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Effects of Particulate Matter Exposure on Memory Test Responses as a Function of Particle Size, Sex, Diet, and Mouse Model

THESIS

submitted in partial satisfaction of the requirements

for the degree of

MASTER OF SCIENCE

In Environmental Health Sciences

by

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LIST OF ABBREVIATIONS

PM	Particulate Matter
UF	Ultrafine
IL	Interleukin
TNF-alpha	Tumor Necrosis Factor-alpha
ORM	Object Recognition Memory
OLM	Object Location Memory
Tau	Tubulin associated unit
Aβ	Amyloid-beta
ApoE KO	Apolipoprotein E knockout
Ldlr KO	Low-density lipoprotein receptor knockout
3xTg	Triple transgenic
AD	Alzheimer's Disease
HF	High Fat diet
Chow	Normal chow diet
M	Male
F	Female
NTg	Non-transgenic

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ABSTRACT OF THE THESIS

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by

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Professor Masashi Kitazawa, Chair

Exposure to elevated levels of ambient air pollution particulate matter (PM), is associated with cognitive decline. In this study we examined the impacts of different sizes of PM (concentrated coarse ($D_p \leq$ 10 μm), concentrated fine ($D_p \leq$ 2.5 μm), and ultrafine PM ($D_p \leq$ 200 nm)) on memory in mice using two standard behavioral tests; the object location test (OLM) and the novel object recognition test (ORM). Secondary variables that were examined via behavioral testing included: sex (M vs. F), diet (high fat vs. normal chow diet), and mouse genotype. We used C57BL/6 mice from 2 studies, CEDARS (3xTg-AD mice) which were genetically modified to develop Alzheimer's Disease, and RESTORE (ApoE KO mice and Ldlr KO mice) which can be hyperlipedemic and develop arterial plaques. These models were chosen to examine the potential impacts of ambient PM on neurodegenerative changes in exposed normal and compromised mice. Both studies were examined individually with respect to impact of PM exposure on behavioral test outcomes and were then compared with respect to the secondary independent variables, genetic modification and sex. There were no statistically significant changes in the Cedars study as a function of PM size. There were, however, statistically significant changes in OLM and ORM memory tasks based on exposure length, sex, genetic predisposition, and diet. The data suggests that these factors can interact

with environmental PM exposure to impact memory performance. This analysis did have some limitations including; multiple people analyzed the behavior data, which could have increased variability in the results and there were losses of animals in some groups due to attrition, which reduced the power of the statistical analyses.

INTRODUCTION

Ambient particulate matter (PM) is a complex mixture of solids and liquids suspended in air, which varies greatly by source. Primary sources of particles include natural sources (sea salt, soil dust, etc.), fossil fuel consumption for transportation, power generation, heating, and industrial operations; and dust generated from road abrasion, tire wear, and brake wear. Secondary particles are also created by chemical and photochemical reactions between gaseous species in the atmosphere (Adams et al., 2015). PM is categorized and operationally defined by its size: coarse PM (PM10, <10 μm), fine PM (PM2.5, <2.5 μm), and ultrafine PM (PM0.1, <0.1 μm). Coarse PM is primarily generated by mechanical action (friction or abrasion) from road surfaces and natural sources like salt and crustal minerals (Mukherjee & Agrawal, 2017). Fine and UF PM mainly derive from combustion-related sources (Adams et al., 2015) and secondary formation in the atmosphere (Huang et al., 2019). Particle size is a major determinant of where these particles deposit in the body (Phalen et al., 2010). Most coarse particles are deposited in the upper airways (nasopharynx, larynx), while fine and ultrafine particles can penetrate deeper and deposit into much deeper regions of the lungs, such as the tracheobronchial tree and the gas exchange region of the lung, reaching the alveoli (Phalen, 2004). Ultrafine particles can also deposit in the upper airways as well, where they can transport via the olfactory nerves, allowing direct access into the brain (Elder et al., 2006).

Within the respiratory tract, macrophages can phagocytize PM or its various components and transport them through interstitial tissues allowing components of the PM to translocate throughout the body. These PM components can reach the brain via the bloodstream and lymphatic system. UF & coarse PM deposition in the upper airways can also reach the brain via axonal transport from the nasal region via the olfactory and trigeminal nerves to reach the olfactory bulb and brainstem respectively (Boyes & van Thriel, 2020). PM 2.5 exposure is associated with increased brain infarcts (Wilker et al., 2015), suggesting that exposure can affect the microvasculature in the brain. PM exposure is associated with systemic inflammation and oxidative stress (Abdul-Rahman et al., 2023). Many inflammatory cytokines, which are

elicited by PM exposure, have been shown to cross the blood-brain barrier; including interleukin (IL)-1 alpha, IL-1 beta, IL-1 receptor antagonist (IL-1ra), IL-6, and tumor necrosis factor-alpha (TNF-alpha) (Banks, 2005). The free radicals generate oxidative stress and have been associated with damage to the blood-brain barrier, increasing its permeability to toxic materials present in circulation (Rosenberg, 2012).

Exposure to such toxic substances can impact cognitive function, such as memory. Object Recognition Memory (ORM) and Object Location Memory (OLM) tasks have been used to study long-term memory in mice (Intlekofer et al., 2013; Vogel-Ciernia et al., 2013). Each of these tests targets different brain regions. The OLM task requires extensive use of the hippocampus (Haettig et al., 2011; Mumby et al., 2002). Regions critical to the ORM task include the insular cortex (Bermudez-Rattoni et al., 2005), perirhinal cortex (Balderas et al., 2008), and ventromedial prefrontal cortex (Akirav & Maroun, 2006). The role of the hippocampus in ORM, particularly in long-term memory, is less clear (Haettig et al., 2011). These tests are used over other memory tests as they are less stressful to other memory tests like the Morris water maze test, and thus more representative of typical human memory (Vogel-Ciernia et al., 2013). ORM and OLM can be used to study either short-term or long-term memory, depending on the time between training/acquisition and testing. Long-term memory testing is examined 24-48 hours post-training (Akirav & Maroun, 2006).

The secondary factors in this study: mouse model, sex, and diet are also associated with memory deficiencies. The mouse models used in the study have each been shown to impact memory. 3xTg-AD (Billings et al., 2005), ApoE KO (Gordon et al., 1995), and Ldlr KO mice (Mulder et al., 2004) have all shown statistically deficits in memory in multiple behavioral studies. 3xTg-AD is a model of familial Alzheimer's Disease (AD) who develops memory deficits after the accumulation of intraneuronal Aβ (Billings et al., 2005). ApoE KO and Ldlr KO mice are models of hyperlipidemia, which has been associated with cognitive decline (Anstey et al., 2008). As Ldlr KO mice require a high-fat diet to induce hyperlipidemia (Ishibashi et al., 1993), diet is also associated with this association between hyperlipidemia and cognitive decline.

Some studies have shown sex does not impact long-term memory in C57BL/6 mice (Allen et al., 2014; Melgar-Locatelli et al., 2024), but others have seen differences based on sex (Cory-Slechta et al., 2018) so further study is needed. Age is also an important factor, as studies have also shown that older mice had more long-term object location memory deficits (Wimmer et al., 2012). Our objective with this analysis is to determine the effects of PM exposure on mice as a function of exposure, sex, mouse model, and diet using the OLM and ORM tests to examine potential impacts on memory. Given existing data, I hypothesize that PM exposure will lead to worsened long-term memory in both tests, which will be exacerbated in older mice and hyperlipidemic mouse models but will not be impacted by sex.

METHODS

Exposure

Animals were exposed to ultrafine (UF) particulate matter (PM), Fine PM, or purified air using the versatile aerosol concentration enrichment system (VACES) (Misra et al., 2001) and custom-designed exposure chambers (Oldham et al., 2004). Particles were drawn through a water vapor saturation chamber and then chilled to allow condensation of water vapor onto the particles, which allows UF PM to grow to a sufficient mass to be separated via inertia and concentrated using virtual impaction. These concentrated particles were then separated via cascade impactors into either UF PM or Fine PM. The virtual impactors operate with a major flow of 100 L/min and a minor flow of 10L/min, which results in a nominal concentration factor of 10x. Gaseous components were not concentrated. Exposures begin at 7:30 am and finish at either 12:30 pm or 1:30 pm, depending on the study, to collect PM generated by the morning and lunch commuter periods. The major sources of PM were nearby major roads, the 55, 73, and 405 freeways, as well as the nearby Santa Ana airport (SNA).

The mice used in this study were from 2 different research projects designed to examine the potential effects of ambient PM on the development or exacerbation of neurodegenerative changes in the exposed

mice. The first study (CEDARS) tested whether mice genetically modified to overexpress tau and Aβ proteins would be susceptible to buildup of plaques in their brains and suffer behavioral changes as a result. The second study (RESTORE) tested whether mice genetically altered to be hyperlipidemic (ApoE KO or Ldlr KO with a high-fat diet) would have altered behavioral activities. These have modifications in their ability to process and metabolize cholesterol that could cause inflammatory changes in the brain or blockages of micro blood vessels that could alter blood and oxygen supply to the brain, potentially impacting long-term memory.

For the CEDARS study, there were two cohorts, which allowed us to test the effects of exposure duration and age. Cohort 1 was exposed for 6 months (August 2021 - February 2022) and Cohort 2 was exposed for 12 months (September 2021 – September 2022). The mice in both cohorts were 3xTg-AD Female mice (Oddo et al., 2003), a model of familial Alzheimer's Disease who progressively develop Aβ plaques and tau tangles alongside synaptic dysfunction. Male 3xTg-AD mice do not consistently develop AD pathology to the same degree as females, so they were not used in the study. 3xTg-AD air-exposed mice were the controls for this study to look at the effects of various sizes of PM exposure on transcriptional regulation in the brain as well as the development of AD pathology. They both started exposures at around 7 weeks of age, meaning the Cohort 1 mice were around 8 months old at the end of the exposure and the Cohort 2 mice were around 14 months old at the end of the exposure. Mice were exposed 5 hours/day, 4 days/week. This resulted in 520 hours of exposure for Cohort 1 and 1040 hours of exposure for Cohort 2. Nine animal deaths occurred during the study, primarily in the 12-month exposure cohort. However, the causes of death were not consistent, so no overall conclusion was reached about why these deaths occurred.

CEDARS Mice Count		
Exposure	**Cohort 1**	**Cohort 2**
Air	10	9
Coarse	9	6
Fine	9	10
UF	10	8
Total	38	33

Table 1: Total number of mice in each exposure for both cohorts in the CEDARS study. *All groups started at n=10, but some animals died due to unknown causes over the course of the study.*

	Cohort 1 CPC (particle count/cm2)	**Cohort 2 CPC (particle count/cm2)**
Air	1.52e3 ± 2.05e2	1.46e3 ± 1.71e2
Ambient	1.43e4 ± 3.14e2	1.44e3 ± 3.87e2
Coarse	1.20e4 ± 8.47e2	9.54e3 ± 6.19e2
Fine	5.16e4 ± 5.48e3	5.02e4 ± 3.61e3
UF	5.71e4 ± 6.30e3	5.78e4 ± 5.13e3

Table 2: PM counts for each cohort and exposure group over the course of the CEDARS study. *Results shown in mean ± standard error*

For the UCLA RESTORE study, there were two cohorts. The first cohort was to test the impact of mouse model alone, comparing the ApoE KO and Ldlr KO mice, both with a normal chow diet. The ApoE KO mice have increased total plasma cholesterol levels (Piedrahita et al., 1992) with additional studies also showing impaired spacial awareness and memory (Gordon et al., 1995). The Ldlr KO mice have slightly elevated serum cholesterol levels (Ishibashi et al., 1993) but are not a model of hyperlipidemia unless fed a high-fat diet (Towler et al., 1998). The second cohort was to test the impact of a high-fat diet on Ldlr KO mice, which makes them a mouse model of hyperlipidemia, and to test if there are different effects of sex in these mice. The overall goal of this study was to dissect how PM leads to systemic effects in the vasculature by looking at pathways involved in liver inflammation. Behavioral studies were added to look at the impact of this vascular damage on memory. This is part of an ongoing study, so groups like wildtype animals could be looked at later, but these groups were done first to see the largest impacts. Both cohorts were exposed for 18 weeks (August 2023 – November 2023). All animals started exposure at approx. 6 weeks old, making them almost 6 months old near the end of the study, when behavior tests were performed. Mice were exposed 6 hours/day, 5 days/week. This results in 540 hours of exposure for both Cohorts 1 & 2.

	Ldlr KO HF chow				Ldlr KO normal chow		ApoE KO normal chow	
	PM		Air		PM	Air	PM	Air
	Male	Female	Male	Female	Male	Male	Male	Male
Cohort 1					11	13	13	13
Cohort 2	13	13	13	12				

Table 3: Groups for the UCLA RESTORE Study. *All groups started at n=13, but a few animals died due to unknown causes over the course of the study.*

	Cohorts 1 & 2 CPC (particle count/cm^2)	**Cohorts 1 & 2 DustTrak PM Mass Concentration (ug/m^3)**
Air	2.61 ± 6.31	2.31 ± 1.55
Ambient	8.65e3 ± 7.12e2	Not collected
Ultrafine	7.09e4 ± 4.72e3	96.74 ± 48.35

Table 4: PM counts for each cohort and exposure group over the course of the UCLA RESTORE study. *Results shown in mean ± standard error.*

Behavior

All mice were behaviorally assessed using object location memory (OLM) and object recognition memory (ORM) tests that were conducted based on a procedure from Dr. Wood's lab (Vogel-Ciernia & Wood, 2014). These tests were run during the last weeks of exposure (Jan-Feb 2022 [Cohort 1] & Aug-Sept 2022 [Cohort 2] for CEDARS and Nov 2023 for RESTORE). Approximately 1 hour after the exposure (so they had time to rest) animals were transferred from the vivarium to the behavior room. The walls of the behavior room were covered in white tarp to minimize distractions for the mice, the room was dark, lit only by one lamp near the behavior area, and white noise was playing to mask outside noise. The arenas differed for each task; the OLM test was run in a white, square box arena whereas the ORM test was run in a dark blue, round bucket arena. Both had a 1cm layer of bedding so that it just covered the floor of the arena without being deep enough to encourage digging behavior.

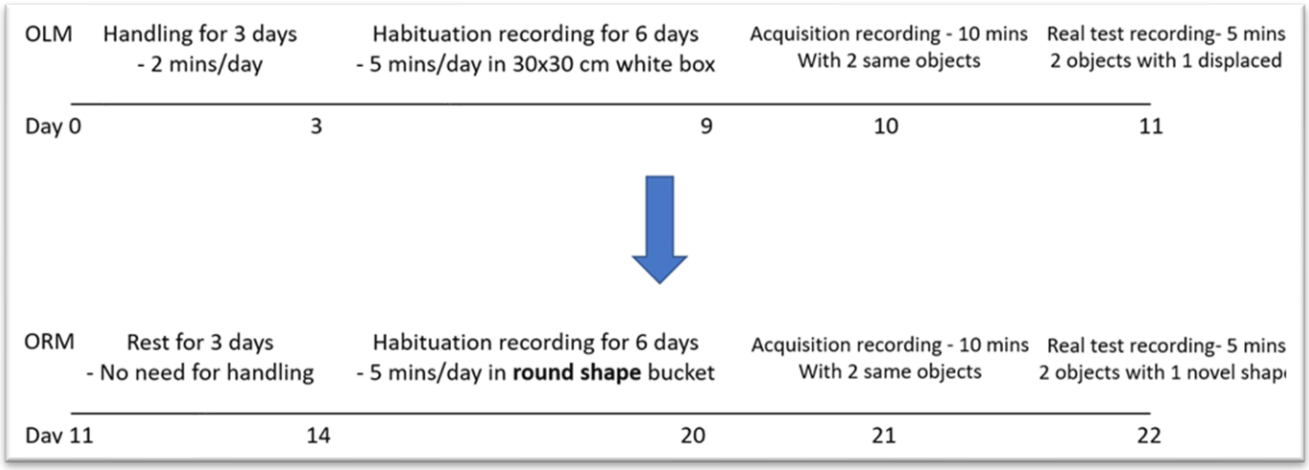

Figure 1: Behavior Schedule. *The cycle on which all our behavior studies were scheduled, with slight differences for each study. We generally use 5 days for habituation instead of the 6 shown. Provided by Jay Hsu.*

Both the OLM behavior begins with 3 days of handling in which animals were simply held in the hands for 2 minutes and allowed to explore freely to get them used to the handler and reduce stress. This is followed by 5 days of habituation in which animals were placed into the empty arena and recorded for 5 minutes each day. This is then followed by 1 day of acquisition, which is recorded. For acquisition (also known as training), animals were placed into the arena with 2 of the same objects and recorded for 10 minutes. This is to test if they prefer either object, in which case they must be removed from further analysis, and also to familiarize them with the objects so that there is a difference when one object is replaced or moved during the test (Lueptow, 2017). The last day in this cycle is the test, in which animals were placed into the same arena from acquisition, but with one of the training objects moved or replaced with a different object. After this, there were 3 days of rest followed ORM behavioral testing using the same full cycle of handling, habituation, acquisition, and test; but now for the other behavioral test. This is to separate the two tests in the memory of the mice to avoid overlap between the two tests. The CEDARS study used this exact cycle, with both cohorts randomized together into one group. The RESTORE study chose to skip the handling step and instead of a resting step for 3 days, the two cohorts were cycled so that one was undergoing behavioral testing for 7 days (5 habituation, 1 acquisition, 1 test) while the other rested. The cycle was as follows:

Cohort 1 OLM, Cohort 2 OLM, Cohort 1 ORM, Cohort 2 ORM. This was done not only to allow for resting, but also to minimize differences in exposure times between cohorts. This did cause slight differences in exposure times between OLM & ORM, but hopefully a 2-week difference is not large enough to impact the results.

Data Analysis

CEDARS Cohorts 1 & 2 were analyzed by Elizabeth Choy, RESTORE Cohort 1 OLM was analyzed by Ashish Sandhu, and all remaining RESTORE was analyzed by Bishop Bliss (Cohort 1 ORM, Cohort 2 ORM & OLM). Acquisition was first analyzed for discrimination index, a measure comparing how often an animal explored the left object versus the right object. The test is then analyzed for discrimination, instead comparing the novel object and the familiar object ((difference between time exploring novel object/combined time exploring novel and familiar objects) *100). A program provided by the Wood lab involved tapping a right and left key every time an animal explored the corresponding object to measure how long each object was explored. Total time exploring each object was then collected from acquisition and test videos. All videos were randomized, and all analysts were blinded as to what group each animal belonged to. All data was then collected by Bishop Bliss and used to calculate discrimination indices. Animals with a discrimination index of above 20 or below -20 were removed from further analysis due to having a pre-existing location bias. For the analysis of the test day itself, both total exploration time and discrimination index were measured and analyzed.

$$DI = \frac{(Time\ exploring\ left\ object - Time\ exploring\ right\ object)}{(Time\ exploring\ left\ object + Time\ exploring\ right\ object)} \times 100$$

Figure 2: Discrimination Index formula for Acquisition. *The formula for Test is very similar, it is ((novel-familiar)/(novel+familiar)*100)*

RESULTS

CEDARS Behavior

CEDARS Mice Count (n)						
Exposure	**Cohort 1**	**ORM**	**OLM**	**Cohort 2**	**ORM**	**OLM**
Air	10	10	8	9	7	6
Coarse	9	7	4	6	5	3
Fine	9	7	8	10	8	5
UF	10	8	9	8	6	5
Total	38	32	29	33	26	19

Table 4: CEDARS Mice Pre- and Post-Exclusion. *Mice with a discrimination index above 20 or below -20 were excluded from the final analyses of the test results. 6 mice were excluded from Cohort 1 ORM, 9 from Cohort 2 OLM, 7 from Cohort 2 ORM, and 14 from Cohort 2 OLM.*

Before analyzing the behavior test data for CEDARS, the acquisition data had to be analyzed to determine if any animals naturally preferred either object. If they did, they would have to be removed from the final analysis as it assumes that the animals have no preference before either one object or location is changed. One animal from Cohort 2 ORM Air was removed due to being an outlier with a discrimination index of -100, this was confirmed by running a Grubb's test for outliers. All other removals post-behavior were due to the acquisition discrimination index. For the CEDARS study, many animals had to be removed due to the acquisition discrimination index, which only compounded the impact of the loss of power from many animals dying during the study. This left many groups with a very small n, weakening the results. The most common cause of object bias is animals being placed too close to one of the objects, though slight differences in the surrounding environment or in the objects themselves may lead to object bias as well.

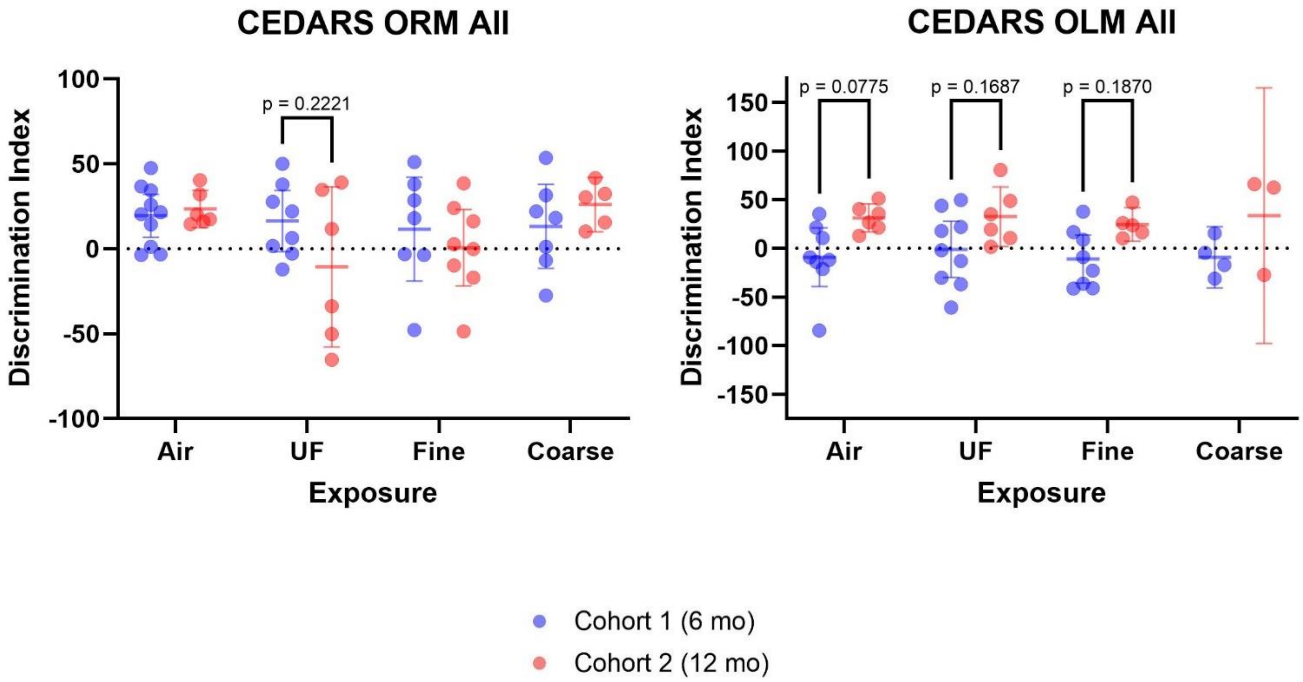

Figure 3: ORM & OLM Discrimination Indices for CEDARS 2021 study. *One-way ANOVA with multiple comparisons. Discrimination indices for both the ORM and OLM tests of the 6-month and 12-month exposures of the 3xTg-AD mice. Results compared to the same exposure in the other cohort. P-values below 0.25 are shown, though none reach p=0.05 significance.*

For the CEDARS 2021 study, all PM exposures were compared to air and the most statistically significant result within a cohort was in ORM Cohort 2 Air vs UF, with a p-value of 0.1809 (graph not shown). Power calculations were done to see what n would cause this result to reach significance (*p<0.05). It was determined that if the n increased from 6 in each exposure, to 9 in each exposure while holding the mean and standard deviation constant then the result would become statistically significant. However, with even this result not reaching any significance in the current experiment we conclude there to be no differences within the cohorts. As such, we compared the cohorts instead.

When comparing ORM results between the 6-month and 12-month exposed cohorts, the group closest to approaching significance was UF (Figure 1) with a p-value of 0.2221. For OLM, Cohort 1 generally had a lower discrimination index for all exposures, with Air (p=0.0775), UF (p=0.1687), and Fine (p=0.1870). This

means that the older mice in Cohort 2 preferred the novel object more in all exposures, even air, than the younger mice in Cohort 1. Still, none of these results alone reach statistical significance ($p<0.05$). 2-way ANOVAs were performed to compare the effects of age/exposure length on the discrimination indices for both ORM and OLM. This revealed that there was a statistically significant difference between the discrimination indices between the 6-month and 12-month exposed mice for OLM ($p=0.0002$), but not for ORM ($p=0.4599$).

RESTORE Behavior Data

	Behavior				ORM/OLM			
	Ldlr KO chow		ApoE KO chow		Ldlr KO chow		ApoE KO chow	
	Male				Male			
	UF PM	Air	UF PM	Air	UF PM	Air	UF PM	Air
Cohort 1	13	11	13	13	13/10	10/10	13/11	13/12
	Ldlr KO HF				Ldlr KO HF			
	UF PM		Air		UF PM		Air	
	Male	Female	Male	Female	Male	Female	Male	Female
Cohort 2	13	13	13	12	12/12	13/13	13/12	12/11

Table 5: RESTORE Mice Pre- and Post-Exclusion. *Mice with a discrimination index above 20 or below -20 were excluded from the final analyses of the test results. No group eliminated more than 3 animals, leaving n=10 or more for every group.*

Just like with CEDARS, before analyzing the RESTORE behavior test data the acquisition data had to be analyzed to determine if any animals naturally preferred either object. If they did, they would have to be removed from the final analysis as it assumes that the animals have no preference before either one object or location is changed. Fewer animals had to be removed from the RESTORE study for their acquisition discrimination index than in the CEDARS study. OLM testing consistently eliminated either the same or more animals than ORM for every group.

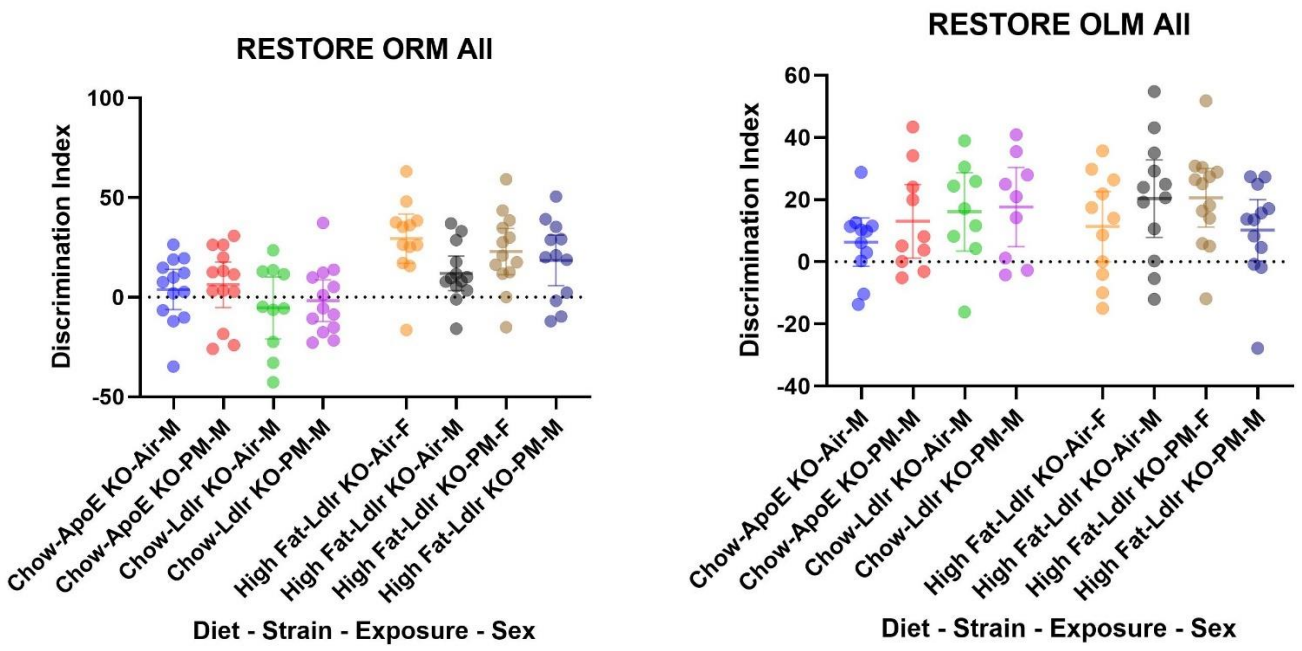

Figure 4: ORM & OLM Discrimination Indices for the RESTORE 2022 Study. *One-way ANOVA with multiple comparisons. Discrimination Indices are shown for Cohorts 1 (normal chow ApoE KO & Ldlr KO) & 2 (high-fat diet Ldlr KO) of the RESTORE 2022 Study. Results were compared between the Air and UF PM exposure of each strain/sex pair. None reached significance p=0.05.*

For the RESTORE 2022 study, we again saw no statistically significant differences within Cohorts 1 or 2 when comparing the discrimination index for air with the UF PM exposure within the corresponding group (Figure 2). No groups had a p-value below 0.4 (graphs not shown). As such, we performed multiple separate analyses to see if there were any differences due to diet, mouse model, or sex.

One notable difference seen in these figures is that the discrimination indices in Cohort 2 appear to be consistently higher than those seen in Cohort 1. Since Cohort 1 had only normal chow mice and Cohort 2 had only high-fat diet mice, we ran a two-way ANOVA comparing the impacts of diet in each cohort on discrimination indices. This analysis shows the overall difference between the cohorts to be very statistically significant for ORM ($p<0.0001$) but not OLM ($p=0.5034$).

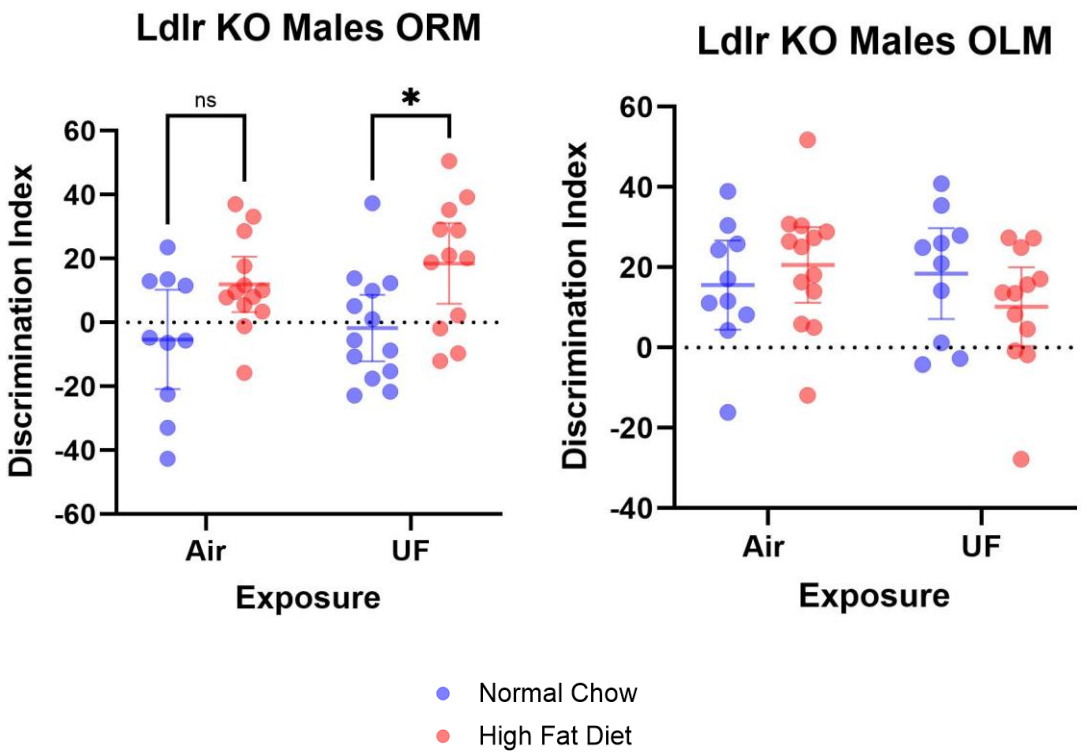

Figure 5: Comparison of the ORM and OLM Discrimination Indices for Ldlr KO males. *Two-way ANOVA with multiple comparisons. Discrimination Indices of ORM & OLM testing for all Ldlr KO mice in the RESTORE 2022 study. Results shown compare differences between these results based on diet. Air exposed mice approached significance (p=0.058) in the ORM study, and UF PM exposed mice showed significance (*p<0.05, p=0.0165).*

To better understand the impact of diet, we followed up on this statistically significant result from the 2-way ANOVA to compare the only mouse model-gender combination present in both Cohorts 1 & 2 directly. For the ORM behavior study, we saw a near statistically significant (p=0.0580) increase in discrimination index between the normal chow and high-fat diet Ldlr KO mice exposed to air and a statistically significant increase (p=0.0165) index between the normal chow and high-fat diet Ldlr KO mice exposed to UF PM. A 2-way ANOVA was run to look at the impact of diet on discrimination specifically on Ldlr KO male mice. The analysis showed that there was a statistically significant impact (p=0.0010) of diet on the discrimination index of the Ldlr KO male mice for the ORM test. These results show an increased preference for the novel object in both Air & UF PM exposures. The OLM behavior study showed no statistically significant changes in the discrimination index, but interestingly the change in the discrimination index for Air exposed mice

went up with the high-fat diet while the change for UF PM exposed mice went down. A 2-way ANOVA looking at the impact of diet showed no statistically significant results (p=0.2690) for the OLM test.

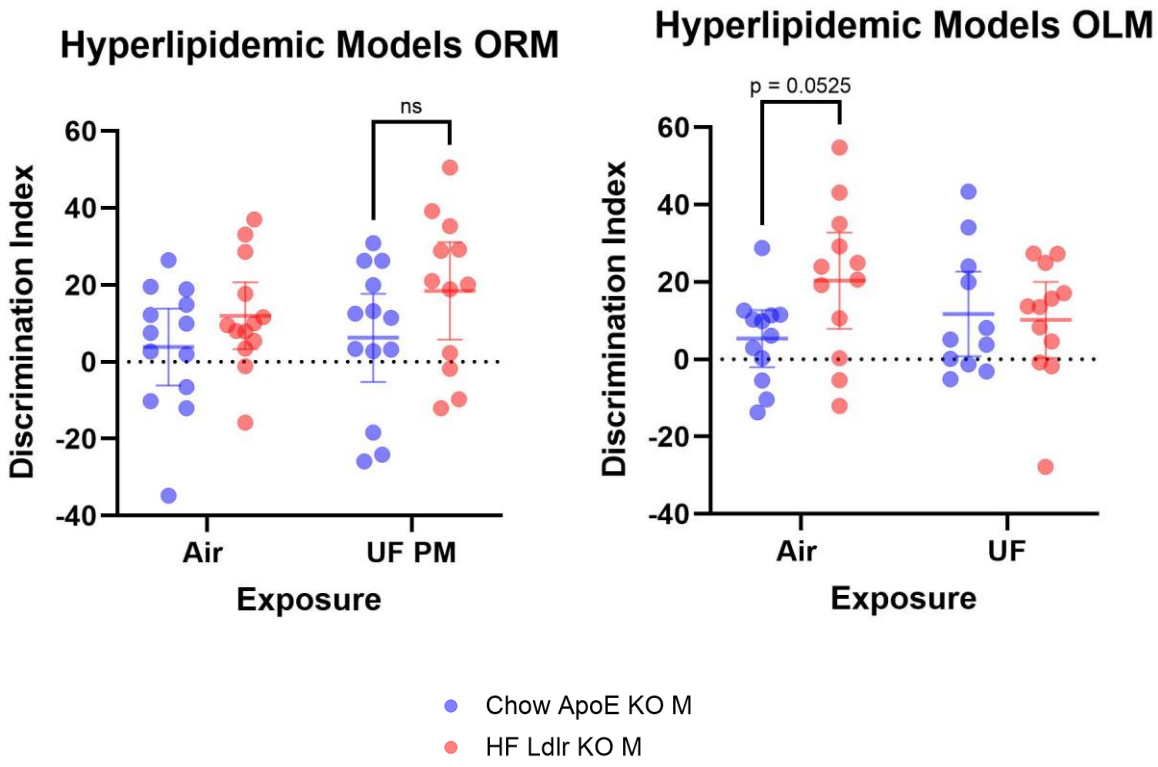

Figure 6: Comparison of the ORM and OLM Discrimination Indices for hyperlipidemic models in the RESTORE 2022 study. *Two-way ANOVA with multiple comparisons. Discrimination Indices of ORM & OLM testing for all hyperlipidemic mice (normal chow ApoE KO & high-fat diet Ldlr KO) in the RESTORE 2022 study. Results shown compare differences between these results based on the two hyperlipidemic models. One result approaching significance was seen, in OLM air exposed mice (p=0.0525).*

We then compared the two hyperlipidemic models to see if they showed different responses in behavioral testing. Both the most statistically significant results here for ORM and OLM show the discrimination indices being higher in the HF Ldlr KO M mice. Results for ORM testing showed a very slight increase in the discrimination index for the UF PM exposure, with a p-value of 0.1691. A 2-way ANOVA comparing the impact of the mouse model on the discrimination index showed a statistically significant increase in the discrimination index (p=0.0442) for ORM. The OLM testing showed a result approaching significance with the air exposure (p=0.0525). A 2-way ANOVA comparing the impact of the mouse model on the

discrimination index did not show a statistically significant increase in the discrimination index (p=0.1568) for OLM.

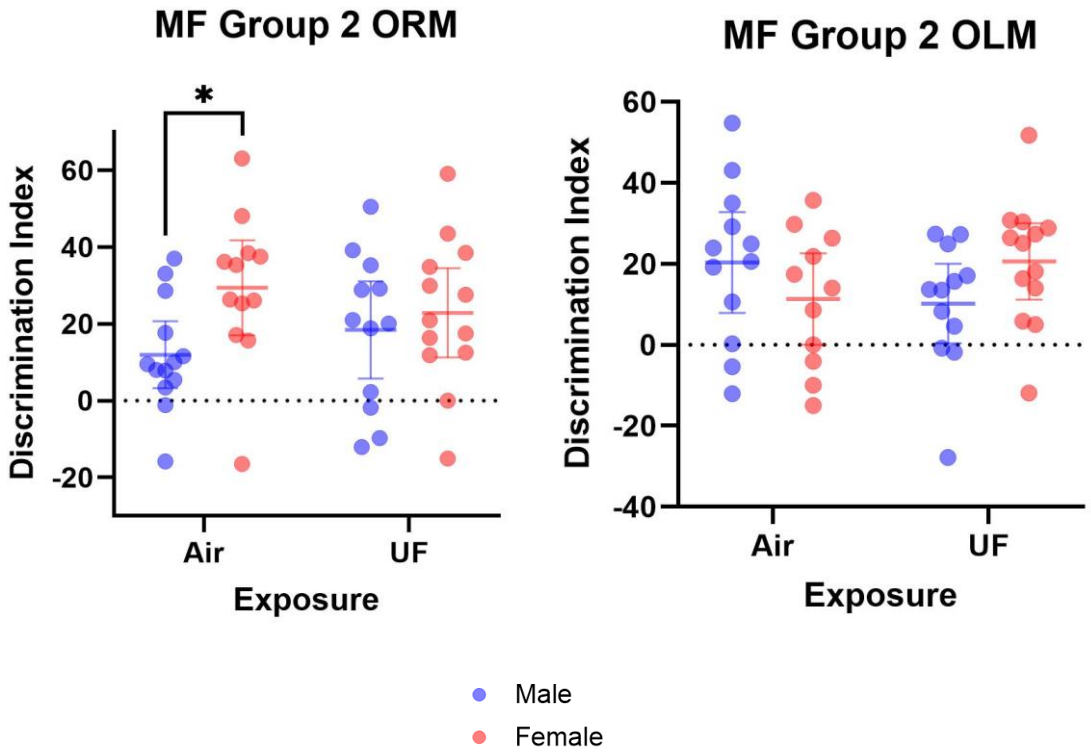

Figure 7: Discrimination Indices of ORM & OLM testing for all mice in Cohort 2 of the RESTORE 2022 study. *Two-way ANOVA with multiple comparisons. All Cohort 2 mice from this study are HF Ldlr KO mice. Results shown compare differences in discrimination index based on sex. Female air exposed mice showed a statistically significant increase in discrimination index (*p<0.05, p=0.0433) in the ORM test compared to the male air exposed mice.*

The final comparison of RESTORE mice is a sex comparison (male vs female). As Cohort 2 has all Ldlr KO mice with a high-fat diet, we were able to look at differences in sex for the Air and UF PM exposures. The only group where male and female mice showed a different discrimination index is Air exposed mice during the ORM testing (p=0.0433). All other groups did not show significance. However, a 2-way ANOVA run to look at the impact of sex on the ORM discrimination indices of the HF Ldlr KO mice showed a statistically significant increase in the discrimination index for the female mice (p=0.0409).

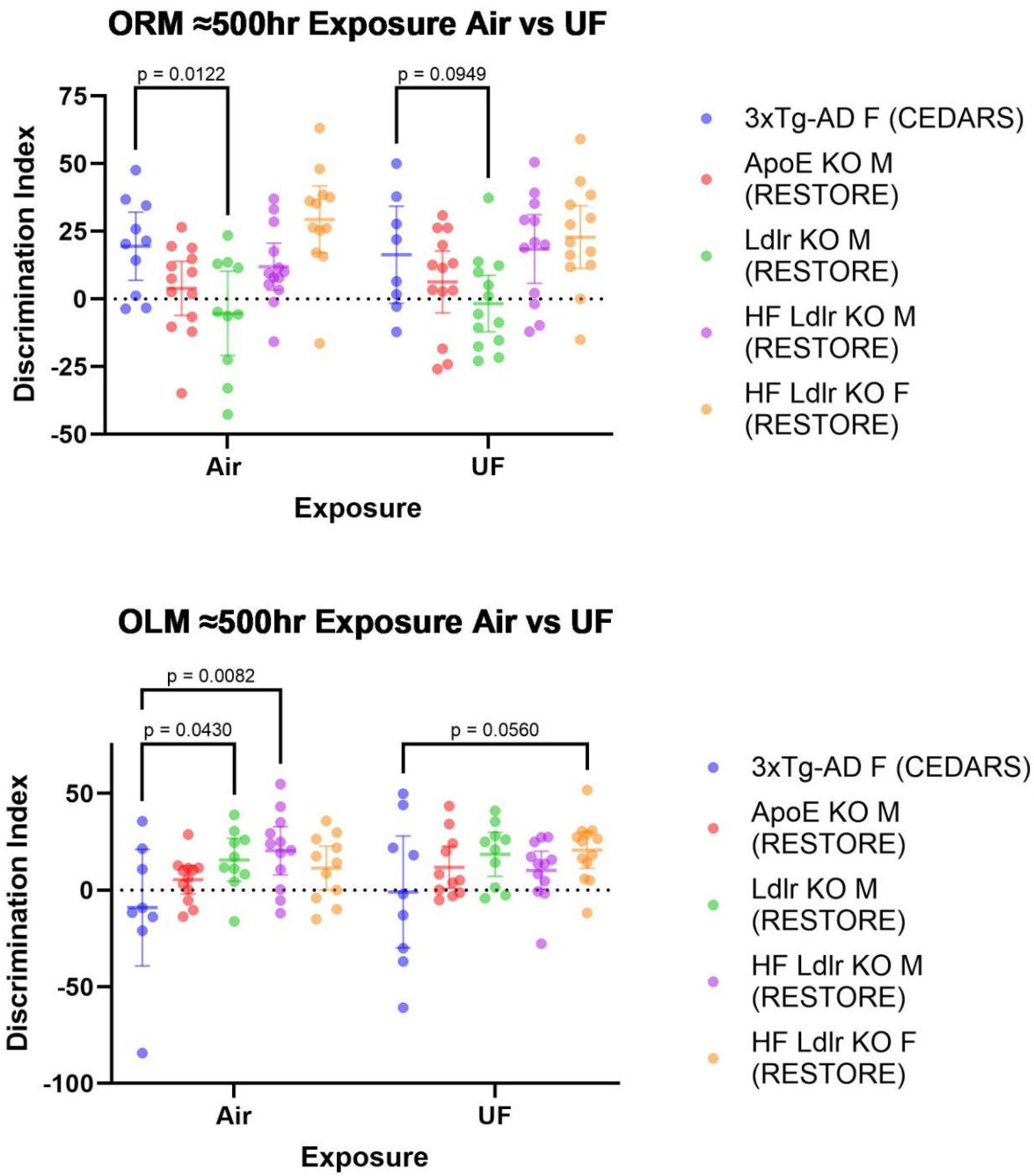

Figure 8: Discrimination Indices of ORM & OLM testing for all mice with approx. 500 hours of exposure. *Two-way ANOVA with multiple comparisons. Comparison of discrimination indices for mice in RESTORE 2022 study and Group 1 of CEDARS 2021 study. Results were compared between CEDARS & RESTORE groups within each exposure. 3 results reached significance ($p<0.05$): ORM Air 3xTg-AD F & ORM Air Ldlr KO M ($p=0.0122$), OLM Air 3xTg-AD F & OLM Air Ldlr KO M ($p=0.0430$), and OLM Air 3xTg-AD F & OLM Air HF Ldlr KO M ($p=0.0082$). Two UF comparisons approached but did not reach significance.*

We were able to make one comparison between both CEDARS and RESTORE, taking advantage of their commonalities in having an UF PM exposure and both having groups with similar lengths of exposure. Despite CEDARS being a 6-month study and RESTORE being an 18-week study, the differences in exposure schedules caused them to both have similar exposure time to either Air or UF PM. CEDARS was 5 hours/day for 4 days/week and RESTORE was 6 hours/day for 5 days per week. This leads to 520 hours of exposure and 540 hours of exposure, respectively. As such, these groups can be compared. As shown in Figure 2, none of the RESTORE groups were statistically significantly different from each other, so they were each compared to CEDARS Cohort 1.

For ORM, the CEDARS mice showed a statistically significant difference in 1 of the 4 air groups, Ldlr KO M ($p=0.0122$). For the UF PM exposed mice, only one group approached significance when compared to the CEDARS mice, Ldlr KO M ($p=0.0949$) again. For OLM, the CEDARS mice showed statistically significant differences in 2 of the 4 air groups, Ldlr KO M ($p=0.043$) and HF Ldlr KO M ($p=0.0082$). For the UF PM exposed mice the only group that approached significance when compared to CEDARS Cohort 1 was the only other female group in the comparison, HF Ldlr KO F ($p=0.056$). An interesting note is that while the CEDARS group discrimination index mean falls in the middle of the other groups for ORM, it does generally fall below all the OLM groups.

2-way ANOVAs comparing the impact of mouse models with around 500 hours of exposure on the discrimination index showed that the mouse model does have an impact on the discrimination index for both ORM ($p<0.0001$) and OLM ($p=0.0079$).

DISCUSSION

Ambient particulate matter has been associated with cognitive decline in several studies (Guxens et al., 2014; Harris et al., 2015; Lertxundi et al., 2015; Porta et al., 2016). In particular, memory deficits related to PM exposure have become an increasingly studied topic as PM exposure has been associated with an increased risk of dementia (Oudin et al., 2016). Studies have also shown that the effects of PM exposure can vary with factors such as the size of PM (Ilango et al., 2023), length of exposure/age of mouse post-exposure (Wimmer et al., 2012), sex of the mice, the mouse model used in the study (Billings et al., 2005; Gordon et al., 1995; Mulder et al., 2004), and diet (Anstey et al., 2008). ORM and OLM tests are commonly used behavioral tests to assess long-term memory (McNulty et al., 2012; Vogel-Ciernia et al., 2013). As such, I hypothesized that I could use results from ORM and OLM to analyze and compare the impacts of PM exposure on memory as a function of exposure, sex, mouse model, and diet.

To test this hypothesis, I first looked at the male 3xTg-AD mice from the CEDARS study. These mice started exposure at 7 weeks of age and were exposed to coarse, fine, and ultrafine PM for either 6 months (Cohort 1) or 12 months (Cohort 2), meaning they were almost 8 (Cohort 1) and 14 (Cohort 2) weeks old post-exposure. There were no statistically significant differences when comparing any of the PM exposures to the air exposure, though a power analysis showed that an increase in 3 animals in both groups for ORM Cohort 2 Air vs UF could bring the p-value of 0.1809 to a level of significance (*$p<0.05$). A 2-way ANOVA comparing the impacts of the exposure length/post-exposure age on the discrimination index showed a statistically significant difference between the discrimination indices between the 6-month and 12-month exposed mice for OLM ($p=0.0002$), but not ORM. Another study looking at the impacts of short-term (2 weeks) UF PM exposure on the memory of 12.5-month-old male NTg C57BL/6 and 3xTg-AD mice (Jew et al., 2019) saw no impact on ORM testing for the 3xTg-AD mice, similar to our study. However, they did see memory deficits in other behavioral tests, such as radial arm maze, independent of the mouse model. This study suggests that looking at multiple behavioral tests may be beneficial and that memory deficits can

occur even with shorter exposures. Our study suggests that age and exposure length play an important role in memory for this mouse model. In another study on a different model of AD, App^{NL-G-F} knock-in mice showed statistically significant decreases in discrimination index for both ORM and OLM. These mice were exposed in our lab for 12 weeks to ultrafine PM in similar concentrations to both CEDARS and RESTORE. The only differences were mouse age, length of exposure, and the person who ran the behavioral testing. As we did not see different discrimination indices in CEDARS or RESTORE, it would follow that the most likely difference is the mouse model itself.

For the RESTORE test, we looked at air & UF PM exposed groups consisting of combinations of mouse models (Ldlr KO and ApoE KO mice), sexes (M or F), and diets (Chow of High-Fat). Cohort 1 looked at male normal chow mice of both models and Cohort 2 looked at M & F High-Fat Ldlr KO mice. No differences were seen comparing Air and UF PM exposures. An analysis comparing the two cohorts shows the overall difference between the cohorts to be very statistically significant for ORM ($p<0.0001$). This difference suggests an effect of diet but because the sex and mouse models between the cohorts aren't the same this needs further analysis. As such I looked at the sex-model combination present in both cohorts, male Ldlr KO mice, and compared the impact of diet on the discrimination index for just these mice and saw a statistically significant impact ($p=0.0010$) of diet on the discrimination index of the Ldlr KO male mice for the ORM test.

Another comparison was to look at the mouse models, comparing the two models of hyperlipidemia to see if they had different impacts on how PM exposure affects memory. A 2-way ANOVA comparing the impact of these mouse models on the discrimination index showed a statistically significant difference in the discrimination index ($p=0.0442$) for ORM, suggesting that how they impact PM exposure's effect on memory is different between both models of hyperlipidemia. Looking at sex impacts on PM exposure is important as it is a growing field in animal research in need of more data. A study on young C57BL/6 mice with short exposures to air or UF PM exposures as pups, then again as adults showed a statistically significant

reduction in ORM discrimination index for both male and female mice (Allen et al., 2014), suggesting that PM has an impact on memory but that it has nothing to do with sex. Another study (Cory-Slechta et al., 2018) shows a decrease in the discrimination index in ORM, but only for males. This study was similarly looking at post-natal development with an early UF exposure and a later UF exposure. These two studies have very different results for the ORM test, suggesting opposite conclusions about the impacts of sex on the effect of PM exposure to influence memory. However, they did use different mouse models, exposure length, and mouse age. They also did behavior tests significantly after exposure. For our study, we see a statistically significant impact comparing M & F for ORM ($p=0.0409$), with females having a higher discrimination index. In OLM, the results are more mixed suggesting that the impact of sex may only be on the ORM test. This lines up with results seen looking at sex in similar studies (Cory-Slechta et al., 2018; Stollery & Christian, 2016).

Comparing the two studies was difficult as they don't share mouse models, but the 6-month exposure CEDARS mice do share the total time exposed with the RESTORE mice as both have just over 500 total hours of exposure. 2-way ANOVAs comparing the impact of each group that has around 500 hours of exposure on the discrimination index showed that the mouse group does have an impact on the discrimination index for both ORM ($p<0.0001$) and OLM ($p=0.0079$). This is the one result in this study showing statistical significance in both ORM & OLM. ORM and OLM target different regions of the brain, with ORM being influences primarily be various cortical regions (with some influence from the hippocampus) and OLM being most influenced by the hippocampus. The sub-analyses suggest that each individual factor is only affecting one test, but that when comparing different studies these differences are no longer restricted to one test and thereby one brain region.

Comparing the overall results to similar studies, a study with $App^{NL\text{-}G\text{-}F}$ knock-in mice showed mean discrimination indices for air exposed mice (as a control group) at around 25 for OLM and 15 for ORM (Kilian et al., 2023). Control groups for a ORM and OLM study done by the Vogel-Ciernia & Wood labs on C57BL/6

mice saw mean discrimination indices between 25-30 (McNulty et al., 2012). For ORM, we see similar results for all CEDARS groups and only 1 group in RESTORE (HF Ldlr KO Air F). For OLM, we see similar discrimination indices in the air-exposed control group results in none of the CEDARS groups and for both air groups in Cohort 2 RESTORE (Figures 2 & 3). This suggests that the discrimination indices for our air groups have a lot of variability compared to other ORM and OLM studies, which in turn suggests that these behavior tests with different discrimination indices in their controls may not have been performed correctly. The comparable CEDARS study group is lower than all the RESTORE study groups, which suggests that given the same exposure length, the 3xTg-AD mice have an overall worse memory than the ApoE KO and Ldlr KO mice. This is not a comparison that has been made directly before, as the 3xTg-AD is a model of early-onset AD, ApoE KO is a model of late-onset AD, and Ldlr KO is neither; only potentially causing memory impairment after an insult such as PM exposure.

It is important to look at the limitations of this study when attempting to reach conclusions about its data. Firstly, due to time constraints, there were several different video analysts, with Elizabeth analyzing the CEDARS videos, Ashish analyzing the RESTORE Cohort 1 OLM videos and myself analyzing the RESTORE Cohort 1 ORM and Cohort 2 OLM & OLM videos. All behavioral testing was still performed by me, but as the scoring of the videos can be a somewhat subjective process with what counts as exploration of an object, this can introduce some variability into the data. A sample of 5 animals from each of Elizabeth and Ashish's analyses were re-analyzed to see if my scoring of the videos differed significantly. This is particularly relevant for the RESTORE ORM comparisons looking at both cohorts and the comparison between CEDARS and RESTORE groups. A t test was performed comparing the analyses and while most results did not show a statistically significant difference ($p=0.2198$ to 0.5402), CEDARS Cohort 2 OLM did show a statistically significant difference ($p=0.0126$). Luckily this group did not end up in direct comparison with any groups with a different video analyst.

Having different video analysts also may have had an impact on total mice in each group, as the CEDARS analysis done by Elizabeth had statistically significantly more mice excluded due to preference during acquisition. This could simply be a difference between the CEDARS and RESTORE study as well. Regardless, it did combine with the many mouse deaths during the CEDARS study to produce some very small groups that weaken the power of the analyses. Another concern here is survivor bias, that perhaps the animals who died were the ones with more severe pathology and the surviving animals were healthier, skewing the results. There were some factors that could have contributed to the within group variability in the data. 1. The two studies compared in this analysis were very different. 2. The hyperlipidemic mouse genetic backgrounds were different. 3. The studies were done at different times of the year so there could have been seasonal differences in PM composition. 4. The behavior tests were performed in different rooms under slightly different conditions.

Overall, this study provides a few interesting insights into some factors (diet, sex, strain) that may be impacting the effects of PM on memory. Statistically significant results were seen in the ORM test for the cohort's impact on how PM exposure impacts the discrimination index for both CEDARS and RESTORE, though no impact of PM itself was seen. Mouse model, sex, and diet were all shown to have an impact on the effect of PM on the discrimination index for ORM as well. Some individual comparisons were shown to be statistically significant across the various analyses as well. However, these results are somewhat unclear due to a variety of differences between the studies such as time, room, and different video analysts. It is important to note that these ORM results involved two cohorts whose videos were scored by different people. Also, the studies themselves were not powered for behavior. Overall, some potentially interesting data was gathered, but future behavior studies would be needed to provide further clarification on these results.

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