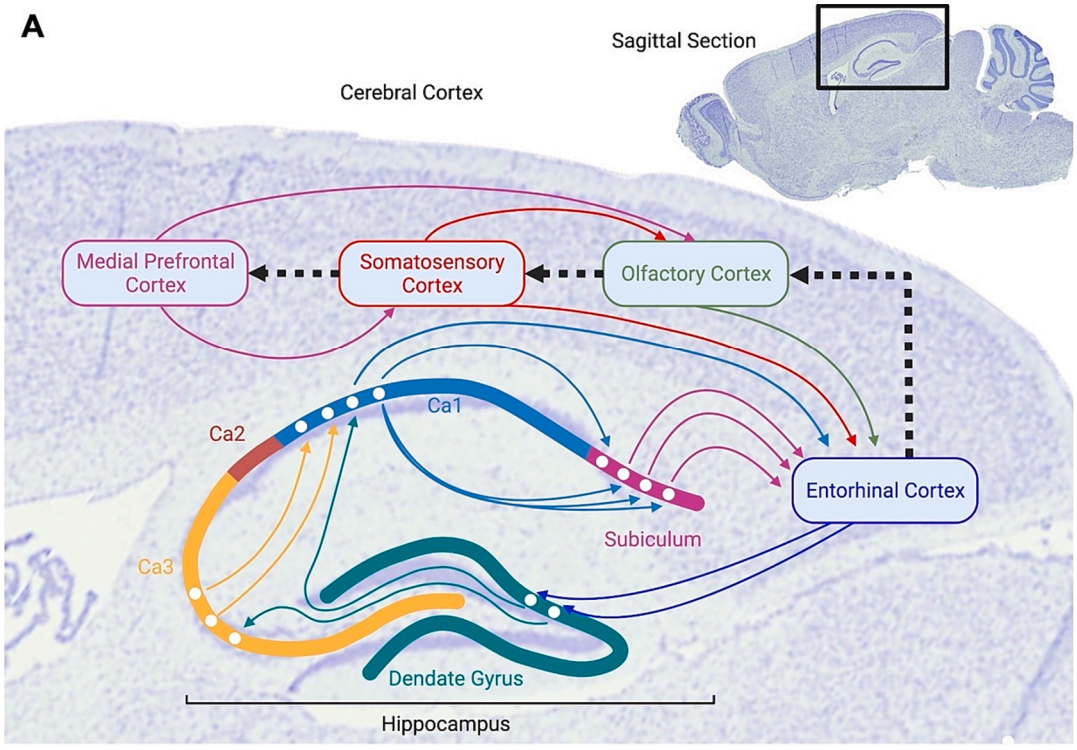

Fig. 2. *In vivo* analysis of neuronal cell loss following exposures to the air pollutant particles in the hippocampus DG, Ca3 and Ca1 regions as well as the somatosensory region (SSC 2, 3, 4, 5, 6a in the parietal lobe in cerebral cortex). Brain samples were collected from W/T and APP/PS1 mice exposed to saline, iron oxide (IRON), diesel (DE) or magnetite particles (MAG) and stained with cresyl violet as described in Methods. (**A**) A schematic showing the hippocampus and SSC regions as well as the perforant pathway. (**B**) Brightfield microscopy images of the hippocampus – first column shows the overall hippocampus regions, with a zoomed in DG, Ca3, Ca1 regions in subsequent columns, and the SSC regions of the W/T-Saline, W/T-MAG, A-Saline and A-MAG mice. (**C, D, E, F**) Cell counting for particle-exposed W/T mice; in the DG region *p = 0.0102 (W/T-MAG) with respect to W/T-Saline; in the Ca3 region **p = 0.0010 (W/T-MAG) vs. W/T-Saline; in the Ca1 region ***p = 0.0005 (W/T-MAG) vs. W/T-Saline; in the SSC region *p = 0.0394 (W/T-DE), ***p = 0.0008 (W/T-MAG) vs. W/T-Saline. (**G, H, I, J**) Cell counting for the particle-exposed AD-predisposed mice; in the DG region, *p = 0.0369 (A-DE) vs. A-Saline; in the Ca3 region *p = 0.0428 (A-MAG) vs. A-Saline; in the Ca1 region **p = 0.0048 (A-MAG) vs. A-Saline; in the SSC region *p = 0.0115 (A-DE), **p = 0.0057 (A-MAG) vs. A-Saline. All error bars indicate mean ± SEM (n = 5–8). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

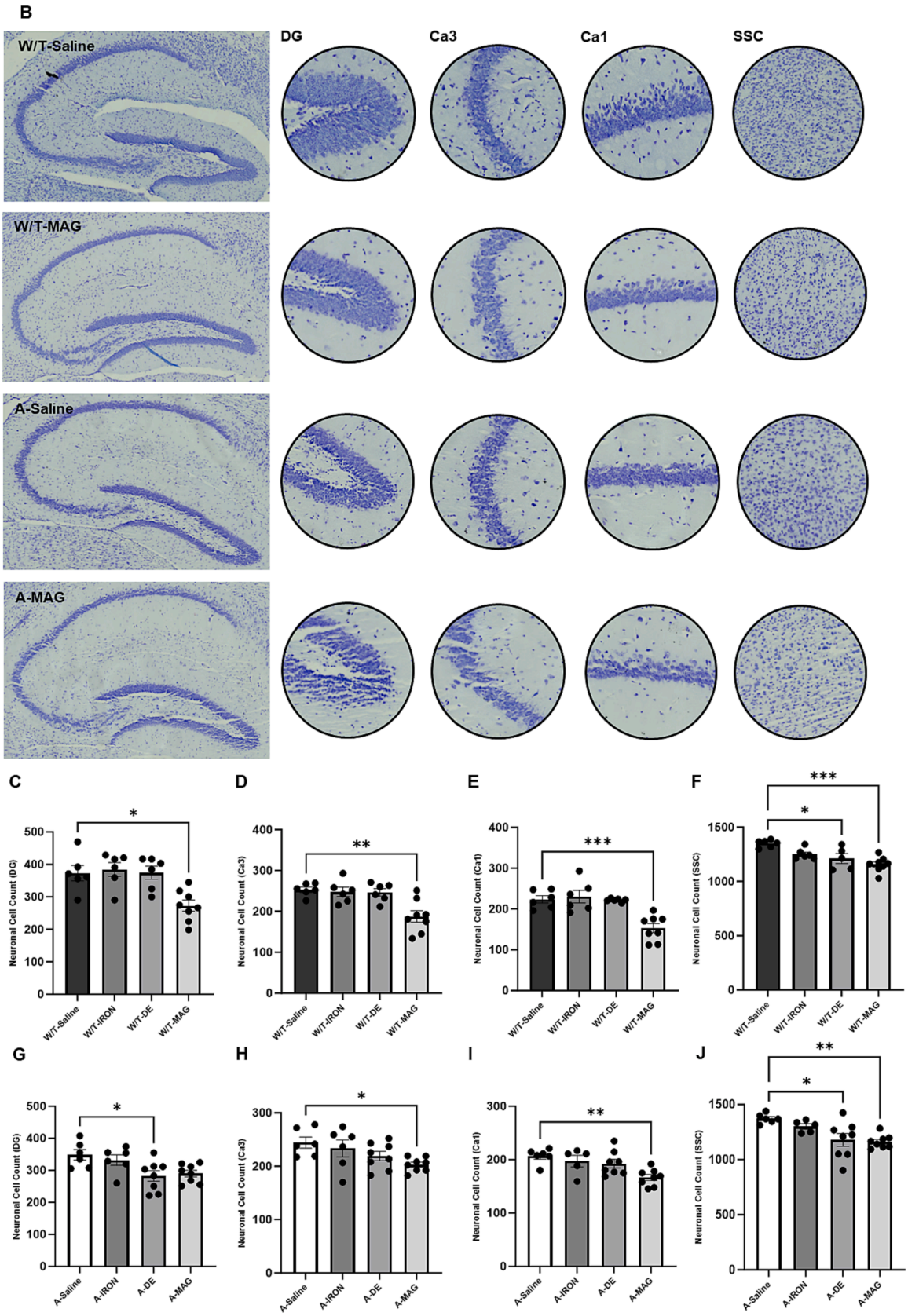

Fig. 2. (*continued*).

observed short-term memory impairment with the mice (the NOR test).

In summary, both WT and AD-predisposed mice showed adverse behavioural changes and neuronal cell loss when exposed to the pollutant particles. All the tested particles increased anxiety and stress levels in WT mice, with no further particle-induced exacerbation in the already anxious and stress AD-predisposed mice. The particles were

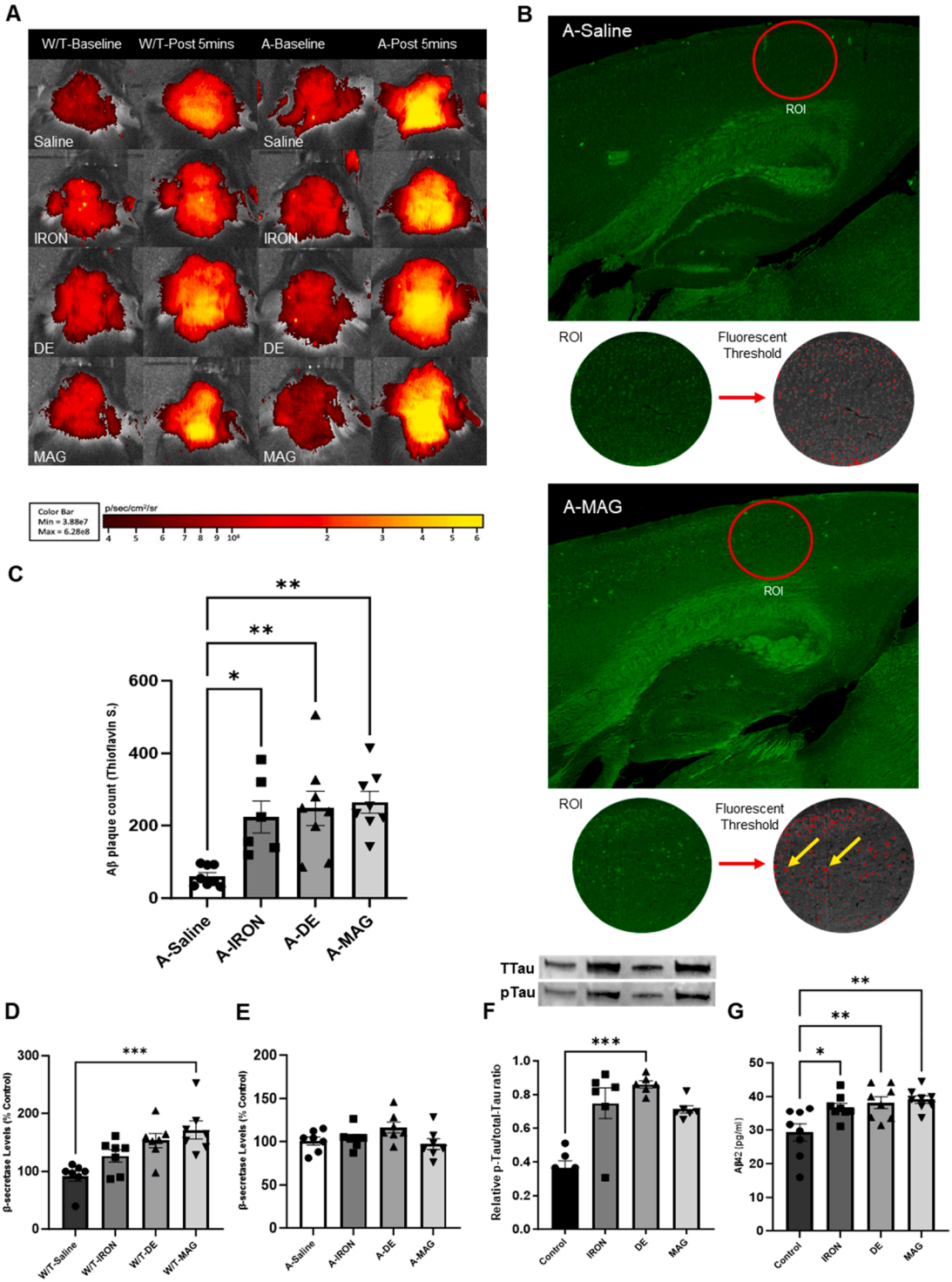

Fig. 3. *In vivo*, *ex vivo* and *in vitro* studies of hallmark AD characteristics. (**A**) Representative images of Aβ42 aggregates detection in the brain of the particle-exposed wild-type and AD-predisposed mice, showing fluorescent radiance before (baseline) and after (post 5 mins) intravenous injection of the NIRF CRANAD probe. The average radiance scale bar provides an indication of the presence of Aβ aggregates/fibrils formation. Note that the W/T mice are not genetically disposed to form the amyloid plaque (as also indicated by the CRANAD probe), therefore further plaque quantification were only performed in the AD mice. (**B**) Thioflavin S stained fluorescence imaging of amyloid β plaque in the whole brain of the particle-exposed AD-predisposed mice, shown are the plaque detection for the A-Saline and A-MAG (with zoomed-in hippocampus images). (**C**) Aβ plaque counting (based on thioflavin S staining) for AD-predisposed mice *p = 0.0242 (A-IRON), **p = 0.0036 (A-DE), **p = 0.0014 (A-MAG) vs. A-Saline, (n = 8). Percentage increase of plasma β-secretase (BACE-1) levels, for (**D**) wild-type mice (n = 7) ***p = 0.0006 (W/T-MAG) vs. W/T-Saline, and (**E**) AD-predisposed mice (n = 7), note that there is no statistical significance for the particle-exposed groups relative to the A-Saline. (**F**) *In vitro* detection of phosphorylated Tau/total Tau protein in SH-SY5Y cells (n = 6) ***p = 0.0003 (DE) vs. Control. (**G**) Aβ42 peptide levels in SH-SY5Y cells (n = 8) *p = 0.0244 (IRON), **p = 0.0022 (DE), **p = 0.0017 (MAG) vs. Control. (**H**) Schematic showing Aβ plaque formation mediated by β-secretase. All error bars indicate mean ± SEM. For the *in vitro* SHSY5Y studies, the experiments were carried out with *n* biological replicates (cells from different passage rounds).

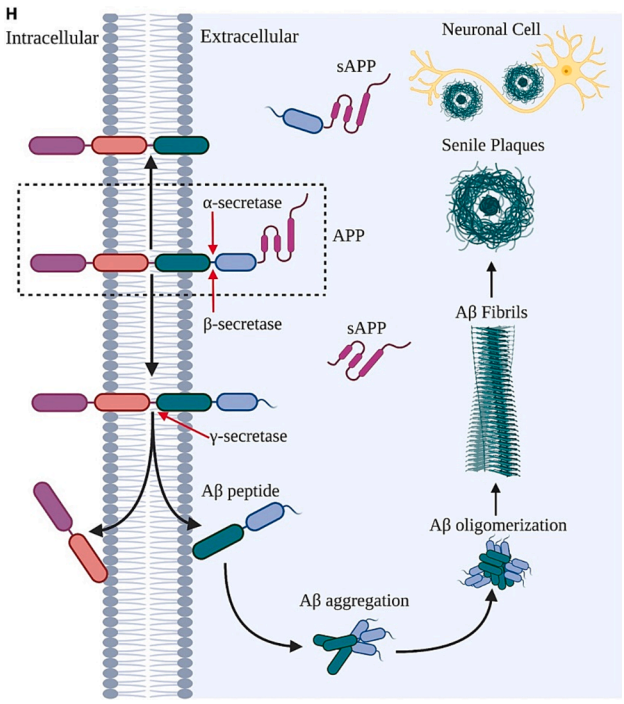

Fig. 3. (*continued*).

found to cause short-term memory impairment in the AD-predisposed mice. However, it remains unclear as with the non-observable memory impairment in the WT mice. Nonetheless, the observed cell loss in the hippocampal and SSC regions suggests particle-induced disruption of the stress- and anxiety-related as well as memory-related perforant pathway. Previous studies have reported disruptions of the perforant pathway in early onset AD patients, increasing anxiety and stress levels, affecting memory, including their spatial recognition (Benice et al., 2006; Padurariu et al., 2012; Trinchese et al., 2004). Recalling the earlier mentioned link between behavioural changes and neuronal cell loss to amyloid β formation, we next looked for the development of the characteristic AD pathologies in the brain of the mice.

3.2. *Exposure to air pollutant particles increased Aβ plaque formation and tau phosphorylation*

We assessed the potential formation of amyloid β (Aβ) plaques using *in vivo* and *ex vivo* whole brain imaging on the WT C57BL/6 and the AD-predisposed APP/PS1 mice. *In vivo* fluorescence NIRF CRANAD staining detects the presence of the insoluble Aβ42 peptide aggregates (Ran et al., 2020), while the *ex vivo* fluorescence thioflavin S staining probes for the characteristic β-pleated sheets of amyloid plaque (Picken and Herrera, 2015). A generally more intense CRANAD fluorescence radiance was observed with the particle-exposed APP/PS1 mice brains, relative to the corresponding saline-administered mice (Fig. 3**A**). The indicated increase in plaque formation was verified by the thioflavin S staining (Fig. 3**B–C**), with IRON ($p = 0.0242$), DE ($p = 0.0036$) and MAG ($p = 0.0014$), showing higher fluorescence counts in the AD-predisposed mice, relative to the saline mice. WT mice, which are not genetically disposed to form the plaque, showed elevated β-secretase 1 (BACE1) enzyme levels in the plasma, with MAG ($p = 0.0006$, Fig. 3**D**). The AD-predisposed mice already showed higher plasma BACE1 levels in the saline group (relative to WT, $p = 0.0028$, Figure S2E), and the particle exposure did not further increase the enzyme levels in these mice compared to the saline group (Fig. 3**E**). BACE1 catalyses the cleavage of APP to form Aβ peptides (including Aβ42), which further aggregate to form plaques (Fig. 3**H**) (Vassar, 2004).

The findings support the hypothesis that the characteristic plaque formation correlates with neuronal cell loss and behavioural changes. The latter were also observed herein, in both the WT and AD-predisposed mice, indicative of early onset AD. Our *in vitro* studies with the pollutant particles also detected increased presence of phosphorylated tau protein, another hallmark of AD pathology.

Aβ plaque formation has been known to associate with the so-called neurofibrillary tangles (NFTs), whereby tau protein abnormally accumulates inside neuron cells (Medeiros et al., 2011). Toxic to neuron cells, these cellular NFTs can be released into extracellular environments, which then also degenerate surrounding neurons (He et al., 2018). Research has indicated that Aβ plaque destabilises tau protein (a microtubule-associated protein found predominantly in axons), rendering it prone to extensive phosphorylation, and in turn, aggregation into bundles of filaments, forming the fibrillary tangles inside neuron cells (Medeiros et al., 2011). More specifically, hyperphosphorylated tau contains a unique double-site phosphor-epitope AT100, which consists of both anti-phospho Thr (Thr212) and anti-phospho Ser (Ser214) (Schneider et al., 1999; Wu et al., 2020). We detected an increase in phosphorylation of Ser214-to-total tau protein ratios in SH-SY5Y cells upon exposure to the particles ($p = 0.0003$ for DE vs control, Fig. 3**F**). While the presence of phosphorylated Ser214 tau does not necessarily mean that NFTs are present or that they will form, phosphorylated Ser214 tau is important as it alone can potentially disrupt the binding of tau to microtubules, which can destabilize the neuron cells cytoskeleton, leading to cell death (Liu et al., 2011). Indeed, a decrease in cell viability was observed in the particle-exposed SH-SY5Y ($p < 0.0001$ for IRON, DE, MAG vs control, **Fig S7**). We also detected elevated presence of Aβ42 peptide in the particle-exposed cell samples ($p = 0.0244$ for IRON, $p = 0.0022$ for DE, $p = 0.0017$ for MAG, Fig. 3**G**), relative to the control.

3.3. *Air pollutant particles increased levels of immune system biomarkers and inflammatory molecules*

To understand the impact of air pollutant particles on AD pathologies, we studied how they affect the expression of relevant biomarkers and inflammatory molecules produced by microglia and astrocyte cells, which are major immune system cells of the central nervous system

(CNS). Microglia and astrocytes work together when the brain is exposed to foreign entities, whereby microglia enhances their phagocytic capacity to remove debris, while astrocytes increase their size to physically guard the blood brain barrier and protect the brain (Vainchtein and Molofsky, 2020). In the case of AD progression, microglia and astrocytes have been known to upregulate specific biomarkers and inflammatory cytokines (Hensley, 2010; Wang et al., 2015), herein assessed in brain samples of the WT C57Bl/6 and AD-predisposed APP/PS1 mice. We also evaluated biomarkers associated with the MAPKs (mitogen-activated protein kinases) pathway, a major signalling network that prompts physiological responses including immune system inflammation in the CNS upon exposure to external stimuli (Iroegbu et al., 2021).

We first tested the effects of pollutant particles on relevant biomarkers in the cerebral cortex of both WT and APP/PS1 mice. In particular, we assessed brain-derived neurotrophic factor (BDNF), a growth factor released by microglia to repair neuron cells (Miranda et al., 2019) and intercellular adhesion molecule-1 (ICAM-1), a transmembrane glycoprotein presented on the surface of both microglia and astrocytes to facilitate interactions of immune system cells (Marlin and Rothlein, 1998). Particle exposures increased levels of BDNF ($p = 0.0001$ for IRON, DE and MAG vs saline, Fig. 4**A**) and ICAM-1 ($p = 0.010$ for MAG vs saline, Fig. 4**C**) in the cerebral cortex of WT. For the APP/PS1 mice, the particle exposures also increased the cerebral cortex BDNF ($p = 0.021$ for IRON, Fig. 4**B**) and ICAM-1 levels ($p = 0.010$ for IRON, Fig. 4**D**), relative to the saline-administered mice. Studies on AD cases have reported increased BDNF levels in damaged brain regions, as well as increased ICAM-1 levels (in serum), when compared to healthy individuals (Angelucci et al., 2009; Faria et al., 2014; Laske et al., 2006; Wennström and Nielsen, 2012). Further, increased presence of the characteristic Aβ plaque, as herein also observed with the particle-exposed mice (the APP/PS1 mice, whole brain imaging), has been linked to upregulations of BDNF (with presence of the growth factor close to the plaque deposits) (Miranda et al., 2019), as well as to increasing surface presentation of ICAM-1 in mice models (Frohman et al., 1991). The particle exposures further detected higher levels of cerebral cortex Iba-1 (ionised calcium-binding adaptor protein, $p = 0.0385$ for IRON, Fig. 4**F**) in the AD-predisposed mice, relative to the saline mice. Iba-1 is expressed in microglia, with studies indicating the role of the protein in the actin (cytoskeletal protein) cross-linking, involved in membrane ruffling of microglia (Ito et al., 2001). The latter is a process essential for microglia activation and in turn, its phagocytic activity (Ito et al., 2001). Increased cerebral cortex Iba-1 levels have been detected in AD cases, which are linked to activation of microglia (Waller et al., 2019). Previous studies have also indicated increased Iba-1 levels in the brain of APP/PS1 mice upon exposure to $PM_{2.5}$ (25.8 μg/m^3, 3 months) (Sahu et al., 2021). Note that herein, there was no significant difference in the BDNF, ICAM-1 and Iba-1 cerebral cortex levels observed between the AD-predisposed and WT saline mice (Figure S2F–H, respectively).

The results so far indicate that particle exposures increased the activity of the CNS immune system, evident from the detected higher levels of microglia and astrocyte biomarkers in the mice cerebral cortex, for both the WT and AD-predisposed mice. Next, we looked for the potential upregulation of inflammatory molecules. During the early stages of the CNS immune response, both microglia and astrocytes have been shown to upregulate cytokine synthesis (Wang et al., 2015). Herein, we detected higher TNF (tumour necrosis factor) and IL-6 mRNA expressions in the particle-exposed WT mice, with DE ($p = 0.035$ for TNF) and MAG ($p = 0.0111$ for TNF and $p = 0.0048$ for IL-6, Fig. 4**G and I**), in the mice hippocampal regions, relative to the saline-administered group. The observations agree with earlier studies reporting increased expressions of TNF and IL-6 in AD patients, more specifically in the hippocampal regions (Wang et al., 2015). Previous studies have also observed increased levels of inflammatory cytokine interferon-gamma in the brain and periphery of wild-type mice (JAXC57BL/6J) when exposed to ultrafine PM (89.5 μg/m^3, 2 weeks) (Saveleva et al., 2022). Inflammation is a biological response of the immune system, and these inflammatory cytokines have been known to induce a number of physiological responses in AD pathologies, including oxidative stress, as later described (Agostinho et al., 2010). No statistically significant upregulation of TNF and IL-6 was observed with the particle-exposed APP/PS1 mice, relative to the saline group (Fig. 4**H and J**).

Ultimately, we tested the biomarkers associated with the major physiological MAPK signalling pathway involved in the immune system response. Activation of MAPK pathway, as has been seen in AD cases, involves activation of the NFκB, JNK and p38 MAPK modules *via* phosphorylation (Huang et al., 2009). We found that exposure to the pollutant particles increased the levels of biomarkers for these modules. Our *in vitro* studies with the SH-SY5Y cell line detected higher levels of phosphorylated nuclear-factor κB (p-NFκB subunit 65) ($p = 0.0035$ for DE and $p = 0.0159$ for MAG vs cell-only control, Fig. 4**K**), phosphorylated c-Jun N-terminal Kinase (p-JNK) ($p = 0.030$ for MAG, Fig. 4**L**) and phosphorylated (p38) MAPK ($p = 0.007$ for IRON, $p = 0.030$ for DE and MAG, Fig. 4**M**) in the particle-exposed samples, relative to the control. The protein complex NFκB are a family of transcription factors that control the expression of genes that are involved in immune responses, including inflammatory cytokine production (Liu et al., 2017). JNK and p38 are protein kinases that are also involved in cytokine production (Hepp Rehfeldt et al., 2020; Yarza et al., 2016). The levels of these biomarkers, more specifically, the p65 NFκB, p-JNK and p38 MAPK, have been found to increase in AD cases (Du et al., 2019; Snow and Albensi, 2016). NFκB has been indicated to upregulate the synthesis of the plaque formation BACE1, herein also seen with the particle-exposed mice (the WT mice) (Jones and Kounatidis, 2017; Snow and Albensi, 2016). Further, recalling the observed short-term memory impairment in the AD-predisposed mice (the NOR behavioural test), research inquiries have linked cognitive decline in mice to activation of the JNK pathway (Du et al., 2019). Studies have also linked the activation of the JNK and p38 MAPK pathway to tau phosphorylation, the latter also seen herein (the particle-exposed SH-SY5Y samples), with p-JNK for instance, often found to localise within the NFT (Ploia et al., 2011; Solas et al., 2023).

In conclusion, it is clear that the pollutant particle exposures increased a number of AD-relevant brain microglia and astrocyte immune responses, upregulating the synthesis of neuro-protective growth factor (BDNF) and surface glycoprotein (ICAM-1), the latter for immune cell interactions, as well as protein that involves in immune cell activation (Iba-1). These responses were seen in both the WT and AD-predisposed mice. The studies further observed upregulation of inflammatory cytokines (increased expressions of TNF and IL-6). The immune responses are likely to associate with the activation of the central MAPK pathway, the latter with detection of a number of biomarkers (p65 NFκB, p-JNK and p38 MAPK from the *in vitro* studies). Next, in our final studies, we looked into the oxidative stress phenomena, with studies been linking immune responses to oxidative stress in AD pathologies. Immune system stimuli, including cytokines, and the quite recently suggested NFκB, have been indicated to mediate generation of reactive oxygen species (ROS) (Kaur et al., 2015; Kinney et al., 2018; Selva et al., 2016).

3.4. Air pollutant particles induce oxidative stress

We studied the levels of the oxidative stress-related microglia and astrocyte enzymes, iNOS and Cox2, in brain samples of the WT and APP/PS1 mice. Exposure of WT mice to the pollutant particles saw an increased cerebral cortex iNOS levels, in particular with DE ($p = 0.0406$) and MAG ($p = 0.0014$), relative to the saline-administered group (Fig. 5**A**). No statistically significant cerebral cortex iNOS increase was observed in the particle-exposed APP/PS1 mice, relative to the corresponding saline mice (Fig. 5**B**). Note that the AD-predisposed mice were associated with higher (cerebral cortex) iNOS levels even prior to the

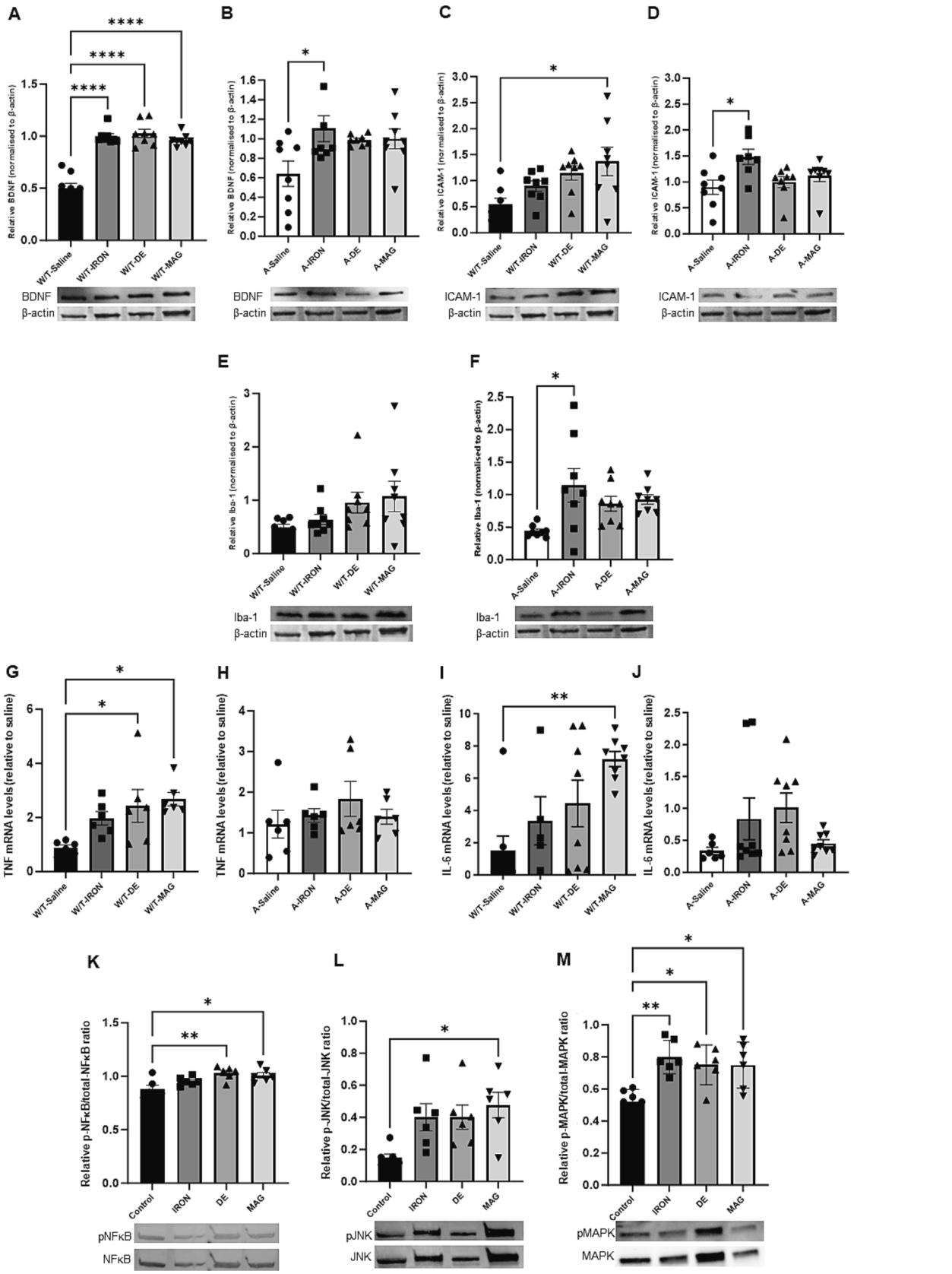

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Fig. 4. *In vivo* and *in vitro* studies of immune response biomarkers and inflammatory molecules. (**A, B**) Relative BDNF protein levels in the cerebral cortex of the particle-exposed wild-type mice (n = 8) ****p = 0.0001 (for all particle groups) vs. W/T-Saline; for the particle-exposed AD-predisposed mice (n = 8) *p = 0.021 (A-IRON) vs. A-Saline. (**C, D**) Relative ICAM-1 protein levels in the cerebral cortex, wild-type mice (n = 8) *p = 0.01 (W/T-MAG) vs. W/T-Saline; for the AD-predisposed mice (n = 8) *p = 0.010 (A-IRON) vs. A-Saline. (**E, F**) Relative Iba-1 protein levels in the cerebral cortex, wild-type mice (n = 8), note that there is no statistical significance for the particle-exposed groups relative to the W/T-Saline; for the AD-predisposed mice (n = 8) *p = 0.0385 (A-IRON) vs. A-Saline. (**G, H**) TNF mRNA expression in the hippocampus, wild-type mice (n = 6) *p = 0.035 (W/T-DE), *p = 0.0111 (W/T-MAG) vs. W/T-Saline; for the AD-predisposed mice (n = 6), no statistical significance for the particle-exposed groups relative to the A-Saline. (**I, J**) IL-6 mRNA expression in the hippocampus, wild-type mice (n = 6–8) **p = 0.0048 (W/T-MAG) vs. W/T-Saline; for the AD-predisposed mice (n = 6–8), no statistical significance for the particle-exposed groups relative to the A-Saline. (**K**) Relative pNFκB/NFκB in SH-SY5Y cells (n = 6) **p = 0.0035 (DE), *p = 0.0159 (MAG) vs. Control. (**L**) Relative pJNK/JNK in SH-SY5Y (n = 6) *p = 0.030 (MAG) vs. Control. (**M**) Relative pMAPK/MAPK in SH-SY5Y (n = 6) **p = 0.007 (IRON), *p = 0.030 (DE and MAG) vs. Control. All error bars indicate mean ± SEM. mRNA levels were normalized to GAPDH. For the *in vitro* SHSY5Y studies, the experiments were carried out with *n* biological replicates (cells from different passage rounds).

particle exposures (p = 0.0451, relative to saline WT, Figure S2I). An inducible nitric oxide synthase, iNOS catalyses the synthesis of nitric oxide (NO) from the amino acid *L*-arginine. Increased presence of iNOS and consequently NO levels, have been documented in neurodegenerative cases, including AD (Mander and Brown, 2004). While NO is an important cellular signalling molecule for neural development, its excess presence (with upregulation of iNOS) have been observed in neuron cells during inflammation (Rajapakse and Mattson, 2009; Togo et al., 2004). Herein, higher levels of NO were detected in particle-exposed SH-SY5Y cells (p = 0.018 for IRON, p = 0.0001 for DE, p = 0.0217 for MAG vs cell-only control, the assay measures secreted NO from cells, Fig. 5**E**), as well as higher levels of ROS (p = 0.0003 for IRON, p = 0.0001 for DE, p = 0.0147 for MAG, general oxygen species, cellular presence, Fig. 5**F**). For the latter, a number of AD studies have also reported increased cellular ROS detection, being linked, at least in part, to Cox2 upregulations (Guan et al., 2019; Nogawa et al., 1998; Wang et al., 2014). Cox2 is a cyclooxygenase that mediates ROS generation, with studies indicating upregulation of the enzyme in response to excess presence of cellular NO (Guan et al., 2019; Nogawa et al., 1998; Wang et al., 2014). Indeed, Cox2 levels were elevated in the cerebral cortex of the particle-exposed WT mice (p = 0.040 for IRON, p = 0.0236 for DE, p = 0.0155 for MAG vs saline group, Fig. 5**C**). As with iNOS, no statistically significant increase in the Cox2 cerebral cortex levels were detected in the APP/PS1 mice upon particle exposures when compared to the corresponding saline mice, which, implies no particle exacerbation effects on the AD-predisposed mice (Fig. 5**D**). Neurons are sensitive to ROS and oxidative stress has been linked to cell loss, as also seen in this study (in the hippocampal and SSC regions) with the particle exposures (Chen et al., 2012). ROS can also react with NO, forming peroxynitrite, which has been implicated in neuronal damage (Ramdial et al., 2017). Ultimately, to better understand the ROS generation mechanisms and to verify its link with immune responses, we studied the potential effect of an NFκB inhibitor on the ROS generation. As shown in Fig. 5**G and H**, less ROS (general oxygen species, cellular presence, p = 0.0001 for IRON, p = 0.0021 for DE, p = 0.0076 for MAG vs particle-treated cells without the inhibitor), and less NO (secreted presence, p = 0.0481 for IRON, p = 0.0013 for DE, p = 0.0167 for MAG), were detected when in the presence of the inhibitor for all particle-treated SH-SY5Y samples. The observations are thought to result from impeded transcription of iNOS and Cox2 in the presence of the NFκB inhibitor (Kaltschmidt et al., 2002), indicating roles of the transcription factor in ROS generation.

3.5. *A working model on the biological effects of air pollutant particles on AD pathologies*

The present *in vivo* and *in vitro* studies seek to find evidence on the potential roles of air pollutant particle exposure on the early onset pathologies of AD. The *in vivo* studies were carried out on the wild-type (C57Bl/6) and AD-predisposed (APP/PS1 transgenic) mice models, being subjected to intranasal administration of the model pollutant particles. We found that the tested pollutant particles, in particular DE and MAG, increased the formation of the characteristic amyloid β plaque in the brain of the AD-predisposed mice (note that the WT mice are not genetically disposed to form the plaque). The observations are in line with the elevated levels of the 'plaque formation' BACE-1 enzyme herein detected in the mice plasma (Cole and Vassar, 2008; Peters et al., 2019), as well as with our *in vitro* studies (SH-SY5Y cells) that saw increased phosphorylation of tau protein in the pollutant particle-exposed samples, with the phosphorylation (which ultimately lead to NFT) often associated with plaque formation in AD cases (Hampel et al., 2010; Medeiros et al., 2011; Schneider et al., 1999). Earlier studies have shown that these hallmark AD pathologies are likely to cause neuronal cell loss (Liu et al., 2011), which were also seen in the present work, more specifically in the hippocampus (DG, Ca3, Ca1 regions) and somatosensory cortex (SSC) regions of the particle-exposed WT and AD-predisposed mice. The cell loss was further correlated to the observed AD-associated behavioural changes, with evidence of increased anxiety and stress within the WT mice, while the particle exposures seemed to prompt short-term memory impairment in the AD-predisposed mice. The particle exposures are thought to disturb the outer brain lobes (including the SSC)-to-hippocampus neuron signalling perforant pathway with the detected cell loss, which supports working memory (Anand and Dhikav, 2012; Setti et al., 2017). Impairment of the pathway has also been linked to increased anxiety and stress (Justice, 2018).

Next, in the present work, we delved into the molecular basis of the amyloid plaque accumulation, to gain insights into how inflammation and oxidative stress responses contribute to the plaque build-up (Fig. 6). For this, we first have a look on how inflammation links to oxidative stress in the current context of the air pollutant exposures. It was deduced from the study that the pollutant particles triggered AD-relevant inflammation and oxidative stress responses, particularly evident with the WT mice. The particle exposures were associated with upregulations of the TNF and IL-6 inflammatory cytokines (sampled from the hippocampus). Our *in vitro* data (with SH-SY5Y cells) linked the inflammation responses, at least in part, to the MAPK signalling pathway, that is, to the indicated activation of the NFκB, JNK and p38 MAPK signaling modules, seen with the particle exposures. Earlier studies have indeed observed upregulations of TNF and IL-6 with activation of the modules (*via* phosphorylation) in neurodegenerative cases (human AD brain samples) (Grammas and Ovase, 2001; Wang et al., 2015). Studies have also indicated that inflammatory cytokines, including TNF and IL-6, can in turn facilitate the activation (*via* phosphorylation) of the NFκB, JNK and p38 MAPK modules (Du et al., 2019; Snow and Albensi, 2016; Wang et al., 2015), denoting a 'cyclic' cause and consequence immune system mechanisms. The transcription factor NFκB has also been found to upregulate the synthesis of the immune cell biomarkers BDNF, ICAM-1 and Iba-1, herein detected in cerebral cortex samples, already seen in neurodegenerative cases (Angelucci et al., 2009; Faria et al., 2014; Laske et al., 2006; Takeda et al., 2016; Waller et al., 2019; Wennström and Nielsen, 2012). In this study, we also deduced the role of NFκB in the generation of ROS. The particle exposures were found to increase the levels of NO (as well as ROS in general). As indicated by our work with an NFκB inhibitor, it is likely that the transcription factor controls the expression of the NO-synthesizing enzyme iNOS, and possibly of Cox2 as well, the latter mediates ROS generation (Hickey et al., 2021; Jiang et al., 2004; Nogawa et al., 1998). Elevated presence of these enzymes were detected in the particle-exposed mice (cerebral cortex samples, in particular with the WT

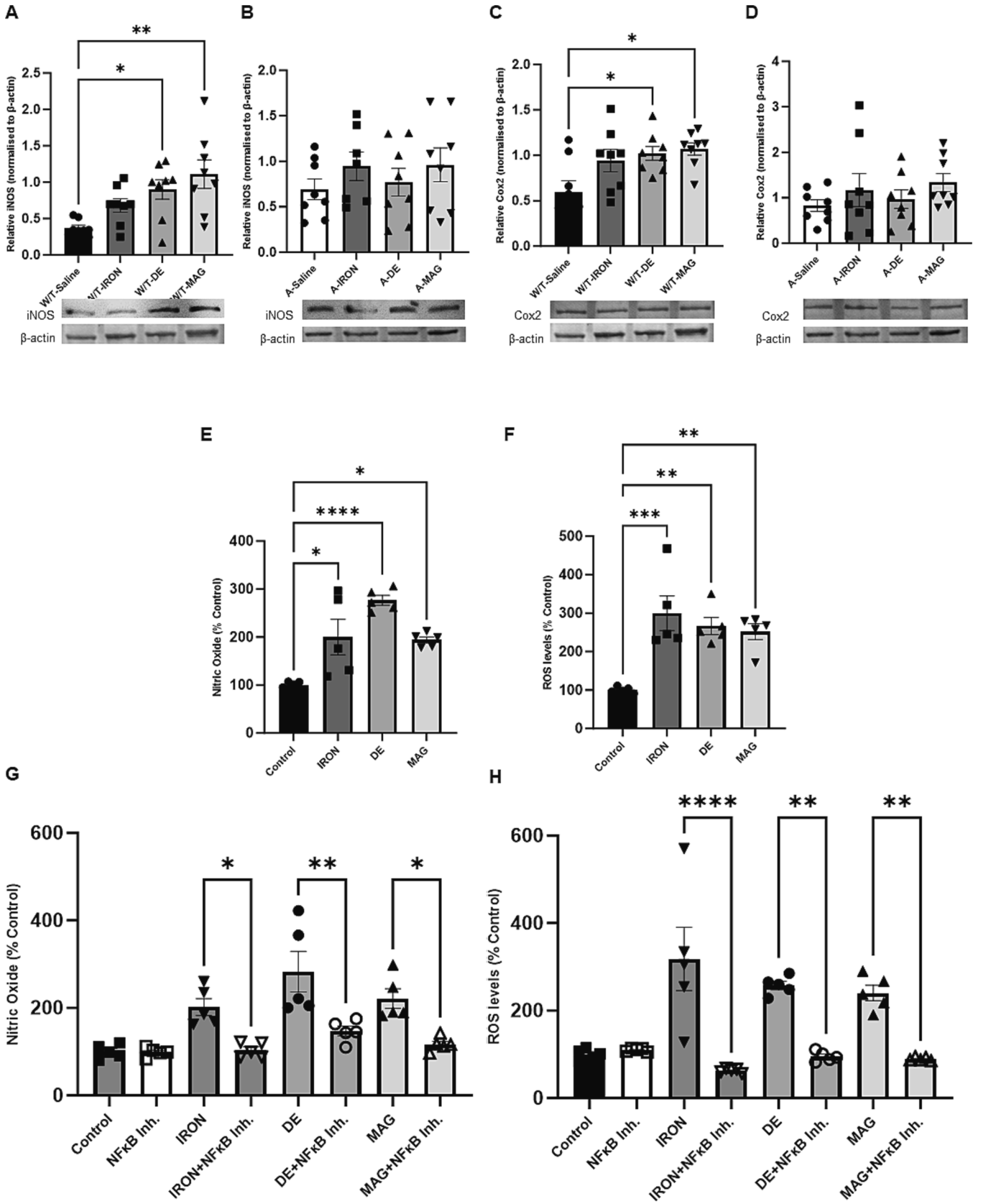

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Fig. 5. *In vivo* and *in vitro* studies of oxidative stress responses. (**A, B**) Relative iNOS levels in the cerebral cortex of the particle-exposed wild-type mice (n = 8) *p = 0.0406 (W/T-DE), **p = 0.0014 (W/T-MAG) vs. W/T-Saline; for the particle-exposed AD-predisposed mice (n = 8), note that there is no statistical significance for the particle-exposed groups relative to the A-Saline. (**C, D**) Relative Cox2 levels in the cerebral cortex, wild-type mice (n = 8) *p = 0.040 (W/T-IRON), *p = 0.0236 (W/T-DE), *p = 0.0155 (W/T-MAG) vs. W/T-Saline; for the AD-predisposed mice (n = 8), no statistical significance for the particle-exposed groups relative to the A-Saline. (**E**) NO levels (secreted from cells) in SH-SY5Y-particle samples, relative to the cell-only control (n = 5) *p = 0.0180 (IRON), ****p = 0.0001 (DE), *p = 0.00217 (MAG) vs. Control. (**F**) ROS levels (cellular presence) in SH-SY5Y-particle samples, relative to the cell-only control (n = 5) ***p = 0.0003 (IRON), **p = 0.0001 (DE), **p = 0.0147 (MAG) vs. Control. (**G**) NO levels in SH-SY5Y-particle samples in the presence of NFkB inhibitor (n = 5) *p = 0.0481 (IRON), **p = 0.0013 (DE), *p = 0.0167 (MAG) vs. NO levels without the inhibitor. (**H**) ROS levels in SH-SY5Y-particle samples in the presence of NFκB inhibitor (n = 5) ****p = 0.00001 (IRON), **p = 0.0021 (DE), **p = 0.0076 (MAG). All error bars indicate mean ± SEM. For the *in vitro* SH-SY5Y studies, the experiments were carried out with *n* biological replicates (cells from different passage rounds).

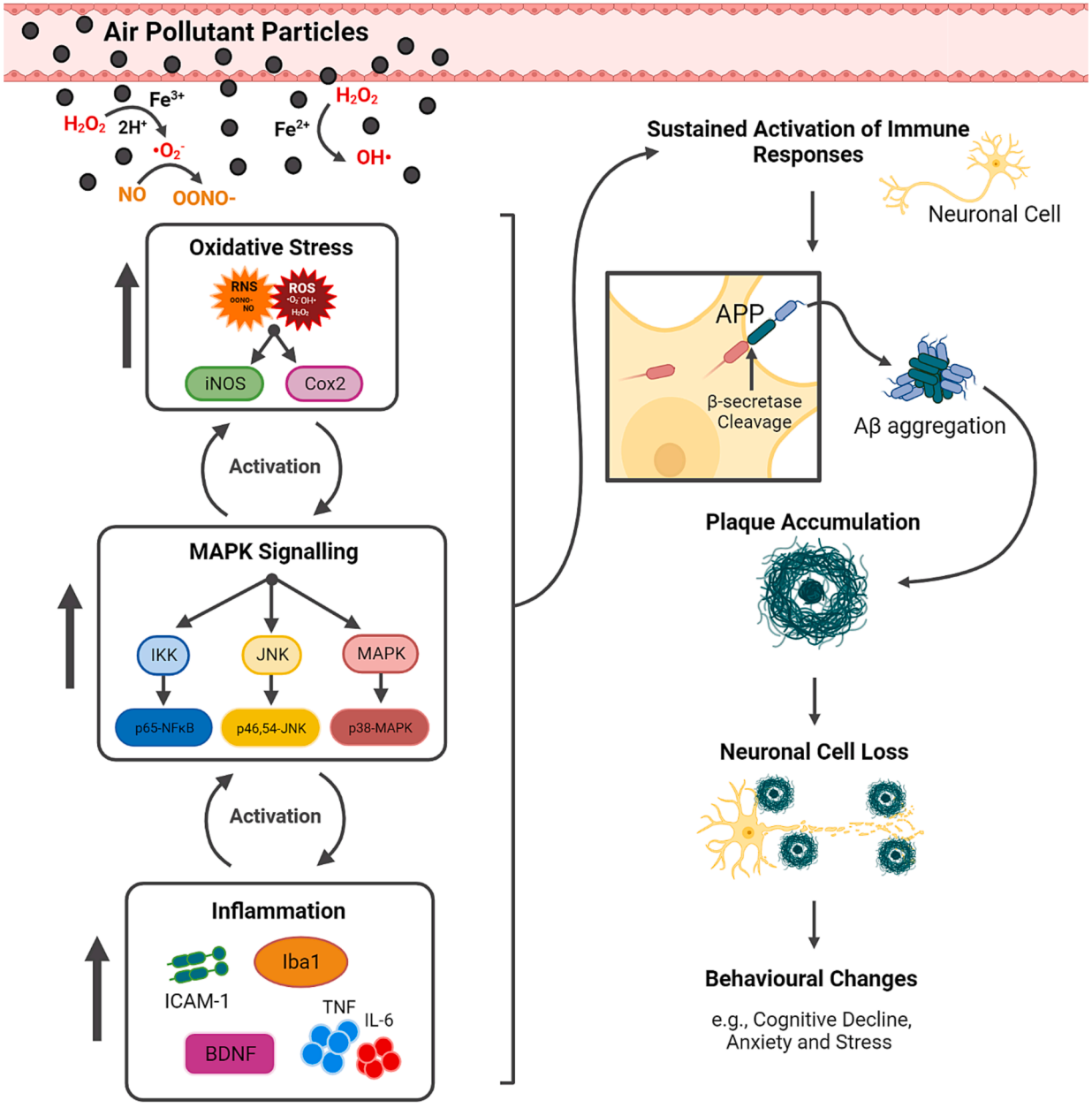

Fig. 6. A working model on the biological effects of air pollutant particulates in the early onset pathologies of AD. Particle exposures induce 'cyclic' stimulation of inflammation and oxidative stress responses through MAPK signalling in the brain. The sustained activation of immune responses leads to accumulation of amyloid plaque, and subsequently, neuronal cell loss and behavioural changes. The inhaled sub-micron sized particulates can enter the brain via the nasal olfactory bulb then travel across the blood brain barrier, or through the paracellular space across the nasal epithelium, then through the perineural space, into the brain (Xie et al., 2019).

mice). Again, denoting a 'cyclic' mechanism, studies have also shown that oxidative stress can activate the NFκB module (reviewed in (Gloire et al., 2006)), which in turn, upregulate the inflammatory cytokines, or in other words, oxidative stress could be a cause and consequence of inflammation in the context of the pollutant particle exposures. Next, addressing the question of how the particle-induced inflammation and oxidative stress responses can lead to plaque build-up, the apparent 'cyclic' stimulation/re-stimulation of the MAPK pathways by the

inflammatory cytokines and ROS suggest a prolonged activation of the immune responses with the particle exposures. Research has indicated exacerbation of AD pathologies as a result of sustained activation of the immune responses. Sustained activation of microglia for instance, can reduce their ability to phagocytose the amyloid β plaque, leading to plaque accumulation (the activated microglia after some time, become enlarged and unable to further phagocytose the plaque) (Wang et al., 2015), with the accumulation consequently leading to NFT build-up (Liu et al., 2011). The sustained activation of immune responses has also been linked to neuronal cell loss, herein also observed, which apart from the plaque accumulation, is also thought to result from the continual generation of the inflammatory cytokines (Liu et al., 2014). Further, a continual generation of ROS has also been indicated to lead to amyloid plaque accumulation, with the phenomenon being correlated to the activation of NFκB (Sun et al., 2022). Studies have reported the upregulation of the plaque formation enzyme BACE-1 by the growth factor (Cole and Vassar, 2007).

Finally, we discuss the apparently different pollutant particle effects on the model mice. Magnetite seemed to inflict the most pathological changes, more specifically, increased anxiety and stress, neuronal cell loss, as well as inflammation and oxidative stress, on the wild-type mice. It is thought that the magnetite, with its sub-micron smaller size, can enter the brain more readily via the earlier mentioned, BBB and paracellular epithelium-perineural pathways, with this hypothesis being consistent with the < 200 nm size magnetite detected by Maher et al. in the AD brain (Maher et al., 2016). The trends however, were rather inconclusive with the AD-predisposed mice, with no clear tendencies as on which particles induced the most effects. It can be further deduced that in general, the pollutant particles did not exacerbate the biological effects, including the inflammation and oxidative stress responses, in the AD-predisposed groups when compared to the wild-type groups. Previous studies have indicated a number of specific neurological effects of iron-based particles. As also herein seen with the WT mice, exposures to iron-based particles have been associated with increased anxiety and stress levels; for instance that observed by Maaroufi et al. in adult rats following $FeSO_4$ exposures (1.5–3 mg/kg/day for 5 days) (Maaroufi et al., 2009). The behavioural changes were thought to correlate with the indicated ability of iron-based particles to modulate neurotransmitters levels that facilitate the neuron-to-neuron synaptic communication (Ferreira et al., 2019). Other studies detected concentrated presence of iron moieties in and around the vicinity of amyloid plaque (Banerjee et al., 2014; Tahmasebinia and Emadi, 2017; Zhao 2019b). Iron(III) (Fe^{3+}) has been indicated to interact with the plaque, being reduced to the Fenton-active iron(II) (Fe^{2+}), which can further react with cellular H_2O_2 to generate the highly damaging hydroxyl radical (Rival et al., 2009; Zhao 2019a). In relation to the present study, it is reasonable to suggest that magnetite with presence of Fe(III) and Fe(II) in its lattice, can interact with the plaque and generate oxygen radicals. Note that, herein, we detected the highest plaque presence with the magnetite exposures in the AD-predisposed mice, when compared to the other tested pollutant particles. Indeed, the MAG exposures were also associated with increased (cerebral cortex) levels of the oxygen radical-generating enzymes iNOS and Cox2, as well as increased presence (*in vitro*) of the respective radical NO and (general) ROS. Neurodegenerative studies have also reported increased β-secretase levels (SH-SY5Y cells) in the presence of iron (synthetic flavone negletin, known to chelate iron) (Banerjee et al., 2014), herein particularly seen with MAG exposures (plasma, with the WT mice), which could associate with the earlier mentioned ROS-induced NFκB activation, leading to upregulation of the enzyme. Further, iron accumulation in neurons has also been indicated to cause cell death. The so-called ferroptosis is an iron-dependent programmed cell death, with mounting evidence correlating the phenomenon to neuronal cell loss in neurodegenerative cases, being linked, among other stress responses, to ROS-induced lipid peroxidation in the brain (Zhao 2019b). The notion is consistent with the herein observed neuronal cell loss (hippocampal DG, Ca3, Ca1 and SSC regions), particularly with MAG, in both the WT and the AD-predisposed mice.

As for the carbon-based diesel particles, earlier studies have also noted their effects on neurodegenerative pathologies. Hullmann et al. reported less motor coordination in AD-predisposed mice (5xFAD) upon exposures to diesel engine exhaust (950 $\mu g/m^3$, 13 weeks), which, as also deduced in the present work, were associated with the observed higher plaque loads in the brain (Hullmann et al., 2017). Studies have also observed changes in inflammation and oxidative stress responses with diesel particle exposures. Mice acute exposures to diesel exhaust particles ($PM_{2.5}$ at 250–300 $\mu g/m^3$, 6 h) saw elevated levels of inflammatory cytokines in the hippocampus region (Cole et al., 2016). Other studies detected increased cytokine expressions (midbrain samples), along with increased presence of amyloid plaque (frontotemporal region), in a rat model that were subjected to chronic exposures of aerosolised diesel emissions (up to 992 $\mu g/m^3$, 6 months) (Levesque et al., 2011). These observations were also herein seen with the diesel exposures, in particular with the WT mice (hippocampal samples for cytokines, whole brain imaging for plaque). Other *in vitro* studies (BV2 cells) with carbon-containing pollutant ($PM_{2.5}$) detected elevated presence of NO (Haghani et al., 2020), also seen in the present work with the diesel exposure (SH-SY5Y cells). At this stage, it is still not clear as to how the different pollutant particles, *i.e.,* the iron-based *versus* carbon-based particles, exhibit unique biological roles in the early onset of AD development. Regardless, it is apparent that the iron-based particles, in particular magnetite, are herein associated with higher extent of the pathological features.

While the findings from our study contributes to valuable insights into the different air pollution particles, it is crucial to acknowledge the constraints associated with this study. First, there are a myriad of approaches in research used for the study of air pollution particles (Shang and Sun, 2018). Inhalation exposure via the nose has long been used and accepted in exposure to environmental pollutant studies due to the availability of resources for this method, ease of delivery, cost and consistent dosage delivery. However, real-world scenarios involve a more complex interaction between the respiratory system and airborne pollutants. Therefore, the outcome for exposure to the air pollutant particles administered via intranasal administration would need to be confirmed using exposure chambers. Second, the AD-predisposed APP/PS1 mouse model more likely mimics the genetic form of AD as to a sporadic form often thought to be associated with environmental factors (Eid et al., 2019). There are currently no consistent transgenic mouse models that can mimic sporadic AD that develops all the hallmarks often observed in patients such as having the deposition of both Aβ and tau similar to sporadic AD patients. The mouse model used in this study for example is not an ideal model to study tau. Future work can therefore include different models of AD that can include *in vivo* studies of the NFT (although very challenging as there are currently no specific fluorescent probe for tau currently available), currently performed *in vitro* for the present study, to first gain a validation on the particle-induced tau phosphorylation that underlies the NFT formation.

In summary, the present work reported the ability of air pollutant particles to induce pathological features that are implicated in early onset AD. In both wild-type C57BL/6 and AD-predisposed APP/PS1 mice models, exposures to the iron-based and carbon-based particles of varying sizes led to behavioural changes and different trends of neuronal cell loss, immune system responses, including inflammation, as well as stimulation of oxidative stress, with magnetite particles appearing to induce the most consistent changes in the AD-related pathological features. The particle exposures, however, do not seem to exacerbate the immune system and oxidative stress responses in the APP/PS1 mice, with pre-existing AD pathological vulnerabilities, despite the more abundant presence of the AD hallmark Aβ plaques in the latter. Future studies are to investigate the exact triggers of the air pollutant particle-induced AD pathologies, which will also give insights into the no-exacerbation effects currently observed with the AD-predisposed cases.

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CRediT authorship contribution statement

Cindy Gunawan: Writing – review & editing, Writing – original draft, Supervision, Funding acquisition, Formal analysis, Conceptualization. **Charlotte Fleming:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Peter J. Irga:** Writing – review & editing, Resources, Formal analysis. **Roong Jien Wong:** Resources, Data curation. **Rose Amal:** Writing – review & editing, Resources, Formal analysis. **Fraser R. Torpy:** Writing – review & editing, Resources, Formal analysis. **S. Mojtaba Golzan:** Methodology, Supervision, Formal analysis, Writing – review & editing. **Kristine C. McGrath:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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