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Long-term impact of oral gestational PM10 exposure on morris water maze performance and hippocampal gene expression

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ABSTRACT

Environmental exposure to air pollution, specially to particulate matter (PM) during pregnancy, plays a significant role in increasing the risk of neurodevelopmental disorders such as autism. This study investigated the long-term impact of oral gestational PM exposure (200 μg/kg/day) on memory in aged rats using the Morris Water Maze (MWM). After completing MWM, hippocampal tissue was collected and analyzed for gene expression. Our findings indicate that gestational PM exposure caused no major developmental alterations, aside from slightly poorer performance in the adherence test. No differences were detected in standard MWM manipulations, however, PM-offspring showed reduced latency in the test session, suggesting a possible compulsive-like behavior. Additionally, hippocampal gene expression revealed downregulation of several genes, including NMDA and GABAergic subunits. These effects depended on exposure and sex. The behavioral effects might reflect cognitive inflexibility linked to gene alterations. Further research is needed to clarify these outcomes.

1. Introduction

Environmental conditions and, specifically, air pollution, have been related with many diseases like lung and liver cancer (Sun et al., 2023; Xue et al., 2022), stroke (Kulick, Kaufman and Sack, 2023), and dementias like Alzheimer's (AD; Hussain et al., 2023), Parkinson's (PD; Murata, Barnhill and Bronstein, 2022), and other neurodegenerative disorders (NDDs; Costa et al., 2020). Back in 2014, the World Health Organization (WHO) warned about seven million premature deaths associated with air pollution that occur daily (Clifford et al., 2016). The United States Environmental Protection Agency (EPA) recommends the use of the Air Quality Index (AQI) to determine if the environmental conditions are suitable to outdoor activities. Specifically, this AQI is calculated with different molecules concentration such as Particulate Matter (PM), Second Organic Aerosols, Polycyclic Aromatic Hydrocarbon, Diesel Exhaust, and other molecules (Kumar and Yadav, 2021). Specifically, PM is divided according the molecule size: coarse PM (PM_{10}), fine PM ($PM_{2.5}$), and ultrafine PM ($PM_{0.1}$) (Becker and Soukup, 2003). The primary origin of these molecules is a combination of industrial activity alongside with human activity (López-Granero et al., 2023).

The possible impact that PM might have on the Central Nervous System (CNS) vary depending on various aspects. First, it varies according to the molecule size. Smaller particles are able to cross through the nasal pathway developing CNS inflammation and the release of different cytokines, affecting the CNS cells by neuroinflammation and oxidative stress (Genc et al., 2012; Costa, 2017). Second, it varies depending on the exposure time; recent literature suggests that the developing brain is a vulnerable target to these compounds, particularly during gestational stages. There is not a consensus about the effects of PM on gestational brain, but a combination of neuroinflammation, oxidative stress, and an impaired neurotransmitter secretion have been related to CNS diseases and NDDs (Sunyer and Dadvand, 2019; Cory-Slechta et al., 2019; Nway et al., 2017; Win-Shwe et al., 2016;

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Haghani et al., 2020).

Interestingly, neurodevelopmental and NDDS share some interesting similarities (altered cerebral flow, blood brain barrier integrity, or angiogenesis, among other variables [Ouellette and Lacoste, 2021]). NDDs are understood as a multifaceted condition that is characterized by cognition, communication, behavior and or motor skills impairments (Mullin et al., 2013). Specifically, functional alterations in NDDs can manifest in various ways. For example, in AD some patients show behavioral changes including agitation, aggression, wandering, apathy and social withdrawal, anxiety, paranoia, hallucinations, delusions, and sleep disturbances (Wu et al., 2024). However, the most affected domains are learning and memory [short-term and long-term memory; Nowrangi et al. (2023)].

Regarding this aspect, literature is showing that air pollutants (closer to school-related zones) impair academic performance scores (Chandra et al., 2022), problem solving abilities (Gartland et al., 2022), grade point average, and executive functions (An et al., 2021). Furthermore, PM molecules are also related with lower learning and memory outcomes in children, and it has also been related with reduced working memory's (WM) performance (Thompson et al., 2023; Rivas et al., 2019). Furthermore, studies using older cohorts also found that $PM_{2.5}$ exposure is associated with poorer cognitive performance in episodic memory (total recall), language skills (sematic fluency) and executive functions (Zare Sakhvidi et al., 2022a; 2022b; Semmens et al., 2023). In addition, preclinical evidence also shows that other air pollution compounds like the diesel exhaust can impar spatial learning performance (Ehsanifar et al., 2021; Zanchi et al., 2010) when exposed in adulthood. Nevertheless, it is essential to analyze these potential impacts of PMs on the developmental brain (at the gestational period). Woodward et al. (2018) found impaired hippocampal-dependent memory when exposed to PMs from gestational day 1 (GD1) to postnatal week 25; interestingly, Oliver et al. (2024) detected a sex-dependent effect on memory outcomes (exposing their animals 6 weeks before mating until ending the lactation period). Along the same lines, other researchers also detected impaired hippocampal-dependent memory using the Morris Water Maze (MWM; Zhang et al., 2023a; Ehsanifar et al., 2019). However, all this preclinical evidence has been focused only in $PM_{2.5}$ without studying the effects of PM_{10} on cognitive functions. Ruiz-Sobremazas et al. (2023)'s review showed the absence of evidence analyzing the effects of larger PM particles on cognitive performance, and to our knowledge, there is few articles analyzing this effect. Also, most of the evidence analyze the effects of PM2.5 on memory in adolescence or youth rodents, while no article, to our knowledge, have analyzed the impact of gestational exposure on memory in older-adult rodents. Previous results from our work showed that PM_{10}-exposed offspring had higher recognition index than control group in the Novel Object Recognition task (Ruiz-Sobremazas et al., 2025).

As explained before, the effects of air pollution could be mainly explained by three different components: increased oxidative stress, increased neuroinflammation and altered neurotransmission systems (Myhre et al., 2018). Specifically, Zhang et al. (2023a) detected an increased presence of microglia in the cerebellum in adolescence when exposed gestationally to $PM_{2.5}$; furthermore, higher levels of 8-Hydroxyguanosine (marker of oxidative stress) miRNA were detected in microglia and Purkinje cells, higher levels of TNF-α and IL-8 protein concentration and mRNA relative expression were present in the cerebellum, as well as higher levels of lipid peroxidation. In addition, Xu et al. (2022) evidenced higher microglia activation in the hippocampus and in the dorsal Prefrontal Cortex (dPFC) in aged mice exposed to traffic related air pollution (TRAP) for 12 weeks. They also found an increase in other neuroinflammatory markers in the mRNA expression of *Il-18, IL-1β, CAT,* and *SOD2* in the hippocampus; and *IL-18* and *CAT* in the dPFC. In addition to neuroinflammation and oxidative stress data explained, Ehsanifar et al. (2019) detected a reduced *NR2a* and *NR3b* mRNA relative expression in the Hippocampus (HCC), which is closely related to spatial learning in the MWM (Wierońska et al., 2023). Previous results from our lab have shown that gestational exposure to PM10 is related with reduced *Maoa* and *Gabrg2* expression in pups (Ruiz-Sobremazas et al., 2024), a reduced expression in *Gad1*, *Aches*, and *PTEN* in the cerebellum; an increase mRNA expression of *Aif1*, *Gad2, Grin2a, Grin2b, Grin2c,* and *Gabrb2* in the hypothalamus, and a reduced expression in the frontal cortex of *Trh, Pon2, Gad2, 5-Htr2c, 5-Htr2a* (Ruiz-Sobremazas, *under review*).

However, most of these studies exposed animals to PM via inhalation, often using whole-body exposure chambers or similar methods (Ruiz-Sobremazas et al., 2023). Nonetheless, PM can settle on land (Islam and Saikia, 2020) or dissolve in drinking water (Pramanik et al., 2020), where it may be absorbed by plants, vegetables, and fruits (Rathebe and Mosoeu, 2023; Rodríguez-Rodríguez et al., 2023; Paull et al., 2020). Through ingestion, these particles can enter the human body, representing a potential route of exposure that may harm human health. Despite this, oral exposure to PM during gestation and its possible link to NDDs remains underexplored. Similarly, other routes, such as dermal exposure, have received little attention, leaving a significant gap in the field of environmental toxicology.

Based on early information, the present study formulates three hypotheses. To begin with, we expect that gestational exposure to PM_{10} will affect neurodevelopmental variables, replicating previous results (Ruiz-Sobremazas et al., *under review*; Ruiz-Sobremazas et al., 2025; Ruiz-Sobremazas et al., 2024). Secondly, we expect that gestational exposure will reduce spatial learning ability in late-adult rodents, impairing the MWM performance. Lastly, we expect that gestational exposure to PM_{10} would result in higher mRNA gene expression of specific genes related with neuroinflammation and oxidative stress, as well as an increase in mRNA expression of some subunits of NMDA receptors, and GABA receptors, replicating Ruiz-Sobremazas et al. (*under review*) and Ruiz-Sobremazas et al. (2024) results in a late-adulthood developmental stage.

2. Material and methods

2.1. Experimental animals

The experimental animals that formed the F0 generation were 11 female Wistar rats (ENVIGO, Barcelona, Spain), aged 3 months. All subjects were housed with three other rats in our facilities in clear polycarbonate cages (50x35x20cm; total volume of 437.5 cm^2). Housing conditions were controlled to a 12-h light cycle starting at 8 p.m., 50 ± 10 % of humidity, and 22 ± 2 ºC for temperature. All rats remained untouched for 14 days to facilitate their habituation to the room conditions; both food and water were provided *ad libitum* to the F0. The female's estrous cycle was monitored daily, and once in late diestrus/early proestrus, each female was paired with one male for 24 h. Sperm presence was checked to confirm mating, and if present, each female was housed individually to start their gestational period (considered gestational day 0; GD0). One day after pregnancy confirmation (GD1), F0 pregnant rats were randomly allocated into PM_{10} exposed or control group. Between GD18 and GD20, a cotton swab was placed in every home cage to allow the rats to build nests. All F0 generation remained undisturbed during the gestational period. All pups (F1 generation; PBS-Males [n = 12], PBS-Females [n = 7], PM-Males [n = 13], PM-Females [n = 11]) were born on the expected days (postnatal day 0; PND0). F1 generation was weighted every two days starting at PND1 to PND31, and monthly from PND210 to PND480. All F1 generation was maintained with a restricted diet (15 g for females, and 18 g for males) until sacrifice at postnatal month (PNM) 17. Water was provided *ad libitum*. F1's environmental conditions were the same as those present for F0. The ARRIVE Guidelines for animal testing were followed (Fig. 1). Also, in Table 1 is depicted the dam and offspring distribution between tasks.

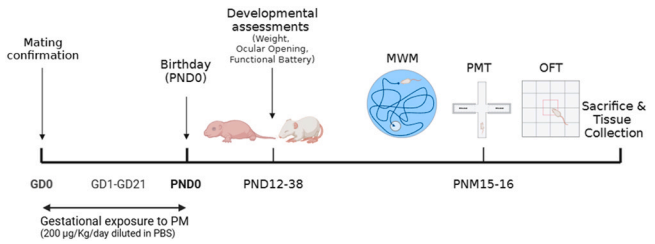


Fig. 1. Image representation of the experimental timeline. Gestational days (GD); Postnatal day (PND), Postnatal month (PNM).

Table 1
Animals used in the present study.

	Development		Behavioral Tasks		RT-qPCR	
	PBS	PM	PBS	PM	PBS	PM
Males	n = 12 (6)	n = 13 (6)	n = 10 (6)	n = 11 (6)	n = 6 (4)	n = 5 (4)
Females	n = 7 (3)	n = 11 (4)	n = 7 (3)	n = 11 (4)	n = 5 (2)	n = 4 (3)

The main number refers to the number used in each group while the number between brackets refers to the number of dams used in each group

2.2. Toxic agent

Half of the pregnant rats were randomly exposed to PM10 (experimental group), controlling body weight to avoid potential biases, whilst the remaining were assigned to the control condition. A specific dosage of 200 µg/kg/day (Standard Material 2787; NIST, Lot: 110,626) was diluted in 5 mL of PBS (Ph7.4), and animals were exposed oral free from GD1 to GD21, where control rats received the same vehicle at the same volume. Standard Material 2787 is a mixture of several components commonly found in air pollution. The main constituents of this material include polycyclic aromatic hydrocarbons, polybrominated diphenyl ethers, various inorganic elements such as metals (aluminum, iron, calcium, lead, etc.), sugars, and dioxins and furans. A detailed summary is provided in Supplementary Material 1. This PM10 dilution and the control condition were presented in water dispensers with steel balls to avoid dripping. All dispensers (with PM_{10} dilution or only PBS) was placed every day in their home cages with the specific dosage. All F0 generation drank the solution in less than two minutes. PM_{10} exposure was performed between 13:00–14:30. This dosage was calculated based on the results reported by Rovira et al. (2020). Specifically, the TecNAtox group detected doses ranging from 25 to 40 µg/m^3 of PM_{10}, depending on climatic conditions and the season of the year. Considering that an adult human (70 kg) breathes approximately 20 m^3 of air per day, the estimated daily intake is about 19–20 µg/kg/day. Based on this reference exposure dose, and applying a safety margin of 10 × to allow comparison with the human population, we calculated a total dose of 200 µg/kg/day. Previous works already used this dosage (Ruiz-Sobremazas et al., 2024, 2025). This exposure route was selected for several reasons, as outlined in the introduction. In summary, most studies primary investigate the effects of PMs via the inhalation pathway. However, larger particles have also been identified in vegetables (Noh et al., 2019), milk (Douglas et al., 2016), and water (Cowen and Ollison, 2006). Also, further explanation can be seen in Ruiz-Sobremazas et al. (2025), (2024).

2.3. Behavioral analyses

2.3.1. Dams and pup developmental assessment

Dams remained untouched until the PND1, where dams' pups were sexed and adjusted to n = 10 per mother (n = 5 per sex when possible). Also, at PND1, pups were randomly distributed between mothers with the same exposure condition. Pups were weighted every two days from PND1 to PND31, and monthly starting at PND210 to PND480. Ocular opening was assessed between PND12 and PND15, with direct scores of 0, 1, and 2, and converted into percentage (0, 50, 100) depending on the percentage of eyes opened. Furthermore, different neuromotor tests were performed at PND16. These tests consist on: grip capacity, adherence to an inclined test and climbing capacity. The first test was the grip capacity. The pup was placed in the rear and it was pulled to the researcher. The score varied depending on the resistance (difficulty to move the animal from the rear by pulling) from 0 to 1 or 2 (none, slight, or strong resistance). The second test was the adherence to an inclined slope. The pups were placed in the middle of the rear with a slope of 60º. The animals that immediately fell received a score of 0, while 1 or 2 were given to rodents that fall after a brief period of 10 s or did not fall at all. Lastly, the climbing capacity was also assessed with the rear and with the same 60º slope. The rear was divided into three different parts and rats were punctuated according to the part reached by climbing (bottom, middle or top; 1, 2, or 3 respectively). All PND16's tests were performed in their own homerooms with dim light and same environmental conditions as explained earlier.

2.3.2. Morris water maze task (learning, spatial and working memory, and inhibitory control)

A black pool (50 cm height, 150 cm diameter) was used to perform the MWM. This pool was filled with tap water to around 1.5 cm over the top one black platform. This platform was always placed in the same position for each animal; however, two different positions (east and west) were used (half animals of each sex and exposure conditions were trained in one position and the other half in another) in order to control any possible bias. Different external stimuli were distributed around the pool to facilitate contextual cues. Water temperature was maintained at 22 ± 1.5ºC. Light conditions were set and controlled to 15–25 luxes in every part of the pool and > 50 luxes in the contextual cues. All animals were moved to the experimental room 30 min before the beginning of the procedure.

All animals performed the task at PNM15, and finished it approximately at PNM16. The MWM protocol described in de Bruin et al. (1994), but with some changes. The experiment was divided into different phases: *Pre-training*, *Acquisition*, *Probe*, *Reinstatement*, *Reversal Learning*, *Visual Control*, and *Working Memory*. The order in which animals performed the task was counterbalanced to avoid any hour-bias. The *Pre-training* phase consisted in one session of 16 trials (4 times in every release point). The *Acquisition* (spatial learning) phase consisted in 8 consecutive session of 4 trials each; the *Probe* phase consisted of an unique session lasting 60 s single-trial where the platform was removed from the pool; the *Reinstatement* phase consisted of a four trial session; the *Reversal Learning* was a three session with four trials each, the platform was moved to the opposite site of the pool; the *Visual cue* phase consisted of a three session with four trials where the level of water was reduced to 1.5 cm lower to the platform and this was covered with white tape to allow the animal to see it; lastly, the *Working Memory* phase consisted of six sessions of two trials each, where the platform position moved across sessions and not across trials. Each trial consisted of releasing the rat in a pseudo-randomly order in some of the four quadrants. The release order was different between sessions but remained unchanged within sessions. The natural rodent behavior in this paradigm is trying to escape, but the only opportunity to scape is finding the hidden platform. All rodents had 90 s to reach the platform; if they did not find the platform, the researcher put them in the platform. All animals stayed 15 s at the platform, whether they found it or not. Once the time finished, all animals were removed from the pool setting and a fixed inter-trial-interval of 15 s started in a placed where the animals had no eye contact with contextual cues.

The main variables analyzed were escape latencies (sec) as the crucial variable in every phase, time in quadrants (sec) which is truly important in the *Probe* phase, heading to platform (mean) that is

important in every phase, and distance moved (cm) and velocity (cm/s) for locomotor state activity. Furthermore, an index of periphery/total time (P/TT) ratio was also calculated. Lastly, we sum the average latencies in every session and calculate total mean latencies (sec). Rat's behavior was recorded using OBS Studio (v27.2.3; OBS Project) and analyzed with Ethovision v16 (Noldus).

2.3.3. Locomotor activity (Motricity)

Motricity was assessed recording each animal's locomotor activity. A plexiglass activity chambers (AccuScan Instruments, Inc., USA/Canada) with dimensions 39 × 39 x 15 cm were used. An array of 16 × 16 x 16 photo beams spaced at 2.5 cm is included to track animal behavior. Rodent behavior was analyzed for 30 min divided in 6 blocks of 5 min each. All experimental chambers were controlled with a computer with VersaMax 4.12. Before recording the locomotor activity, all rats were driven to the experimental room and then isolated for 1 h in individual boxes with *ad libitum* food and water. The selected variables were total distance (cm), vertical time (s), margin distance (cm), center and margin time (s), ratio time (periphery vs center), and velocity (cm/s). Data was extracted with VersaData (PLC Control System SL). Environmental conditions were set at 22 ± 2 ºC for temperature, 50 ± 10 % for humidity, and dim-light condition for environmental light.

2.3.4. Plus maze test (Anxiety)

The Plus Maze test was performed after the Open Field test. The Plus Maze test is an elevated platform (90 cm above the ground) with 4 arms, of which two are enclosed with walls while the other two do not have any walls. All arms are connected by a central square, place where the animal is placed. The rodent, once placed inside, must decide whether to explore the closed arms, where higher anxiety rates can be found, or to explore the open arms, which shows the classical rodent exploring behavior. All animals were moved to the experimental room one hour before starting the procedure. Once time finished, each rat was placed individually into the center square of the apparatus and let it freely explore for 5 min. Animal behavior was recorded using OBS Studio (v27.2.3; OBS Project), and analyzed with Ethovision (v16; Noldus) software. The selected variables were: distance moved (cm), velocity (cm/s), time in each arm (s), entries to each arm (n), anxiety index {AI = [(CA - OA)]/[(CA + OA)]}. 70 % Ethanol was used to clean the apparatus between rodents. Experimental order was counterbalanced between rodents to avoid any possible bias. Environmental conditions were set at 22 ± 2 ºC for temperature, 50 ± 10 % for humidity, and dim-light condition for environmental light.

2.4. Brain reverse transcription quantitative polymerase chain reaction (RT-qPCR)

All rats were sacrificed after completing the Plus Maze test. They were anesthetized with equithesin and quickly decapitated. The brain was dissected into the frontal cortex, hypothalamus, hippocampus, and cerebellum. Only the hippocampus was used in this experiment. All samples were immediately flash-frozen to prevent RNA and protein degradation. All materials were treated with RNAse ZAP (Invitrogen), and samples were stored at −80°C until use. RNA isolation followed the Trizol method (Ambion), and RNA total concentration was determined using a Qubit fluorometer (Invitrogen). RNA was normalized to 100 ng/µL for cDNA synthesis, which was then diluted in RNAse-free water (1:10 factor) for RT-qPCR. RT-qPCR reactions were carried out using SYBR green master mix, primers, RNAse-free water, and cDNA. Samples were added in duplicates and analyzed using a thermocycler (Step One v2.2.2, Applied Biosystems). Melting curves and Ct values were examined for abnormalities (e.g., multiple peaks), with primer efficiency evaluated using a standardized dilution. *GAPDH* primers targeting exons served as the housekeeping gene, whilst *GAPDH* primers designed into an intron section of the gene were used to quantitatively control gDNA contamination of our samples. Only genes with Ct values below 30 and without melt curve abnormalities were included for statistical analysis. Ct values were normalized to the housekeeping gene (ΔCt) and to the subject with the highest ΔCt (ΔΔCt). ΔΔCt values were transformed to calculate fold changes (2ΔΔCt). Primer characteristics are represented in the Table 2.

2.5. Statistical analyses

Both sexes and exposure groups were chosen as the main factors for all the analysis. All variables that involved more than 1 evaluation (weight, ocular opening, MWM comparisons when performed between sessions or trials, and Locomotor activity comparisons) were analyzed using RM-Bayesian ANOVAs, while discrete variables (birthweight, ocular opening in each day, functional battery variables, PMT variables, and RT-qPCR fold change) were analyzed with a two-way Bayesian ANOVA. RT-qPCR comparisons were performed using fold change (relative expression). A Principal Component Analysis was performed within the sessions of the learning phase (mean latency in each session). Lastly, Bayesian correlations were performed between gene expression and S9 outcomes.

Following Ruiz-Sobremazas et al. (2025) analysis, and Golub and Sobin (2020) recommendations, litter was included in the analysis as random factor to control intralitter likeness. The experimental unit is, therefore, the F1 pups. Evidence supporting hypothesis H1 (indicating a potential effect of PM on the variable) was considered when $BF10 > 1$, while evidence for H0 (suggesting a null effect of PM on the variable) was indicated when $BF10 < 1$. Interpretation of BF10 was categorized as follows: $1 < BF10 < 3$, anecdotal evidence for H1; $3 < BF10 < 10$, moderate evidence; $10 < BF10 < 30$, strong evidence; $30 < BF10 < 100$, very strong evidence; $BF10 > 100$ extreme evidence (Lee et al., 2013). Due to the lack of sufficient prior evidence, we set a uniform 0.5 prior which reflects unbiased assignment of probabilities between both hypotheses. Anecdotal evidence refers to observed effects in the data that do not reach the threshold that is set in the Bayesian analysis to be considered as moderate evidence. Those anecdotal evidence should be interpreted as illustrative (Quintana and Williams, 2018), offering little support for the alternative hypothesis. All statistical analyses were conducted using JASP (Amsterdam, Netherlands), software version 0.18.0.0. Images were created using GraphPad Prism 8.

3. Results

Weight evolution analyses were divided into birthweight (PND1), preweaning (PND1-PND21), postweaning (PND23-PND38), and adult development (PNM7-PNM16). First, birthweight analyses revealed that neither exposure nor sex ($BF_{10} \approx 1$) affected birthweight (Fig. 2a), while litter achieved extreme evidence ($BF_{10} = 311710.528$). Furthermore, same results were present for the preweaning stage, where an expected effect of PNDs was evidenced ($BF_{10} = 6.928 \times e^{307}$; Fig. 2a) without any sex or exposure effect, while litter remained with anecdotal evidence ($BF_{10} = 2.091$). Next, for the postweaning period, an expected PNDs effect was found ($BF_{10} = 1.409 \times e^{+114}$) while exposure and sex did not affect weight evolution, and litter remained with anecdotal evidence ($BF_{10} = 1.310$). Lastly, in terms of adult development, an extreme effect of sex was detected ($BF_{10} = 3.503 \times e^{+14}$; Fig. 2a), where males achieved higher weights. In addition, PNDs also reached extreme evidence ($BF_{10} = 728.153$), but exposure seems not affect adult weight evolution, and this effect is not affected by litter ($BF_{10} < 1$).

Regarding ocular opening (PND12-PND15), an expected extreme PNDs effect was present ($BF_{10} = 4.135 \times e^{+34}$; Fig. 2b), but neither exposure nor sex neither litter seems to affect ocular opening, with the only exception of exposure, which marginally reached anecdotal evidence in percentage eyes opened at PND12 ($BF_{10} = 1.296$; Fig. 2b), showing that PM-offspring marginally opens their eyes faster than control. Lastly, no effect of sex neither exposure nor litter was detected in the grip and climbing tests from the functional battery performed at

Table 2
Primer Selection for Gene Expression Analysis Using the NCBI Tool.

Gene name	NCBI name	Forwad (3' > 5')	Reverse (3' > 5')	Origin
Glyceraldehyde-3-phosphate dehydrogenase	*GAPDH* (Intron)	ctgggtggctcaaggaata	cacacgcatcacaaaaaggt	Authors' design
	GAPDH (Exon)	cttcaccaccatggagaag	catggactgtggtcatgag	Authors' design
Forkhead Box P1	*FOXP1*	ccctctgtcatcaccaccac	ggtggtctaacttccgcgtt	Coca et al. (2024)
Glutamate receptor subunit 1a	*Grin1a*	atggcttctgcatagacc	gttgtttacccgctcctg	Lau et al. (2013)
Glutamate receptor subunit 2a	*Grin2a*	agttcacctatgacctctacc	gttgatagaccacttcacct	Lau et al. (2013)
Glutamate receptor subunit 2b	*Grin2b*	aagttcacctatgacctttacc	catgaccacctcaccgat	Lau et al. (2013)
Glutamate receptor subunit 2c	*Grin2c*	ggcccagcttttgaccttagt	cctgtgaccaccgcaagag	Lau et al. (2013)
Serotonin receptor 2a	*HT2a*	aacggtccatccacagag	aacaggaagaacacgatgc	Kindlundh-Högberg et al. (2006)
Serotonin receptor 2c	*HT2C*	ttggactgagggacgaaagc	ggatgaagaatgccacgaagg	Kindlundh-Högberg et al. (2006)
Gamma-Aminobutyric Acid Type A Receptor Subunit Alpha2	*GABRA2*	ccaggatgacggaacattgc	ggaaagtcctccaagtgcatt	Fujimura et al. (2005)
Paraoxonase 2	*PON2*	tagaccttccaaactgccgc	acgctaaagaatgccagacca	Authors' design
Allograft inflammatory factor 1	*AIf1*	agaatgatgctgggcaagaga	tagctttcttggctggggg	Authors' design
Thyrotropin Releasing Hormone	*TRH*	ccaggggacttgtggtcaaa	cttgtgggctttgcttcacc	Authors'design
Dopamine receptor 2	*DRD2*	cagtcgagctttcagagcca	ccaattctccgcctgttcact	Authors' design

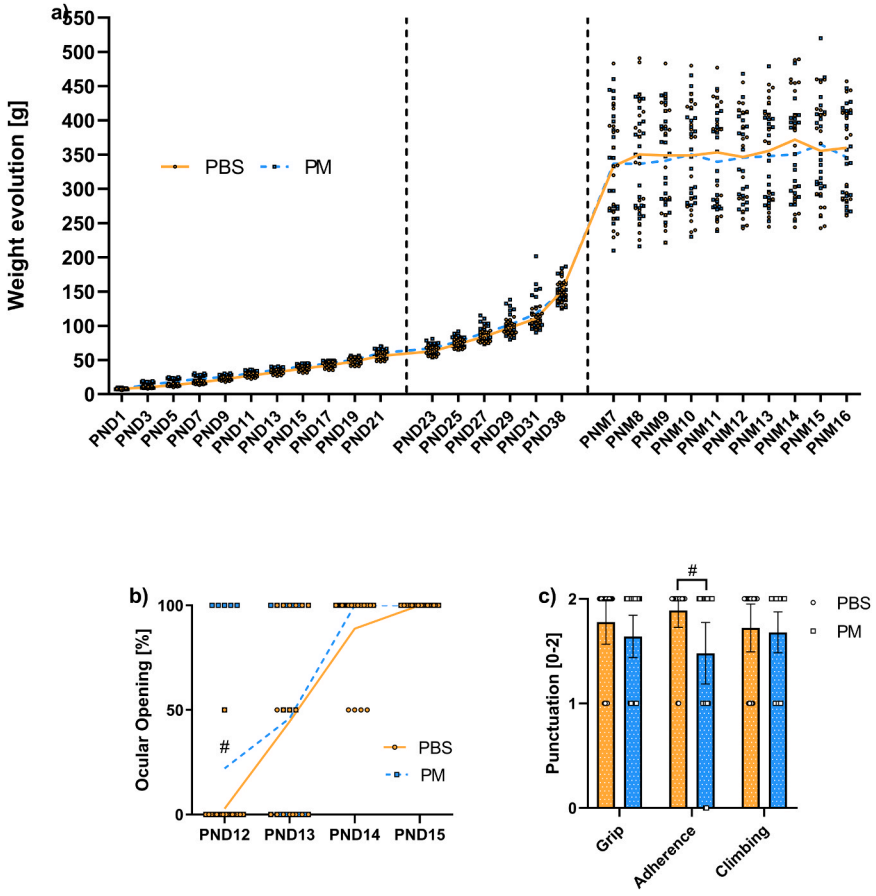


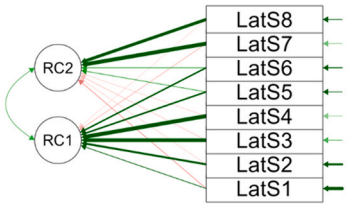
Fig. 2. Graphical representation of developmental variables. Figure a) shows weight evolution from birth to PNM16; image b) depicts percentage ocular opening between PND12 and PND15; image c) represents the punctuation obtained at PND16 in the Grip, Adherence, and Climbing tests. Individual values are represented in all images. # Anecdotal evidence. Total n in each group is as follows: PBS-Males (12), PBS-Females (n = 7), PM-Males (n = 13), PM-Females (n = 11).

PND16. However, anecdotal evidence was present for exposure towards H1 in the adherence test for exposure (BF_{10} = 2.208), where PM-offspring seems to perform worse this task (PBS; M = 1.889; 95 %CI = 1.728 – 2.050; PM; M = 1.480; 95 %CI = 1.185 – 1.775; Fig. 2c). No effect towards H1 was detected for sex nor litter.

3.1. MWM spatial learning

All animals learned the platform position by the end of the Spatial Learning sessions. Platform position was analyzed as a covariable to determine the presence of any bias, but no effect was present regarding the platform position counterbalanced (BF_{10} < 1). Furthermore, a PCA model was carried out with all the Spatial Learning sessions (S1-S8) with average latency to see if the rodent behavior, within the MWM paradigm, can be explained with one model alone. The PCA revealed one significant model (p = 0.007) with two main components: RC1 (S1 to S6) and RC2 (S7 and S8). Component loadings and uniqueness can be seen in Fig. 3.

Furthermore, the Spatial Learning phase analyses were performed with this difference. The RM-Bayesian ANOVA revealed expected extreme evidence towards H1 in latency to platform between S1-S6 for sessions (BF_{10} = 4.478 e^{+15}; Fig. 4b) and for trials (BF_{10} = 202652.731; Fig. 3b). All rats required less time to reach the platforms across sessions and across trials. However, no evidence towards H1 was present for



PCA main components, their loadings, uniqueness, Eigenvalues, and proportion of variance			
	RC1 (Learning)	RC2 (Plateu)	Uniqueness
LatS1	0.422	-	0.830
LatS2	0.627	-	0.633
LatS3	0.888	-	0.261
LatS4	0.930	-	0.179
LatS5	0.562	-	0.401
LatS6	0.547	-	0.459
LatS7	-	0.923	0.211
LatS8	-	0.792	0.402
RC1 Eigenvalue = 3.098; Proportion variance = 0.387; RC2 Eigenvalue = 1.526; Proportion variance = 0.191			

Fig. 3. Graphical representation and PCA main components, loadings and uniqueness, Eigenvaluess and proportion of variance of all the learning sessions from the MWM.

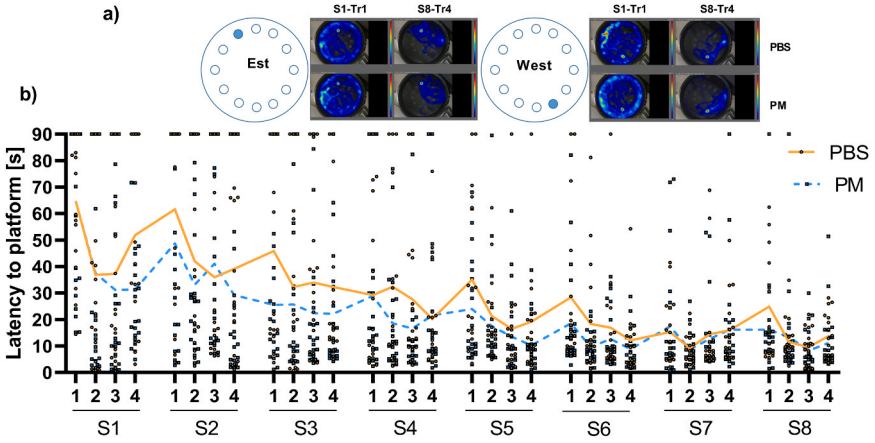


Fig. 4. Spatial Learning phase. Figure a) represents the PCA main components and heatmap representation of the MWM performance change divided by platform position. Image b) shows latency to platform across sessions (S) and trials. PBS-Males (n = 10), PBS-Females (n = 7); PM-Males (n = 11); PM-Females (n = 11).

sessions in latency to platform between S7-S8, while trials achieved moderate evidence ($BF_{10} = 7.634$; Fig. 4b). No effect of exposure neither sex nor litter was present between S1-S6 and S7-S8. Motor variables were also analyzed, finding expected extreme evidence of session ($BF_{10} = 3.205\ e^{+13}$) and trial ($BF_{10} = 183500.580$) for total distance between S1-S6, without being affected by sex, exposure, or litter (Supplementary Material 2, Fig. 1a). Rats needed to travel less distances to reach the platform position (Fig. 4a, heatmap representations). On the contrary, no effect of session, trial, sex, exposure, or litter was present for total distance between S7-S8 (Supplementary Material 2, Fig. 1a). Regarding velocity, moderate evidence towards H_1 was found for session ($BF_{10} = 6.641$) between S1-S6 and extreme evidence was present for trials ($BF_{10} = 880.444$). Sex, exposure, and litter did not affect velocity. For S7-S8 comparisons, no evidence of sessions nor trials was present for velocity; furthermore, sex achieved anecdotal evidence ($BF_{10} = 1.462$), while exposure and litter did not affect velocity, being the females the fastest group. Heading to platform revealed no effect neither for session, trial, exposure, sex, or litter between S1-S6; however, sex reached anecdotal evidence ($BF_{10} = 1.699$) between S7-S8, while sessions, trials, and exposure did not reached evidence towards H_1. Both results show that female rats may face the platform more directly and with higher velocity. Ratios analyses revealed extreme evidence for session ($BF_{10} = 8716.886$) and trial ($BF_{10} = 1017.239$), while sex achieved anecdotal evidence ($BF_{10} = 1.765$) for S1-S6. Lastly, for the sum of latencies, we found anecdotal evidence for exposure ($BF_{10} = 1.557$), while sex and litter did not reach any H_1 degree of evidence (Supplementary Material 2, Fig. 1b). Exposed offspring required less time to reach the platform (PBS; M = 197.770; 95 % CI = 155.208 – 240.333; PM; M = 153.298; 95 % CI = 129.862 – 176.734; Fig. 5a).

In terms of S7-S8 comparisons, trial, session, and litter did not achieve evidence towards H_1; however, sex reached strong evidence ($BF_{10} = 23.369$). Sex*trial interaction did not reach H_1 evidence ($BF_{incl} < 1$). Female offspring spent more time in the periphery than in the center area (Males; M = 22.857; 95 %CI = 14.940 – 30.775; Females; M = 35.408; 95 %CI = 25.805 – 45.012; Fig. 4d). In terms of the sum of latencies, no effect was found for exposure neither sex (Fig. 5b).

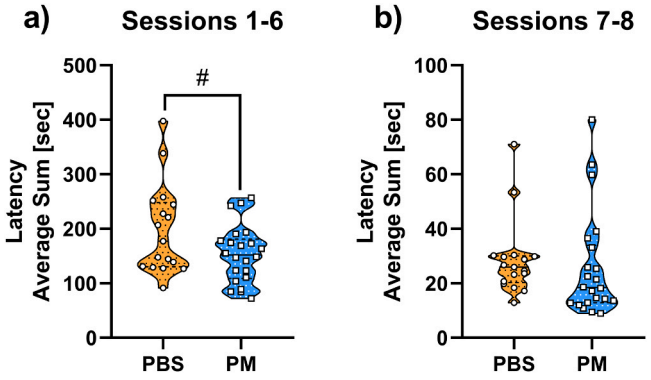


Fig. 5. Average sum of the latencies to reach the platform across all the learning sessions within the first MWM procedure. Fig. a represents the average latency sum across sessions 1–6 and Fig. b depicts average latency sum across sessions 7–8. PBS-Males (n = 10), PBS-Females (n = 7); PM-Males (n = 11); PM-Females (n = 11).

3.2. MWM probe and spatial memory consolidation

After the Spatial Learning phase, a probe session followed. Regarding the first 30 s analyzed, no effect of sex neither exposure was detected for time spent in any of the quadrants: Target, Opposite, North or South (Fig. 6b); however, litter reached moderate evidence ($BF_{10} = 8.897$) in time spent in west quadrant. However, sex reached strong evidence ($BF_{10} = 13.085$) towards H_1 for the ratio periphery-center, while litter and exposure did not achieve anecdotal evidence. Females spent more time in the periphery than males (Males; M = 24.822; 95 %CI = 19.230 – 30.415; Females; M = 42.050; 95 %CI = 31.637 – 52.464). Same result was found for the 60 s probe (Fig. 6c); but sex increased their evidence to very strong ($BF_{10} = 30.238$) in the periphery-center ratio, and litter increase its evidence in time in west quadrant to very strong ($BF_{10} = 81.700$). No difference was detected in frequency in each quadrant for the 30 s probe.

As expected, an extreme effect of platform position was present, both in the 30 s ($BF_{10} = 2.525\ e^{+11}$) as well as in the 60 s probe ($BF_{10} = 6.625\ e^{+17}$). In both probes, rats spent more time in the target quadrant than in the opposite (30 s probe; Target; M = 16.400, 95 %CI = 14.324 – 18.475; Opposite; M = 4.596; 95 %CI = 3.065 – 6.127; 60 s probe; Target; M = 31.461; 95 %CI = 28.495 – 34.428; Opposite; M = 8.335; 95 %CI = 6.188 – 10.482; Figs. 6b and 6c). Same effect was detected for frequency in each quadrant for 30 s probe ($BF_{10} = 4.881\ e^{+8}$) and 60 s probe ($BF_{10} = 8.222\ e^{+18}$). In both probes, rats enter more times the target quadrant than the opposite (30 s probe; Target; M = 5.821; 95 % CI = 4.767–6.875; Opposite; M = 2.462; 95 %CI = 1.814–3.109; 60 s probe; Target; M = 12.744; 95 %CI = 11.034–14.454; Opposite; M = 4.256; 95 %CI = 3.314–5.199; Data not shown). Litter remained without evidence in most of the variables but in frequency to the opposite quadrant in the 60 s probe achieved moderate evidence ($BF_{10} = 3.739$). No effect of sex neither exposure was detected for frequencies in any quadrant.

3.3. Morris water maze test (Reinstatement; S9) and probe effect

Regarding the MWM test session, a strong effect towards H_1 was detected for exposure ($BF_{10} = 11.804$) in latency to platform with the RM-Bayesian ANOVA, but litter achieved moderate evidence ($BF_{10} = 7.415$); nevertheless, the model composed by exposure + litter remained with moderate evidence ($BF_{10} = 5.438$); this effect might be affected, but is not explained, by the intralitter likeliness because exposure ($BF_{incl} = 3.073$) and trial ($BF_{incl} = 2.689$) are the highest effects detected. PM-offspring had lower latency than control exposed animals (PBS; M = 18.281; 95 %CI = 9.141 – 27.421; PM; M = 9.185; 95 %CI = 5.777 – 12.593). Trial reached anecdotal evidence ($BF_{10} = 1.410$), while sex did not reached evidence for H_1 (Fig. 7a). Specifically, the Bayesian ANOVA performed with the average latency across trials at S9 revealed a very strong effect of exposure ($BF_{10} = 35.394$), without sex affecting this variable, nevertheless, litter reached strong evidence ($BF_{10} = 10.046$), and the exposure + litter model also reached strong evidence ($BF_{10} = 15.030$); however, exposure is the highest effect detected ($BF_{incl} = 3.397$). PM-offspring had lower mean latency to platform than PBS-offspring (PBS; M = 18.281; 95 %CI = 13.491 – 23.070; PM; M = 9.185; 95 %CI = 6.379 – 11.990; Fig. 7a). Furthermore, in total distance, trial achieved moderate evidence ($BF_{10} = 9.902$), while exposure achieved anecdotal evidence ($BF_{10} = 1.431$; Fig. 7b and c). In terms of velocity (Fig. 7d), neither sex, exposure, trial nor litter showed any effect towards H_1. However, sex achieved moderate evidence ($BF_{10} = 3.199$) for heading to platform while trial only achieved anecdotal evidence ($BF_{10} = 1.796$), and exposure, nor litter did not show any effect towards H_1 (Fig. 7e). That is, probe testing phase seemed to affect control rats' performance, as expected, but did have little influence on exposed animals. Female rats oriented differently to the platform position than males (Males; M = 14.896; 95 %CI = - 16.982 – 21.951; Females; M = −6.334; 95 % CI = −28.894 – 12.467). Also, female rats had higher periphery-center index compared to male rodents (Males; M = 26.490; 95 %CI = 18.768 – 34.213; Females; M = 40.031; 95 %CI = 31.355 – 48.708).

To corroborate our hypothesis, S8-S9 comparison was performed to clarify any probe effect. The comparison revealed a moderate effect of trials ($BF_{10} = 9.708$), and anecdotal exposure effect ($BF_{10} = 2.236$; Fig. 8a), and moderate litter evidence ($BF_{10} = 4.158$) while sex and session remained without H_1 evidence for latency to platform. This effect is driven by the trial effect ($BF_{incl} = 2.003$), and the complete interaction session*trial*exposure*sex ($BF_{incl} = 2.668$), and in a lesser way by litter ($BF_{incl} = 1.538$). Both exposure groups in S8 had the same latency to platform ($BF_{10} < 1$). In addition, trial reached strong evidence ($BF_{10} = 10.126$) in total distance (Fig. 8b), without the impact of sex, exposure, and session, while litter remained with anecdotal evidence ($BF_{10} = 1.353$). In terms of heading to platform, sex achieved anecdotal evidence ($BF_{10} = 1.703$), while session, trial, exposure, and litter did not achieve H_1 evidence.

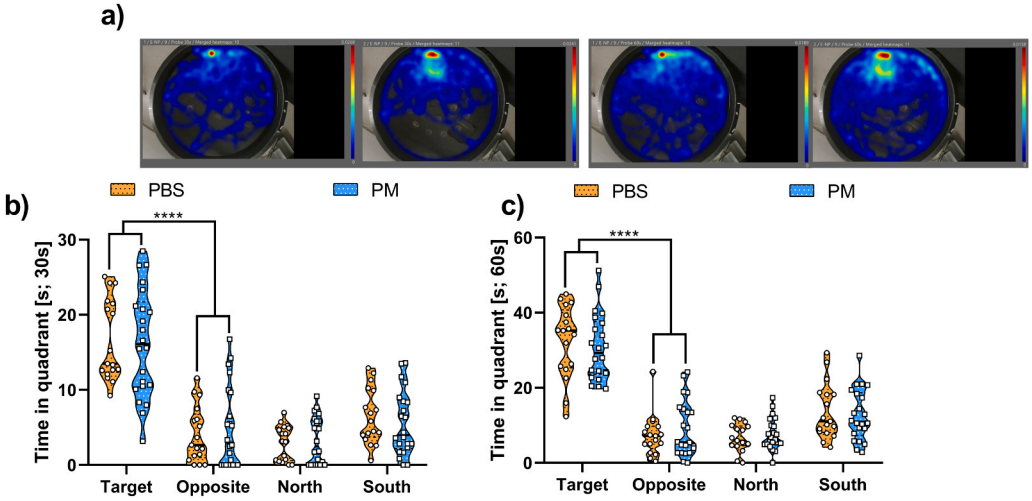


Fig. 6. Probe Phase. Figure a) show two heatmap (left heatmaps correspond to 30 s probe; right heatmaps correspond to 60 s probe; both heatmaps were taken from the same target position for PBS [left] and PM [right] groups). Figs. b and c correspond to time in each quadrant in 30 s and 60 s probe respectively. Individual data is shown with median and quartiles. **** extreme evidence. PBS-Males (n = 10), PBS-Females (n = 7); PM-Males (n = 11); PM-Females (n = 11).

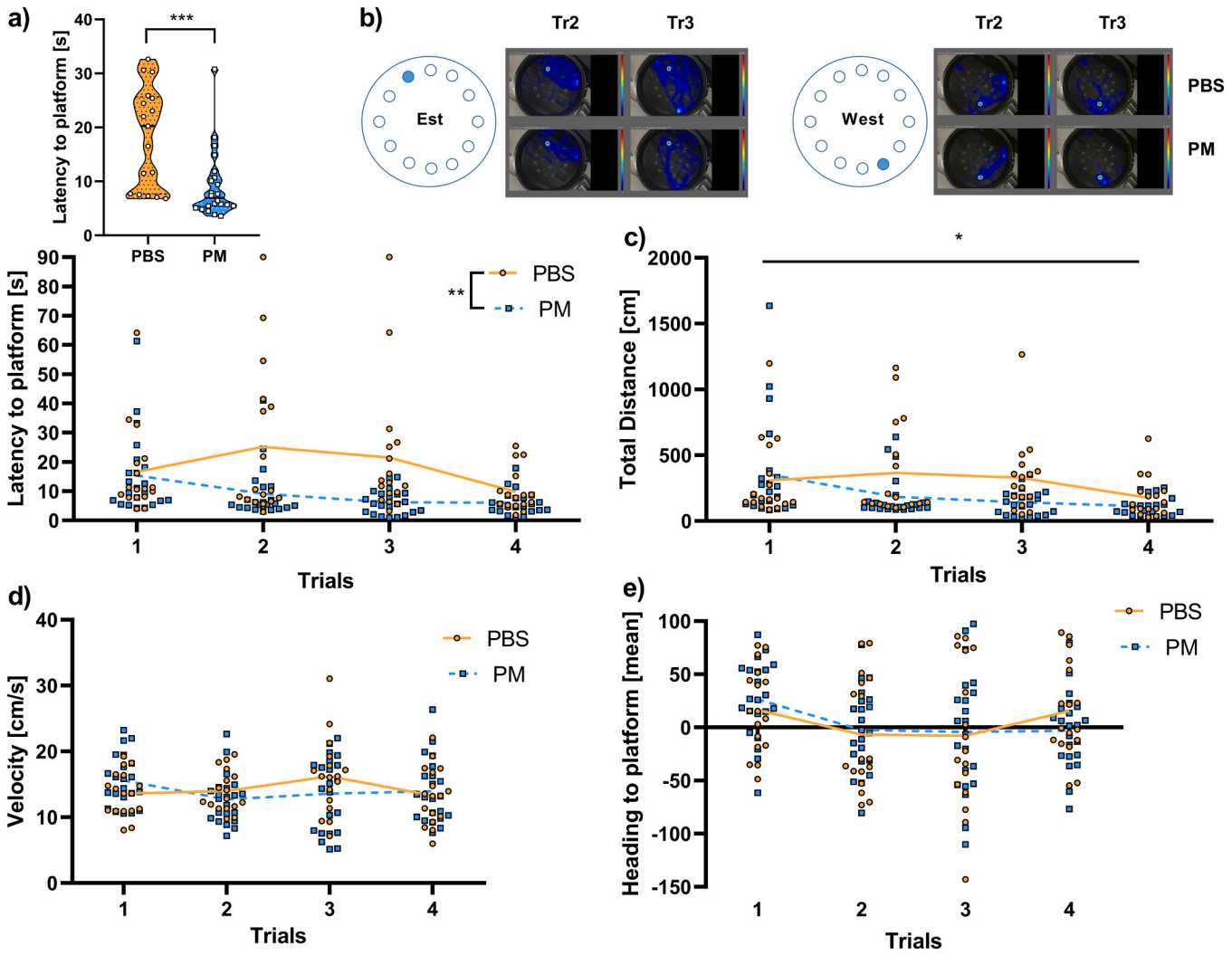


Fig. 7. Test/Reinstating session. Fig. a shows mean and total latency to platform across S9 trials. Figure b) depicts heatmaps regarding platform position in S9. Fig. c shows total distance travelled across trials in S9. Figure d depicts velocity in the S9. Figure e shows heading to platform maintained in S9 trials. * Moderate evidence; ** Strong evidence; *** Very strong evidence. PBS-Males (n = 10), PBS-Females (n = 7); PM-Males (n = 11); PM-Females (n = 11).

3.4. Reversal phase

Regarding the reversal phase, expected extreme evidence was found for session (BF_{10} = 78691.010) and trials (BF_{10} = 2.707 e^{+6}) in latency to platform (Fig. 9a). However, neither sex, exposure, or litter seem to impact this variable (BF_{10} < 1). Same results were found for session (BF_{10} = 1.181 e^{+12}) and trial (BF_{10} = 2.555 e^{+9}), and exposure, sex, and litter (BF_{10} < 1) for total distance moved in the pool (Fig. 9b). In terms of velocity (Fig. 9c), exposure, sex, and litter achieved evidence towards H_0, while session reached moderate evidence (BF_{10} = 3.826), and trial reached anecdotal (BF_{10} = 1.232). No evidence towards H_1 was present for meander, heading, turn angle, and angular velocity. However, an extreme effect of trial (BF_{10} = 35860.708) was present for Periphery/center ratio (Fig. 9d), while sex remained anecdotal (BF_{10} = 2.446), and session achieved very strong evidence (BF_{10} = 34.065) towards H_1. Males had lower ratio than females and this gets lower as the reversals are performed. All animal's behavior varies depending on sessions and trials, without being affected by gestational exposure to PM_{10}. Thus, exposure did not seem to influence inhibitory control behavior regarding perseveration or cognitive flexibility.

3.5. Visual task phase

In terms of visual phase, an expected extreme effect of trial was evidenced (BF_{10} = 828.763; BF_{incl} = 192.676), while session and sex reached moderate evidence (BF_{10} = 5.400; BF_{incl} = 1.400; BF_{10} = 5.700; BF_{incl} = 2.411 respectively) for latency to platform (Fig. 10b). Neither sex*trial or sex*session reached evidence for H_1 (BF_{incl} < 1). Females required more time to locate the visual platform than males (Males; M = 6.632; 955CI = 6.214 – 7.439; Females; M = 12.695; 95 %CI = 10.501 – 14.884). Regarding total distance moved (Fig. 10a), session achieved anecdotal evidence (BF_{10} = 1.317), while trial, exposure, and litter did not show any degree of evidence towards H_1. However, sex reached moderate evidence (BF_{10} = 5.520), where females traveled further distances (Males; M = 129.07; 95 %CI = 95.716 – 162.424; Females; M = 224.275; 95 %CI = 102.622 – 345.927). For velocity (Fig. 10c), neither exposure, sex, session, and litter reached evidence towards H1, but trials achieved extreme evidence (BF_{10} = 4.640 e^{+7}). No effect of sex, trial, exposure, session, or litter was detected for meander. On the contrary, sex reached moderate evidence (BF_{10} = 4.238) for the heading to platform (Fig. 10d), while session, trial, exposure, and litter did not achieve any evidence. Sexes oriented differently to the platform (Males; M = 10.609; 95 %CI= −6.413 – 27.630; Females; M = −7.136; 95 %CI = −27.218 – 12.947), being the females the most accurate one. In

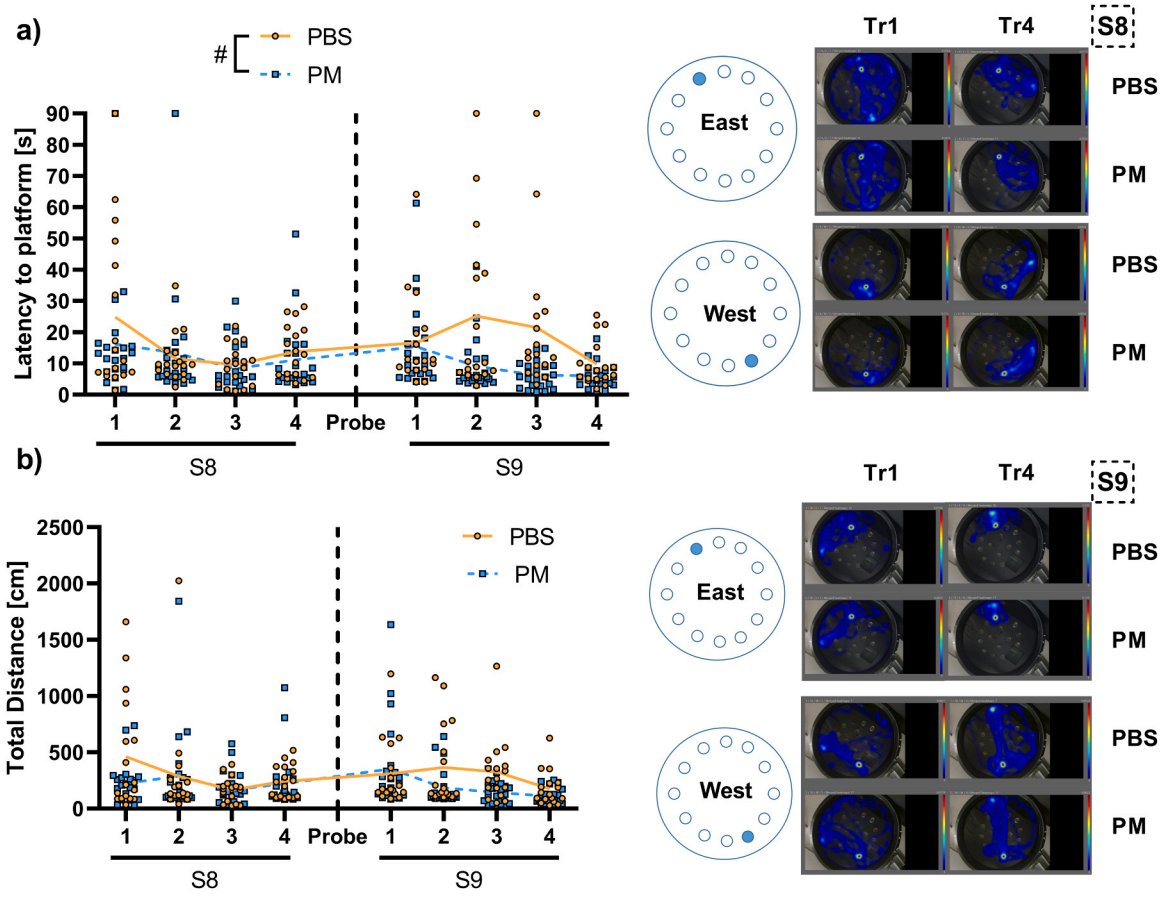


Fig. 8. Graphical representation of Latency to platform in S8 and in S9 (a), and Total distance in S8 and S9 (b). Heatmaps, with mean behavior, is also shown. PBS-Males (n = 10), PBS-Females (n = 7); PM-Males (n = 11); PM-Females (n = 11).

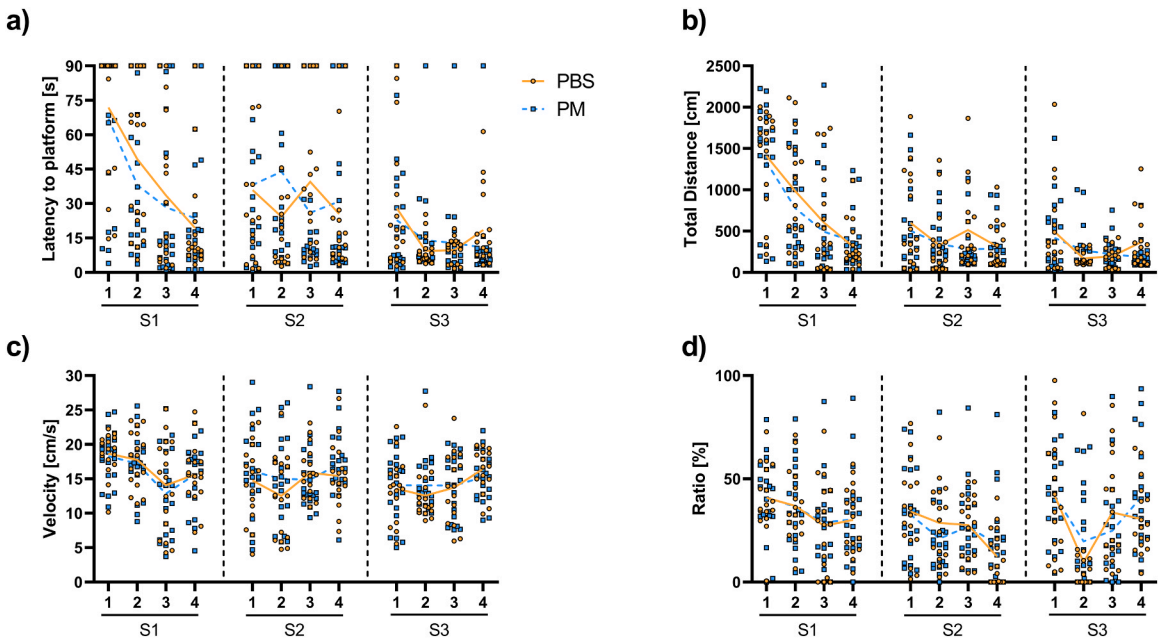


Fig. 9. Graphical representation of the main variables analyzed in the reversal phase. Image a) represents latency to platform; b) total distance; c) velocity; and d) Center/Periphery Ratio. Individual data is depicted in all images. PBS-Males (n = 10), PBS-Females (n = 7); PM-Males (n = 11); PM-Females (n = 11).

addition, sex remained with anecdotal evidence for H1 in turn angle, and angular velocity, but achieved strong evidence in P/TT ratio (BF_{10} = 32.289), while session, exposure, or litter did not. In this variable (ratio P/TT; Fig. 10e), trial also achieved very strong evidence (BF_{10} = 1677.856); all groups had lower ratio as they are performing the task. Sex*trial interaction showed evidence towards H_0. Males showed

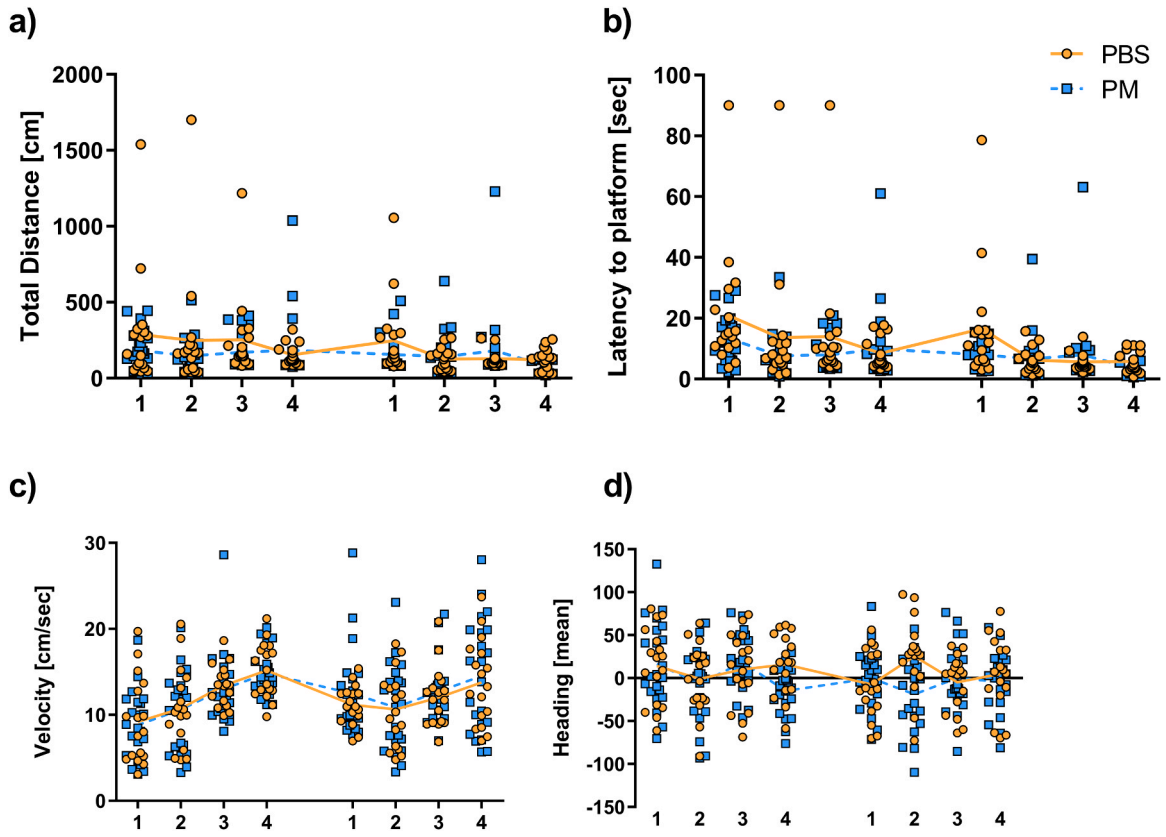


Fig. 10. Graphical representation of the main variables analyzed in the visual task of the MWM. Picture a) shows total distance traveled inside the pool; image b) depicts latency to reach the platform; image c) shows velocity inside the MWM pool; image d) depicts mean heading to platform and image e) shows the Periphery/Center ratio. All images show individual data. PBS-Males (n = 10), PBS-Females (n = 7); PM-Males (n = 11); PM-Females (n = 11).

reduced ratio when compared to females (Males; M = 22.135; 95 %CI = 14.805 – 29.465; Females; M = 34.639; 95 %CI = 23.963 – 43.940).

3.6. Working memory

Regarding working memory part, no effect of exposure, sex trial, session, or litter were detected in mean latency to reach platform (BF_{10} < 1; Fig. 11a, 11b). Same evidence for all variables was found in total distance moved (Supplementary Material 2, Fig. 2a), velocity (Supplementary Material 2, Fig. 2b), meander, and heading (Supplementary Material 2, Fig. 2c). Sex achieved anecdotal evidence for turn angle (BF_{10} = 1.451) and angular velocity (BF_{10} = 1.497); females headed more accurately to the platforms than males. When the percentage variation is compared, no effect of session, exposure or sex was detected neither for mean latency to reach the platform, total distance moved, velocity, meander, heading, turn angle or angular velocity.

3.7. Plus maze test

In terms of behavior in an anxiogenic paradigm, an extreme effect was present for Sex (BF_{10} = 830.571), while exposure neither litter did not achieved evidence towards H_1. Same results were found for velocity, with extreme evidence for Sex (BF_{10} = 1519.743) without exposure affecting the variable. Females moved further distances (Males; M = 1370.774; 95 %CI = 4.191 – 4.964; Females; M = 1817.902; 95 %CI = 1663.145 – 1972.658) with higher velocity (Males; M = 4.578; 95 %CI = 4.191 – 4.964; Females; M = 6.115; 95 %CI = 5.612 – 6.618; Fig. 12a and 12b).

Regarding anxiety behaviors, no effect towards H_1 was present for sex nor exposure nor litter for time spent in open arms (BF_{10} < 1), while sex achieved anecdotal evidence (BF_{10} = 1.018), and exposure did not

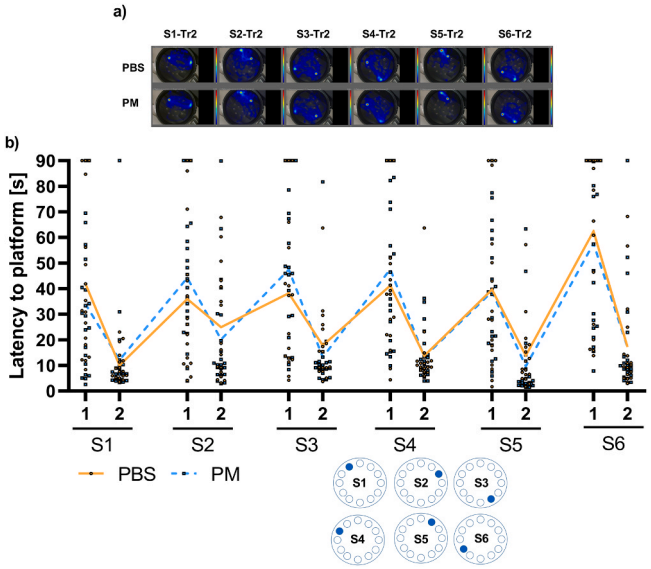


Fig. 11. Graphical representation of the main variables analyzed in the working memory phase of the MWM. Picture a) shows a heatmap representation of the groups, session and trials; b) shows mean latency to reach the platform. All images show individual data. PBS-Males (n = 10), PBS-Females (n = 7); PM-Males (n = 11); PM-Females (n = 11).

affect. In addition, no effect of exposure nor sex neither litter was present for frequency to the open arm, while sex achieved moderate evidence towards H1 (BF_{10} = 6.252), where females entered more times the closed arms than males (Males; M = 11.789; 95 %CI = 8.967 – 14.612;

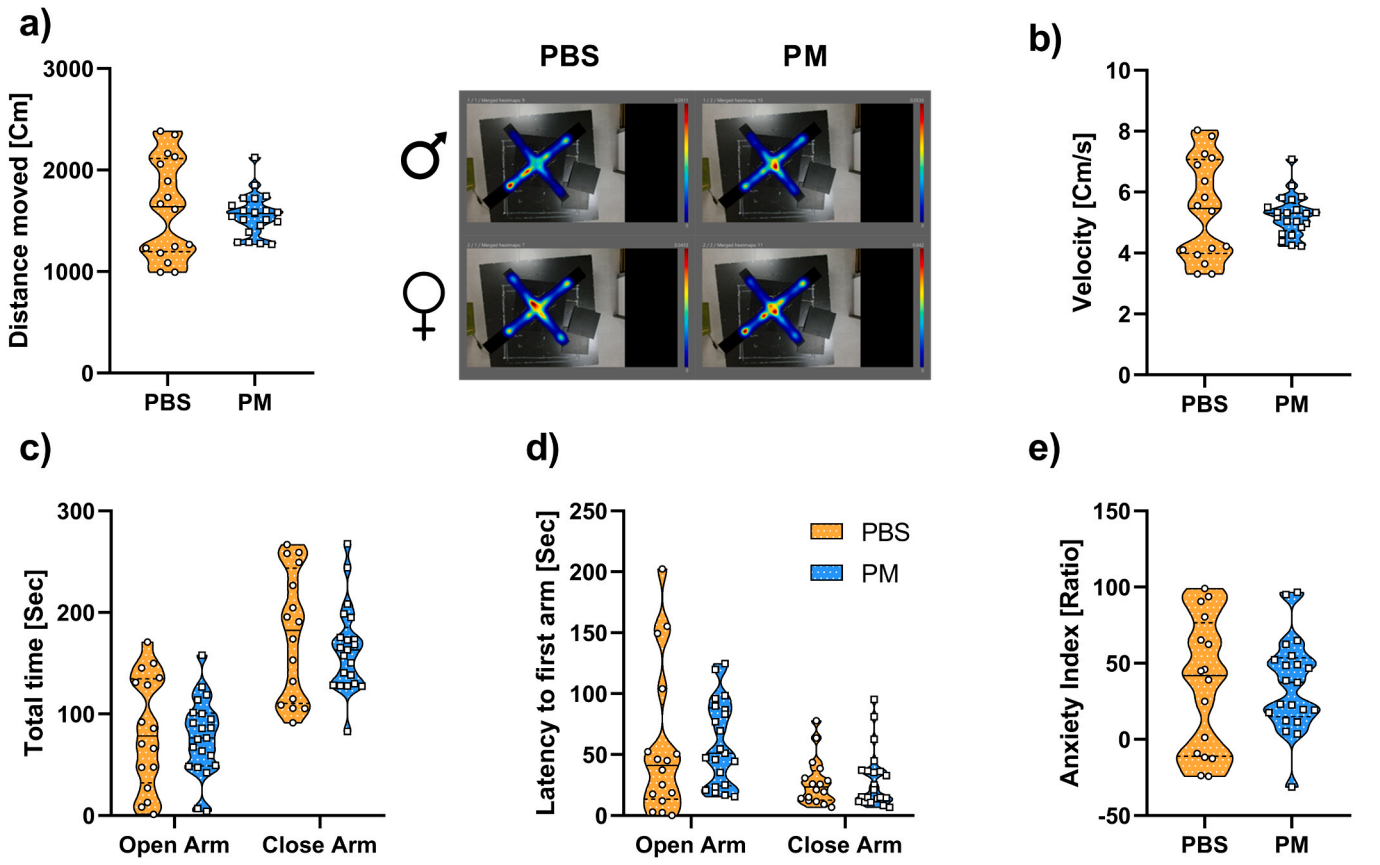


Fig. 12. Graphical representation of the PMT variables. Image a) shows total distanced moved in the PMT alongside four heatmaps showing mean behavior. Image b) depicts velocity within the paradigm; image c) and d) show total time spent in both arms and latency to first arm (open and close). Lastly, image e) shows the Anxiety Ratio results. Violin plots represent individual values with the median and quartiles. PBS-Males (n = 10), PBS-Females (n = 7); PM-Males (n = 11); PM-Females (n = 11).

Females; M = 16.556; 95 %CI = 14.486 – 18.625). In terms of latency to first arm (closed or open), exposure and sex did not achieved evidence towards H_1. Lastly, the A/I ratio revealed no sex or exposure effect (Figs. 12c to 12e).

3.8. Open field test

Regarding all open field variables, cycle reached extreme evidence for horizontal activity (BF_{10} = 1.575 e^{+41}), total distance (BF_{10} = 8.013 e^{+29}; Fig. 13b), movement time (BF_{10} = 1.613 e^{+38}), rest time (BF_{10} = 1.619 e^{+38}), vertical activity (BF_{10} = 1.006 e^{+27}), vertical time (BF_{10} = 6.264 e^{+25}; Fig. 13e), margin distance (BF_{10} = 2.158 e^{+26}), margin time (BF_{10} = 18690.374; Fig. 13d), center distance (BF_{10} = 1.022 e^{+26}), center time (BF_{10} = 18665.939), rearing activity (BF_{10} = 8.776 e^{+26}), and P/C ratio (BF_{10} = 18689.762; Fig. 13c). In addition, exposure, sex, and litter did not reach H1 evidence for any variable mentioned before. On the contrary, sex reached extreme evidence for H1 for velocity (BF_{10} = 257.555; Fig. 13c), while cycle, exposure, and litter did not affect velocity. Females moved faster than males (Males Cycle1; M= 9.514; 95 %CI = 8.680–10.348; Females Cycle1; M= 11.902; 95 %CI = 11.315–12.488; Males Cycle2; M= 10.560; 95 %CI = 9.636–11.485; Females Cycle2; M= 11.924; 95 %CI = 10.949–12.899; Males Cycle3; M= 9.758; 95 %CI = 8.420–11.096; Females Cycle3; M= 13.130; 95 % CI = 12.015–14.246; Males Cycle4; M= 9.291; 95 %CI = 7.404–11.179; Females Cycle4; M= 12.285; 95 %CI = 10.580–13.989; Males Cycle5; M= 8.894; 95 %CI = 6.838–10.949; Females Cycle5; M= 14.680; 95 % CI = 12.467–16.893; Males Cycle6; M= 10.086; 95 %CI = 8.155–12.017; Females Cycl6; M= 11.758; 95 %CI = 9.572–13.944).

3.9. RT-qPCR results

Fig. 14 provides an overview of the differential gene expression observed in the hippocampus, highlighting various findings from a Two-Way Bayesian ANOVA. First, related with the neurotransmission-related genes, no effect was seen in *Drd2* for exposure nor sex, but litter remained with anecdotal evidence (BF_{10} = 1.042). Same as with dopamine, no evidence for exposure or sex was found for serotonin receptors, neither for litter. For Glutamate system, sex (BF_{10} = 4.516) and exposure (BF_{10} = 3.129) achieved moderate evidence for *Grin1a* (Fig. 14a), however, the interaction between Exposure*Sex (BF_{incl} = 2.835) did not reach moderate evidence, and litter remained without H_1 evidence. Control group showed higher fold change compared to PM-offspring (PBS; M = 2.095; 95 %CI = 1.744–2.446; PM; M = 1.511; 95 %CI = 1.130–1.892). In addition, females exhibited higher fold changes compared to male (Males; M = 1.552; 95 %CI = 1.327–1.776; Females; M = 2.175; 95 %CI = 1.679–2.672). For *Grin2b* (Fig. 14b), exposure achieved anecdotal evidence (BF_{10}= 2.256) and sex achieved moderate evidence (BF_{10}= 4.788), and litter remained without evidence (BF_{10} < 1). Females had higher fold changes than males (Males; M = 1.671; 95 % CI = 1.338–2.004; Females; M = 2.520; 95 %CI = 1.879–3.162). For *Grin2c* (Fig. 14c) exposure reached strong evidence (BF_{10} = 12.986) while sex achieved anecdotal, and litter evidence remained under 1. Control group had higher gene expression than exposed group (PBS; M = 1.793; 95 %CI = 1.561–2.024; PM; M = 1.284; 95 %CI = 1.048–1.521). Lastly, no evidence for exposure neither sex, nor litter was detected for *Grin2a*. The GABAergic system was also analyzed with *Gabra2* (Fig. 14d). Exposure provided moderate evidence (BF_{10} = 3.650) while sex and litter did not show any evidence. PM-offspring had higher gene

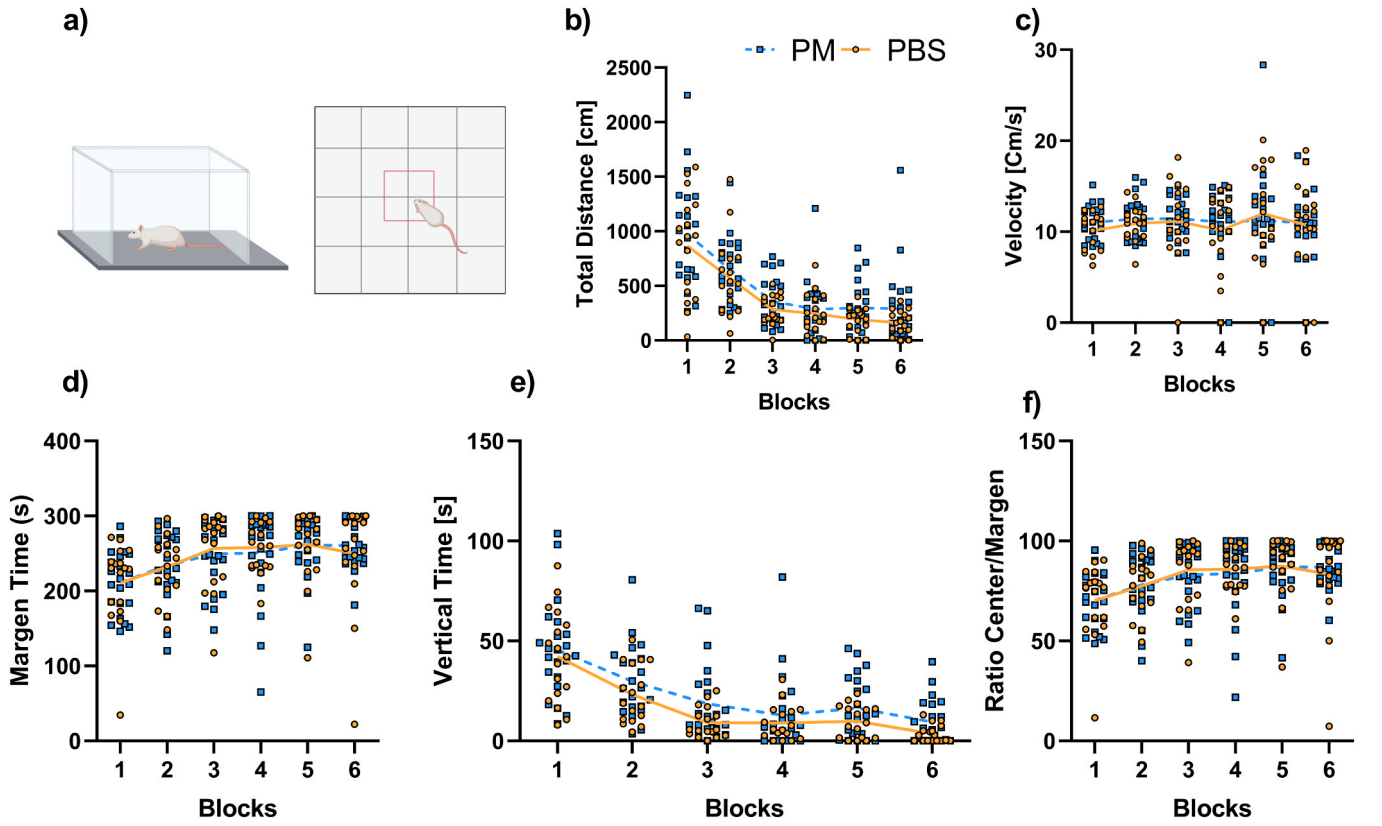


Fig. 13. Graphic representation of the OFT variables. Image a) shows an example created with Biorender Software of a rat within an OFT. Image b) shows total distance, c) velocity, d) margin time, e) vertical time, and f) Center/Margin Ratio. PBS-Males (n = 10), PBS-Females (n = 7); PM-Males (n = 11); PM-Females (n = 11).

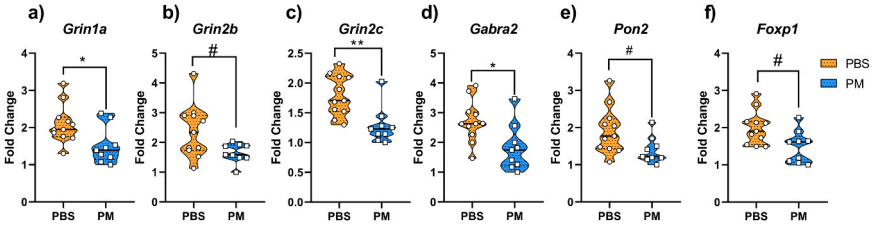


Fig. 14. Graphical representation of the genes that achieved evidence towards H1. All graphics represent individual plots with median and quartiles. Total n = 20 (PBS n = 11; PM n = 9). # Anecdotal Evidence; * Moderate Evidence; ** Strong Evidence. PBS-Males (n = 6), PBS-Females (n = 5), PM-Males (n = 5), PM-Females (n = 4).

expression than control (PBS; M = 1.827; 95 %CI = 1.226–2.428; PM; M = 2.707; 95 %CI = 2.235–3.180).

Other processes analyzed were inflammation, oxidative stress and cell function. Regarding inflammation, neither exposure, sex, nor litter reached evidence towards H_1. In terms of oxidative stress, anecdotal evidence (BF_{10} = 2.056) was detected for exposure at *Pon2* (Fig. 14e) while sex and litter did not. Control group had higher gene expression to PM-exposed offspring (PBS; M = 1.995; 95 %CI = 1.685–2.304; PM; M = 1.488; 95 %CI = 1.154–1.822). Lastly, cell function with *Foxp1* (Fig. 14f), both exposure (BF_{10} = 2.969) and sex (BF_{10} = 2.353) provided anecdotal evidence, while litter did not reach anecdotal evidence. PM group had lower gene expression than control (PBS; M = 1.995; 95 %CI = 1.685–2.304 M PM; M = 1.488; 95 %CI = 1.154–1.822). To conclude, no effect of exposure or sex neither litter were seen in *TRH*. Bayesian correlations between gene expression and S9 outcomes revealed no link between them (Supplementary material 3).

4. Discussion

This study aims to clarify the potential effects of gestational exposure to PM_{10} on neurodegenerative processes. Our findings indicate that PM_{10} exposure during gestation did not influence body weight at any of the three measurement points (birth, pre-weaning, and post-weaning). As expected, sex differences were observed in body weight. Regarding other developmental variables, neither exposure nor sex had an effect on eye-opening, although anecdotal evidence suggested poorer performance in the adhesion test for the PM group. Regarding learning, spatial memory, anxiety and locomotor activity, no significant effects of sex or exposure were found. Interestingly, a sex effect emerged during the probe phase (ratio P/TT), where females spent more time swimming near the periphery. Specifically, in S9, we detected that PM-exposed offspring seems to be less cognitive flexible than the control group, PM-offspring reached the platform faster than control. Anecdotal evidence of exposure effects was noted between S8 and S9 during the probe test. However, that effect found in the reinstatement phase was not

detected in the reversal phase, where only anecdotal sex differences were observed in the P/TT ratio. In the visual phase, moderate effects were detected for both platform latency and the P/TT ratio for sex. No significant effects of exposure or sex were found in the working memory phase. In the PMT, strong effects were evident in both velocity and distance, with moderate effects in the number of entries into closed arms. Finally, in the OFT, strong sex effects were detected in velocity, with females being the fastest group. Interestingly, we detected long-term effects of gestational exposure to PM on hippocampal gene expression, detecting that gestational PM exposure downregulates glutamate- and GABA-related genes and some genes related to oxidative stress and cell functioning.

There is not abundant information about the effects of air pollutants (and specifically PM_{10}) on developmental assays. We found anecdotal evidence of worsen adherence capacity in our exposed animals. The link between air pollutants and physical development remains contentious. In the present research we did not find any difference in body weight (not birth neither weight gain), but in previous research from Ruiz-Sobremazas et al. (*Under Review*), we detected differences in birthweight using in both works the same exposure procedure. Same as before, few information is available about air pollutants and muscle strength. Available information links PMs exposure to sarcopenia (Zhang et al., 2023b), but the evidence found is anecdotal. Gestational exposure to PMs might influence hand grip strength, but further research is needed to clarify these effects. Ruiz-Sobremazas et al. (2025); under review) found some effects of oral gestational exposure in grip and adherence capacity using two different commercial houses. Further research is needed, and more tests (like the rotarod), should be performed to determine the possible link between gestational air pollution and muscular development. Also, few evidence can be found in oral exposure of PMs and developmental milestones. Some evidence (Miranda et al., 2018) detected an increase of birthweight, but our data do not show any influence of oral PM exposure in birthweight or weight development in any comparison performed.

Next, previous studies have analyzed the effects of gestational exposure to different air pollutants during critical periods such as prenatal or early life. Ehsanifar et al. (2019) exposed mice to DE throughout gestation, finding that higher exposure times were associated with different learning outcomes in the MWM. Additionally, they detected a reduced time spent in the correct quadrant during the Probe phase. They also observed higher mRNA expression of pro-inflammatory molecules (*IL-6, IL-1β, TNF-α*) and reduced *NR2a* and *NR3b* levels. Lastly, CA1 morphology changed compared to controls when exposed for more than 2 h to air pollution. Zhang et al. (2023a) exposed mice to $PM_{2.5}$ throughout gestation, finding that PM-exposed offspring spent less time in the target quadrant during the Probe phase and exhibited higher TNF-α protein and mRNA levels in the cerebellum. Zheng et al. (2019), Zhang et al. (2021), and Wang et al. (2019) also found that higher $PM_{2.5}$ dosages were associated with reduced swimming distances and fewer entries into the target quadrant during the Probe phase. Woodward et al. (2018) explored hippocampal-dependent memory using another task (Novel Object in Context Recognition), finding that exposed offspring to TRAP had 15 % less discrimination, which correlated to CA1 microbleeds. Zanchi et al. (2010) exposed the rats prenatal and early life, finding in another hippocampal-dependent task a reduced discrimination index and an increase in the habituation index, as well as higher levels of oxidative stress in the cortex.

Nevertheless, even though these articles explored the influence of critical stages exposure to air pollutants and memory, they mainly focus on the short-term effects of that exposure, testing memory and learning performance in adolescence or early youth. However, our main objective was to determine the possible effects, if present, of gestational exposure to PM_{10} on late-adulthood memory and learning performance. We detected a long-term effect of gestational exposure to air pollutants (mainly in cognitive flexibility in the reinstatement phase), and to our knowledge, this is the first study that has found that long-term vulnerability of gestational-PM exposure.

It is worth noting the effect we found in the MWM. Our animals learned the task perfectly, without any significant differences between the two groups during the learning phase. Interestingly, most studies analyzing the effects of exposure to air pollutants during critical stages have found differences in various variables during the Probe phase (Ehsanifar et al., 2019; Zhang et al., 2023a, 2021; Zheng et al., 2019; Wang et al., 2019). However, we did not detect any differences in the Probe phase; instead, our differences were observed after the Probe phase, during the test/reinstatement session (S9). Following the Probe, all animals performed the test/reinstatement session. The PBS-offspring were affected by the removal of the platform during the Probe phase, while the PM-offspring remained unchanged. To our knowledge, this is the first study to find this effect after PM exposure, although it has been observed before with other toxicants. Perez-Fernandez et al. (2021) detected the same effect after CPF exposure between PND10-PND15. This effect might indicate compulsive-like behavior, or, more precisely, an unsensitivity to contextual disruptions during learning. However, we did not detect any differences in the reversal phase, which might complicate the explanation. Furthermore, this effect can be considered as a form of extinction. Several studies have linked NMDA glutamate receptors across different brain structures (such as the amygdala, mPFC, and hippocampus) with the extinction procedure (Myers et al., 2011). Regarding the hippocampus, recent evidence shows a potential link between sex-hippocampal NMDA and fear extinction (Glavonic et al., 2023). They found differences in fear extinction and recovery between females and males, as well as in the concentrations of grin1a, grin2a, grin2B, and grin4a proteins during adolescence. Interestingly, we found a clear down-regulated pattern of *grin2c* and *grin1a* in the hippocampus. This data goes in accordance with previous literature linking these genes and the compulsive-like behaviors.

Even though we detected NMDA downregulated pattern (*Grin1a, Grin2c*) and GABAergic upregulation (*Gabra2*), there was no correlation with behavioral outcomes (specifically those obtained in S9; Supplementary Material 2). As explained before, glutamatergic neurotransmission is crucial in MWM learning and fear extinction. The probe phase is a procedure where the animal cannot escape from the pool for a specific period of time (60 s), therefore, it can be considered as an extinction-like condition in an aversive environment. It is not clear how those variables are related because there was only one work that detected the same pattern (Perez-Fernandez et al., 2021). However, we might hypothesize that PM exposure reduces HCC plasticity that might affect S9 outcomes (in terms of higher latency to reduced latency to reach the platform). However, further studies with other techniques should be performed to determine if gestational PM exposure is related with compulsive-like outcomes (like with the Schedule Induced Polydipsia) and if it is linked with altered long-term potentiation and long-term depression in the HCC. In addition, we also detected sex differences in some mRNA NMDA subunits, which goes in accordance with previous findings linking sex and aging with different glutamatergic system outcomes (Clayton et al., 2002; Giacometti and Barker, 2020; Ridge et al., 2009; Hönack and Löscher, 1993). NMDA receptors are influenced by different hormones (estrogen and corticosterone); corticosterone favors diffusion while estrogen restrain cellular diffusion (Dupuis et al., 2023). Furthermore, brain plasticity dependent on NMDA receptor is also affected due to cyclic hormones fluctuations (Hyer et al., 2018).

In addition to the glutamatergic system, the GABAergic system is also influenced by sex (Tabatadze et al., 2015; Wolf et al., 2022). Moreover, evidence shows that the aging process affects both systems (Burnyasheva et al., 2023a; Burnyasheva et al., 2023b; Behuet et al., 2019). Other processes such as oxidative stress or development might also be affected by our exposure, but further research is needed to clarify these effects. Regarding locomotor and anxiety activity, we did not detect any effect of gestational exposure to PMs. Nevertheless, we observed the expected effect of sex, with females being more active than

males (Bernstein et al., 2024; Mead et al., 1996; Díez-Noguera and Cambras, 1990).

Alternative interpretations to our results might clarify those effects detected. First, there are several differences between the available studies using the MWM paradigm after exposing to air pollutants through gestation. First, the exposure route is completely different. Most articles used whole-body exposure chambers to expose the pregnant dams to air pollutants, while we used the ingestion pathway, which has been proven as one of the absorption routes for air pollutants. Second, the dosage varies between studies. To this date, there is no consensus about the critical dosage that should be used to assess developmental neurotoxicology outcomes; furthermore, higher dosages might show dose–related effects. Third, timing of the behavioral screening. Most articles analyze memory, learning and other cognitive outcomes in earlier stages (adolescence or youth) where the animals are on their "cognitive peak", while we tested them on late-stage life. Also, those articles do not study the impact of the aging process, which might affect toxic effects due to glial activation, brain plasticity dynamics, memory decline, etc.); furthermore, it would be interesting if future research analyzes the effects of gestational PM exposure on memory and learning at different developmental stages, being able to clarify possible aging-like influences that might affect both control and exposed groups. And lastly, the behavioral procedure is also different. Most articles analyze only learning and finish their behavioral screening with the probe phase, while we analyze more deeply all those cognitive domains. Researchers should conduct a more detailed analysis of animal behavior using the MWM with various manipulations to obtain the most comprehensive information possible about other cognitive domains that might be affected. Nevertheless, our work has some limitations that need to be mentioned. Future research should also perform the MWM in youth to establish a baseline performance and determine if the outcomes differ, potentially indicating the effects of PM exposure. Regarding the MWM analysis, it would be interesting to include several IA pathway analyses in future research to clarify the putative relationships. Future works should also analyze the putative influence of trans-organ regulation on the effects of oral PM gestational exposure. In addition, future studies should also include platform-crossing as a variable to analyze in the probe phase, which may add more information to time and frequency in quadrants studied in the present work. Lastly, the PBS-Females group had lower sample size compared to the others. Future works should determine if the present results are biased due to the reduced sample size in that specific group.

5. Conclusion

In conclusion, our work supports that gestational exposure to PM is not associated with memory deficiencies in older adults. Nevertheless, it is noteworthy that we observed similar outcomes using a different toxic component and another critical developmental exposure period, as reported by Perez-Fernandez et al. (2021). Furthermore, these behavioral results are in accordance with previous from our laboratory (Ruiz-Sobremazas et al., 2025) in terms of behavior (memory), and with the literature in terms of sexual differences in hippocampal gene expression. Our down-regulated pattern in some NMDA subunits in the hippocampus, alongside with sex differences in those subunits, are two widely study phenomena in the scientific literature. Histological and inmunohistological analysis should be performed to determine whether these differences are located in a specific area of the hippocampus. This consistency across studies highlights the robustness of the findings and suggests that future research could explore these effects further using specific models of compulsivity. Additionally, our study contributes further evidence to the understanding of sex differences in brain development, emphasizing the necessity of considering the aging process differently for males and females. This distinction is crucial for developing targeted interventions and improving overall neurological health outcomes. Lastly, the PBS-Female group has a smaller sample size compared to the other groups analyzed. While the data obtained provided valuable insights, the results should be interpreted with caution. Future works should analyze this aspect with higher sample size.

CRediT authorship contribution statement

Coca Mario: Writing – review & editing, Visualization, Methodology, Investigation. **Morales-Navas Miguel:** Writing – review & editing, Supervision, Software, Methodology, Investigation, Formal analysis, Conceptualization. **Ruiz-Sobremazas Diego:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis. **Perez-Fernandez Cristian:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Data curation, Conceptualization. **López-Granero Caridad:** Writing – review & editing, Supervision, Software, Resources, Project administration, Funding acquisition, Conceptualization. **Sanchez-Santed Fernando:** Writing – review & editing, Supervision, Software, Resources, Project administration, Funding acquisition, Conceptualization. **Rodulfo-Cárdenas Rocío:** Writing – review & editing, Visualization, Methodology, Investigation. **Colomina Maria Teresa:** Writing – review & editing, Supervision, Software, Resources, Project administration, Funding acquisition, Conceptualization. **Nerea Rios Nieto:** Writing – review & editing, Investigation.

Declaration of Generative AI and AI-assisted technologies in the manuscript preparation process

During the preparation of this work, the author(s) used ChatGPT-3.5 to enhance text comprehension. After using this tool/service, the author (s) reviewed and edited the content as necessary and take full responsibility for the final version of the published article.

Declaration of Competing Interest

The authors declare that there is no competing financial interests or personal relationships tha could have appeared to influence the work reported in the present manuscript.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.etap.2025.104874.

Data availability

Data will be made available on request.

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