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Efeitos da exposição ao material particulado ($MP_{2,5}$) no sistema nervoso central de ratas Wistar e sua prole durante os períodos gestacional e lactação.

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Pelotas, abril 2022

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Resumo

FERREIRA, Ana Paula da Silva. **Efeitos da exposição ao material particulado ($MP_{2,5}$) no sistema nervoso central de ratas Wistar e sua prole durante os períodos gestacional e lactação.** 2022. 110f. Tese (Doutorado) - Programa de Pós-Graduação em Bioquímica e Bioprospecção da Universidade Federal de Pelotas, Pelotas, 2022.

A exposição a poluição ambiental representa um importante fator de risco a saúde, o material particulado ($MP_{2,5}$) presente na atmosfera tem sido implicado no desenvolvimento e agravamento de diversas patologia do sistema nervoso central (SNC). A interação entre $MP_{2,5}$ e o SNC pode ocorrer pela via olfatória, estando relacionada com os processos neurodegenerativos. A exposição ao $MP_{2,5}$ pode interferir no desenvolvimento do SNC embrionário resultando em alterações comportamentais de ratos Wistar. A partir das amostras de $MP_{2,5}$ coletadas, foram preparadas duas diluições 1:50 e 1:25 utilizadas na instilação intranasal das fêmeas prenhes durante os períodos de gestação e lactação (39 dias), alocadas em três grupos experimentais, branco e diluições 1:50 e 1:25. Os objetivos deste estudo foram avaliar a exposição ao $MP_{2,5}$ em relação ao comportamento, detectar e quantificar os componentes metálicos do $MP_{2,5}$ no hipocampo e córtex pré-frontal, avaliar a formação de espécies reativas de oxigênio (EROs) e a atividade da enzima superóxido dismutase (SOD). Duas tarefas comportamentais foram realizadas, o reconhecimento de objetos em campo aberto e o labirinto em Y. Os resultados obtidos no reconhecimento de objetos demonstraram diferenças significativas, entre as fêmeas o grupo 1:25 (4.8 ± 1.3) e o grupo branco (9.1 ± 1.3) ($p = 0.061$) em relação a interação com o objeto novo. Entre os machos uma diferença significativa ($p = 0.0128$) foi encontrada em relação a interação com o objeto familiar entre os grupos branco (3.7 ± 1.0) e 1:50 (7.2 ± 1.0). Uma diferença significativa também foi encontrada ($p = 0.0207$) entre o grupo branco (7.5 ± 1.0) e o grupo 1:25 (4.2 ± 1.0) na interação com o objeto novo, indicando uma redução no comportamento exploratório e um declínio na memória de curto e longo prazo, especialmente nos machos. No labirinto em Y, as ninhadas apresentaram diferenças significativas entre fêmeas e machos, grupo branco fêmeas (67,02 ± 5,41) machos (39,60 ± 5,41) ($p < 0,0001$), grupo 1:50 fêmeas (62,62 ± 5,41) machos (42,47 ± 5,41) ($p = 0,0014$) e 1:25 fêmeas (64,25 ± 5,41) machos (40,22 ± 5,41) ($p = 0,0002$) indicando uma redução da memória espacial nos machos. Os dados comportamentais obtidos apontam para uma maior suscetibilidade dos machos em relação as fêmeas quando expostos ao $MP_{2,5}$. A detecção e quantificação no hipocampo e córtex pré-frontal (CPF) revelou a presença de componentes metálicos do $MP_{2,5}$. Em relação a formação de EROs não houve diferença significativa entre os grupos ($p > 0,05$), já a análise da atividade da SOD no CPF das ninhadas foi encontrada uma diferença significativa ($p = 0,0095$) entre os grupos branco (50, 62 ± 10,84) e 1:50 (91,86 ± 10,84) indicando um aumento na atividade antioxidante. No hipocampo das matrizes uma diferença significativa ($p = 0,0001$) foi encontrada entre os grupos branco (79 ± 13,18) e 1:25 (6,10 ± 13.18). Os resultados obtidos indicaram a presença do $MP_{2,5}$ nos tecidos nervosos, e alteração na memória espacial dos machos das ninhadas, e também foi observada alteração no balanço redox.

Palavras chave: poluição ambiental, neurodegeneração, comportamento

Abstract

FERREIRA, Ana Paula da Silva. **Effects of particulate matter ($PM_{2.5}$) exposure in the central nervous system of Wistar female rats and their offsprings during the pregnancy and nursing periods.** 2022. 110f. Thesis (Doctorate degree) – Biochemistry and Bioprospection Post Graduate Program. Universidade Federal de Pelotas, Pelotas, 2022.

Exposure to environmental pollution represents an important risk factor for health, the particulate matter ($PM_{2.5}$) present in the atmosphere has been implicated in the development and worsening of various central nervous system (CNS) pathologies. The interaction between $PM_{2.5}$ and the CNS may occur through the olfactory pathway, being related to neurodegenerative processes. Exposure to $PM_{2.5}$ may interfere with the development of the embryonic CNS resulting in behavioral changes in Wistar rats. From the collected $PM_{2.5}$ samples, two dilutions 1:50 and 1:25 were prepared for intranasal instillation of pregnant females during the periods of pregnancy and lactation (39 days), allocated in three experimental groups, blank and dilutions 1:50 and 1:25. The objectives of this study were to evaluate exposure to $PM_{2.5}$ related to behavior, detect and quantify the metallic components of $PM_{2.5}$ in the hippocampus and prefrontal cortex, evaluate the formation of reactive oxygen species (ROS) and the activity of the enzyme superoxide dismutase (SOD). Two behavioral tasks were performed, the novel objects in the open field and the Y maze. The results obtained in the recognition of objects showed significant differences, between the females the group 1:25 (4.8 ± 1.3) and the blank group (9.1 ± 1.3) ($p = 0.061$) related to new object interaction. Among males, a significant difference ($p = 0.0128$) was found related to the interaction with the family object between the blank (3.7 ± 1.0) and the 1:50 (7.2 ± 1.0) groups. A significant difference was also found ($p = 0.0207$) between the blank group (7.5 ± 1.0) and the 1:25 group (4.2 ± 1.0) in the interaction with the new object, indicating a reduction in exploratory behavior and a decline in short and long-term memory, especially in males. In the Y maze, the offsprings showed significant differences between females and males, blank group females (67.02 ± 5.41) males (39.60 ± 5.41) ($p < 0.0001$), group 1:50 females (62.62 ± 5.41) males (42.47 ± 5,41) ($p = 0.0014$) and 1:25 females (64.25 ± 5.41) males (40.22 ± 5.41) ($p = 0.0002$) indicating a reduction in spatial memory in males. The behavioral data obtained point to a higher susceptibility of males in relation to females when exposed to $PM_{2.5}$. The detection and quantification in the hippocampus and prefrontal cortex (PFC) revealed the presence of metallic components of $PM_{2.5}$. Regarding the ROS formation, there was no significant difference between the groups ($p > 0.05$), while the analysis of SOD activity in offspring PFC was found a significant difference ($p = 0.0095$) between the blank (50, 62 ± 10.84) and 1:50 (91.86 ± 10.84) groups, indicating an increase in antioxidant activity. In the hippocampus of the matrices a significant difference ($p = 0.0001$) was found between the blank (79 ± 13.18) and 1:25 (6.10 ± 13.18) groups. The results indicated the presence of $PM_{2.5}$ in the nervous tissues, and alteration in the spatial memory of the offspring males, and an alteration in the redox balance was also observed.

Key words: Environmental pollution, neurodegeneration, behavior

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Lista de Abreviaturas e Siglas

$MP_{2,5}$ – Material Particulado com diâmetro de até 2,5

SNC – Sistema Nervoso Central

EROs – Espécies Reativas de Oxigênio

ON – Óxido Nítrico

SOD – Superóxido Dismutase

PCBs – Bifenilos policlorados

PAHs – Hidrocarbonetos aromáticos policíclicos

SNP – Sistema nervoso periférico

PMAP – Padrões moleculares associados a patógenos

PMAD – Padrões moleculares associados a danos

TLR – Receptores tipo Toll

ATPR – Receptores de adenosina trifosfato

LPS – Lipopolissacarídeos

IFN-γ – Interferon-γ

IL-1β – Interleucina-1β

IL-6 – Interleucina-6

TNF-α – Fator de necrose tumoral-α

CCL2 – Quimiocina CCL2

IL-4 – Interleucina-4

IL-13 – Interleucina-13

IL-10 – Interleucina-10

CAT – Catalase

GSH – Glutationa reduzida

GSH-Px - Gluationa peroxidase

HO-1 – Heme oxigenase-1

Cyp-1b1 – Citocromo P450 1b1

HSP 70 – Proteína de choque térmico 70

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1. Introdução

A exposição à poluição atmosférica tem sido alvo de inúmeros estudos que indicam a associação desta condição ambiental ao desenvolvimento ou ao agravamento de condições pré-existentes. Uma fração crítica da poluição atmosférica, é o material particulado (MP), constituído por partículas sólidas e líquidas de diversas granulometrias, formas e composição química e está relacionada a ações deletérias à saúde (CHENG et al., 2017a; COLE et al., 2016; CORY-SLECHTA et al., 2018; COSTA et al., 2019a; HULLMANN et al., 2017; KU et al., 2017; RIBEIRO et al., 2016).

A origem e a fonte do MP pode ser natural (*spray* marítimo, atividade vulcânica e matéria biológica) ou antropogênica (biomassa industrial, uso de combustíveis fósseis) e ainda resultar de reações químicas atmosféricas (CHENG et al., 2017; KU et al., 2017; RIBEIRO et al., 2016). A composição do MP inclui, poeira, compostos orgânicos e inorgânicos, metais, íons, entre outros, sendo classificado em três categorias principais, baseadas no diâmetro das partículas: MP ultrafino (<0,1µm), MP fino ($MP_{2,5}$ entre 0,1 µm e 2,5 µm) e MP grosso (PM_{10} entre 2,5 µm e 10 µm) (ALLEN et al., 2014; BLOCK; CALDERÓN-GARCIDUEÑAS, 2009; KIM et al., 2020). A fração $MP_{2,5}$ apresenta um maior espectro patológico devido a sua facilidade de ingressar no organismo, comprometendo a funcionalidade tecidual (KU et al., 2017a; MINGUILLÓN et al., 2008).

De acordo com a Organização Mundial de Saúde (OMS, 2016) a poluição do ar é um dos mais importantes fatores relacionados as alterações em diversos sistemas orgânicos como cardiovascular, respiratório, hematológico, reprodutivo e imunológico vem sendo reportadas ao longo dos anos, indicando que a exposição crônica a poluentes representa um fator crítico no âmbito patológico (CALDERÓN-GARCIDUEÑAS et al., 2008, 2011; DI DOMENICO et al., 2020a; HAGHANI et al., 2020a, 2020b; KAMPA; CASTANAS, 2008; KU et al., 2017; RIBEIRO et al., 2016).

Neste contexto o sistema nervoso central (SNC) emerge neste contexto como um importante alvo da exposição à poluição atmosférica, implicando em alterações no desenvolvimento e nas funções nervosas. Além disso, o MP vem sendo relacionado à neurodegeneração, neuroinflamação, modificações morfológicas, alterações comportamentais e também no declínio na aprendizagem e na memória (BLOCK;

CALDERÓN-GARCIDUEÑAS, 2009b; BURSTEIN, 2021; DE PRADO BERT et al., 2018; HAHAD et al., 2020; HARRISON; YIN, 2000).

O $MP_{2,5}$ pode atingir o SNC através da via sistêmica sanguínea após a inalação ou pela absorção pelo epitélio olfatório. A interação do $MP_{2,5}$ com a barreira hematoencefálica gera um dano que altera a permeabilidade seletiva da mesma, permitindo que ocorra bioacumulação de componentes do MP nos tecidos nervosos (BLOCK; CALDERÓN-GARCIDUEÑAS, 2009; CHEW et al., 2020; DI DOMENICO et al., 2020; KONG et al., 2012; LIU et al., 2019).

A neurotoxicidade do $MP_{2,5}$ está relacionada a ativação microglial, liberação de mediadores e de citocinas pró-inflamatórias, aumento na produção de espécies reativas de oxigênio (EROs), alteração na liberação de fatores de crescimento neural e secreção de óxido nítrico (ON), estes mecanismos podem ocorrer nas mais diversas combinações, agravando ou iniciando processos patológicos (CACCIOTTOLO et al., 2020; CHENG et al., 2016; KIM et al., 2020; WOODWARD et al., 2017).

As injúrias provocadas pelo $MP_{2,5}$ compreendem mielinização deficiente, espectro autista, demência, redução cognitiva, ansiedade e depressão, proteopatologias, alterações comportamentais e decréscimo na memória (ALLEN et al., 2014; CHENG et al., 2016; HAGHANI et al., 2020a; WOODWARD et al., 2017).

A elucidação das vias e mecanismos envolvidos na patogênese das enfermidades causadas pela exposição ao $MP_{2,5}$ pode fornecer subsídios para ações de redução da emissão de poluentes. Da mesma forma, o entendimento dos desfechos ocasionados pela exposição ao $MP_{2,5}$ pode auxiliar no desenvolvimento de terapias para minimizar os danos orgânicos.

Para avaliar a exposição ao $MP_{2,5}$ a utilização de modelos animais fornece valiosos indícios a respeito dos mecanismos envolvidos na fisiopatologia das alterações nervosas, sobre o período e a forma de exposição e as prováveis manifestações patológicas (CHEN et al., 2017; EMAM et al., 2020a; KLOCKE et al., 2018; LI et al., 2020; SMITH; WHITE III; VILLEDA, 2018).

2. Objetivos

2.1 Objetivos geral

Avaliar os efeitos no comportamento de fêmeas adultas Wistar e suas ninhadas à exposição ao material particulado ($MP_{2,5}$) por instilação, durante o período gestacional e de lactação, detectar e quantificar a presença de bioacumulação dos componentes metálicos do MP e o desbalanço redox em hipocampo e córtex pré-frontal.

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2.2 Objetivos específicos

- Revisar as alterações morfofisiológicas ocasionadas no desenvolvimento embriológico de ratos Wistar expostos ao $MP_{2,5}$;
- Estabelecer protocolo para determinar o volume e concentração para utilização do método de instilação do $MP_{2,5}$ em ratos Wistar fêmeas;
- Determinar a composição do $MP_{2,5}$, bem como, a bioacumulação de $MP_{2,5}$ no córtex pré-frontal e hipocampo das matrizes e suas respectivas proles;
- Avaliar o comportamento e a memória das matrizes e da prole exposta ao $MP_{2,5}$ durante a gestação e lactação nas tarefas de labirinto em Y e de reconhecimento de objetos;
- Avaliar parâmetros bioquímicos relacionados ao estresse oxidativo (EROs e SOD) no córtex pré-frontal e hipocampo de ratos Wistar expostos ao $MP_{2,5}$.
- Comparar o comportamento entre machos e fêmeas das ninhadas expostas ao $MP_{2,5}$.

3. Revisão de literatura

Para o desenvolvimento deste estudo foi realizada uma busca em três bancos de dados, PubMed, Web of Science e Science Direct, utilizando os descritores relacionados ao tema proposto, sistema nervoso central, material particulado fino, acumulação, estresse oxidativo e comportamento. Inicialmente cada descritor foi pesquisado individualmente, logo após combinados, com a finalidade de estabelecer o embasamento teórico do estudo.

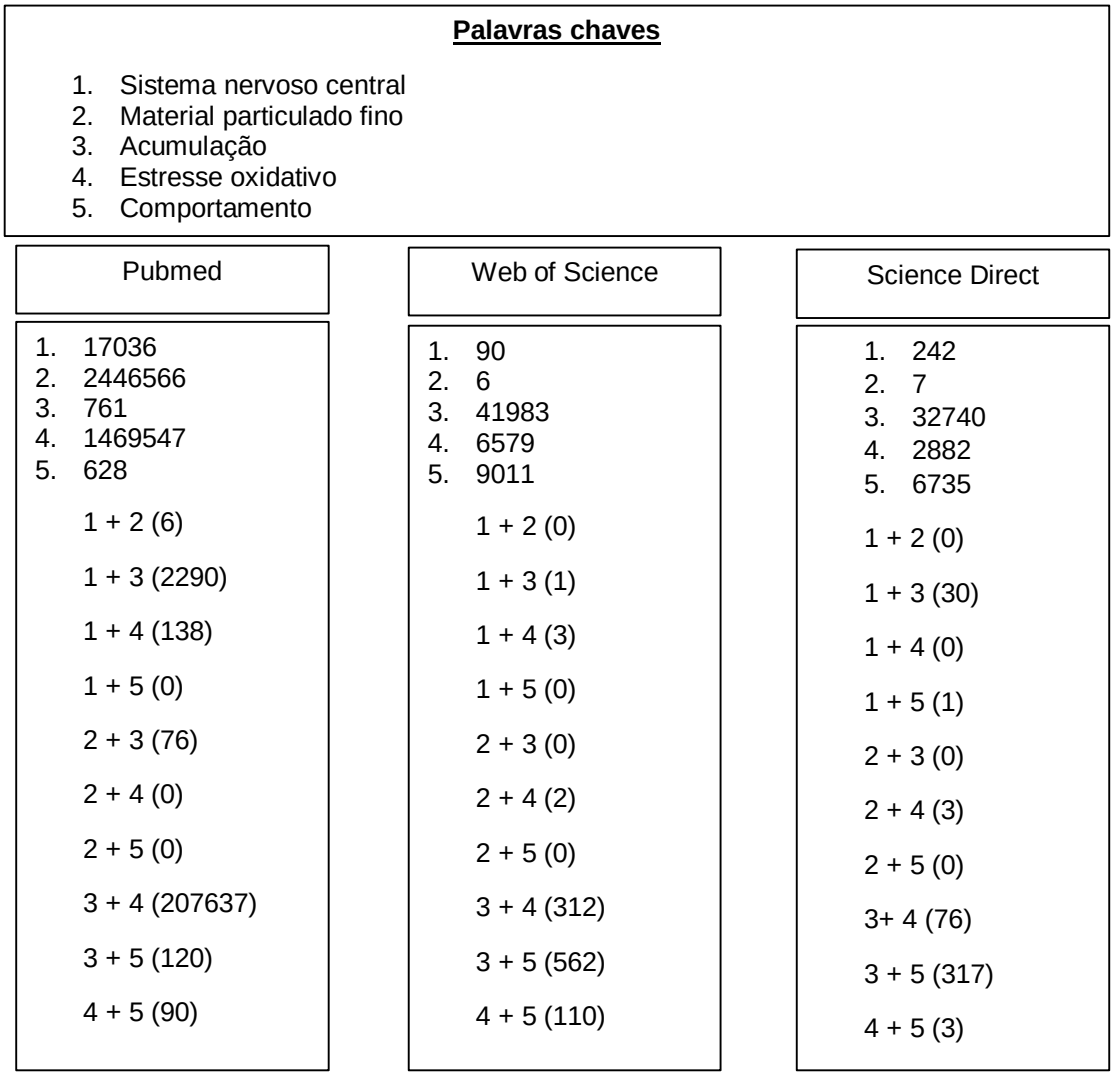

Figura 1: Fluxograma da busca literária realizada nas três bases de dados, com o número de artigos encontrados em cada uma delas.

3.1 Material Particulado 2,5 (MP2,5)

A poluição atmosférica resulta das atividades antropogênicas, em especial daquelas relacionadas as emissões resultantes da queima de combustíveis fósseis e das atividades industriais, onde o aumento destes componentes altera consideravelmente a qualidade do ar respirado (CSERBIK et al., 2020; MATEUS et al., 2013).

Estudos epidemiológicos e experimentais reportam a associação da exposição ao $MP_{2,5}$ a doenças como Alzheimer, Parkinson, espectro autista, câncer, doenças pulmonares, entre outras (AHLERS; WEISS, 2021; COSTA; CHANG; COLE, 2017; KIM et al., 2020; LJUBIMOVA et al., 2018; SHIH et al., 2018; WU et al., 2019a).

O material particulado (MP) dependendo da sua concentração e capacidade de bioacumulação nos tecidos pode causar danos ao organismo, alterando assim, as condições homeostáticas, e estando relacionado ao desenvolvimento de patologias (FONKEN et al., 2011; KU et al., 2017; RIBEIRO et al., 2016).

O material particulado é classificado de acordo com seu diâmetro aerodinâmico em $MP_{2,5}$ (diâmetro entre 0,1 µm e 2,5 µm), MP_{10} (diâmetro entre 2,5 µm e 10 µm) e $MP_{0,1}$ (diâmetro menor que 0,1 µm) (Figura 2) (BATES et al., 2019; BLOCK; CALDERÓN-GARCIDUEÑAS, 2009; CÁCERES QUIJANO et al., 2022).

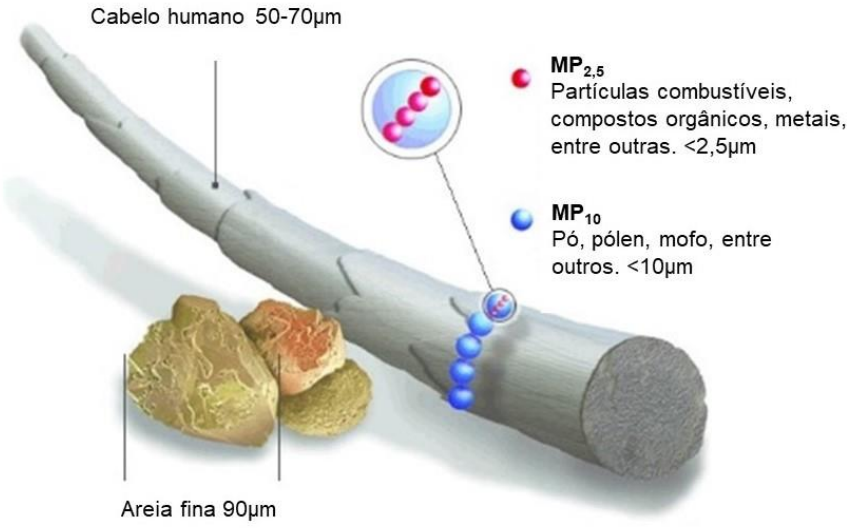

Figura 2: Tamanho relativo do material particulado. Fonte: Modificado de U.S. Environmental Protection Agency (US EPA).

O tamanho e as propriedades físico-químicas das partículas facilitam a sua penetração no organismo através de variadas formas de exposição, como por exemplo, inalação, ingestão ou absorção. De uma forma geral, partículas menores apresentam uma maior possibilidade de acumulação nos tecidos e, por isso, uma maior toxicidade (BALASUBRAMANIAN et al., 2013; KU et al., 2017).

O $MP_{2,5}$ representa uma importante fração da poluição atmosférica relacionada a ações deletérias à saúde (Figura 3). A composição do $MP_{2,5}$ compreende partículas sólidas (poeira, metais, nitritos e sulfatos), gotículas líquidas, compostos gasosos orgânicos (compostos orgânicos voláteis não metano, bifenilos policlorados (PCBs), hidrocarbonetos aromáticos policíclicos (PAHs) originados de inúmeras fontes (CHENG et al., 2017; KU et al., 2017).

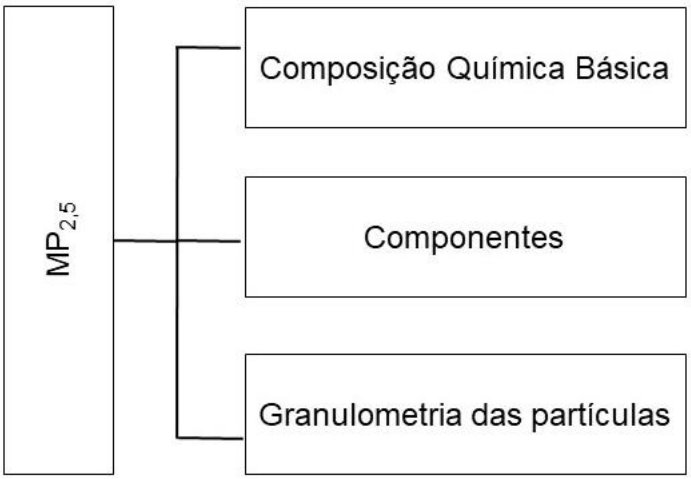

Figura 3: Composição básica do $MP_{2,5}$ relacionadas aos danos orgânicos. Fonte: Produzida pelos autores.

Embora os gases atmosféricos (óxidos de nitrogênio, monóxido de carbono, ozônio, dióxido de enxofre) não sejam considerados componentes do MP, suas interações com as biomoléculas podem resultar em partículas secundárias potencializando a perturbação da homeostasia (EMAM et al., 2020; KU et al., 2017; LIU et al., 2019)

A exposição ao MP causa neuroinflamação, aumento do estresse oxidativo e ativação glial, sendo estes os mecanismos implicados na sua patogenicidade. A exposição durante o desenvolvimento embrionário pode implicar em alterações na organogênese, resultando em deficiência funcional, como dificuldade de aprendizagem e memória (ALLEN et al., 2014; KU et al., 2017). Sua composição heterogênea promove inúmeras alterações em processos tais como, síntese proteica alterada, metilação do DNA, peroxidação lipídica e disfunção mitocondrial (CORY-SLECHTA et al., 2018; KU et al., 2017; PARK et al., 2021).

3.2 Sistema Nervoso

O sistema nervoso (SN), como um todo, compreende o sistema nervoso central (SNC) e o sistema nervoso periférico (SNP), essas estruturas se integram com a função de permitir o ajuste do corpo humano aos meios interno e externo, ou seja, garantir a homeostase (GUYTON & HALL, 2017).

O SNC dos vertebrados possui um isolamento representado pela barreira hematoencefálica. Seu funcionamento normal é fundamental para a manutenção da homeostasia, sendo responsável pelo aporte de nutrientes e pela remoção dos metabólitos entre o tecido nervoso e o sangue, além de restringir a infiltração de células do sistema imune e a penetração de xenobióticos (MADSEN et al., 2013; MAŁECKI et al., 2017; PILAKKA-KANTHIKEEL et al., 2013; SAWICKI et al., 2019).

A exposição ao MP pode modificar a característica funcional da barreira hematoencefálica, levando a perda da permeabilidade seletiva permitindo a passagem e a bioacumulação dos componentes do MP no tecido nervoso (KONG et al., 2012; MORRIS-SCHAFFER et al., 2018a; SAWICKI et al., 2019).

Em estudo realizado por Kong et al. (2012), os autores demonstraram que componentes do MP podem penetrar nos neurônios e mover-se ao longo dos axônios ou dendritos para os neurônios adjacentes, podendo ser transportados também através das conexões sinápticas, endocitose e difusão passiva.

O SNC possui uma defesa inata residente, a micróglia, representada por dois fenótipos de atividade. Os fenótipos M1, denominado de pró-inflamatório, e o M2, denominado de anti-inflamatório, em condições fisiológicas estão em equilíbrio.

Entretanto, a exposição o $MP_{2,5}$ promove a ativação do fenótipo M1 que está relacionado a neuroinflamação (BABADJOUNI et al., 2018; COLE et al., 2016; KIM et al., 2020; PATTEN et al., 2020).

As funções imunorregulatórias dos fenótipos microgliais M1 e M2 são ativadas por padrões moleculares associados a patógenos (PMAP) ou padrões moleculares associados a danos (PMAD). A ativação dos fenótipos se dá via receptores tipo Toll (TLR) ou receptores de adenosina trifosfato (ATPR) (NAKAGAWA; CHIBA, 2014).

Os padrões PMAP e PMAD estão relacionados a presença de lipopolissacarídeos (LPS) e interferon-γ (IFN-γ). A produção de citocinas e mediadores incluindo Interleucina-1β (IL-1β), Interleucina-6 (IL-6), Fator de necrose tumoral-α (TNF-α), quimiocina CCL2 (CCL2), espécies reativas de oxigênio (EROs) e óxido nítrico estimula a ativação do fenótipo pró-inflamatório (NAKAGAWA; CHIBA, 2014).

A ativação anti-inflamatória do fenótipo M2 ocorre via estimulação de interleucinas 4 e 13 (IL-4 e IL-13) resultando na produção de Interleucina-10 (IL-10) com objetivo de regulação do processo inflamatório (NAKAGAWA; CHIBA, 2014).

A interação do $MP_{2,5}$ com o SNC pode promover alterações morfofuncionais, relacionada a modificações na via de sinalização proteína kinase A (PKA)/cAMP proteína de ligação ao elemento (CREB)/fator neurotrófico derivado do cérebro (BDNF) (LIU et al., 2021).

A exposição ao $MP_{2,5}$ promove um desequilíbrio entre a ativação dos perfis anti e pró-inflamatórios microgliais, a atividade do fenótipo M2 torna-se insuficiente frente a exacerbada atividade do fenótipo M1 favorecendo o estabelecimento da neuroinflamação, resultando em injúrias no SNC (BLOCK; CALDERÓN-GARCIDUEÑAS, 2009; KIM et al., 2020; NAKAGAWA; CHIBA, 2014).

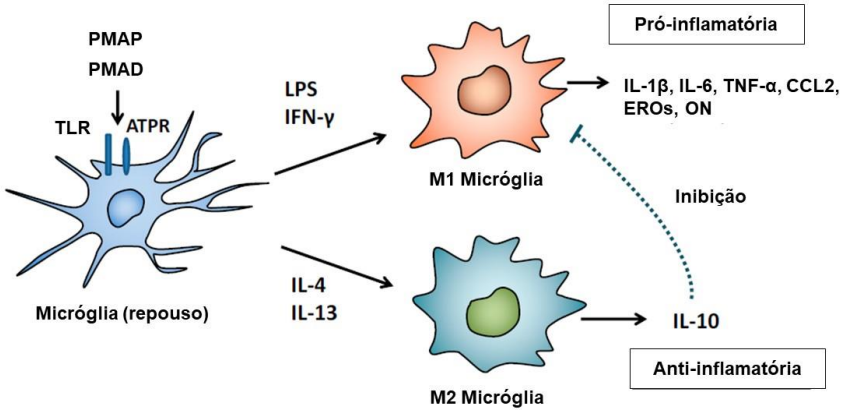

Figura 4: Vias clássicas de ativação dos fenótipos microgliais M1 e M2. Fonte: modificado de Pharmaceuticals **2014**, 7, 1028-1048; doi:10.3390/ph7121028.

A plasticidade sináptica também pode ser alterada pela exposição ao $MP_{2,5}$, provocando um desequilíbrio na neurotransmissão excitatória e/ou inibitória, através da alteração da expressão gênica neuronal, esta desestabilização pode incrementar o risco de desenvolvimento de doenças neurodegenerativas (KU et al., 2017).

Além disso, a exposição pode afetar a densidade celular do tecido nervoso, impactando negativamente as funções nervosas. Estudos indicam alterações morfológicas do bulbo olfatório, do hipocampo, corpo caloso, córtex pré-frontal, núcleo paraventricular e arqueado hipotalâmicos em decorrência da exposição ao $MP_{2,5}$ (BABADJOUNI et al., 2018; CAMPOLIM et al., 2020; CHENG et al., 2016; COLE et al., 2016; LJUBIMOVA et al., 2018; PATTEN et al., 2020; TSENG et al., 2019; WOODWARD et al., 2017).

Os neurônios piramidais da zona CA1 do hipocampo, a qual está intimamente relacionada a cognição, apresenta uma maior vulnerabilidade ao desenvolvimento de processos neurodegenerativos, como a doença de Alzheimer, indicando que o $MP_{2,5}$ deve ser considerado como um importante fator desencadeante da neurodegeneração (BABADJOUNI et al., 2018; WOODWARD et al., 2017).

Além de afetar a densidade celular, evidências apontam que o processo de mielinização neuronal também sofre alterações. A exposição gestacional a agentes ambientais afeta o desenvolvimento da substância branca nervosa e a maturação adequada dos oligodendrócitos, que resulta em uma redução da mielinização. Estes

aspectos estão associados a etiologias das doenças do neurodesenvolvimento, como por exemplo o espectro autista (CORY-SLECHTA et al., 2018; KLOCKE et al., 2018).

Desta forma, o SNC emerge como um alvo importante da exposição a poluição atmosférica, em particular da fração $MP_{2,5}$, a qual pode alcançar os tecidos nervosos superiores produzindo alterações funcionais, morfológicas e comportamentais (CHENG et al., 2016; COLE et al., 2016; KU et al., 2017; MORRIS-SCHAFFER et al., 2018).

3.3 Exposição gestacional e neonatal ao $MP_{2,5}$

A sequência celular do desenvolvimento ontogenético do SNC deve ser precisa, iniciando com a proliferação celular e subsequente diferenciação, migração e maturação das células. Seguido da formação das redes neuronais/axonais, há a modificação ou eliminação de conexões, através da sinaptogênese, brotamento e crescimento axonal, continuando com a mielinização e a poda sináptica (BABIKIAN et al., 2011; CORY-SLECHTA et al., 2018) (Figura 5).

O desenvolvimento do SNC pode ser afetado pela exposição a fatores não genéticos, como a poluição atmosférica, a exposição gestacional ao $MP_{2,5}$ pode comprometer funções nervosas resultando em alterações comportamentais exploratórias, redução no aprendizado e o comprometimento da memória (ALLEN et al., 2017; CORY-SLECHTA et al., 2018; JIA et al., 2018; ZHANG et al., 2018).

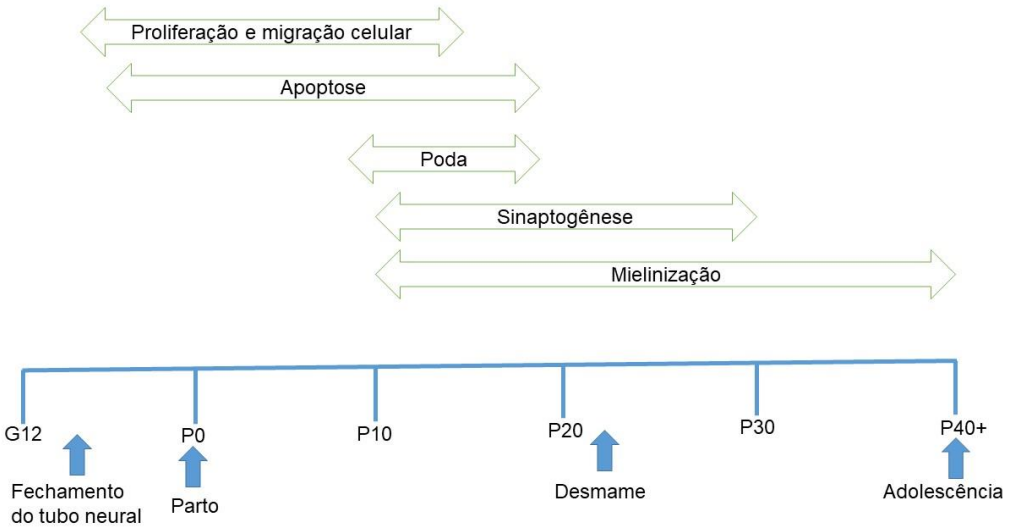

Figura 5: Desenvolvimento do SNC em ratos. G12: 12º dia gestacional, P10, P20, P30 e P40 dias pós-parto. Fonte: Modificado de Dev. Neurosci., 2010;32:431–441 DOI: 10.1159/000320667.

A utilização de modelos animais, como os roedores, em estudos de exposição ao $MP_{2,5}$ nos períodos gestacional e neonatal indicaram alteração nos níveis de neurotransmissores, neuroinflamação, perda de memória, comportamento depressivo e/ou ansiedade nas ninhadas. Essas alterações, por sua vez, afetam as funções do córtex pré-frontal e de estruturas como o estriado, hipocampo, hipotálamo, amigdala, cerebelo e tronco cerebral (CHURCH et al., 2018; CORY-SLECHTA et al., 2019; LIU et al., 2019a; RAO et al., 2019).

Evidências apontam que o $MP_{2,5}$ pode ser translocado através da barreira transplacentária de acordo com o tamanho, a concentração, a dissolução e a composição das partículas. A interação do MP com os tecidos embrionários pode provocar diferentes alterações no neurodesenvolvimento, em razão da via e da forma de exposição, do período gestacional e do modelo animal experimental (AHLERS; WEISS, 2021b; BONGAERTS et al., 2020; EHSANIFAR et al., 2019; EMAM et al., 2020a).

3.4 Bioacumulação

O potencial tóxico do $MP_{2,5}$ para os tecidos orgânicos está relacionado a sua capacidade de penetração e bioacumulação. Evidências apontam alterações morfofuncionais no SNC quando os componentes do MP inalados ou absorvidos, pelas vias sistêmica e/ou olfatória, interagem com os tecidos nervosos, podendo promover uma perturbação da homeostasia celular (HAHAD et al., 2020; KUMAR et al., 2021; MORRIS-SCHAFFER et al., 2018).

A composição química do $MP_{2,5}$ inclui espécies inorgânicas (por exemplo, sulfatos e nitritos), compostos orgânicos (por exemplo, ácidos carboxílicos, aminoácidos e hidrocarbonetos aromáticos policíclicos) e metais como, arsênico (As), cálcio (ca), cobre (Cu), níquel (Ni), estrôncio (Sr) e titânio (Ti) (BONGAERTS et al., 2020; MATEUS et al., 2018; VENTURA-LIMA et al., 2009).

A bioacumulação dos metais provenientes da exposição ao MP pode promover toxicidade no SNC, estando relacionado ao desequilíbrio redox e ao estabelecimento da condição inflamatória, contribuindo assim para o desenvolvimento e/ou agravamento de injurias nervosas (BONGAERTS et al., 2020; MATEUS et al., 2018; RIBEIRO et al., 2016).

O acesso dos componentes do $MP_{2,5}$ ao SNC, pode ocorrer pela via olfatória através da absorção dos compostos pelo epitélio olfativo das cavidades nasais, constituindo-se em uma via de comunicação direta entre o ar atmosférico e as estruturas nervosas, como o córtex, cerebelo e hipocampo (CHEW et al., 2020; COLE et al., 2016; COSTA et al., 2019b; HAHAD et al., 2020).

A concomitância entre as vias sistêmica e absortiva favorecem o processo de bioacumulação do $MP_{2,5}$ no SNC, implicando em interações MP e as biomoléculas (Figura 6). Os metais em concentrações e forma química adequada podem participar de mecanismos fisiológicos, porém quando em excesso sofrem reações que alteram suas propriedades gerando toxicidade (KAMPA; CASTANAS, 2008; VENTURA-LIMA et al., 2009)

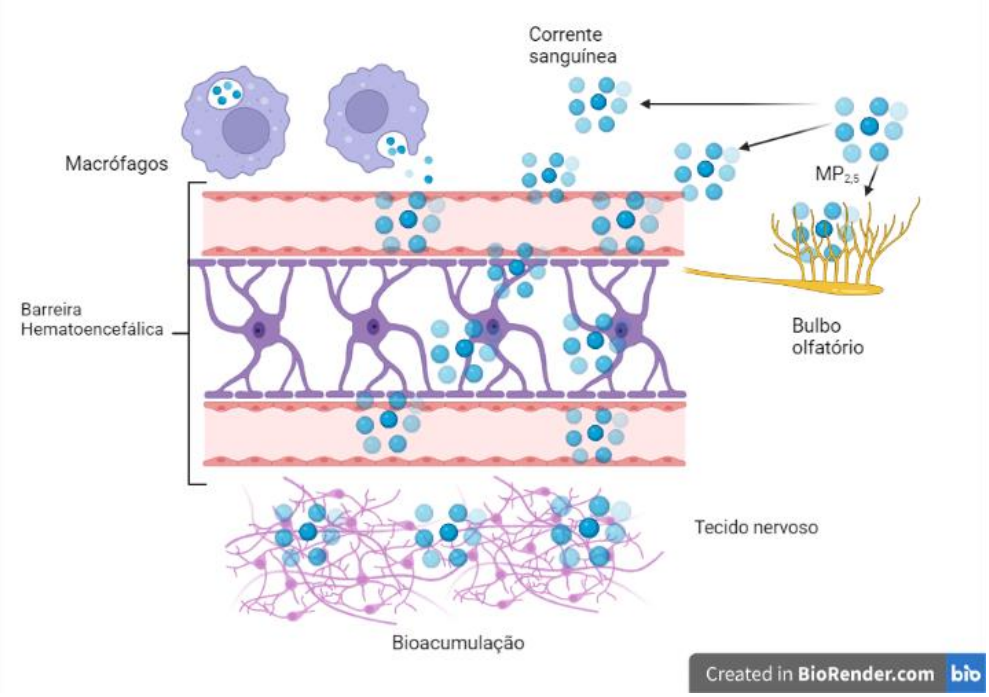

Figura 6: Vias de acesso do $MP_{2,5}$ ao SNC, resultando em bioacumulação.

Os mecanismos patológicos da bioacumulação do $MP_{2,5}$ estão relacionados por exemplo, a alteração da permeabilidade seletiva da barreira hematoencefálica, a liberação de mediadores inflamatórios, a ativação microglial, o aumento na produção de EROs, ao desequilíbrio sináptico e a desregulação hormonal, podendo ocorrer simultaneamente, nas mais variadas combinações (EMAM et al., 2020; HAHAD et al., 2020; NAKAGAWA; CHIBA, 2014; SAWICKI et al., 2019).

3.5 Estresse oxidativo e o Material Particulado

Após a exposição ao $MP_{2,5}$, dois processos cruciais se desenvolvem, o estresse oxidativo e a neuroinflamação. Ambos contribuem para a toxicidade dos componentes do MP ao SNC, indicando assim o comprometimento funcional das estruturas nervosas (CHURCH et al., 2018; COLE et al., 2016; MORRIS-SCHAFFER et al., 2018).

O SNC apresenta características singulares que o torna particularmente suscetível ao estresse oxidativo, como o alto consumo de oxigênio, a abundância lipídica e uma reposta antioxidante reduzida que representam a tríade de fatores básicos desta suscetibilidade (CHENG et al., 2016; RAO et al., 2019; WANG et al., 2019).

O estresse oxidativo ocorre quando a concentração de EROs supera a capacidade antioxidante, e a alteração do estado redox modifica biomoléculas como o ácido desoxirribonucleico (DNA), as proteínas e os lipídios levando a danos teciduais ou morte (BATES et al., 2019; HAHAD et al., 2020; ØVREVIK, 2019; TRABOULSI et al., 2017).

As EROs, o peróxido de hidrogênio (H_2O_2), o radical superóxido ($O_2^{\cdot-}$) e o radical hidroxila ($OH^{\cdot}$) apresentam um ou mais elétrons não pareados o que pode torna-los reativos as biomoléculas (BATES et al., 2019; COLE et al., 2016; JENG, 2010; SPAAS et al., 2021), desta forma a exposição ao $MP_{2,5}$ pode incrementar a produção, bem como, depletar as defesas antioxidantes (BATES et al., 2019; DE OLIVEIRA ALVES et al., 2020; HAHAD et al., 2020).

A ação deletéria das EROs é inibida e/ou reduzida pelo sistema de defesa antioxidante. Usualmente, esse sistema é dividido em não-enzimático (substâncias

endógenas ou exógenas) e enzimático, representado pelas enzimas superóxido dismutase (SOD), catalase (CAT) e glutationa peroxidase (GSH-Px). O desequilíbrio entre o potencial oxidativo e a defesa antioxidante é relatado como um dos principais fatores de agravamento ou desencadeador de alterações neurológicas relacionadas a exposição ao $MP_{2,5}$ (ALLEN et al., 2017; DE OLIVEIRA ALVES et al., 2020; PARK et al., 2021; ZHANG et al., 2018a).

O desequilíbrio redox também está relacionado com alterações na expressão gênica no SNC, foram reportadas reduções na expressão da SOD1, GSH e GSH-Px relacionadas a exposição ao $MP_{2,5}$, indicando deficiência na resposta antioxidante e aumento na síntese proteica de heme oxigenase-1 (HO-1), citocromo P450 1b1 (Cyp-1b1) e proteína de choque térmico 70 (HSP 70), as quais estão associadas a condições de estresse no SNC (KIM et al., 2020d; MILANI et al., 2020)

As regiões nervosas apresentam diferentes comportamentos frente ao dano oxidativo das EROs, estando relacionado a capacidade de resposta antioxidante e as interações entre o $MP_{2,5}$ e as biomoléculas. As características teciduais podem desencadear a toxicidade através da metabolização dos compostos do MP resultando em distintos níveis de estresse oxidativo nas regiões cerebrais, tais como hipocampo e córtex pré-frontal (DE OLIVEIRA ALVES et al., 2020; MILANI et al., 2020; SALVI; LIU; SALIM, 2020).

3.6 Respostas comportamentais

Segundo Quillfeldt (2006) a memória pode ser classificada por vários critérios, dentre eles a função (trabalho e referência), conteúdo (declarativa/explícita e de procedimento/implícita), duração (imediata ou de curto prazo e remota ou longo prazo), natural (associativa e não associativa) e motivação (recompensa e aversão). A compreensão dos mecanismos envolvidos na memória vem sendo objeto de estudo por várias décadas. No entanto, ainda permanecem vários questionamentos acerca das bases fisiológicas, moleculares, genéticas e bioquímicas relacionadas a este tema (POO et al., 2016).

A relação entre diversas redes neurais de diferentes estruturas nervosas formam a base anatômica e fisiológica que fornecem informações importantes para o entendimento das funções dos diferentes padrões de memória, como por exemplo, a formação hipocampal, o estriado e o córtex pré-frontal, associadas ao aprendizado, aos movimentos corpóreos e a memória de procedimento (KIM; MARTIN, 2015).

O hipocampo e as transmissões sinápticas excitatórias são essenciais para o aprendizado e a memória espacial, além de promover a plasticidade neuronal (WIN-SHWE et al., 2008).

O córtex pré-frontal desempenha um papel fundamental para inúmeros processos cognitivos e de execução, tais como a atenção, a tomada de decisão, a memória de procedimento e o comportamento. Em roedores, o córtex pré-frontal medial apresenta similaridades funcionais com a humana, organizado por diferentes áreas, incluindo os córtex pré e infralímbico. Ambos envolvidos no comportamento, na impulsividade e na formação de hábitos, respectivamente (VAN AERDE & FELDMEYER, 2015).

O comportamento dos animais frente a estímulos apresenta uma característica estereotipada, mesmo considerando as variações individuais, sendo possível identificar o significado dos movimentos em relação ao ambiente. Os experimentos de Pavlov em 1927, forneceram a base desta constatação (THOMPSON et al., 2017).

Deste modo, as avaliações comportamentais e dos processos de aquisição, consolidação e evocação da memória, podem fornecer informações que auxiliem no entendimento dos circuitos e mecanismos de codificação e armazenamento das informações (POO et al., 2016).

No caso de roedores, o comportamento exploratório representa uma condição fundamental para a sobrevivência da espécie, permitindo a compreensão dos mecanismos de interação inter e intraespecíficas e as relações com o ambiente. A utilização dos roedores, como objetos de estudo comportamental permite avaliar, entre outros fatores, a resposta frente a novidade, os mecanismos neurais implicados em determinados comportamentos e o efeito de diferentes tratamentos no que diz respeito ao SNC (ALLEN et al., 2014 b).

A atividade exploratória dos roedores pode ser analisada em uma variedade de tarefas que incluem, labirintos de múltipla escolha, cilindros e campo aberto. Tais

tarefas fornecem informações relevantes sobre as principais características comportamentais em diferentes situações (THOMPSON et al., 2017).

Dentre as respostas comportamentais mais comumente analisadas estão o *crossing* (número de cruzamentos nos quadrantes), o *grooming* (número de manipulações na face) e o *rearing* (número de permanência nas patas posteriores) e a interação com os objetos (QUILLFELDT, 2006; THOMPSON; BERKOWITZ; CLARK, 2018).

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A função de explorar o ambiente, característica dos roedores, é frequentemente descrita em termos conceituais como, medo e ansiedade, curiosidade e coleta de informações, além de aquisição de mapas espaciais do ambiente (THOMPSON; BERKOWITZ; CLARK, 2018)

A exposição ao MP tem sido associada ao aumento dos fatores de risco para autismo, deficiência cognitiva, isquemia, esquizofrenia e depressão em humanos. No comportamento animal observam-se alterações nos parâmetros relacionados a recompensa, aumento da ansiedade e redução da característica exploratória dos ratos. A avaliação comportamental dos animais é uma ferramenta bastante importante para o estudo de doenças do sistema nervoso em humanos (ALLEN et al., 2014 a).

4. Capitulos

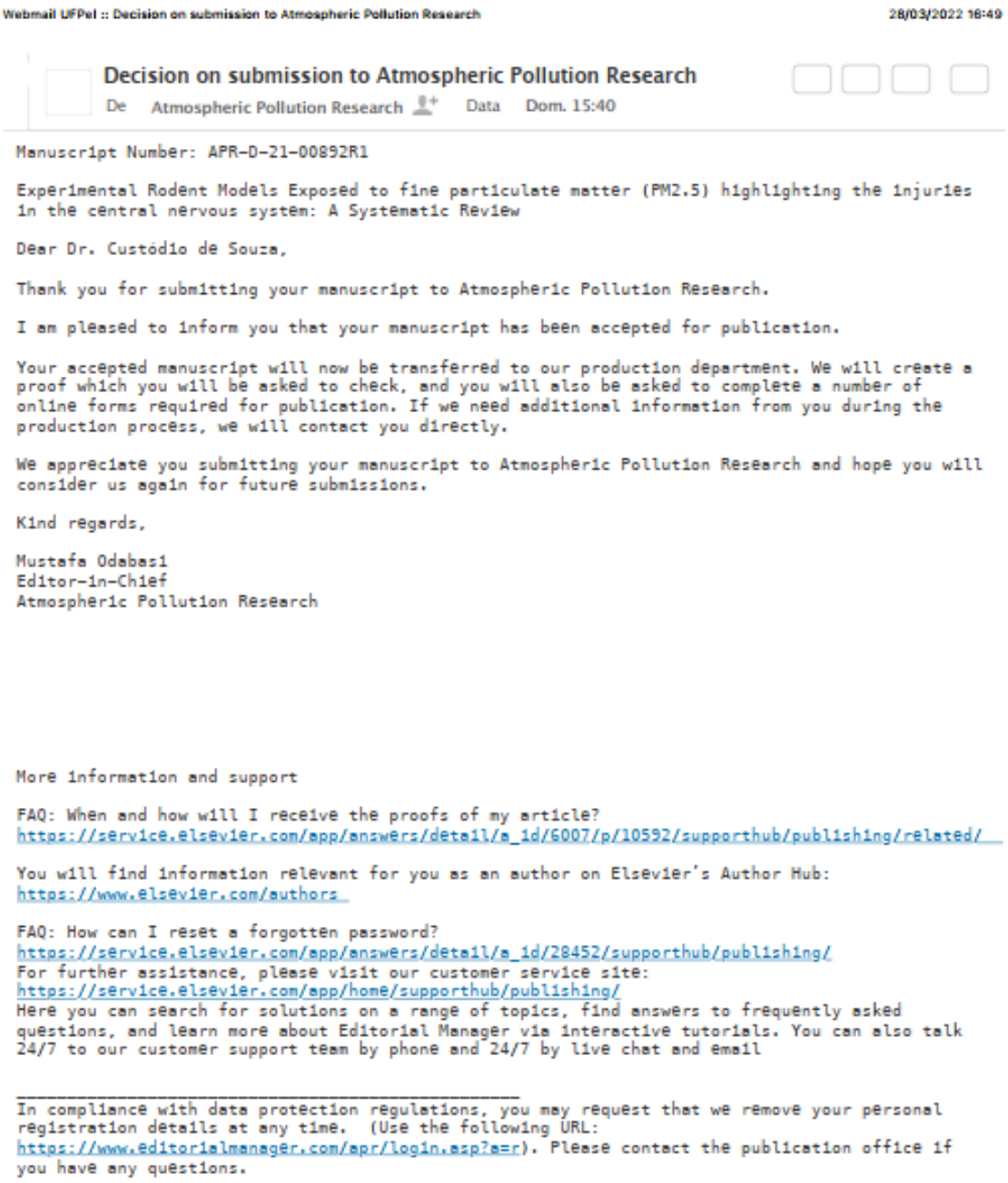
Os resultados que fazem parte do trabalho desenvolvido durante o período de Doutorado e estão apresentados sob a forma de um artigo de revisão e de um manuscrito original. As seções sobre os materiais e métodos, resultados, discussão e referências bibliográficas encontram-se nos respectivos artigos e representam a integralidade desse estudo.

As referências bibliográficas observadas no final da tese são referentes a introdução e revisão da literatura do corpo da tese.

Os artigos estão estruturados de acordo com as revistas as quais foram submetidos, respectivamente: *Atmospheric Pollution Research* (Artigo de revisão) e o *Behavioural Brain Research* (manuscrito original).

4.1. Artigo de revisão aceito para publicação pela revista *Atmospheric Pollution Research*

Confirming acceptance for publication Atmospheric Pollution Research



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Experimental Rodent Models Exposed to fine particulate matter (PM2.5) highlighting the injuries in the central nervous system: A Systematic Review

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4.1.1 Experimental Rodent Models Exposed to fine particulate matter ($PM_{2.5}$) highlighting the injuries in the central nervous system: A Systematic Review

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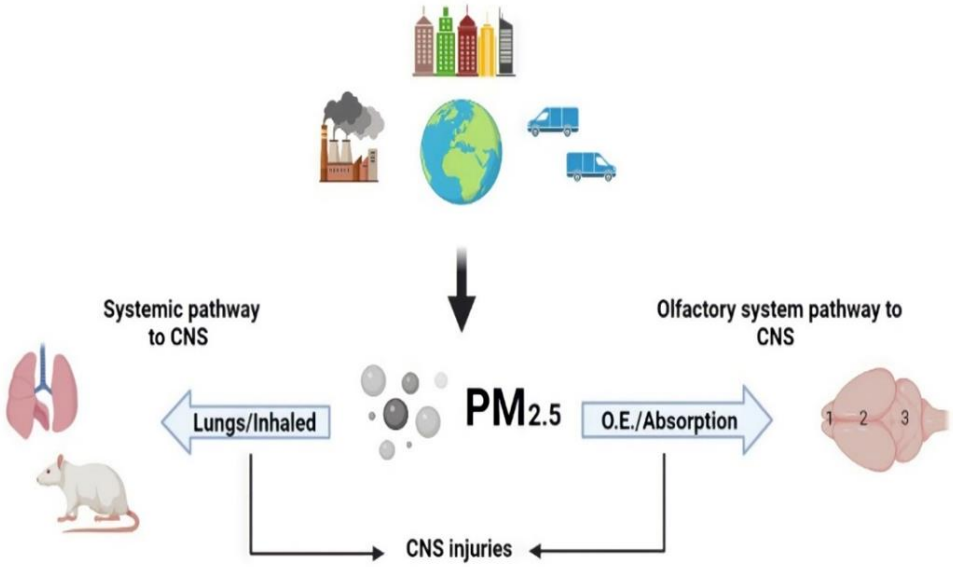

Introduction

Studies have suggested the association between exposure to air pollutants with the development of respiratory and cardiovascular system diseases (Cole et al., 2016; Hullmann et al., 2017). As exposure is constant, potential damage to health has become an important research topic to understand the mechanisms involved in the various changes in organic functions (Chuang et al., 2020; Haghani et al., 2020b; Ku et al., 2017b; Tyler et al., 2016). There are a large number of components present in the complex mixture of air pollution. These components can be derived from different sources such as fuel combustion by vehicles, diesel-powered transport, and local industrial processes (Cacciottolo et al., 2020; Ku et al., 2017b). The critical fraction of air pollution related to health problems in the population is called particulate matter (PM). The composition of PM includes, soil dust, organic and inorganic compounds, metals, ions, among others. Currently, PM is divided into three major size categories based on particle diameter: ultra-fine PM (UFPM, < 0.1 μm), fine PM ($PM_{2.5}$, < 2. 5 μm), and coarse PM (PM_{10}, < 10 and > 2.5 μm) (Allen et al., 2017; Kim et al., 2020). $PM_{2.5}$ is the major harmful component of air pollution, as it can reach the central nervous system (CNS) via absorption, which occurs in the olfactory system, and via inhalation, through the respiratory/circulatory system (Allen et al., 2017; Kim et al., 2020). Studies indicate that the interaction of $PM_{2.5}$ elements with the CNS can promote alterations in the morphophysiological functions, e.g. the signaling pathway evolving the protein kinase A (PKA)/cAMP element binding protein (CREB)/brain-derived neurotrophic factor (BDNF) (Liu et al., 2021). Alterations in the molecular mechanisms can be able to interferer on typical neurogenesis and synaptogenesis development, which are related to alteration of survival of primary neurons, morphology and strength of synaptic transmission. These morphophysiological disruptions are associated to expression of anxiety/depression like behaviors and impaired spatial learning and memory abilities in rodents (Allen et al., 2017; Chuang et al., 2020; Haghani et al., 2020a; Kim et al., 2020; Liu et al., 2021). One of the exposure pathways is the morphological changes in the nasal and olfactory epithelium resulting of the interaction of $PM_{2.5}$ with biological molecules present in cells. From the absorption in the olfactory tract the modifications can reach and alter the blood-brain barrier by compromising the permeability of cell membranes and, consequently, altering the flow of substances (Cheng et al., 2016; Kim et al., 2020). In the CNS, the stimuli produced by $PM_{2.5}$ activate the first line of defense via microglia cells (microgliosis), which release pro-inflammatory mediators and cytokines promoting the increase of reactive oxygen species (ROS), nerve growth factors, nitric oxide (NO), and toxicity-related chemotactic cytokines (Cacciottolo et al., 2020; Cheng et al., 2016; Kim et al., 2020; Woodward et al., 2017). In addition to the microgliosis, astrocytes are also activated (astrogliosis). These reactive cells are closely related to neuroinflammation state in rodent model exposed to $PM_{2.5}$ and are consistent to the human neurodegenerative conditions (Kulas et al., 2018; Onoda et al., 2020). The oligodendrocytes also plays an important role to the neurodevelopment, since the differentiation and maturation of this cells are critical to myelinization, ensuring the saltatory conduction of potential propagation (Klocke et al.,

2018a). The neurotoxicity of air pollution, especially the PM component is related to several injuries as myelination deficiency, risk of autism, dementia, cognitive failure, neuroinflammation, amyloidogenesis, glutamatergic impairment, and increase of anxiety and/or depressive behaviors (Haghani et al., 2020a; Woodward et al., 2017). Studies performed with animal models appoint that pre and postnatal exposure to the $PM_{2.5}$ is related to imbalance of synaptic pathways, altered neuronal morphology and density, and also neurotransmitter release which are compatible to that alterations found in central nervous system injuries (Allen et al., 2017; Cheng et al., 2017a; Woodward et al., 2017). The CNS has emerged as a mean target for the harmful actions of air pollution (Cole et al., 2016; Hullmann et al., 2017; Klocke et al., 2017). Exposure to $PM_{2.5}$ has been identified as an important factor related to adverse neurological outcomes such as neuroinflammation, neurodegenerative diseases, and impaired cognitive function (Chuang et al., 2020; Haghani et al., 2020a; Ku et al., 2017a; Tyler et al., 2016). To understand and clarify the mechanism involved in the $PM_{2.5}$ exposure and brain injuries the use of the rodent models is crucial. The results from experimental studies allowed the translational correlation with the human alterations, since some techniques cannot be performed in humans, the findings in the rodent exposure underlying the pathways of brain damage. Therefore, this review aimed to explore the findings of the knowledge of the interaction of $PM_{2.5}$ and biological molecules in the CNS, which is essential to understand the pathways that lead to morphophysiological changes related to neurological disorders.

2. Methods

2.1 Strategy for literature search

A search was conducted in the following electronic scientific literature databases: PubMed, Science Direct, SpingerLink and Web of Science, until July 2021.The terms used for the search included “air pollution”, “particulate matter”, “$PM_{2.5}$”, “central nervous system”, “CNS”, “brain”, “neurodegeneration”, “rodent”, “rat” and “mouse” that were based on Medical Subject Headings (MeSH®) terms (https://www.ncbi.nlm.nih.gov/mesh/), generating the search string: [(air pollution OR particulate matter OR $PM_{2.5}$ AND central nervous system OR CNS OR brain AND neurodegeneration AND rodent OR rat OR mouse)]. Refine filters were applied as follow, free full text, six years, not human, English were applied to narrow the search. Two researchers independently, performed the first stage of the selection strategy analyzing the titles and abstracts to identify the eligible studies. Subsequently, a third researcher reviewed the initial screening to define the final selection and performed the full text analyzes according to the applied inclusion and exclusion criteria. 47 articles were selected to compose this review.

2.2. Inclusion and exclusion criteria

After the initial screening of the search results recovered (n=161), the remaining articles (n=75) were reviewed carefully to identify potentially eligible studies through the application of the

inclusion and exclusion criteria. The inclusion criteria of the studies in this review were as follows: **(1)** Article published between 2016 to 2021; **(2)** Free full text available in the scientific databases; **(3)** Experimental data related to CNS morphophysiology; **(4)** Rodent experimental model; **(5)** Exposure to PM2.5 target the CNS. The exclusion criteria were as follows: **(1)** Reviews, duplicate, cohort studies; **(2)** Only *in vitro* experimental model; **(3)** Human exposure to $PM_{2.5}$. Studies that fulfill all the requirements were selected to compose this review (n=47). The review process flow diagram follows the PRISMA guidelines (Figure 1) (Page et al., 2020).

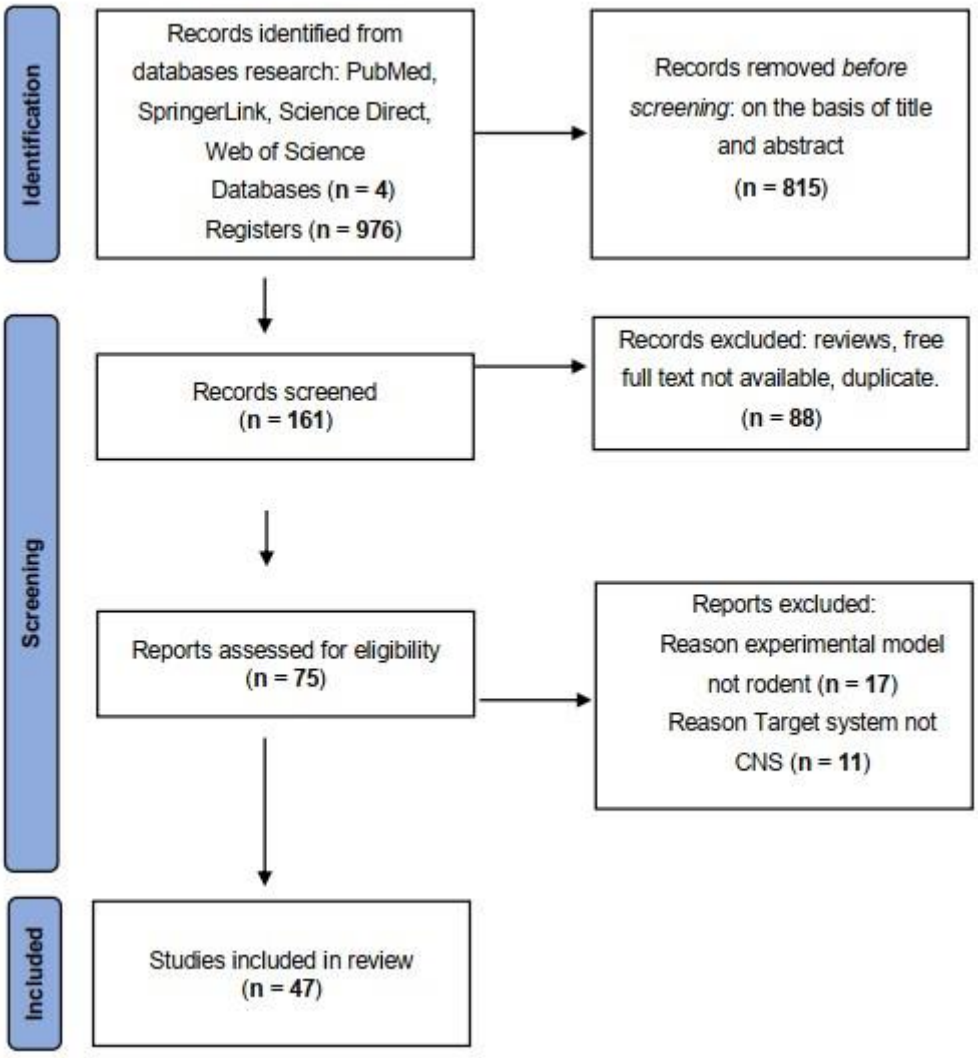

Figure 1: Flow chart describing the selection of the articles.

3. Results

3.1 Fine particulate matter ($PM_{2.5}$) toxicity

$PM_{2.5}$ represents an important fraction of the air pollution related to deleterious health issues, its heterogeneous composition promotes several alterations in the biological molecules, inducing damages and misfunction compromising the morphophysiology of the organic systems (Cory-Slechta et al., 2018; Ku et al., 2017a). $PM_{2.5}$ is basically compound by solid particles (e.g., soil dust, soot, pollen, etc.), liquid droplets, organic compounds (e.g., polychlorinated biphenyls, and polycyclic aromatic hydrocarbons) from many sources. Although the atmospheric gases (e.g., oxides of nitrogen, carbon monoxide, ozone, sulfur dioxide) are not considered as PM component, they can react with the compounds resulting in secondary particles, related to molecular alterations (Emam et al., 2020; Ku et al., 2017a; Liu et al., 2019). Each component can interact with the organic cells by different mechanisms inducing the homeostasis disruption. The major findings of the altered mechanisms related to exposure to $PM_{2.5}$ in experimental rodent model CNS are summarized in **Table 2**. $PM_{2.5}$ consist in a mixture of components, for this reason to understand the mechanisms associated to the CNS injuries a variety of experimental exposures are performed using different compounds (Ehsanifar et al., 2019; Morris-Schaffer et al., 2018b; Onoda et al., 2020; Tyler et al., 2016; Wen et al., 2021; Woodward et al., 2017; Yang et al., 2017). The experimental methodologies provide evidences that $PM_{2.5}$ exposure is indeed an important health risk factor to development, deterioration and misfunction of the CNS, in whole, partial or combined form (Babadjouni et al., 2018; Cheng et al., 2017; Cole et al., 2016; Klocke et al., 2017; Ljubimova et al., 2018; Morris-Schaffer et al., 2019; Nephew et al., 2020; Shih et al., 2018; Yang et al., 2017). Epidemiological data collected worldwide indicate that exposure contribute negatively to human health and is related to neurodegenerative diseases, behavioral disorders, decline in cognitive, memory and learn abilities (Allen et al., 2017; Cole et al., 2016; Ehsanifar et al., 2019; Hullmann et al., 2017; Patten et al., 2020; Wen et al., 2021). The rodent model were exposed as the follow characteristics **(i)** time duration (acute and chronic), **(ii)** gender (male and female), **(iii)** timelife (pre and postnatal), and also the $PM_{2.5}$ compound (**Figure 2**). The olfactory system is connected to CNS being responsible for olfative sense, therefore represents an important pathway to absortion of $PM_{2.5}$ allowing the particles traffic from de enviromnent to neural ambient (Cheng et al., 2016; Klocke et al., 2018a; Tyler et al., 2016). Evidences appointed to the disruption of the olfactory epithelium caused by $PM_{2.5}$ as a key factor for the neurological disorders, in addition the substances inhaled in the respiratory process can also reach the CNS promoting decrease of the brain-blood barrier protective permeability (H. H. Chen et al., 2018; Park et al., 2020; Rao et al., 2019). The homeostatic disturbance caused by the $PM_{2.5}$ is related to a variety of outcomes as, neuroinflammation, synaptic imbalance, altered neurodevolpment, increase of oxidative stress, apoptosis, morphophysiological

alterations, behavioral alterations and imparement in leraning, memory and cognite functions (Haghani et al., 2020b; Jia et al., 2018; Klocke et al., 2018a; Park et al., 2020).

Table 2. Description of the experimental procedures reported in the evaluated studies. Rodent models, exposure conditions, pathway (P)*, chamber (C), oropharyngeal aspiration (OA), intranasal instillation (InI), intratracheal instillation (InT), oral gavage (OG), dilution (D), suspension (S) and stomach tube (ST). Time (T)**, weeks (wks), days (d) and hours (h) and PM concentration*** ([PM]) and major findings mechanisms in the CNS related to $PM_{2.5}$ exposures. Altered mechanisms reported in the evaluated studies, microglial activation (MA), increase pro-inflammatory citokines (IPC), oxidative stress (OE), protein aggregation (PA), synaptic imbalance (SI), apoptosis (A), morphological changes (MC), memory and behavior impairment (MBI) and other changes (OC).

		Exposure conditions			Major findings mechanisms								
Reference	**Rodent model**	**P***	**T****	**[PM] *****	**MA**	**IPC**	**OE**	**PA**	**SI**	**A**	**MC**	**MBI**	**OC**
Cheng et al., 2016	C57BL/6J adult male	C	3 wks	150 µg mL	X	X	X	-	-	-	-	-	-
Cole et al., 2016	C57BL/6 and ApoE-/- male	C	6 h 30 d	300 µg m^{-3}	X	X	X	-	-	-	-	-	-
Tyler et al., 2016	C57Bl/6 male and female	C	6 h	250–300 µg m^{-3}	-	X	-	-	-	-	-	-	-
Allen et al., 2017	C57BL/6 adult male and female	C	13 d	96 µg m^{-3}	X	-	-	-	X	-	X	-	-
Cheng et al., 2017	Sprague-Dawley male	C	28 d	595 µg m^{-3}	-	-	-	-	-	-	X	-	-
Hullmann et al., 2017	5XFAD female	C	3 and 13 wks	0.95 µg m^{-3}	-	-	-	X	-	-	-	-	-
Klocke et al., 2017	B6C3F1 male and female	C	1 mth	92.69 µg m^{-3}	X	-	-	-	-	-	X	-	-
Ku et al, 2017	C57BL/6 mice	OA	4 wks	1 and 5 mg/kg bw	-	X	-	-	X	-	-	X	-
Ku et al., 2017 a	C57BL/6 young male	InI	28 d	10 mg mL^{-1}	-	-	-	-	-	X	-	X	-
Woodward et al., 2017	C57BL/6J young and adult female	C	10 wks	342 µg m^{-3}	X	-	-	-	X	-	X	-	-
Yang et al. 2017	Wistar young male	InT	10 d	500 µg m^{-3}	-	-	-	X	-	-	-	-	-
Babadjouni et al., 2018	C57BL/6J adult male	C	10 wks	305 µg m^{-3}	X	-	-	X	-	-	-	-	-
Chen et al., 2018	$IKK2^{Neu-KO}$	C	4 mths	3.6 and 71.6 µg m^{-3}	-	X	-	-	-	-	-	-	-
Church et al., 2018	B6C3 pregnant female	C	30 d	3.1 and 135.8 µg m^{-3}	-	-	-	-	-	-	-	X	-
Cory-Slechta et al., 2018	C57Bl6/J male litter	C	13 d	40 to 60 µg m^{-3}	-	-	-	-	-	-	-	X	-

Jia et al., 2018	C57 BL/6 J male	C	4 mths	91.3 and 17.9 μg m^{-3}	-	X	-	-	-	-	X	X	X
Klocke et al., 2018	B6C3F1 male and female	C	1 mth	92.7 and 3.5 μg m^{-3}	-	-	-	-	-	-	X	-	-
Klocke et al., 2018 a	B6C3F1 male and female	C	16.5 d	92.69 μg m^{-3}	-	-	-	-	-	-	X	-	-
Kulas et al., 2018	FVB male and female	C	22 d	46.70 μg m^{-3}	-	-	-	-	-	-	X	-	X
Ljubimova et al., 2018	Fisher adult rats	C	2 wks, 1, 3 and 12 mths	0.46 μg m^{-3}	-	X	-	-	-	-	-	-	X
Morris-Schaffer et al., 2018	C57BL6 newborn	C	13 d	48.1 μg m^{-3}	-	-	-	X	-	-	-	X	X
Shih et al., 2018	SD adult rats	C	24 wks	16.3 μg m^{-3}	-	X	-	-	-	-	X	-	-
Zhang et al., 2018	(SPF) Kunming female	InT	20 d	0.25, 1.5 and 3.45μg μL^{-1}	-	-	-	-	-	X	X	-	-
Cory-Slechta et al., 2019	C57Bl6/J male litter	C	13 d	22, 44, 53, 96 and 121 μg m^{-3}	-	-	-	-	-	-	X	X	-
Ehsanifar et al. 2019	NMRI pregnant female and male offspring	C	12 wks	350–400 μg DEPs m3	-	-	-	-	-	-	-	X	-
Jew et al., 2019	3xTgAD and NTg	C	8 d	57 μg m^{-3}	-	-	-	-	-	-	-	X	-
Liu et al., 2019	Sprague Dawley pregnant female and newborn	InI	60 d	180 μg m^{-3}	-	-	-	-	X	-	X	X	X
Morris-Schaffer et al., 2019	C57BL/6 J newborn	C	13 d	100 μg m^{-3}	-	-	-	-	-	-	-	-	X
Rao et al., 2019	$Adra2b^{Tg}$ male	C	12 wks	93.1 and 12.2 μg m^{-3}	-	X	X	-	X	-	-	X	-
Tseng et al., 2019	Sprague-Dawley	InT	21 d	0 and 2.5 mg kg^{-1}	X	X	-	-	-	-	X	X	-
Wang et al., 2019	SPF normal ICR pregnant female and offspring	InT and OG	28 d	3.456 μg μL^{-1}	-	X	X	-	-	-	X	X	-
Cacciottolo et al. 2020	J20- hAPPswe male	C	10 wks	300 μg m^{-3}	-	-	X	-	-	-	-	-	X

Campolin et al., 2020	C57BL/6 J or WT mice, and TLR4-deficient male	C	5 d and 12 wks	600 µg m^{-3}	X	-	-	-	-	-	-	-	X
Chuang et al., 2020	SH young male	C	3 and 6 mths	8.6 and 10.8 µg m^{-3}	-	-	X	X	-	X	-	-	-
Emam et al., 2020	Wistar rats pregnant and offspring	C	41 d	43.8 µg m^{-3}	-	-	-	-	-	-	-	X	-
Haghani et al., 2020	C57BL/6J pregnant female	C	3 wks	27 µg m^{-3}	-	-	-	-	X	-	-	-	X
Haghani et al., 2020 a	C57BL/6NJ young male	C	3 wks	100, 200, and 300 µg m^{-3}	-	-	-	-	-	-	-	X	-
Kim et al., 2020	Sprague-Dawley pregnant	D	24 h	5, 25, 50, and 100 µg mL^{-1}	X	X	X	-	-	-	-	-	-
Liu et al., 2020	Sprague-Dawley adult male and female	C	6 mths	100 µg m^{-3}	-	X	-	-	-	-	-	-	-
Milani et al, 2020	BALB/cOlaHsd young male	InT	6.5 d	50 µg of UFPs in 100 µL	-	-	X	-	-	-	-	-	-
Nephew et al., 2020	Sprague-Dawley pregnant female and male offspring	C	29 d	200 µg m^{-3}	-	-	-	-	-	-	X	X	-
Onoda et al., 2020	ICR pregnant female and male offspring	InI	12 wks	95 µg mL^{-1}	-	-	-	X	-	-	-	-	-
Park et al., 2020	C57BL6 adult male	C	3 wks	1000 µg m^{-3}	-	-	X	X	-	-	-	-	-
Patten et al., 2020	Sprague-Dawley male and female rats gestational and early postnatal	C	50 d	23.58 µg m^{-3}	X	-	-	-	-	-	-	-	-
Liu et al., 2021	Sprague-Dawley pregnant	S	3 d	50 µg mL^{-1}	-	-	-	-	X	X	-	-	-
Park et al., 2021	BALB/c young male	ST	12 wks	500 µg m^{-3}	-	X	X	-	X	X	-	X	-
Wen et al., 2021	C57BL/6 pregnant female and offspring	InT	22 d	250 and 2500 µg kg^{-1} bw	-	-	-	-	-	-	-	X	X

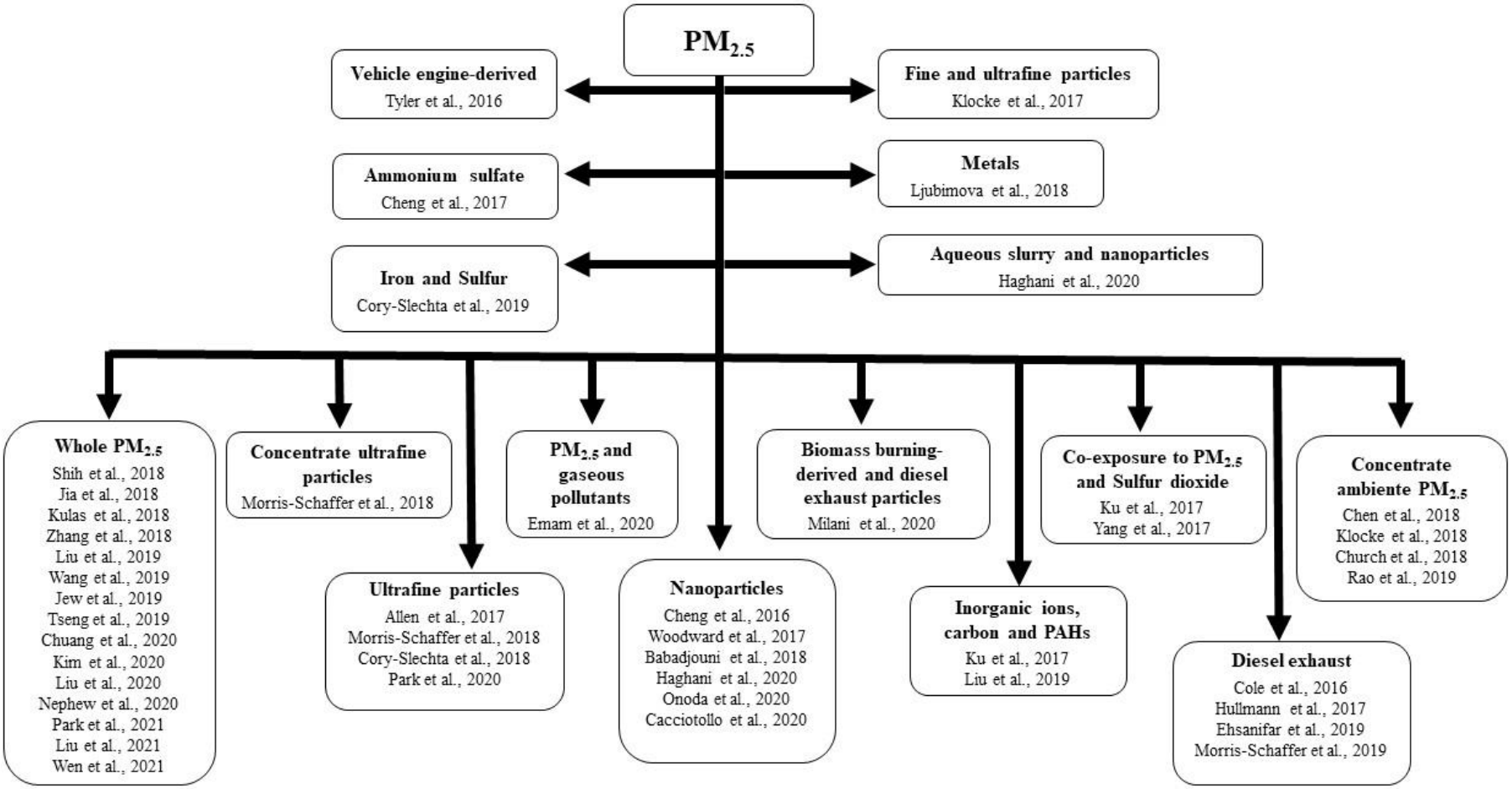

Figure 2. $PM_{2.5}$ components used to rodent exposure protocols.

The $PM_{2.5}$ compounds interaction with nervous tissue still remains unclear in many aspects, however some findings are described in reports of experimental studies. As the PM can be used in different composition, as showed in the Figure 2, the focused mechanism varies according the experimental techniques applied. The following sections describes the major mechanism regarding to $PM_{2.5}$ toxicity.

3.2 Microglial cells activation

The CNS possess resident innate immune cells, the microglia, these cells present an important role in the neuroprotective condition, however the microglial activation is also related to the neuroinflammation which may lead to the neurotoxic effects (Cole et al., 2016; Kim et al., 2020). Two microglial phenotypes are present in CNS, the M1 responsible for pro-inflammatory responses and the M2 related to anti-inflammatory actions (Kim et al., 2020; Patten et al., 2020). The imbalance in the microglial phenotypes increasing the M1 activation contributes to establishment of the neuroinflammation, to elucidate the role of the microglia activation some molecules related to inflammation were analyzed (Babadjouni et al., 2018; Tseng et al., 2019; Woodward et al., 2017). To evaluate the microglial activation as result of $PM_{2.5}$ exposure in rodent brain, the IBA1 microglial activation biomarker was analyzed and showed increase in the olfactory bulb and hippocampus (Cole et al., 2016), olfactory bulb and neuroepithelium (Cheng et al., 2016); in the CA1 hippocampal regions, stratum oriens and dentate gyrus polymorphic layer (Patten et al., 2020; Woodward et al., 2017), medial corpus callosum (Babadjouni et al., 2018), and in paraventricular nucleus and arcuate nucleus on the hypothalamus (Campolim et al., 2020). In addition to IBA1 analyses, other examinations were performed, the translocator protein 18 kD (TSPO) is considered a neuroinflammation biomarker produced in astrocytes and microglial cells, it was quantifying in different CNS regions, a discrete increase in the cerebellum and in multiple cortical regions, was observed in male, although no significate difference was presented, when associate with the result of IBA1 from the same study, indicate the microglia activation (Cole et al., 2016; Tseng et al., 2019) evaluated the expression of macrophages/microglia CD68 in the cortex and hippocampus, the exposure to $PM_{2.5}$ increased this inflammatory marker, providing evidences of phenotype M1 activation. The morphological aspect of the microglia in the hippocampus and corpus callosum (CC) associated to the myelination of CC provides indication of inflammatory microglial state, the changes in microglia morphology indicate microglial pro-inflammatory activation (Klocke et al., 2017), in

addition Allen et al. (2017) reported a size decrease of CC region also related to inflammatory state. The astrocytes activation combined to microgliosis, also plays an important role in the neuroinflammatory condition. In CNS injuries the astrocytes became reactive, increasing in size and number to protect the nervous tissue, however the astrogliosis is strongly related to a pro-inflammatory condition and to enhance the microglial activation (Kulas et al., 2018; Onoda et al., 2020). Rodents exposed to $PM_{2.5}$ presented an increase in the glial fibrillary acidic protein (GFAP) and activating transcription factor 6 (ATF6), reactive astrocytes markers, which indicates an upregulation in the expression of neuroinflammatory state. In addiction evaluation of oligodendrocytes morphology is related to altered neurodevelopment. Rodent exposure to $PM_{2.5}$ presents abnormal oligodendrocytes differentiation and maturation resulting in neurons hypo or hypermyelination, of some brain areas interfering in the conduction of potential propagation and cells density. (Klocke et al., 2018a). These findings support the hypothesis that $PM_{2.5}$ exposure is related to neuroinflammation in rodent model, by the combination of glial cells activation and altered morphology.

3.3 Increase Pro-inflammatory cytokines

Pro-inflammatory cytokines play an important role in the neuroinflammation state, being intimately related to microglial M1 phenotype activation, this protein set is predominantly activated by microglia/macrophages cell (Cole et al., 2016; Kim et al., 2020; Tseng et al., 2019). To demonstrate the relation between the $PM_{2.5}$ exposure and the CNS inflammatory state the level of expression of the pro-inflammatory cytokines (Cheng et al., 2016; Cole et al., 2016; Park et al., 2021; Tyler et al., 2016; Wang et al., 2019a; Yang et al., 2017) and its respective gene (H. H. Chen et al., 2018; Jia et al., 2018; Kim et al., 2020; Liu et al., 2020; Ljubimova et al., 2018; Rao et al., 2019; Tseng et al., 2019; Tyler et al., 2016; Yang et al., 2017) were measured. The studies focused predominantly in hippocampus, olfactory bulb, whole brain, and as the cytokines includes a variety of inflammatory mediators a detailed description is presented in the Table 3. Underlying the increase of cytokines level, some gene expressions were also finding, increase of hippocampal mRNA expression of Il-1β (Liu et al., 2020; Park et al., 2020; Tyler et al., 2016), mRNA TNFα, mRNA Interleukin-1α (IL-1α), mRNA IL-6 and mRNA NF-κB (H. H. Chen et al., 2018; Cheng et al., 2016; Jia et al., 2018; Kim et al., 2020; Liu et al., 2020; Park et al., 2021; Wang et al., 2019a), mRNA TNFα, IL-6 and TGF-β1 in the cortex suggesting an imbalance between pro and anti-inflammatory profile, (Yang et al., 2017).

Ljubmova et al. (2018) reported an upregulation of the expression of mRNA of IL-16 and IL-13α1, in addition, mRNA IL-6 and mRNA IL-12 increase in whole brain analyzes (Rao et al., 2019). The measurement of cytokines level and genes expression provides robust evidences that $PM_{2.5}$ exposure is strongly related to development of inflammation in the CNS, which represents an important pathway to disruption of neurological homeostasis.

Table 3. Description of the pro-inflammatory cytokines implicated in the neuroinflammation related to $PM_{2.5}$ exposure studies.

	Pro-inflammatory cytokines				
Reference	**IL**	**CHK**	**TGF**	**TNF**	**CNS region**
Cole et al 2016	1α, 1β, 3, 6	-	-	α	Olfactory bulb and hippocampus
Tyler et al 2016					
$ApoE^{-/-}$mice*	1β, 6 *	- *	β *	α *	Hippocampus
C57BL16 mice*	1β *	Ccl5, Cxcl1*	-*	α *	Hippocampus
$ApoE^{-/-}$mice**	6 **	Ccl5, Cxcl1**	β **	-**	Hippocampus
C57BL16 mice**	- **	-**	-**	-**	Hippocampus
Cheng et al 2016	-	-	-	α	Olfactory bulb and neuroepithelium
Yang et al 2017	6	-	-	α	Brain cortex
Shih et al 2018	6				Hippocampus
Ljubimova et al 2018	13-Rα1, 16	-	-	-	Brain
Chen et al 2018	6	-		α	Hypothalamus
Jia et al 2018	19	Cxcl1, Cxcl15, Cxcl17, Ccl2, Ccl25	-	-	Hippocampus
Wang et al 2019	1β	-	-	α	Hippocampus
Rao et al 2019	6,12	-	-	-	Brain
Tseng et al 2019	1β, 5	-	-	α	Brain
Kim et al 2020	1β, 6	-	-	α	Brain
Liu et al 2020	1β, 6	-	-	α	Brain
Park et al 2020	-	-	-	α	Olfactory bulb, hippocampus, and cerebellum

Abreviations: IL (interleukin); Chk (chemokines); TGF (*transforming growth factor*); TNF (tumor necrosis factor); CNS (central nervous system). *acute exposure, **chronic exposure

3.4 Oxidative stress

To sustain the CNS normal function, the biochemistry integrity is fundamental. The exposure to $PM_{2.5}$ is an important pathway that produces biochemical impairment, through the increase of oxidative stress (Cole et al., 2016; Park et al., 2020; Wang et al., 2019a). The high oxygen consumption, lipid-rich content and a poor antioxidant response system characterize the CNS high susceptible to the oxidative stress, which are related to neurodegenerative and neuropsychiatric disorders (Cheng et al., 2016; Rao et al., 2019; Wang et al., 2019a). The mitochondrial adenosine triphosphate (ATP) synthesis is the

major source of free radical, as reactive oxygen species (ROS), reactive nitrogen species (RNS) and carbon- and sulfur-centered radicals, under physiological conditions the free radicals are neutralized by the antioxidative system (Milani et al., 2020; Park et al., 2021; Wang et al., 2019a). The exposure of experimental models to the $PM_{2.5}$ results in oxidative imbalance increasing the stress conditions. Measurement of malondialdehyde (MDA) and 4-HNE trans-4hydroxy-2noneal levels are used as a marker of lipid peroxidation which are related to increase of oxidative stress, the rodent models exposed to $PM_{2.5}$ exhibit an increase of MDA (Chuang et al., 2020; Cole et al., 2016; Park et al., 2021; Wang et al., 2019a) and 4-HNE trans-4hydroxy-2noneal (Cacciottolo et al., 2020; Cheng et al., 2016) levels in the CNS. The ROS are considered the major component of the redox imbalance, however the RNS emerged as an important factor in the oxidative stress condition, the $PM_{2.5}$ exposure demonstrate the increase of 3-nitrotyrosine (3-NT) a marker of RNS, that indicate the important role of these free radicals in the biochemical impairment (Cheng et al., 2016). The redox imbalance affects the gene expression related to stress in the CNS, a decrease of superoxide dismutase 1 (SOD1), glutathione peroxidase (GSH and GSH-Px) expression indicates a decline of antioxidant response as result to the $PM_{2.5}$ exposure (Rao et al., 2019; Wang et al., 2019a). To maintain the cellular homeostasis some proteins are synthetized in injuries conditions, an increase in Heme oxygenase-1 (HO-1), Cytochrome P450 1b1 (Cyp1b1), Heat shock protein (HSP 70) levels as result to the $PM_{2.5}$ exposure indicates the establishment of oxidative stress conditions (Kim et al., 2020; Milani et al., 2020). The increase of oxidative stress in the CNS represents a crucial pathway to aggravate the neurological misfunction.

3.5 Protein aggregation

Proteogenesis represents a critical process to maintain the organic homeostasis, to achieve the accurate folding and stable protein conformation is necessary the correct function of the cells machinery as the signaling, transcription, translation, post translational modifications, degradation and the support of the chaperones, alterations in any phase could lead a disruption in the proteostasis resulting in health issues (Babadjouni et al., 2018; Hullmann et al., 2017; Onoda et al., 2020; Park et al., 2020; Yang et al., 2017). Neurotoxicity is often related to aggregation of misfolded proteins or altered expression of this biomolecules, Table 4.

Table 4. Description of altered proteins related to $PM_{2.5}$ exposure

Reference	Proteins	CNS region
Yang et al 2017	**Aβ**	cortex, hippocampus
Hullmann et al 2017	**Aβ**	brain
Babajouni et al 2018	**C5**	corpus callosum
Chaung et al 2020	**Tau**	olfactory bulb
Onoda et al 2020	**B-sheet-rich**	brain
Park et al 2020	**Aβ**	olfactory bulb, cortex, hippocampus, cerebellum

The exposure to $PM_{2.5}$ represents an important mechanism of altered protein formation, this process is closely associated to neurodegenerative diseases, as Alzheimer (Chuang et al., 2020; Hullmann et al., 2017; Park et al., 2020; Yang et al., 2017), ischemic conditions (Onoda et al., 2020) and neuroinflammation (Babadjouni et al., 2018). Aβ and Tau protein aggregation indicates the development of Alzheimer disease phenotype in the experimental models exposed to $PM_{2.5}$ revealing the connection between the air pollution and neurological disfunction (Chuang et al., 2020; Hullmann et al., 2017; Park et al., 2020; Yang et al., 2017). The complement system is a set of proteins related to immunological response, C5 and C5α proteins are ones of these immunoproteins, the C5 and C5α overexpression and accumulation in corpus callosum was associated to microglial activation in the experimental model expose to $PM_{2.5}$ increasing the neuroinflammation (Babadjouni et al., 2018). β-sheet-rich proteins represents a group of misfolded proteins, overexpression and accumulation of these altered proteins around the brain blood vessels related to $PM_{2.5}$ exposure resulting in ischemic conditions which could initiate or aggravate neurodegenerative diseases (Onoda et al., 2020). These findings suggest that proteopathologies are related to $PM_{2.5}$ exposure resulting in neurodegenerative diseases.

3.6 Synaptic imbalance

CNS activity is sustained by a network of synaptic excitatory and inhibitory balance that regulates neuronal plasticity, excitability and connectivity, thus disturbance in this system results in neurological misfunction (Haghani et al., 2020a; Liu et al., 2021, 2019; Morris-Schaffer et al., 2018b; Woodward et al., 2017). Excitatory and inhibitory imbalance (E/I imbalance) modify the neural coding process resulting in neurodegenerative diseases, behavioral alterations, memory and learning impairment and anxiety /depression state (Allen et al., 2017; Haghani et al., 2020a; Ku et al., 2017a; Park et al., 2021; Rao et al., 2019). Neurotransmitters promotes the neuronal communication (chemistry synapsis)

through the binding on its receptor, the E/I imbalance is related to neurotransmitter release and receptor expression regulation (Allen et al., 2017; Haghani et al., 2020a; Morris-Schaffer et al., 2018b; Park et al., 2021; Rao et al., 2019). Glutamate (GLU) represents the main excitatory neurotransmitter related to several neural functions, the exposure to $PM_{2.5}$ modify the hippocampal release and expression of the GLU receptors, increase in GLU release indicates the association with excitotoxicity related to E/I imbalance in neuronal disfunction (Allen et al., 2017; Woodward et al., 2017), in addition the receptor expression contributes to the glutamatergic profile in the misfunction of CNS (Allen et al., 2017; Ku et al., 2017a; Woodward et al., 2017). Dopamine is an important regulatory neurotransmitter between the striatum and prefrontal cortex, the E/I imbalance on this transmitter arising as a pathway related to behavioral and motor functions (Haghani et al., 2020a; Morris-Schaffer et al., 2018b), the increase of the expression of α2B-adrenergic brain receptor was also related to behavioral alterations (Rao et al., 2019). Cholinergic system presents misfunction in the experimental model exposed to $PM_{2.5}$ an increase of Acetylcholinesterase activity leading a faster degradation of the Acetylcholine in the synaptic cleft resulting in cognitive impairment (Park et al., 2021). Decrease in protein expression related to neuronal development, e.g., synaptophysin and PKA/CREB/BDNF pathway, indicate CNS misfunction, resulting in E/I imbalance abnormal neural tissue formation (Haghani et al., 2020a; Liu et al., 2021, 2019). The synaptic imbalance represents an important issue to comprehend the involvement of chemistry synapsis and the neurological disorders.

3.7 Apoptosis

In physiological conditions the apoptosis represents a control mechanism of cellular growth, however injuries in the CNS provokes an apoptotic imbalance resulting in increase of cell death (Chuang et al., 2020; Ku et al., 2017a; Park et al., 2021). Caspases comprehend a endoproteases family related to apoptosis, an activation of these Caspases indicates an increase in the cell loss (Chuang et al., 2020). The $PM_{2.5}$ exposure is associated to neuronal death associated to Caspase-3; Caspase-8 and Caspase-9 activation resulting in neurodegeneration, morphological and behavioral alterations, memory and learning impairment (Chuang et al., 2020; Ku et al., 2017a; Liu et al., 2021; Park et al., 2021; Zhang et al., 2018).

3.8 Morphological changes

The morphological analyzes are important to evaluate the damages caused by the $PM_{2.5}$ exposure, the loss of the normal anatomy is related to misfunction of the CNS. Table 5

Table 5. Summarized morphological alterations in CNS related to $PM_{2.5}$ exposure

Reference	Alterations
Klocke et al 2017	Ventriculomegaly, increased corpus callosum, reduced hippocampal area, frontal cortex thickness, early myelinization
Allen et al 2017	Ventriculomegaly, reductions in size of the corpus callosum (CC), hypomyelination, aberrant white matter development and/or structural integrity
Woodward et al 2017	Neurite atrophy and hypomyelination of CA1 hippocampal and dentate gyrus,
Cheng et al 2017	decrease in maturation of newborn neurons of hippocampal subgranular zone
Shih et al 2018	Increased volumes of whole-brain and hippocampal volumes, spongiosis and neuronal shrinkage in the cortex, cerebellum, and hippocampus
Klocke et al 2018	ventriculomegaly, hypermyelination
Jia et al 2018	impaired the neuron structure in mice hippocampus
Klocke et al 2018 a	ventriculomegaly, periventricular hypermyelination, and enlargement of the corpus callosum
Zhang et al 2018	impairments on brain tissue
Kulas et al 2018	brain development may be impaired
Liu et al 2019	fewer synapses, thinner post- synaptic density, and shorter active zone in immature and mature rats
Wang et al 2019	Hippocampal neurodevelopmental impairment
Cory-Slechta et al 2019	Ventriculomegaly
Tseng et al 2019	Decreased dendritic branches in CA1 and CA3 hippocampal
Nephew et al 2020	neurite atrophy in the hippocampus

The evaluation of the structural alterations represents an important tool to understand the nervous tissue response to the $PM_{2.5}$ exposure. Many studies report hippocampal analysis since this area is related to depression-like and anxiety-like behaviors (Cheng et al., 2017a; Jia et al., 2018; Klocke et al., 2017; Nephew et al., 2020; Shih et al., 2018b; Tseng et al., 2019; Wang et al., 2019b; Woodward et al., 2017). The hippocampal findings as atrophy (Klocke et al., 2017; Nephew et al., 2020; Shih et al., 2018b; Tseng et al., 2019; Woodward et al., 2017), immature cells formation (Cheng et al., 2017a; Jia et al., 2018), hypomyelination (Klocke et al., 2017; Woodward et al., 2017) and impaired neurodevelopment formation (Allen et al., 2017; Jia et al., 2018; Klocke et al., 2017; Kulas et al., 2018; Shih et al., 2018b; Wang et al., 2019b; Zhang et al., 2018) were observed in histological techniques as immunohistochemistry, hematoxylin and eosin (HE) stained slides, micrographs, transmission electron microscopy (TEM) and magnetic resonance imaging (MRI). The authors related the $PM_{2.5}$ exposure toxicity to some

mechanisms as alteration BBB permeability, increase of oxidative-stress and altered antioxidant capacity, pro-inflammatory state, immune and hormonal response disruption, which ones affected the neurogenesis and synaptogenesis resulting in abnormal brain morphology. The $PM_{2.5}$ toxicity compounds were related to increase of metal, that is similar to the autism spectrum disorder in humans including persistent ventriculomegaly, initial increases followed by sustained loss of white matter and gray matter, loss of myelin connectivity across the hemispheres, persistent brain inflammation and excitatory (glutamate)-inhibitory (GABA) imbalance resulting in behavior alterations and oligodendrocytes maturation and myelinization alteration (Cory-Slechta et al., 2019; Jia et al., 2018; Klocke et al., 2018a, 2018b, 2017; Tseng et al., 2019). Morphological alteration is also related to injuries to structural plasticity altering the synaptic ultrastructure promoting signaling deficit between pre and postsynaptic membranes (Liu et al., 2019; Tseng et al., 2019; Wang et al., 2019b; Zhang et al., 2018). Also mitochondrial ultrastructural damages were reported, as broken and partly blurred mitochondrial cristae, fuzzy and broken nuclear membrane, and autophagic bodies in the cytoplasm, those findings are related to apoptosis increase (Zhang et al., 2018). The traffic-related air pollution (TRAP) and $PM_{2.5}$ exposure induced decrease in neurogenesis, BBB permeability alteration and impairs neuronal differentiation (Liu et al., 2021; Nephew et al., 2020).

3.9 Cognitive and Behavioral impairment

Cognitive and behavioral functions are closely related to the normal CNS development, exposure to $PM_{2.5}$ induces morphophysiological alteration since the early stages of embryogenic period, until the adult life (Church et al., 2018; Jew et al., 2019a; Liu et al., 2019). Disruption in the nervous structures and synapses network are the major factors associated to decrease of memory and learning functions (Cory-Slechta et al., 2018; Morris-Schaffer et al., 2018b; Park et al., 2021), behavioral alterations, as reduced sociability and anxiety (Church et al., 2018; Emam et al., 2020; Wang et al., 2019a) and depression, autism like behavior (Cory-Slechta et al., 2019; Ehsanifar et al., 2019; Emam et al., 2020; Haghani et al., 2020a; Jia et al., 2018; Liu et al., 2019; Nephew et al., 2020; Rao et al., 2019; Wen et al., 2021). To measure the cognitive and behavioral parameters the experimental models were exposed to $PM_{2.5}$ and submitted to behavioral tasks, the results provide evidences of impairment in the abilities to interact with the environment displaying memory failure, delay learning and increase of anxiety (Cory-Slechta et al.,

2018; Ehsanifar et al., 2019; Emam et al., 2020; Liu et al., 2019; Morris-Schaffer et al., 2018b; Nephew et al., 2020; Park et al., 2021; Rao et al., 2019; Wen et al., 2021), in addition the tasks also indicate the behavior-like depression and autism and reducing of sociability (Church et al., 2018; Cory-Slechta et al., 2019; Haghani et al., 2020a; Jew et al., 2019b; Jia et al., 2018; Liu et al., 2019; Tseng et al., 2019; Wang et al., 2019a). The impairment of cognitive and behavioral decrease highlighting the CNS as an important target to $PM_{2.5}$ exposure and is strongly related to neurological disorders.

3.10 Other changes

The interaction of the organic systems with the CNS function related to $PM_{2.5}$ exposure is crucial to understand the pathways which may lead to neural disruption (Haghani et al., 2020a; Jia et al., 2018; Ku et al., 2017a; Kulas et al., 2018; Wen et al., 2021). Gene expression represents an important molecular tool which provides evidences of altered cellular signaling to establish the relation of genic alterations and CNS misfunction (Ku et al., 2017a; Ljubimova et al., 2018). Evidences indicate the diet type associated to $PM_{2.5}$ exposure affect the body weight and insulin resistance altering the energetic metabolism resulting in impaired CNS neurodevelopment and decreasing the neurological functions (Campolim et al., 2020; Haghani et al., 2020a, 2020b; Morris-Schaffer et al., 2019, 2018a). Endocrine control is a major mechanism to sustain the organic homeostasis, exposure to $PM_{2.5}$ is related to alteration in the hormones release, the adrenal and thyroid glands are controlled by the neuroendocrine axis Hypothalamus-Pituitary forming the HPA and HPT axis, respectively (Jia et al., 2018; Liu et al., 2019; Wen et al., 2021). The experimental animal models exposed to $PM_{2.5}$ presents dysregulation in the CNS response to the hormonal control, as these glands regulates key points of organic functions, impaired morphophysiological functions were observed (Jia et al., 2018; Liu et al., 2019; Wen et al., 2021), resulting in increase of susceptibility of CNS misfunction. The systemic immune response to $PM_{2.5}$ exposure was also implicating in the CNS misfunction, related to the altered development of the immune system in pre-natal exposed rodents, the findings indicate an increase of reactivity of the immune cells, and of the pro-inflammatory pathways in both, systemic and nervous immunological responses altering the immune phenotype in the offspring (Kulas et al., 2018).

4. Discussion

This literature review focused in the findings from the last five years concerning to the $PM_{2.5}$ exposure in rodent experimental model and the implication on the CNS. Forty-seven articles were analyzed to collect the evidences that indicates the mechanisms involved in morphophysiological alterations. $PM_{2.5}$ is a mixture of compounds, fractioned or whole PM were used by the authors to elucidate the $PM_{2.5}$ and CNS interaction, as described in Figure 2. The studies were performed in different rodent models, as C57B1/6 mice, Sprague-Dawle rats, Wistar rats, B6C3F1 hybrid mice, NMRI mice, ICR mice, J20hAPPswe mice, FVB mice, SPF Kunming mice, SPF normal ICR mice, BALB/c mice, Fisher rats, IKK2Neu-KO mice, Nestin-creIKKflox$^{=+}$ mice, Adra2bTg mice and BALB/cOlaHsd mice, each strain display characteristics specifications considered in the studies conduction. The methods used to expose de rodents to the $PM_{2.5}$ were inhalation or instillation, in pre or postnatal stages of life, in both, male and female animals. Regardless these differences among the studies, the CNS alterations reveal a bias to develop. The microglial activation was found by different authors (Allen et al., 2017; Babadjouni et al., 2018; Campolim et al., 2020; Cheng et al., 2016; Cole et al., 2016; Kim et al., 2020; Klocke et al., 2017; Patten et al., 2020; Tseng et al., 2019; Woodward et al., 2017) indicating that $PM_{2.5}$ exposure is a trigger to neuroinflammation trough the microglia M1pro-infammatory phenotype. The activation of the immune cells in the CNS is related to neurotoxicity since the neurons, astrocytes and oligondedrocytes became targets to the microglia actions, resulting in lost of cell homeostasis, which could initiate and/or agravate neurodegenerative conditions. In addiction to the microglial activation the increase of pro-inflammatory citokines was also found in several studies (M. Chen et al., 2018; Cheng et al., 2016; Cole et al., 2016; Jia et al., 2018; Kim et al., 2020; Ljubimova et al., 2018; Park et al., 2021; Rao et al., 2019; Shih et al., 2018a; Tseng et al., 2019; Tyler et al., 2016; Wang et al., 2019a; Yang et al., 2017). This findings are closely related to the microglia action increasing the neuroinflammatory state, the pro-inflammatory citokines plays an important role in the inflammation signaling pathway. The exposure to $PM_{2.5}$ is associated to systemic inflammatory state which may achieve the CNS thru the blood stream, and induce the citokines production (Chen et al., 2018; Cheng et al., 2016; Park et al., 2021; Tseng et al., 2019; Tyler et al., 2016). The disruption of CNS functions is strongly related to the increase of oxidative stress. The free radicals ROS, RNS and carbon- and sulfur-centered react with biomolecules damaging the blood-

brain barrier and plasmatic membrane, altering the CNS environment and intracellular medium, allowing the entrance of abnormal substances (Cacciottolo et al., 2020; Cole et al., 2016; Haghani et al., 2020b; Park et al., 2021). The unique feature of the CNS of high oxygen consumption, content of lipids and an insufficient antioxidant defense promotes a vulnerability to the oxidative stress, relating this process to impairment of CNS functions, development and neurodegenerative diseases (Cacciottolo et al., 2020; Cheng et al., 2016; Kim et al., 2020; Milani et al., 2020; Park et al., 2020; Wang et al., 2019a). As result to $PM_{2.5}$ misfolding proteins were observed (Babadjouni et al., 2018; Hullmann et al., 2017; Onoda et al., 2020; Park et al., 2020; Yang et al., 2017). The proteophatologies represents the core of the neurodegeneration associated to Alzheimer, Parkinson and Huntington diseases among others. These findings suggest that the $PM_{2.5}$ exposure induces the loss of proteostasis in some level of the protein synthesis (Babadjouni et al., 2018; Hullmann et al., 2017; Onoda et al., 2020; Park et al., 2020; Yang et al., 2017). In addiction misfolded proteins is also related to neurotoxicity stimulating the neuroinflammatory state (Babadjouni et al., 2018; Hullmann et al., 2017; Onoda et al., 2020; Park et al., 2020; Yang et al., 2017). Morphophysiological alterations were found impairment in the synaptic network (Haghani et al., 2020b; Liu et al., 2021, 2019; Morris-Schaffer et al., 2018a; Woodward et al., 2017), increase of apoptosis (Chuang et al., 2020; Ku et al., 2017a; Park et al., 2021) and abnormal neurodevelopment (Allen et al., 2017; Cheng et al., 2017a; Cory-Slechta et al., 2019; Jia et al., 2018; Klocke et al., 2017, 2018b, 2018a; Kulas et al., 2018; Liu et al., 2019; Nephew et al., 2020; Shih et al., 2018a; Tseng et al., 2019; Wang et al., 2019a; Woodward et al., 2017; Zhang et al., 2018). The synaptic imbalance, increased apoptosis and abnormal neurodevelopment presents an interrelation development, the pre-natal exposure of the pregnant females results in altered morphology of the CNS areas, decrease and impaired of the neurotransmission and, also induces the cells death. The association of these features compromising the neuro system function. Furthermore, all the findings described above culminate to cognitive impairment and behavioral alterations. The rodent models were submitted to tasks to evaluate the spatial, short- and long-lasting memory, novel objects recognition related to cognitive functions (Cory-Slechta et al., 2019; Morris-Schaffer et al., 2018b; Park et al., 2021). The behavior was analyzed based on anxiety (Church et al., 2018; Emam et al., 2020; Wang et al., 2019a), depression and autism like (Cory-Slechta et al., 2019; Ehsanifar et al., 2019; Emam et al., 2020; Haghani et al., 2020a; Jia et al., 2018; Liu et al., 2019; Nephew et al., 2020; Rao et al., 2019; Wen et al., 2021). The

findings suggest that rodent models exposed to $PM_{2.5}$ presents impairment of these parameters, which can highlight the mechanisms involved in the neuropathological conditions in human, exposed to air pollution. The gene expression (Ku et al., 2017a; Ljubimova et al., 2018), the variation in the body weight and insulin resistance (Campolim et al., 2020; Haghani et al., 2020a, 2020b; Morris-Schaffer et al., 2019, 2018a), the endocrine misfunction of the HPA and HPT axis (Jia et al., 2018; Liu et al., 2019; Wen et al., 2021) and the immune system (Kulas et al., 2018) responses to $PM_{2.5}$ were also analyzed providing evidences regarding the influence of these altered mechanisms in the development of neuroinflammatory state. The understanding of this correlation can indicate in what manner the CNS is affected by inhalation or absorption of $PM_{2.5}$. Two studies are carried out to test substances which one could minimize the effects of $PM_{2.5}$ in the CNS, vitamin B (Wang et al., 2019a) and *Ecklonia cava* (Park et al., 2021), both compounds emerging as promising treatments to relief the damages in the CNS, further studies are necessary to clarify its pharmacological use. The reviewed findings provide important evidences that, exposure of whole or fractions components of the $PM_{2.5}$, by the inhalation or absorption in any stage of life is strongly related to misfunction of the CNS. The studies indicate that alterations emerging as correlated process, displaying a cascade of events, pro-inflammatory state is associated to microglial activation, increase of pro-inflammatory citokines and oxidative stress. The interaction of these mechanisms results in an increment of each other in a looping feature. In addition, the stablishing of the inflammatory conditions, may lead to an altered proteostasis, apoptosis and morphological changes, culminating with the cognitive and behavioral alterations. We concluded that complexity of the mechanisms associated to neurodegenerative and neurological diseases related to $PM_{2.5}$ exposure implicates in further studies, to understanding and clarify the interactions, the molecular and genetic pathways, allowing the future prevention actions and development of novel treatments to minimize the CNS injuries. The epigenetic approach and the relationship between the PM exposure and brain-gut axis emerging as an important studies subject. In addiction the use of different rodent model can provide crucial information about the development of neurological outcomes as result of $PM_{2.5}$ exposure.

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4.2 Manuscrito submetido a revista *Neurotoxicology*

Cover Letter to Neurotoxicology

Pelotas, April , 2022.

Editors - in - Chief

Pamela J. Lein

Remco Westerink

Dear Editors,

We are sending to you the revised version of a manuscript "Memory and behavior study of fine particulate matter (**$PM_{2.5}$) intranasal exposure during pregnancy and nursing** in Wistar rats model." by APS Ferreira and colleagues to be submitted for publication in Neurotoxicology (ISSN: 0161-813X). This manuscript has not and will not be published in whole or in part in any other journal. Our study analyzes the findings of the effects of particulate matter intranasal exposure in female Wistar during pregnancy and nursing and their respective offsprings regarding behavioral, memory outcomes and oxidative stress parameters. And also quantify metal compounds present in the air samples. We would like to publish in Neurotoxicology because we know that publication in this journal presented a good appreciation and relevance.

The authors declare that are no conflicts of interest.

I will await your guidance on the manuscript.

Thank you very much for your attention,

Best regards,

Izabel Cristina Custódio de Souza, PhD
Professor and Researcher at Universidade Federal de Pelotas

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Submission confirmation

Neurotoxicology
Memory and behavior study of fine particulate matter (PM2.5) intranasal exposure during pregnancy and nursing in Wistar rats model.

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Abstract:	Exposure to the air pollution is considered an important health risk factor, particulate matter 2.5 (PM2.5) is referred as harmful component of the environmental contamination. The composition of PM2.5 is heterogeneous and contains solid and liquid particles, biological and non-biological elements. The compounds found in the PM2.5 vary according the localization, particle size, weather conditions, industrial activities, geological characteristics and population density, among others. PM2.5 encompasses fine particles (0.1 to 2.5 μm diameter) that are associate to organic damages, by inhalation and/or translocation to the tissues environment. The central nervous system (CNS) emerged as target to damages related to PM2.5 exposures since the particles can be translocated to nervous tissue through the olfactory nerve pathway inducing function alteration. PM2.5 exposures during embryonic/fetal life period may affect the CNS development of Wistar rats. Adult female Wistar rat were exposed during pregnancy and nursing periods (39 days) by intranasal instillation to PM2.5 solutions. The matrices were assembled in three experimental groups that received the blank solution, that was used as the control for all analysis, and two PM2.5 dilutions 1:50 and 1:25 in daily basis until the end of the experimental time. The aim of this study is verify the maternal exposure to PM2.5, during pregnancy and nursing periods, leads to neurological outcomes in the offspring, through the metal bioaccumulation, memory and behavior tasks responses, and oxidative state evaluation of two brain structures: hippocampus (H) and prefrontal cortex (PFC). Metal components as As, Ca, Cu, Ni, Sr and Ti were detected in H and PCF. Significant difference ($p < 0.05$) between the experimental groups for As, Ca and Ti in both tissues. Cu element was detected only in hippocampus and Ni only in PFC, a significant difference was found ($p < 0,05$) among the experimental groups for these elements. Regarding the oxidative stress, no significant difference was found in reactive oxygen species formation between the groups. An increase of superoxide

	dismutase (SOD) activity in PFC was found among offspring blank and 1:50 groups ($p < 0.05$) and for the mothers a decrease of SOD hippocampal activity was found between blank and 1:25 groups ($p < 0.05$). Novel recognition object (NOR) for PM2.5 experimental treatments (blank, PM2.5 dilutions 1:50 and 1:25) at 24 h post training session. Mothers and their offspring were evaluated for the short and long term memory and exploratory behavior. In the NOR and Y maze tasks the mothers no significant differences were found. For the offspring significant differences ($p < 0.05$) in both tasks, in addition a sex comparison between males and females indicates that the males are more susceptible to the PM2.5 toxic effect. These findings indicate alteration in the CNS development resulting in neurological outcomes due to the PM2.5 exposures in the embryonic/fetal life period.
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4.2.1 Memory and behavior study of fine particulate matter ($PM_{2.5}$) intranasal exposure during pregnancy and nursing in Wistar rats model.

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Introduction

The exposure to environmental factors, such pollution of water and air must be considering as potential hazard for the health issues, since the contaminants can interact with living organisms triggering pathological conditions, as the central nervous system (CNS) disorders (JIA; WANG; LIU, 2018; KILIAN et al, 2018). Air pollution results from a mixture of organic and inorganic compounds, among them the particulate matter emerges as an important mechanism of

homeostatic disruption that can reacting in different pathways with biomolecules (EHSANIFAR et al., 2019; JIA et al., 2018; KILIAN et al, 2018; SHIH et al., 2018). Particulate matter (PM) represents the major air pollution constituent, which is related to anthropogenic activities, such as vehicular and industrial emissions, thermal power plant, residues incineration, and natural sources as, dust and coastal aerosols, geological material (JIA; WANG; LIU, 2018; KILIAN et al, 2018). PM composition is basically solid and liquid particles, biological and non-biological elements. However, the compounds found in the PM vary according the localization, particle size, weather conditions, industrial activities, geological characteristics and population density, among others (JIA; WANG; LIU, 2018; KILIAN; et al, 2018). PM is classified based on particles diameter as ultrafine $PM_{0.1}$ (particles up to 0.1 μm), fine $PM_{2.5}$ (0.1 to 2.5 μm) and coarse fraction PM_{10} (2.5 to 10 μm) (JIA; WANG; LIU, 2018; KILIAN et al, 2018). The organic alterations are related to the diameter of the PM particles, among them the $PM_{2.5}$ plays a critical role organic damages. Exposure to PM2.5 represents a health risk since the particles are easily inhaled, absorbed by the respiratory system and bloodstream, respectively, reaching other organic systems, and may cause tissue bioaccumulation altering cellular functions. (EHSANIFAR et al., 2019; JIA et al, 2017; KILIAN et al, 2018; RIBEIRO et al., 2016). The inhaled $PM_{2.5}$ can reach the CNS through breathing and the bloodstream. However, the olfactory nerves emerge as a pathway related to the absorption of air pollution particles by direct infiltration and translocation directly into the CNS (COSTA et al., 2019; KILIAN et al, 2018; MORRIS-SCHAFFER et al., 2019). The composition of the PM is a heterogeneous mixture, with metallic particles being one of the components related to CNS damage by tissue accumulation which could result in a decrease in the selective permeability of the blood-brain barrier (BBB), resulting in an interaction between the particles and BBB (SHIH et al., 2018, CHENG et al., 2016; MATEUS et al., 2018; RIBEIRO et al., 2016). This interaction is related to increased oxidative stress (CACCIOTTOLO et al., 2020; MILANI et al., 2020; PARK et al., 2021), neuroinflammation (BABADJOUNI et al., 2018; CAMPOLIM et al., 2020; TSENG et al., 2019), altered neurodevelopment, decrease cognitive functions or depressive symptoms, decreased memory, and behavioral impairment (CHURCH et al., 2018; CORY-SLECHTA et al., 2018; JIA et al., 2018; CORY-SLECHTA et al., 2019; NEPHEW et al., 2020; PARK et al., 2021). To understand the mechanisms implicated in the adverse neuropsychological effects reported in the epidemiological studies in humans, the exposure of experimental animal models can provide evidence about the interaction of $PM_{2.5}$ with CNS tissue (CALDERÓN-GARCIDUEÑAS et al., 2011, COSTA et al., 2019; EHSANIFAR et al., 2019). Experimental animal models can provide information about injuries on CNS related to $PM_{2.5}$ exposures. Environmental responses based on memory and behavioral tasks could be associated with some brain impairment such as autism, dementia, neuroinflammation, neurodegeneration, anxiety, and depressive behavior (ALLEN et al., 2017; CHENG et al., 2017; HAGHANI et al., 2020; WOODWARD et al., 2017; KULAS et al., 2018; EMAM et al., 2020). As memory

represents a process related to a complex set of neural networks, different brain areas are responsible for diverse responses to environmental exposure. Two main brain structures are implicated in memory processing: the hippocampus and prefrontal cortex (PFC). The hippocampus is related to memory organization of the context experienced and the PFC is associated with the retrieval of context-appropriate memory control by suppression of decontextualized information (EICHENBAUM et al, 2017; ITSKOV et al, 2011; MOITA et al, 2003; ZHANG et al., 2018a). In rodent models, the hippocampus-PFC pathway connections are identified as a crucial area for remembering and firing patterns concerned to specific objects, spatial and temporal environmental events (EICHENBAUM et al, 2017; ITSKOV et al, 2011; MOITA et al, 2003). The PM exposure during pregnancy and nursing could interfere in the offspring neurogenesis, altering the hippocampus-PFC pathway resulting in impaired brain functions, which are similar to that one found in neurological diseases, such as Alzheimer, Parkinson, depression, autism, among others (ALLEN et al., 2014; COSTA et al., 2019; HEO et al., 2020; JEW et al., 2019; LABBAN et al., 2021; NEPHEW et al., 2020; ZHANG et al., 2018b). Behavioral tasks, such as Novel Object Recognition (NOR), Open field and Y maze, can be applied to access information about the CNS function. Those one could be used to examine different phases of learning and memory (for example, acquisition, consolidation, or recall), to assess different types of memory (e.g., spatial memory), or to assess different retention intervals (short-term versus long-term memory) (ANTUNES; BIALA, 2012; FONKEN et al., 2011; LEGER et al., 2013; LUEPTOW et al, 2017; QUILLFELDT, 2006; WINTERS et al, 2008). The NOR task is frequently used to evaluate recognition memory (ANTUNES et al, 2012; LEGER et al., 2013; QUILLFELDT, 2006; WINTERS et al, 2008). The open field task provides information about the locomotor activity, anxiety-like response (FONKEN et al., 2011; LABBAN et al., 2021), and Y maze measures spatial, working memory, and recognition by making use of a rodent's natural exploratory instincts (EHSANIFAR et al., 2019; HULLMANN et al., 2017; IYASWAMY et al., 2018; QUILLFELDT, 2006). The aim of this study is verify the maternal exposure to $PM_{2.5,}$ during pregnancy and nursing periods, leads to neurological outcomes in the offspring, through the metal bioaccumulation, memory, behavior tasks responses, and oxidative state evaluation of two brain structures: hippocampus and prefrontal cortex.

Material and Methods

Fine Particulate Matter ($PM_{2.5}$) Sampling

Samples of $PM_{2.5}$ were collected in Gávea district localized in the south zone of the Rio de Janeiro city (22◦ 58′ 43.8″ S, 43◦ 13’ 59.7” W). The site has urban features and is far approximately 1.5 km from the Rodrigo de Freitas lagoon and the Atlantic Ocean and less than 1 km from vehicular tunnels, and forest areas. Samples were collected for 24 hours, once a week, using fiberglass

filters (203 × 254 mm, 0.21 mm thickness, 0.3 μm diameter, Millipore, USA), and high-volume samplers (Energética, Brazil). More sampling details can be found in JUSTO et al (2020).

Animal design

Rodent model

For the present study, we used 54 adult female and 80 males Wistar rats (8 weeks old). The animals were provided by Central Animal House of the Federal University of Pelotas.

General conditions

The animals were kept in cages under standard temperature (23 ± 1°C), relative humidity (45–55%), and lighting (12-h light/dark cycle) conditions. The animals had *ad libitum* access to standard rodent pelleted diet and water.

Reproduction period

In order to obtain pregnant females, the adult animals were placed in cages in a ratio of three females to one male during five days. At the end of this period the adult males were removed. All the females were kept in the experimental groups until de pregnancy confirmation by abdominal palpation, mammary growth and weight increase.

Experimental groups

To perform the intranasal instillation three adult females were randomly placed in cages. Six cages for each treatment blank, 1:50 and 1:25 $PM_{2.5}$ dilutions (**n=18 rats/group**). The instillation protocol was performed during 39 days, pregnancy (21 days) and nursing (18 days). Until the birth all females receive the treatments, after that the not pregnant females were removed from the experimental groups.

Offspring

At the end of the nursing period the offspring were randomly separated by sex from the experimental mother groups, the maximum number of male in the offspring was the inclusion criteria to forming the groups. Three cages of females and males (**n=3 rats/cage**) for each treatment blank, 1:50 and 1:25 $PM_{2.5}$ dilutions (**n=9 rats/treatment**). The offspring were hosted under the identical general environmental conditions. All animal procedures were approved by the ethical committee of the Federal University of Pelotas (CEEA-UFPel) under protocol number nº 14673/2018.

Preparation of $PM_{2.5}$ experimental solutions

PM sample (86.36 cm^2) and blank filter (43.18 cm^2) aliquots were cut and weighed (1g) using an analytical balance (Bel M214-AIH, Rio de Janeiro, Brazil, ± 0.0003 g). Metals and other

compounds contained in $PM_{2.5}$ samples were extracted with saline solution (0.9 %), diluted to 1 g/100 mL, mechanically stirred for 1 h, and subsequently filtered through a 30 mm mesh. Next, the saline extracts were filtered again in a 0.45 µm cellulose acetate membrane (Macherey-Nagel, Germany) to eliminate smaller particles and larger compounds (JUSTO et al., 2020; MATEUS et al., 2018; RIBEIRO et al., 2016). Each extract was considered a concentrated solution (CS). Concentration-response curves were generated using the PM in its concentrated form (CS) and from the results two dilutions were used 1:50 and 1:25. To perform the instillation protocol three solutions were used: 1) blank solution (saline solution and unused filter); 2) 1:50 and 3) 1:25 $PM_{2.5}$ solutions (saline solution and the concentrated solution diluted 50X and 25X, respectively).

$PM_{2.5}$ Intranasal instillation

The adult female rats were randomly placed in boxes (**n=3 animals/cage**). The exposure has been initiated five days after the end of mating season, during the gestational and nursing period (39 days). Three experimental groups were assembled according to instillation solution: 1) blank, 2) 1:50 and 3) 1:25 (**n=18 rats/treatment**). The adult female intranasal instillation procedure was performed using a 1-10 µL micropipette daily at the morning period, during pregnancy and nursing period (39 days), the offspring did not receive intranasal instillation, only the same handling of the mothers. 10 µL drop of solutions were delivered in the nasal cavity without penetration to avoid injuries (Figure 1) (DEY et al., 2011; HANSON; FREY, 2008; KAUR et al., 2016; PREDIGER et al., 2012). The animals were carefully handled to minimize the stress.

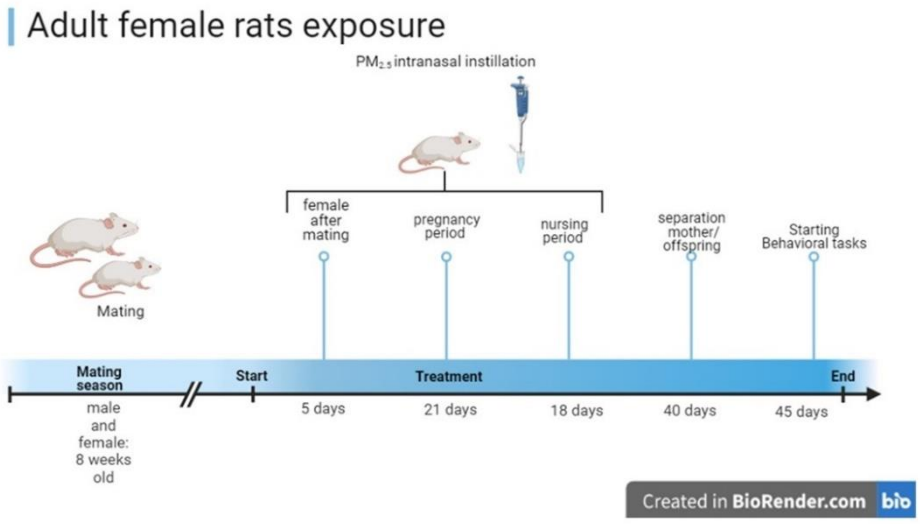

Figure 1. Timeline of intranasal instillation protocol of female rats during pregnancy and nursing period.

Behavioral Tasks

The animals were submitted to two tasks, starting with Novel Object Recognition (NOR) associate to Open Field and two days later to the Y Maze. The behavioral tasks were performed at the age of 20 weeks-old and 22 weeks-old for the mothers and 10 weeks-old and 12 weeks-old for the offspring, respectively to the tasks. In order to realize the NOR associated to open field the animals were evaluated in three different times, training, and 1 hour and a half (1h30min) and 24 hours post training. In the Y maze task the animals were submitted to two sessions, one for the acclimation to the apparatus and a second session, thirty minutes later, for the memory and behavior evaluation. The both apparatus was cleaned up with a 40 % alcohol solution and dried after each individual rat session. All the sessions were recorded to a posterior analysis.

Novel object recognition (NOR)

In order to evaluate the short and long term memory abilities and the exploratory behavior the experimental animals were exposed to two sets of objects in an open field apparatus (ANTUNES et al, 2012; COLE et al., 2019; LABBAN et al., 2021; MAITI et al., 2021). The animals were placed in the procedure room 30 minutes before the task, for acclimation. The task was performed in a wooden rectangular arena (50 cm high X 45 cm X 60 cm) divided into 12 equal squares (15 × 15 cm). Initially the animals were exposed to the empty apparatus during five minutes for habituation. After that the animals were submitted to three experimental sessions, during five minutes each: training, 1 hour and a half (1 h 30 min) and 24 hours post training. To evaluate the objects recognition in the training and 1h30min, two identical objects were placed in the box for interaction. In the 24 h session one of the objects was replaced by a new one, to estimate the interaction index related to an environmental novelty (ANTUNES et al, 2012; BROADBENT et al., 2010; COLE et al., 2019; MAITI et al., 2021). The discrimination index (DI) was calculated according to the equation: DI = (time spent on the novel object – time spent on the familiar object) / (time spent on the novel object + time spent on the familiar object).

Open field

The open field parameters as latency (exploratory behavior), crossing (number of quadrant cross), rearing (orientation response), and grooming (self-cleaning) were collected during the experimental session of NOR task, to provide information about locomotor activity and anxiety-like behavior (BRENES et al., 2009; QUILLFELDT, 2006; WEINSTOCK, 2017; ZANCHI et al., 2010a).

Y maze

After 30 minutes of apparatus acclimation, the animals were submitted to one session of 8 minutes. The animals were placed in the center of the labyrinth allowing the spontaneous choice of the entry arms. This task evaluates the procedure spatial memory providing information about the exploration behavior of the Wistar rat (HULLMANN et al., 2017; IYASWAMY et al., 2018; KRAEUTER; GUEST, 2019; QUILLFELDT, 2006). The sessions were recorded for counting of the arms entries and determining the spontaneous arms alternation percentage, using this formula: spontaneous arms alternations (%) = spontaneous alternations number / total entries number - 2 X100.

Tissue collection

At the end of behavioral tasks, the animals were euthanized using inhalator anesthetic isoflurane. The average age of the mothers was seventy-eight days, and for the offspring forty-eight days. The animals were placed in a hermetic chamber containing gaze soaked with isoflurane (4 %) until the suppression of the reflex movements and loss of voluntary muscles control (1.5 to 2 min), followed by decapitation (NEVES et al., 2013). The cranial bone was opened to the removal of the whole brain. The nervous structure was dissected to harvest the following tissue samples, hippocampus and prefrontal cortex. The samples were frozen at -80 ºC to posterior metal bioaccumulation, and oxidative stress parameters.

Bioaccumulation in the nervous structures

To perform the bioaccumulation analysis, the nervous structures were lyophilized. The frozen samples (-80 ºC) storage in vials were placed in the vacuum chamber for the primary dying stage avoiding the ice crystal formation, followed by the secondary dying stage were occurring the desorption of the water content, in the forty-eight-hour period. The lyophilized extracts were diluted with purified water (MATEUS et al., 2018; MOLNAR et al., 2021), centrifuged and analyzed by inductively coupled plasma-mass spectrometry (ICP-MS) for the determination of the metals present (CÁCERES QUIJANO et al., 2022; JUSTO et al., 2020; MATEUS et al., 2013).

Metal analysis

The lyophilized experimental blank solution (filter not used), the diluted solutions of $PM_{2.5}$ samples (1:50 and 1:25) and nervous tissues were analyzed by ICP-MS. The analysis was performed by ICP-MS (ELAN 6000, PerkinElmer-Sciex, USA) to identify, and quantify the elements. About 40 elements were analyzed. Six elements Arsenic (As), Calcium (Ca), Copper (Cu), Nickel (Ni), Strontium (Sr), Titanium (Ti) were selected for the bioaccumulation analyzes (Table 2). The screening of the elements was based on the following criteria: a) is under the range

of quantification (LQ), and detection (LD) limits, b) not appear only in the blank filter, c) be present in the nervous tissue (prefrontal cortex and hippocampus) in one of the experimental groups. Analytical curves in the range of 50 - 100 µg L^1 were used to quantify the elements (CÁCERES QUIJANO et al., 2022; JUSTO et al., 2020; MATEUS et al., 2013).

Table 1. Metal concentration (mg L^{-1}) detected in the filters, mean concentration ± standard deviation of the measurement of elements (mg kg^{-1}, dry weight) in a pool (n=9) following criteria: a) is under the range of quantification (LQ) and detection (LD) limits, b) not appear only in the blank filter, c) be present in the nervous tissue (hippocampus and prefrontalprefrontal cortex) in one of the experimental groups.

Element	Blank filter (mg L^{-1})	Filter with $PM_{2.5}$ (mg L^{-1})
As	0 ± 0.06	0.26 ± 0.06
Ca	0.36 ± 225.5	554 ± 225
Cu	1.60 ± 1.64	11.10 ± 1.64
Ni	0 ± 0.20	0.76 ± 0.20
Sr	6.6± 3.98	9.11 ± 3.98
Ti	0 ± 0.22	1.48 ± 0.22

Experimental solutions

A total of 9 samples of experimental solutions, three per extract (blank, 1:50 and 1:25 dilutions) were analyzed, for the elements (As, Ca, Cu, Ni, Sr, Ti) selected for the bioaccumulation analyzes to evaluate contaminations. Blank filters were analyzed simultaneously with the samples and the average values discount from the samples.

Bioaccumulation in nervous tissue

The mother and offspring nervous tissues (prefrontal cortex and hippocampus) were analyzed in triplicate for each treatment: blank, 1:50 and 1:25 dilutions (**n=36**). Nitric acid 10% solution was added to each poll and analyzed by ICP-MS. Certified PM reference material (SRM 1648a, urban dust, NIST-USA) and biological reference material (SRM 1577b, bovine liver, NIST-USA) were used to evaluate the extraction efficiency. The reference materials were extracted and analyzed simultaneously with the samples. The analyzed elements (As, Ca, Cu, Ni, Sr, Ti) are stable in the tested solutions (CÁCERES QUIJANO et al., 2022; JUSTO et al., 2020; MATEUS et al., 2013).

Oxidative stress parameters

The samples were stored at − 80 °C until utilization. The hippocampus and prefrontalprefrontal cortex were homogenized in 10 volumes (1:10 w/v) of sodium phosphate buffer, pH 7.4 containing KCl. The homogenates were centrifuged at 3500 rpm for 10 min at 4 °C. The pellet was discarded, and the supernatant was used for the measurements. Protein content was determined using the method developed by (LOWRY et al., 1951) with bovine serum albumin as the standard solution containing Folin-Ciocalteau reagent (molybdate, tungstate and phosphoric

acid). That one reacts with proteins and suffer a reduction reaction in presence of copper (catalyzer) producing compounds with 650nm of maximal absorbance.

Reactive oxygen species assay

Reactive oxygen species (ROS) formation was determined according to Ali et al. (1992), described in (TEIXEIRA et al., 2020) with modifications. In this assay, the oxidation of DCFH-DA to DCF (fluorescent 2′,7′-dichlorofluorescein) was measured for the detection of reactive species of PFC and hippocampus homogenates. The nervous tissue samples were dissolved in TrisHCl 10 mM pH 7.4 (MW 157.60) and adjusted to the protein level (0.8-1 mg/dL). DCF fluorescence intensity emission was recorded at 480-520 nm excitation each 30 s after the addition of DCFHDA to the medium, during 5 min. The results were expressed as μmol DCF per mg of protein.

Superoxide dismutase activity

Superoxide dismutase (SOD) activity was measured using the method described by (MISRA; FRIDOVICH, 1972). This assay is based on the inhibition of superoxide dependent adrenaline auto-oxidation in the samples. The intermediate in this reaction is superoxide, which is scavenged by SOD and is measured in a spectrophotometer adjusted at 480 nm, 15/15 s during 15 min. The specific activity of SOD was reported as units per mg of protein (TEIXEIRA et al., 2020).

Statistical analysis

Data are expressed as mean ± SEM of the mean, and analyzed using two-way ANOVA, followed by Tukey post-hoc tests for adjustments. All analyses were performed with GraphPad Prism 6 software (GraphPad Software,Inc.). The threshold significance level was $\alpha = 0.05$.

Results and Discussion

Behavioral Tasks

Three behavioral tasks were performed to evaluate the neurobehavioral alterations of the adult female Wistar rats exposed to $PM_{2.5}$ during pregnancy and nursing and their respectively offspring: novel object recognition (NOR), open field, and Y maze labyrinth.

Novel object recognition (NOR)

The analyzed parameter in the NOR was the memory abilities at the short and long term for object recognition, observing the exploratory behavior common in rats (ANTUNES; BIALA, 2012; COLE et al., 2019; LABBAN et al., 2021; MAITI et al., 2021). No significant difference was observed among the experimental mother groups ($p > 0.05$) that finding is corroborate by previous studies (HULLMAN et al., 2017; WOODWARD et al., 2017; CORY-SLECHTA et al., 2018; LIU et al., 2019), that reports the mature females did not present memory impairment of the short and long time memory parameter by the $PM_{2.5}$ exposures. The results for the offspring reports significant differences ($p < 0,05$) regarding the interactions with the novel object introduced 24 h after training (Figure 2). For the females (Figure 2A) a significant difference ($p = 0.061$) was found between the blank (9.1 ± 1.3) and 1:25 dilution (4.8 ± 1.3), and for the males (Figure 2B) a very significant difference ($p = 0.0128$) was found among the blank (3.7 ± 1.0) and 1:50 dilution (7.2 ± 1.0) for the familiar object, and a difference (0.0207) was found between blank (7.5 ± 1.0) and 1:25 dilution (4.2 ± 1.0) for the novel object.

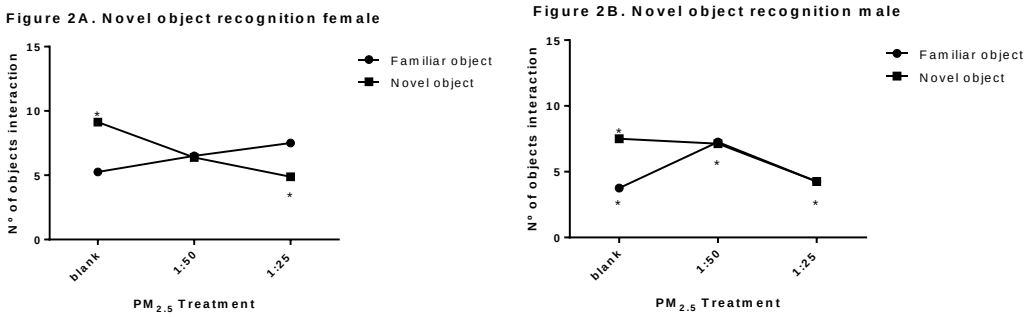

Figure 2. Novel recognition object (NOR) for $PM_{2.5}$ experimental treatments (blank, $PM_{2.5}$ dilutions 1:50 and 1:25) at 24 h post training session. Ten weeks-old females and males (**n=9rats/treatment**) were presented to a novel object in order to evaluated the short and long term memory and exploratory behavior. Significant differences ($p < 0.05$)*. Data represent mean ± SEM of objects interactions.

These results indicate that $PM_{2.5}$ exposure of the mother during the pregnancy and nursing could interfere in the memory network formation (PFC/hippocampus pathway) the long term memory and indicate a decrease of exploratory common in rodents (BROADBENT et al., 2010; COHEN et al., 2013; WINTERS et al., 2008). However, for the males the data suggest that $PM_{2.5}$ 1:50 dilution has an implication also for the short time memory. The $PM_{2.5}$ exposures could interfere in the hippocampal areas altering the memory acquisition, consolidation and recall for the offspring can be affected during brain formation which can be related to the behavioral alteration (COHEN et al., 2013; SHIH et al., 2018). Our findings for the different patterns between females and males in the NOR task agree with other sex comparative studies reports (COLE et al, 2016; HULLMAN et al., 2017; WOODWARD et al., 2017; CORY-SLECHTA et al., 2018; WANG et al., 2019).

Open Field

The open field task evaluates four parameters: time expand to live the first quadrant (latency), locomotor activity (crossing), postural orientation (rearing), and self-cleaning behavior (grooming). Mothers and offsprings were submitted to three sessions (training, 1h30min and 24 h post-training). No significant difference was observed among the experimental mother groups ($p > 0.05$) for all parameters evaluated. These results agree with previous studies could be related to the complete formed brain structures and the age of the adult females (8 weeks-old) at the starting of the instillation, indicating that young females could recovery, at some extent, to the $PM_{2.5}$ exposures (HULLMAN et al., 2017; WOODWARD et al., 2017; CORY-SLECHTA et al., 2018; LIU et al., 2019). The results found in the offspring show no significant difference ($p > 0.05$) among the female experimental groups (Figure3A) for latency parameter. Among the male of the offspring groups a significant difference ($p = 0.0288$) was found between the $PM_{2.5}$ 1:25 dilution group at the training (8.7 ± 4.4) and 24 h post-training (20.8 ± 4.4) sessions, this finding could be related to an increase of depression-like behavior as previously studies reported (HULLMAN et al., 2017; WOODWARD et al., 2017; CORY-SLECHTA et al., 2018; LIU et al., 2019).

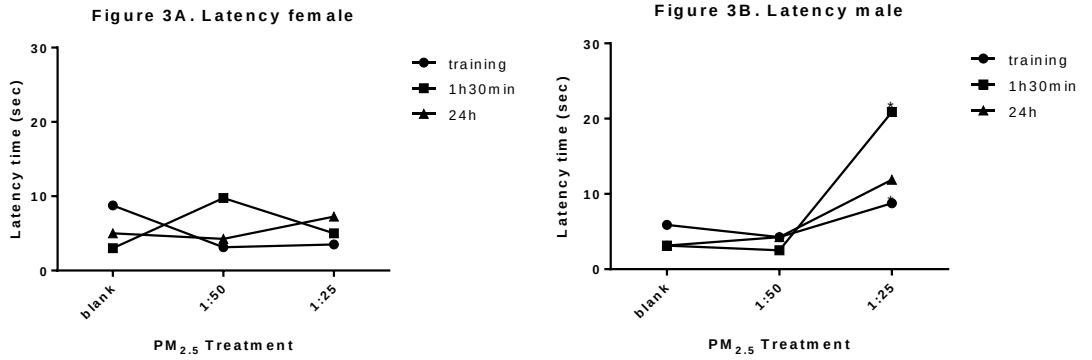

Figure 3: Time spent to live the first quadrant, latency period related to the $PM_{2.5}$ experimental treatments (blank, $PM_{2.5}$ dilutions 1:50 and 1:25) at three different time sessions (training and 1h30min and 24 h post training). Ten weeks-old females and males (**n=9rats/treatment**) were evaluated regarding the time to start the open field exploration. Significant differences ($p < 0.05$) *. Data represent mean ± SEM of latency time in seconds.

The parameters related to postural orientation (rearing) and self-cleaning (grooming) no significant differences ($p > 0.05$) were found within or between all the mothers and offspring experimental groups.

Y maze

The Y maze is frequently used to evaluate the working memory and exploratory behavior in rats, by the spontaneous choice of arms entry. Among the mother experimental groups, no significant difference was found ($p > 0.05$), regarding to the spatial memory as obtained in previous studies

(CHEN et al., 2017; LEE et al., 2021; WEINSTOCK, 2017; WIN-SHWE et al., 2012). The age of the female rats (10 weeks-old) exposed to PM in our study could be a factor that explains the mother experimental groups results. Lee et al. (2021) reported that young age rats present a reduced amount of brain damage as result of a PM exposure. We found no differences ($p > 0.05$) within the offspring groups. However, we found a significant difference between male and female offspring groups ($p > 0.05$). The results for the spontaneous alternations reveals significant differences between all groups by sex, blank groups ($p = 0.0001$) females (67 ± 5.41) and males (39.60 ± 5.41), 1:50 dilution groups ($p = 0.0014$) females (62.62 ± 5.41) and males (42.27 ± 5.41) and 1:25 dilution groups ($p = 0.0002$) females (64.25 ± 5.41) and males (40.22 ± 5.41) these findings indicate that the males presented a memory deficit when compared to the females, as presented in the Figure 4. The majority of the research performed to assess memory use male as an animal model, however, that one who tested female, and found more differences regarding memory alterations (COLE et al., 2016; WEINSTOCK, 2017; YAN et al., 2020). A study conducted by Klocke et al (2018) also found differences in the corpus callosum between male and female, suggesting that mechanisms involved in brain repair could be sexually dimorphic. These results indicate that males are more affected by the PM exposure than females regarding the spatial memory and exploration behavior.

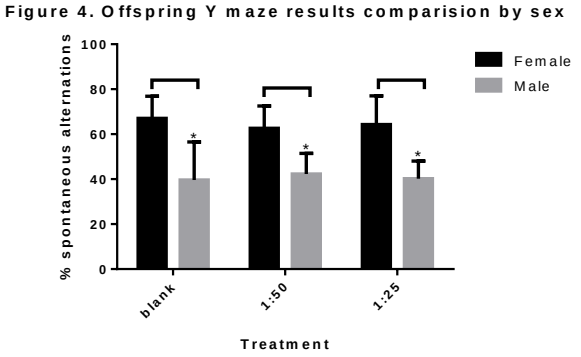

Figure 4. Percentile of spontaneous alternations related to the $PM_{2.5}$ experimental treatments (blank, $PM_{2.5}$ dilutions 1:50 and 1:25) one-time session. Twelve weeks-old females and males (**n=9rats/treatment**) were evaluated regarding the time to start the Y maze exploration. Significant differences ($p < 0.05$) *. Data represent mean ± SEM of spontaneous alternation percentile.

Metal analysis

In the data obtained from the ICP-MS for filters (blank and $PM_{2.5}$), solutions (blank, 1:50 and 1:25 dilutions), and nervous tissue (prefrontal cortex and hippocampus), six elements (As, Ca, Cu, Ni, Sr, Ti) were reliably detected in the samples. Bioaccumulation analyses showed that in the PFC Cu and Sr did not show significant differences among groups, and on the comparison among mother and offspring. The metals present in the PM share many common mechanisms

related to their toxicities, including the production of oxidative stress, reaction with sulfhydryl groups, and interference with essential metals (CÁCERES QUIJANO et al., 2022; VENTURA-LIMA et al., 2009; WANG; FOWLER, 2008). We observed significant differences in As in both tissues. **Figure 5A**, presents the PFC bioaccumulation, no significant differences were found comparing the experimental groups mother and offspring decoupled ($p > 0.005$). However, a highly significant differences were found in the groups comparison ($p < 0.001$) between the blank mother (1.493 ± 0.13), blank offspring (0.33 ± 0.13) and 1:25 offspring (0.47 ± 0.13). Also among 1:50 mother (1.55 ± 0.13), 1:50 offspring (0.47 ± 0.13) and 1:25 offspring (0.40 ± 0.13). And between 1:25 mother (1:26 ± 0.13), 1:50 offspring (0.47 ± 0.13), 1:25 offspring (0.40 ± 0.13). **Figure 5B** presents the hippocampus bioaccumulation, a significant difference ($p = 0.0005$) was found between blank mother (1.19 ± 0.18), blank offspring (0.35 ± 0.18). And also between blank mother and 1:50 offspring (0.39 ± 0.18) ($p = 0.0010$) and blank mother and 1:25 offspring (0.47 ± 0.18) ($p = 0.0040$). A significant difference ($p = 0.002$) among blank offspring (0.35 ± 0.18) and 1:50 mother (1.10 ± 0.18) and between blank offspring and 1:25 mother (1.72 ± 0.18) ($p < 0.0001$). Significant differences ($p = 0.004$) was also found between 1:50 mother (1.10 ± 0.18), and 1:50 offspring (0.39 ± 0.18) and also among 1:50 mother and 1:25 mother (1.72 ± 0.18) ($p = 0.018$). A significant difference ($p = 0.015$) was found between 1:50 mother (1.10 ± 0.18) and 1:25 offspring. A highly significant difference ($p < 0.0001$) was found among 1:50 offspring (0.39 ± 0.18) and 1:25 mother (1.72 ± 0.18) and between 1:25 mother and 1:25 offspring (0.47 ± 0.18) ($p < 0.0001$). The only significant difference ($p = 0.018$) intra group was found between 1:50 mother (1.10 ± 0.18) and 1:25 mother (1.72 ± 0.18).

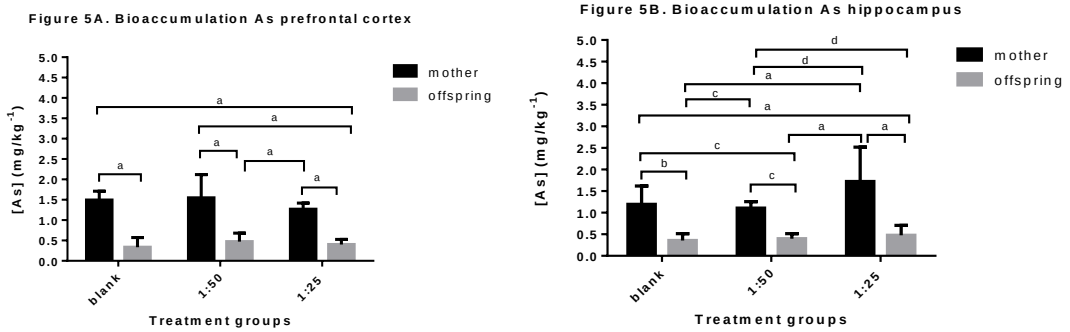

Figure 5. Comparison of the Arsenic (As) bioaccumulation in the prefrontal cortex (PFC) and hippocampus for $PM_{2.5}$ experimental treatments (blank, $PM_{2.5}$ dilutions 1:50 and 1:25) after the mother exposure. **Figure 5A.** Bioaccumulation in PFC and **Figure 5B** Bioaccumulation in hippocampus. Significant differences ($p < 0.05$) are represented by letters according the p values a) $p < 0,0001$, b) $p = 0,0005$, c) $p= 0,0023$ and d) $p < 0,05$. Data represent mean ± SEM As bioaccumulation.

Ca bioaccumulation is presented in the **Figure 6A** for PFC where a significant difference was found between blank mother (7408 ± 2135) and blank offspring (861.5 ± 2135) ($p = 0.04$) and also between blank mother and 1:25 offspring (810.1 ± 1910) ($p = 0.01$). **Figure 6B** on the

hippocampus a significant difference was found among blank offspring (418.3 ± 92.5), 1:50 offspring (130.3 ± 92.5) (p = 0.03) and 1:25 offspring (116.6 ± 92.5) (p = 0.03).

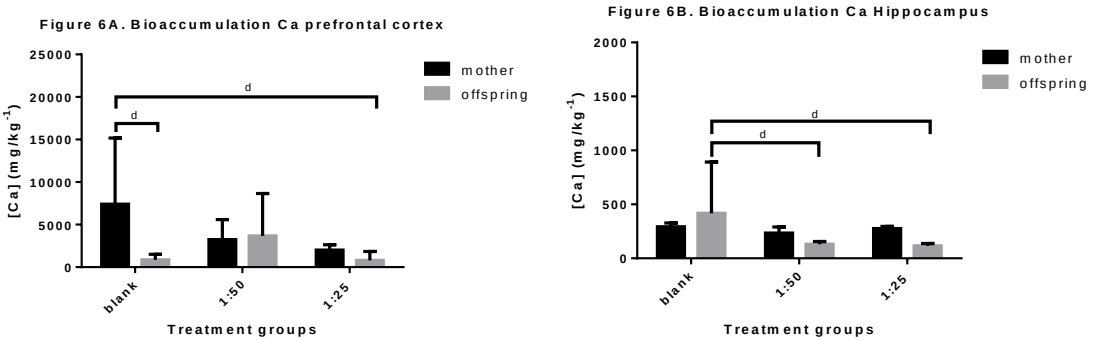

Figure 6. Comparison of the Ca bioaccumulations in the prefrontal cortex (PFC) and hippocampus for the for $PM_{2.5}$ experimental treatments (blank, $PM_{2.5}$ dilutions 1:50 and 1:25) after the mother exposure. **Figure 6A.** Bioaccumulation in PFC and **Figure 6B** Bioaccumulation in hippocampus. Significant differences ($p < 0.05$) are represented by letters according the p values a) $p < 0,0001$, b) $p = 0,0005$, c) $p= 0,0023$ and d) $p < 0,05$. Data represent mean ± SEM Ca bioaccumulation.

For the Cu bioaccumulation a significant difference was found in hippocampus a between blank mother (2.97 ± 0.60), 1:50 mother (1.06 ± 0.60) (p = 0.03) and 1:25 offspring (1.38 ± 0.45) (p= 0.01) for Cu bioaccumulation, as presented in the **Figure 7**.

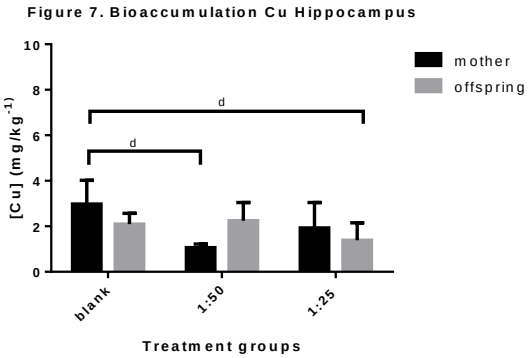

Figure 7. Cu bioaccumulations in the hippocampus for $PM_{2.5}$ experimental treatments (blank, $PM_{2.5}$ dilutions 1:50 and 1:25) after the mother exposure. Significant differences are represented by letters according the p values a) $p < 0,0001$, b) $p = 0,0005$, c) $p= 0,0023$ and d) $p < 0,05$. Data represent mean ± SEM Cu bioaccumulation.

The Ni element in PFC was detected in the blank mother (0.25 ± 0.02), blank offspring (0.20 ± 0.02) and 1:50 offspring (0.12 ± 0.02). A significant difference was found ($p < 0,05$) between blank mother and 1:50 offspring as show in **Figure 8**. In the hippocampus NI was detected in blank mother group (0.15 ± 0.001).

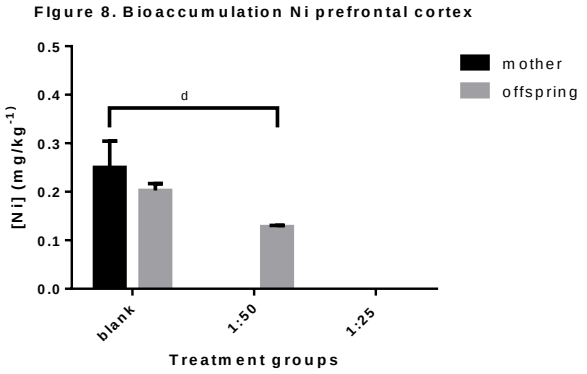

Figure 8. Ni bioaccumulations in the PFC for $PM_{2.5}$ experimental treatments (blank, $PM_{2.5}$ dilutions 1:50 and 1:25) after the mother exposure. Significant differences are represented by letters according the p values a) $p < 0{,}0001$, b) $p = 0{,}0005$, c) $p = 0{,}0023$ and d) $p < 0{,}05$. Data represent mean ± SEM Ni bioaccumulation.

The Sr detected in the hippocampus was only found in the blank offspring group (0.27 ± 0.27). For the Ti bioaccumulation in the PFC a highly significant difference was found between the blank mother (28.96 ± 2.06) blank offspring (17.36 ± 2.06) ($p < 0.0001$). Blank mother (28.96 ± 2.06) also present a significant difference ($p < 0.05$) with 1:25 mother (22.58 ± 2.06) and with 1:25 offspring (20.24 ± 2.06) ($p = 0.001$). In the offspring groups a significant difference was found among blank offspring (17.36 ± 2.06) and 1:50 offspring (23.67 ± 2.06) as presented in **Figure 9A**. In the hippocampus, **Figure 9B**, Ti showed a significant difference ($p = 0.02$) between blank offspring (24.80 ± 1.40) and 1:50 mother (20.28 ± 1.40)

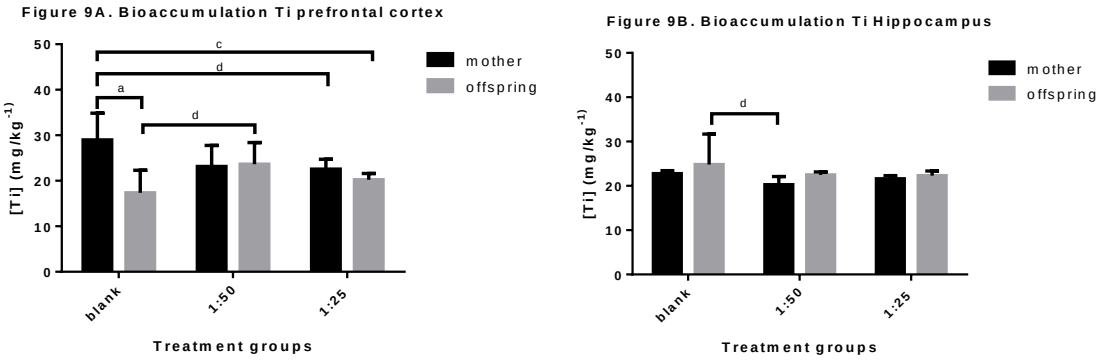

Figure 9. Comparison of the Ti bioaccumulations in the prefrontal cortex (PFC) and hippocampus for the $PM_{2.5}$ experimental treatments (blank, $PM_{2.5}$ dilutions 1:50 and 1:25) after the mother exposure. **Figure 9A.** Bioaccumulation in PFC and **Figure 9B** Bioaccumulation in hippocampus. Significant differences ($p < 0.05$) are represented by letters according the p values a) $p < 0{,}0001$, b) $p = 0{,}0005$, c) $p = 0{,}0023$ and d) $p < 0{,}05$. Data represent mean ± SEM Ti bioaccumulation.

Oxidative stress parameters

Two analyzes were performed in the mother and offspring nervous tissue PFC and hippocampus (**n=5**), ROS assay and SOD activity, to evaluated ROS formation and the antioxidant capacity of

the mother exposed to $PM_{2.5}$ during pregnancy and nursing to blank filter and $PM_{2.5}$ dilutions (1:50 and 1:25) and the respective offspring.

Reactive Oxygen Species

The ROS formation was not significant ($p > 0.05$) between the groups in the PFC and hippocampus, the results are presented in the table 2.

Table 2. Level of ROS formation in the mother (M) experimental groups exposed to the treatments blank filter and $PM_{2.5}$ dilutions 1:50 and 1:25 and the respectively offspring (OS). The results are presented by mean ± SEM of the number of interaction (n = 5). No significant difference was found between the experimental groups ($p > 0,05$).

ROS level				
	PFC		**H**	
	M	**OS**	**M**	**OS**
blank	2016 ± 671.1	2208 ± 479.1	0.62 ± 0.14	0.72 ± 0.09
1:50	1041 ± 671.1	1958 ± 497.1	0.81 ± 0.14	0.71 ± 0.09
1:25	2028 ± 671.1	1312 ± 497.1	0.72 ± 0.14	0.65 ± 0.09

SOD activity

The activity of SOD in PFC was not significant ($p > 0.05$) for the mothers in all groups. For the offspring a significant difference ($p = 0.0095$) was found between the groups blank (50.62 ± 10.84) and 1:50 (91.86 ± 10.84) indicating an increase in the antioxidant activity, as show in **Figure 10**.

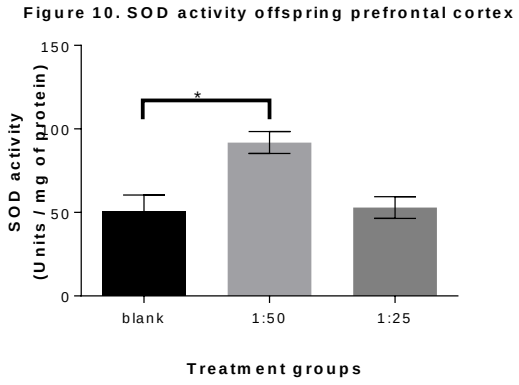

Figure 10. SOD activity in the offspring PFC for $PM_{2.5}$ experimental treatments (blank, $PM_{2.5}$ dilutions 1:50 and 1:25) after the mother exposure. Significant difference was found between blank and 1:50 ($p = 0.0095$) *, indicating an increase of antioxidant defense in the offspring from the mothers exposed to the higher dilution of $PM_{2.5}$. (n = 5). Data represent mean ± SEM SOD activity.

Regarding to hippocampus analyzes among the offspring groups no significant difference was found ($p > 0.05$). However, a significant difference ($p = 0.0001$) was found between mother blank (79.84 ± 13.18) and 1:25 (6.10 ± 13.18) experimental groups, the data are presented in the **Figure 11**.

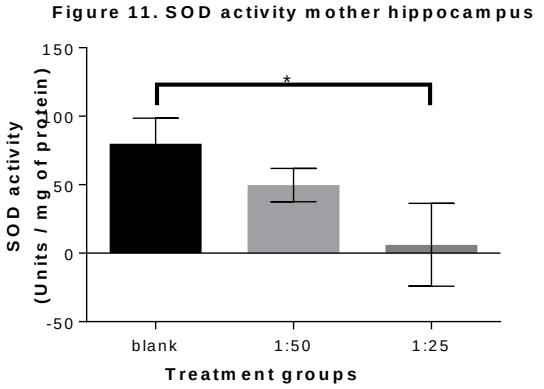

Figure 11. SOD activity in the mother hippocampus for $PM_{2.5}$ experimental treatments (blank, $PM_{2.5}$ dilutions 1:50 and 1:25) after mother exposure. Significant difference was found between blank and 1:25 ($p = 0.0001$) *, indicating a decrease of antioxidant defense in the females exposed to lower dilution (1:25) of $PM_{2.5}$. (n = 5). Data represent mean ± SEM SOD activity.

Discussion

The present study aimed to evaluate the effects of $PM_{2.5}$ exposures by intranasal instillation (during pregnancy, and nursing period) in the females Wistar rats and their offspring regarding the metal accumulation, oxidative stress formation in the nervous tissue, and the behavioral alterations. PM presents a heterogeneous composition varying mass concentration, chemical composition, particles size, and hygroscopic property, among others. For this reason, the identification and quantification of $PM_{2.5}$ components present in samples are important to assess the underlying mechanisms related to the interaction between $PM_{2.5}$ and biomolecules (ALLEN et al., 2017; WANG et al., 2019b; WU et al., 2019). The $PM_{2.5}$ samples used for exposure where analyzed by ICP-MS and indicate presence of metals as As, Ca, Cu, Ni, Sr and Ti. These components are associated with neurotoxicity as they are responsible for promoting an imbalance of oxidative stress and antioxidant defense, in addition to activating apoptotic pathways (LIU et al., 2018; VENTURA-LIMA et al., 2009). Our findings indicate presence of this metals in two brain regions, prefrontal cortex (PFC) and hippocampus. Previous studies report that exposure to air pollution can lead to the metal accumulation in nervous tissue by the olfactory pathway reaching the PFC and hippocampus being associated to tissue damage, increase of oxidative stress

and neuroinflammation, that aggravating the neurodegenerative and neurodevelopmental pathologies conditions and promoting decreased cognitive functions, and depressive symptoms (BLOCK; CALDERÓN-GARCIDUEÑAS, 2009; SALVI; LIU; SALIM, 2020; DI DOMENICO et al., 2020). For the metal bioaccumulation between the mothers experimental groups the results did not present significant difference ($p > 0.05$) for As, Ca and Ni. However, for As a significant difference ($p < 0.0001$) was found between mother groups and offsprings in PFC and hippocampus accumulation, that result may indicate that arsenic in the used dilutions (1:50 and 1:25) reach the mother brain by the intranasal instillation and accumulated in the PFC region but did not cross the placental barrier, as the ROS formation did not show significant difference between the groups. The inorganic arsenic present in the $PM_{2.5}$ could suffer transformation into organic a the less toxic specie by enzymatic activity of the monomethylarsonic acid (MMAV) reductase and methyltransferase in this metabolic pathway requiring GSH as substrate of conjugation (VENTURA-LIMA et al., 2009; WANG; FOWLER, 2008). The PFC Ca accumulation significant difference ($p < 0.05$) was also found only between blank mother and offspring and G25offspring groups, and for hippocampus Ca accumulation significant difference ($p < 0.005$) was found between blank, G25 and G50 offspring groups, as the Y maze results showed difference between the female and male offspring in the spatial memory, that may be related to related to a mitochondrial paroxonase 2 (PON 2) that is higher in females in contrast to males, producing microglia activation, as measured by increased Iba1 (ionized calcium-binding adapter molecule 1) expression, and of TSPO (translocator protein) binding (COSTA et al., 2019; GIORDANO et al., 2011). Cu was detected in the hippocampus in the mother blank and G50 and G25 offspring, however this data not present relation with ROS level and SOD activity found in our results. The Ni and Ti were found in the PFCPFCPFC and hippocampus of blank groups mother and offspring and also in the lower dilution G50 offspring for Ni and for Ti in the G50 mother and offspring groups, that may indicate that Ni and Ti can cross the placental barrier since according the dilution used, we also found an increase in the SOD activity in the PFC of G50 offspring group indicating am antioxidant defense was activated (HAGHANI et al., 2020a; LIU et al., 2018). In our study, the results of NOR task did not show significant difference between the mother experimental groups, which are in agreement with other studies report that the female rat hormonal patterns induces an earlier development and maturity of CNS, generating a protection for PM2.5 exposures damages (HULLMAN et al., 2017; WOODWARD et al., 2017; CORY-SLECHTA et al., 2018; WANG et al., 2019). However, the results reported for the offspring indicate the $PM_{2.5}$ exposures during pregnancy and nursing present some influence in the development of CNS, among the offspring, where the females present a significant difference between the blank and 1:25 experimental groups, while the males the both $PM_{2.5}$ dilutions (1:50 and 1:25) showed significant differences when compared with de blank group, regarding the exploratory behavior and long term memory. These data indicating that the male offspring were more affected by the mother exposure during pregnancy and nursing period, then the female, however the higher concentration of $PM_{2.5}$ (1:25) affect the exploratory behavior of and long term memory, in both sex (SHIH et al., 2018; CORY-SLECHTA et al., 2018; WANG et al., 2019). In addition, Y maze task shows significant differences related to sex. The male offspring performance was worst related to female offspring. This fact may be due to the characteristic of

the task, that uses the hippocampus and PFC. Studies with mice exposure to $PM_{2,5}$ demonstrate changes in hippocampal neurons that could explain the impairment of cognitive abilities (BLOCK; CALDERÓN-GARCIDUEÑAS, 2009; FONKEN et al., 2011; SALVI; LIU; CORY-SLECHTA et al., 2019; SALIM, 2020). At the same way, data from the literature explain the $PM_{2.5}$ and ultrafine particulate matter can enter in CNS via olfactory epithelium, bulb and reach to olfactory cortex and other regions, and altering the motor activity, spatial learning and memory and novel recognition ability (ANTUNES; BIALA, 2012; COSTA et al., 2019; JEW et al., 2019; ZANCHI et al., 2010b). In contrast the oxidative stress in PFC measures was the same in hippocampus but in PFC an increase of SOD may be related to adaptive response to protect the structure, the reaction between reactive oxygen species (ROS) and the biomolecules represents a main pathway that impacts cellular function, such as survival, proliferation, differentiation and apoptosis (HSIEH; YANG, 2013; MADSEN et al., 2013; ZANCHI et al., 2010a). The SOD is the first enzyme activated in ROS cascade. Further studies necessary to clarify and extend these results. In conclusion, this experimental study indicates that exposure to $PM_{2.5}$ during pregnancy and nursing periods, reach the CNS of the embryos altering the offspring neurodevelopment related to brain metal bioaccumulation, altering of antioxidant defense parameters and impairing the spatial memory and the exploratory behavior of rodents.

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5. DISCUSSÃO

Este estudo foi conduzido em dois momentos, a revisão dos principais achados relacionados a exposição ao $MP_{2,5}$ e o SNC nos últimos seis anos, e a exposição experimental ao $MP_{2,5}$ em fêmeas Wistar adultas, durante o período gestacional e lactação, avaliando as respostas comportamentais, a detecção e quantificação dos componentes metálicos do $MP_{2,5}$ e a geração de estresse oxidativo das matrizes e suas ninhadas.

Os resultados obtidos neste estudo evidenciaram a importância do entendimento dos mecanismos envolvidos nas injurias do SNC relacionadas a exposição a poluição ambiental, em particular ao $MP_{2,5}$.

O MP constitui-se em uma fração do ar atmosférico apresentando uma composição heterogênea que inclui, partículas sólidas gotículas líquidas, compostos gasosos orgânicos que se originam das mais variadas fontes (CHENG et al., 2017b; KU et al., 2017a; MORRIS-SCHAFFER et al., 2018a).

O diâmetro aerodinâmico do MP apresenta uma estreita relação com o desenvolvimento e/ou agravamento de condições patológicas nas mais variadas espécies, dentre as frações MP_{10}, $MP_{0,1}$ e $MP_{2,5}$. O $MP_{2,5}$ apresenta o maior potencial danoso à saúde, devido a maior capacidade de absorção nas vias sistêmica e respiratória (BALASUBRAMANIAN et al., 2013; KU et al., 2017a; MINGUILLÓN et al., 2008; RIBEIRO et al., 2016).

As vias de exposição ao $MP_{2,5}$ encontradas nos últimos seis anos compreendem, câmaras de exposição (ALLEN et al., 2017; CHENG et al., 2016, 2017a; COLE et al., 2016; CORY-SLECHTA et al., 2018; HULLMANN et al., 2017; KULAS et al., 2018; MORRIS-SCHAFFER et al., 2018a; PARK et al., 2020; SHIH et al., 2018a; TYLER et al., 2016; WOODWARD et al., 2017), aspiração orofaringeal (KU et al., 2017a), instilação intratraqueal (MILANI et al., 2020; WANG et al., 2019a; WEN et al., 2021; YANG et al., 2017; ZHANG et al., 2018c), gavagem (WANG et al., 2019a), diluição (KIM et al., 2020b), suspensão (LIU et al., 2021), sonda estomacal (PARK et al., 2021) e instilação intranasal (KU et al., 2017a; LIU et al., 2019a; ONODA et al., 2020).

A câmara foi a forma de exposição ao $MP_{2,5}$ mais utilizada nos últimos seis anos, porém dada a especificidade do equipamento utilizado neste processo, observa-se que somente os pesquisadores que já possuem esta tecnologia utilizam este método. No delineamento experimental deste estudo a instilação intranasal foi eleita a forma de exposição das fêmeas Wistar, por se tratar uma via não invasiva e de fácil aplicação, e já reportada por outros autores (KU et al., 2017a; LIU et al., 2019a; ONODA et al., 2020).

Além das vias de exposição, os componentes do $MP_{2,5}$ utilizados nos últimos seis anos também foram revisados, buscando relacionar a composição aos mecanismos envolvidos nas injúrias ao SNC, os resultados obtidos indicaram a utilização do $MP_{2,5}$ na sua integralidade como a forma mais comum para exposição (CHUANG et al., 2020; JEW et al., 2019a; JIA et al., 2018; KIM et al., 2020b; KULAS et al., 2018; LIU et al., 2019a, 2021, 2020; NEPHEW et al., 2020; PARK et al., 2021; SHIH et al., 2018a; TSENG et al., 2019; WANG et al., 2019a; WEN et al., 2021; ZHANG et al., 2018c).

Seguido da utilização da fração nanopartículas (BABADJOUNI et al., 2018; CACCIOTTOLO et al., 2020; CHENG et al., 2016; HAGHANI et al., 2020b; ONODA et al., 2020; WOODWARD et al., 2017), das frações $MP_{2,5}$ concentrado (CHEN et al., 2018a; CHURCH et al., 2018; KLOCKE et al., 2018a; RAO et al., 2019), partículas ultrafinas (ALLEN et al., 2017; CORY-SLECHTA et al., 2018; MORRIS-SCHAFFER et al., 2018a; PARK et al., 2020) e das emissões de diesel (COLE et al., 2016; EHSANIFAR et al., 2019; HULLMANN et al., 2017; MORRIS-SCHAFFER et al., 2019).

Outras composições do $MP_{2,5}$ utilizadas para exposição também foram reportadas na literatura nos últimos seis anos, em menor escala.

A exposição experimental do estudo conduzido no desenvolvimento desta tese, foi realizada com a utilização do $MP_{2,5}$ integral, porém as análises de bioacumulação foram realizadas a partir dos componentes metálicos do MP.

As amostras de $MP_{2,5}$ coletadas no distrito da Gávea, localizado na cidade do Rio de Janeiro, Brasil (22◦ 58′ 43.8″ S, 43◦ 13’ 59.7” W), foram processadas de acordo com a metodologia descrita por estudos anteriores (JUSTO et al., 2020; MATEUS et al., 2013, 2018; RIBEIRO et al., 2016).

Os extratos e os tecidos nervosos foram analisados pelo método de ICP-MS para detecção e quantificação dos metais presentes nas amostras de $MP_{2,5}$ utilizadas no estudo seguindo os procedimentos metodológicos previamente descritos (JUSTO et al., 2020; MATEUS et al., 2013, 2018; RIBEIRO et al., 2016).

Os resultados obtidos indicaram a presença de As, Ca, Cu, Ni, Sr e Ti os metais e metaloides presentes no $MP_{2,5}$ estão relacionados a neurotoxicidade e compartilham mecanismos intrínsecos comuns de injuria ao SNC, tais como incremento do estresse oxidativo, indução da síntese de proteínas relacionadas ao estresse, redução da resposta antioxidante e interferência nas atividades dos metais essenciais (HAGHANI et al., 2020a; LIANG et al., 2018; LIU et al., 2018; LJUBIMOVA et al., 2018; MORRIS-SCHAFFER et al., 2018b; SANGLARD et al., 2018; VENTURA-LIMA et al., 2009; WANG; FOWLER, 2008).

Os principais mecanismos encontrados na revisão de literatura foram ativação microglial (BABADJOUNI et al., 2018; CHENG et al., 2016; COLE et al., 2016; KIM et al., 2020c; KLOCKE et al., 2017; TSENG et al., 2019) , aumento da citocinas pró-inflamatórias (KIM et al., 2020b; LJUBIMOVA et al., 2018; PARK et al., 2021; TSENG et al., 2019; TYLER et al., 2016; YANG et al., 2017), estresse oxidativo (COLE et al., 2016; MILANI et al., 2020; PARK et al., 2021; WANG et al., 2019a), agregação proteica (BABADJOUNI et al., 2018; CHUANG et al., 2020; HULLMANN et al., 2017; PARK et al., 2021), desequilíbrio sináptico (ALLEN et al., 2017; HAGHANI et al., 2020b; LIU et al., 2019a, 2021), apoptose (CHUANG et al., 2020; KU et al., 2017a; LIU et al., 2020; ZHANG et al., 2018c), alterações morfológicas (KLOCKE et al., 2017; LIU et al., 2019a; NEPHEW et al., 2020; SHIH et al., 2018a), déficit de memória e mudanças comportamentais (CORY-SLECHTA et al., 2018; EHSANIFAR et al., 2019; EMAM et al., 2020a; NEPHEW et al., 2020; PARK et al., 2021).

O estudo experimental teve por objetivo avaliar os aspectos relacionados a memória e comportamento, bioacumulação dos componentes metálicos do $MP_{2,5}$ e o estresse oxidativo frente a exposição materna de fêmeas Wistar ao $MP_{2,5}$, como mecanismos associados a danos no SNC.

O uso de modelos animais é uma importante ferramenta para a compreensão dos efeitos da exposição ao $MP_{2,5}$ no SNA, um dos objetivos da

revisão de literatura realizada, foi avaliar a importância da experimentação utilizando roedores.

Os resultados obtidos indicaram que os roedores expostos ao $MP_{2,5}$ pré e pós-natal, fornecem evidências fundamentais para o esclarecimento das possíveis vias alteradas no SNC que são correlacionadas com as patologias desenvolvidas por humanos, como Alzheimer, Parkinson, espectro autista, ansiedade e depressão e perdas cognitivas (ABDELKADER et al., 2017; ALLEN et al., 2017; CACCIOTTOLO et al., 2020; CHURCH et al., 2018; CREED et al., 2019; EHSANIFAR et al., 2019; JEW et al., 2019b; KULAS et al., 2018; NEPHEW et al., 2020; PARK et al., 2021; TEIXEIRA et al., 2020)

As mães e suas respectivas ninhadas foram submetidas a tarefas comportamentais (reconhecimento de objetos, campo aberto e labirinto em Y) para avaliação de parâmetros associados a ansiedade, atividade locomotora e memória.

Os resultados obtidos indicaram que no aspecto relacionado a ansiedade e atividade locomotora não foram observadas alterações em nenhum dos grupos experimentais, entretanto na avaliação da memória espacial quando as ninhadas são comparadas por gênero os machos apresentaram um déficit de memória quando comparados com as fêmeas, a literatura corrobora este achado, indicando que as fêmeas são menos sensíveis a ação deletéria do $MP_{2,5}$ (ALLEN et al., 2014b; ARRUDA et al., 2011; WANG et al., 2019a; WU et al., 2019b).

Em relação ao estresse oxidativo foram realizadas análises do nível de ERO´s e SOD em córtex pré-frontal e hipocampo, não houve diferença significativa na formação de EROs em nenhum dos grupos experimentais.

Os resultados da atividade da SOD indicaram que entre as fêmeas adultas expostas ao $MP_{2,5,}$ aquelas que foram instaladas com a maior concentração de MP, diluição 1:25, apresentaram uma redução na atividade enzimática antioxidante hipocampal, já entre a ninhada o grupo relacionado as mães instiladas com a menor concentração, diluição 1:50, apresentaram um incremento na produção da SOD no córtex pré-frontal.

O resultado obtido na estrutura hipocampal do grupo das fêmeas adultas pode indicar que a menor concentração na diluição 1:50 não afeta a resposta antioxidante, já a resposta do grupo de maior concentração concorda com os

resultados que indicam que o MP pode reduzir a defesa antioxidante enzimática (ALLEN et al., 2017; BATES et al., 2019; DE OLIVEIRA ALVES et al., 2020; HAHAD et al., 2020; PARK et al., 2020; ZHANG et al., 2018c).

Nas ninhadas observamos um aumento na atividade da SOD no córtex pré-frontal no grupo de menor diluição (1:50), este resultado pode indicar esta diluição favorece a translocação pela barreira transplacentária, porém não reduz a resposta antioxidante enzimática, representando uma dose sub crônica, corroborando a ação dose-resposta do MP (GARGOURI et al., 2018; RAO et al., 2019; WANG; FOWLER, 2008; WOODWARD et al., 2017).

6. CONCLUSÃO

Os dados obtidos na revisão de literatura e no estudo experimental conduzido reportam informações relevantes para a compreensão de alguns mecanismos relacionados a exposição ao $MP_{2,5}$ e as alterações no SNC resultantes desta interação, fornecendo também subsídios para o desenvolvimento de outros estudos experimentais no contexto deste tema.

7. PERSPECTIVAS FUTURAS

Devido a relevância do tema nosso grupo de trabalho dará sequência ao estudo, realizando outras metodologias tais como cultura de células, expressão de citocinas pró-inflamatórias e outros parâmetros de estresse oxidativo.

A avaliação de placenta para determinar quais componentes do MP podem translocar para o ambiente uterino interferindo no desenvolvimento embrionário, resultando em organogênese alterada.

A pesquisa nesta área ainda apresenta muitas questões a serem respondidas que podem fornecer não só a elucidação dos mecanismos patológicos, mas também indicar ações de redução de emissão de poluentes e contribuir para o desenvolvimento de terapêuticas que possam reduzir ou até mesmo reverter os danos no SNC.

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ANEXOS

Anexo 1 - Parecer do Comitê de Ética

Anexo 2 - Carta de aceite para publicação Atmospheric Pollution Research

Anexo 1 - Parecer Comitê de ética em experimentação animal – CEEA/ UFPel.

UNIVERSIDADE FEDERAL DE PELOTAS

PARECER Nº **51/2018/CEEA/REITORIA**
PROCESSO Nº 23110.014673/2018-32
INTERESSADO: IZABEL CRISTINA CUSTODIO DE SOUZA

Pelotas, 14 de maio de 2018

Certificado

Certificamos que a proposta intitulada **"AVALIAÇÃO DAS RESPOSTAS MORFOLÓGICAS E DE MARCADORES BIOQUÍMICOS EM MODELO DE CULTURA DE NEURÔNIOS DE RATOS WISTAR EXPOSTOS AO MP2.5"**, processo nº 23110.014673/2018-32, sob a responsabilidade de **Izabel Cristina Custodio de Souza** - que envolve a produção, manutenção ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto humanos), para fins de pesquisa científica (ou ensino) – encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), e recebeu parecer **FAVORÁVEL** a sua execução pela Comissão de Ética em Experimentação Animal, em reunião de 07/05/2018.

Finalidade	(X) Pesquisa ( ) Ensino
Vigência da autorização	09/06/2018 a 15/12/2022
Espécie/linhagem/raça	*Rattus norvegicus*/Wistar
Nº de animais	191
Idade	23 fêmeas adultas e 168 filhotes
Sexo	Machos e fêmeas
Origem	Biotério Central - UFPel

Solicitamos que o Termo de Consentimento Livre e Esclarecido seja assinado pelo Chefe do Biotério como fornecedor dos animais.

Salientamos também a necessidade deste projeto ser cadastrado junto ao *COBALTO* para posterior registro no *COCEPE* (código para cadastro nº **CEEA 14673-2018**).

_____

M.V. Dra. Anelize de Oliveira Campello Felix

Presidente da CEEA

Documento assinado eletronicamente por **ANELIZE DE OLIVEIRA CAMPELLO FELIX**, **Médico Veterinário**, em 14/05/2018, às 09:50, conforme horário oficial de Brasília, com fundamento no art. 6º, § 1º, do Decreto nº 8.539, de 8 de outubro de 2015.

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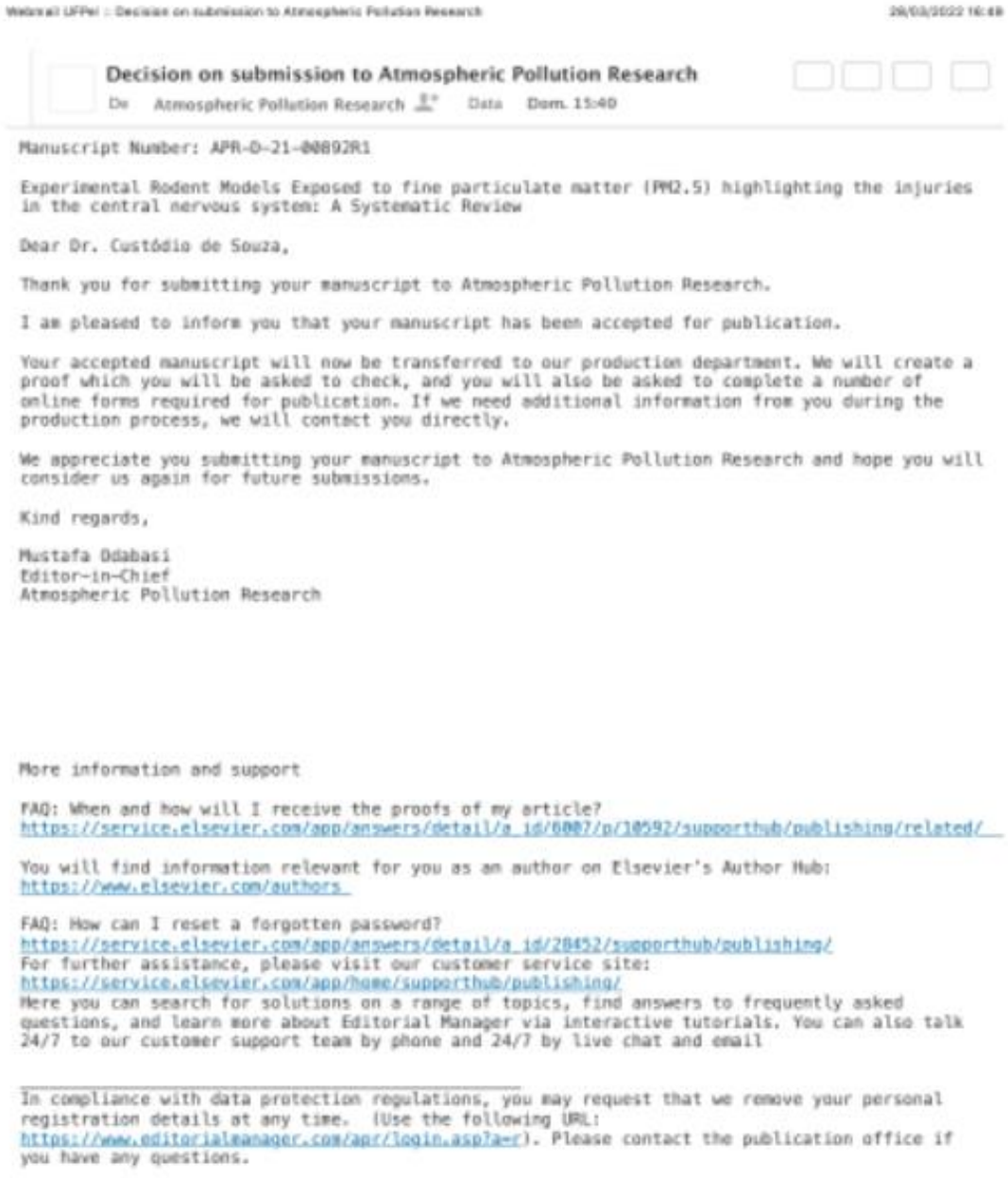
Anexo 2 - Carta de aceite para publicação Atmospheric Pollution Research



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Experimental Rodent Models Exposed to fine particulate matter (PM2.5) highlighting the injuries in the central nervous system: A Systematic Review

Dear Dr. Custódio de Souza,

Thank you for submitting your manuscript to Atmospheric Pollution Research.

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Mustafa Odabasi
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