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Impact of a high-fat diet on spatial learning and memory: The role of sex, *APOE* genotype, and postnatal chlorpyrifos exposure

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ABSTRACT

Environmental factors, such as exposure to neurotoxicants and diet, play a critical role in shaping cognitive function, particularly in genetically susceptible individuals. Chlorpyrifos (CPF), an organophosphate pesticide, and high-fat diets (HFD) have been independently associated with cognitive impairment, yet their combined effects remain poorly understood. Apolipoprotein E (*APOE)* genotype influences vulnerability to cognitive decline, with the *ε4* allele being a major risk factor for neurodegenerative diseases. This study assessed the interplay between *APOE* genotype, sex, early-life CPF exposure, and HFD on spatial learning and memory. Male and female C57BL/6, apoE3- and apoE4-targeted replacement (TR) mice were orally exposed to CPF during postnatal days 10–15 and subsequently subjected to a HFD for 8 weeks. At the end of the HFD challenge, body weight gain was calculated, and spatial learning and memory assessed using the Morris Water Maze test. Results indicate that HFD-driven weight gain was influenced by sex and *APOE* genotype. All groups acquired the spatial learning task, but postnatal CPF exposure affected performance in certain groups. Retention was more variable in females, suggesting increased susceptibility to environmental exposures. Notably, apoE4-TR females showed improved memory retention following either CPF exposure or HFD, whereas apoE4-TR males exhibited impaired long-term memory after HFD exposure. These findings highlight the complex interactions between genetic and environmental factors. Understanding these dynamics is essential for developing targeted nutritional and public health strategies to mitigate cognitive decline. Importantly, dietary recommendations should not be generalized but tailored to individual profiles to optimize cognitive health and disease prevention.

1. Introduction

The environment plays a crucial role in shaping human health, particularly during sensitive periods such as pregnancy, lactation, and childhood. These stages represent windows of heightened vulnerability, during which the brain undergoes fundamental developmental processes that can be significantly influenced by environmental factors, including exposure to neurotoxicants and dietary patterns. Such influences not only disrupt the developing nervous system but also have long-term consequences for the adult brain, potentially affecting cognitive functions such as learning and memory (Rock and Patisaul, 2018; Tamm and Ceccatelli, 2017).

Among environmental neurotoxicants, pesticides are of particular concern due to their widespread use in agriculture, domestic settings,

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and public health interventions. Organophosphate pesticides, such as chlorpyrifos (CPF), have been extensively studied for their neurotoxic effects, especially during early development (Biosca-Brull et al., 2021; Burke et al., 2017; Guardia-Escote et al., 2023). The primary target of CPF toxicity is the central and peripheral nervous system, where it irreversibly inhibits acetylcholinesterase, leading to excessive cholinergic signaling (Pope, 1999). Furthermore, developmental exposure to CPF has been linked to widespread effects beyond the nervous system, such as oxidative stress and liver toxicity (Elsharkawy et al., 2013; Ezzi et al., 2016). Besides occupational exposure, the general population is also at risk of exposure to CPF through dietary residues, raising concerns about its continued impact on human health. Although its use was withdrawn from the European Union market in 2020, it continues to be used in other countries worldwide (Wołejko et al., 2022).

In addition to neurotoxicant exposure, diet is another major environmental factor influencing brain function such as inhibitory control (Ruiz-Sobremazas et al., 2024). A high-fat diet (HFD) has been linked to systemic inflammation (Evans et al., 2024), gut microbiota dysbiosis (Malesza et al., 2021) and insulin resistance (Choi et al., 2015), among others. Moreover, HFD has been associated with cognitive impairment and neuronal degeneration (Wang et al., 2021), and is frequently used as a model for studying cognitive deficits and evaluating the neuroprotective effects of potential therapies in rodents (Wang et al., 2021; Yuan et al., 2019). However, findings in the literature remain inconsistent, with some evidences suggesting that a hypercaloric diet may actually enhance learning and memory under certain conditions (Haleem and Mahmood, 2019; Yoshizaki et al., 2020).

Beyond environmental influences, genetic factors also play a key role in determining individual susceptibility to cognitive decline. One of the most well-characterized genetic factors is the apolipoprotein E (*APOE*) gene, which encodes three major isoforms in humans: apoE2, apoE3, and apoE4 (Huang and Mahley, 2014). While apoE3 is the most common variant, apoE4 has been widely associated with an increased risk of cognitive decline and neurodegenerative diseases, such as Alzheimer's disease (AD) (Fortea et al., 2024; Roses, 1996). ApoE is mainly involved in lipid transport in plasma and the central nervous system, with isoforms influencing its function. Specifically, apoE3 and apoE4 –but not apoE2– bind to low-density lipoprotein receptors with high affinity. However, structural differences in apoE4 lead it to preferentially bind to large lipoproteins, whereas apoE2 and apoE3 show a preference for small high-density lipoprotein particles (Huang and Mahley, 2014; Mahley, 1999; Weisgraber, 1990). These properties underlie the metabolic differences observed among *APOE* genotypes.

Despite growing evidence on the individual effects of *APOE* genotype, CPF exposure, and HFD on cognitive function, their potential interactions —and the role of sex-specific differences— remain poorly understood. Recent studies, including those from our group, have demonstrated that developmental CPF exposure induces metabolic alterations, such as disrupted glucose homeostasis, lipid metabolism, and hepatic function in adult animals (Guardia-Escote et al., 2020b; Pérez-Bermejo et al., 2024; Pinos et al., 2021). These findings suggest that CPF and HFD may share overlapping metabolic targets, potentially leading to synergistic effects on both physiological and cognitive outcomes. Investigating their combined impact offers a more realistic and integrative model of environmental risk, with important implications for public health. This approach could inform risk assessment and support the development of targeted interventions to mitigate cognitive decline associated with environmental exposures, dietary patterns—such as high-fat or obesogenic diets—and related metabolic disorders in human populations.

This study aimed to assess spatial learning and memory performance in targeted replacement (TR) mice expressing human *ε3*, *ε4*, or wild-type (WT) alleles, exposed to CPF from postnatal days (PND) 10–15 (inclusive), and later subjected to a HFD at 3 months of age. We hypothesized that apoE4-TR mice would show greater vulnerability to CPF- and HFD-induced cognitive deficits, with sex-specific modulation of these effects. To our knowledge, this is the first study to integrate these variables, providing new insights into the complex interplay between genetic susceptibility, environmental exposures, and cognitive function.

2. Material and methods

2.1. Animals and care

The apoE-TR animal model, first proposed by Sullivan et al. (1997), has a C57BL/6NTac background and the murine gene has been replaced by the human allele. In the present investigation, we used both male and female apoE-TR mice homozygous for either the *ε3* or *ε4* allele (Taconic Europe, Lille Skensved, Denmark) and the wild-type C57BL/6 J (Charles River, L'Arbresle, France). All the mice were maintained under standard conditions at 22 ± 2 °C and 50 ± 10 % humidity on a 12 h light/dark automatic light cycle (lights on 8:00 – 20:00). Mice were fed normal chow diet (Panlab, Barcelona, Spain) *ad libitum* and had free access to fresh water. The experimental timeline is shown in Fig. 1. All experimental procedures were approved by the Animal Care and Use Committee of the Rovira i Virgili University (Tarragona, Spain) and complied with the Spanish (Royal Decree 53/2013) and European (2010/63/EU) regulations. The present study followed the ARRIVE guidelines (Percie du Sert et al., 2020), and every effort was made to minimize animal suffering.

2.2. Chemical compounds and postnatal treatment

CPF [0,0-diethyl O-(3,5,6-trichloropyridin-2-yl) phosphorothioate], with a purity of 99.5 %, was purchased from Sigma-Aldrich Co. LLC. (Madrid, Spain). CPF was dissolved in corn oil and adjusted to administer 1 mg/kg in 1 μL/g of body weight. The control group received an equivalent volume of corn oil. Both treatments were administered orally using a micropipette from PND 10–15. Animals were randomly assigned to the treatment and control groups. This exposure setting has been previously used (Guardia-Escote et al., 2023, 2020a, 2019). The rationale for this exposure period, which corresponds to the equivalent of birth and the early postnatal period in humans, is that several critical neurodevelopmental processes, such as synaptogenesis and myelination, occur during this time (Semple et al., 2013). The dose administered in this study falls within the lower range of those typically used during this developmental stage. For comparison, doses of 1 or 3 mg/kg have been employed in studies with exposure periods spanning PND 1–4 and 11–14 (Ricceri et al., 2006, 2003), while other studies have used 1 mg/kg at PND 1–4 and 5 mg/kg at PND 11–14 (Aldridge et al., 2004; Dam et al., 2000; Slotkin et al., 2001). Importantly, the dose used in this study has not been associated with systemic toxicity or alterations in brain ChE activity (Basaure et al., 2018; Savy et al., 2015).

2.3. Dietary intervention: high fat diet

At three months of age, animals were staged in pairs according to the sex, genotype and postnatal treatment. Pairing was used to facilitate accurate monitoring of food intake and reduce social isolation-related stress. Body weight and food intake were monitored for one week

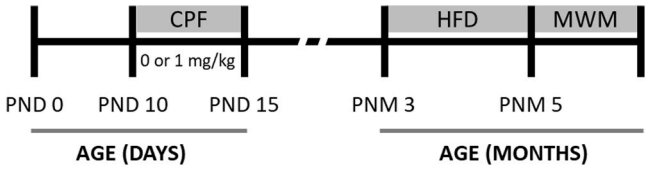

Fig. 1. Experimental timeline including the CPF treatment period from PND 10–15, the feeding of the HFD from PNM 3–5 and the MWM at the end of the dietary challenge. Abbreviations: CPF: chlorpyrifos, HFD: high-fat diet; MWM: Morris Water Maze, PND: postnatal day, PNM: postnatal month.

(week 0). Then, animals were divided in two dietary groups: one continued with the regular chow diet (SAFE A04 diet, Panlab, Barcelona, Spain) whereas the other was fed with a HFD (Purified diet 230 HF, SAFE, Augy, France) for eight weeks. The HFD provides 5.32 kcal/g (13.1 % proteins, 26.3 % carbohydrate and 60.6 % fat), and the regular chow supplies 3.34 kcal/g (19.3 % proteins, 72.4 % carbohydrates and 8.4 % fat). During the dietary intervention, animals were monitored twice a week for body weight (g) and food intake (g). The number of animals in each group is provided in Table 1.

2.4. Morris water maze

After the eight-week HFD challenge, animals were tested in a Morris Water Maze (MWM) test to assess spatial learning and memory. Originally developed by Morris in 1984, the MWM remains one of the most widely used and validated tasks for assessing spatial learning and memory in rodents (Morris, 1984; Vorhees and Williams, 2006). It is considered a robust and reliable method with broad applicability in behavioral neuroscience (D'Hooge and De Deyn, 2001). The test was performed in a circular pool (1 m diameter, 60 cm high), virtually divided in four quadrants. A submerged escape platform (10 cm diameter) was placed in the target quadrant (TQ). External clues were added in the surrounding walls whereas an internal rotating wall was added to the pool and moved after each trial in order to avoid internal clues. Besides, starting positions were changed between trials. During the acquisition period, animals performed 10 sessions, with 2 trials per session, distributed in 5 days. During the acquisition trials, the animals had up to 90 s to reach the escape platform. Otherwise, the experimenter will guide the animal to the platform and place it there for 30 s. The inter-trial time was 1:30 h, and the inter-session time was 4 h for the sessions held on the same day. Hence, two acquisition trials took place in the morning, and two in the afternoon for 5 consecutive days. We monitored the time spent to reach the platform or latency (s), as well as the distance traveled (cm) and swim velocity (cm/s). Cumulative performance data for time and distance were derived from all acquisition sessions. The retention of the task was assessed by two probe trials: 24 h after trial 12 on day 4, and 4 h after trial 20 on day 5. Long-term retention was assessed by a probe trial at 72 h after the last acquisition trial. Probe trials consisted in removing the platform and allowing the animal to freely swim in the pool for up to 60 s, while monitoring the time spent in the TQ, where the platform was formerly placed. Performance in the MWM test was recorded by a video camera (Sony CCD-IRIS model) and analyzed using the video software EthoVision® XT 11.5 (Noldus Information Technologies, Wageningen, The Netherlands).

2.5. Visual reversal

The visual reversal took place the day after the long-term retention probe. This consisted in relocating the escape platform in the opposite quadrant. Moreover, a visible clue was placed on the top of the platform, so the subject could easily see it. The visual reversal trials followed the same protocol as previously outlined for the acquisition period. Each animal performed the visual reversal trial four times, divided into two sessions with two trials per session, all conducted within a single day. The inter-session time was 4 h, and cognitive flexibility was assessed by measuring the time taken to reach the platform (s).

Table 1
Number of animals in each group in the dietary intervention and in the MWM.

Genotype	Treatment	Males	Females
C57BL/6	CNT-CNT	10	7
	CNT-HFD	10	8
	CPF-CNT	8	8
	CPF-HFD	10	8
APOE3	CNT-CNT	8	8
	CNT-HFD	8	8
	CPF-CNT	8	8
	CPF-HFD	8	7
APOE4	CNT-CNT	8	8
	CNT-HFD	9	7
	CPF-CNT	12	8
	CPF-HFD	7	10

Abbreviations: CNT: control, CPF: chlorpyrifos-treated, HFD: high-fat diet

2.6. Statistical analysis

Data were analyzed using the SPSS 27.0 software (IBM Corp, Chicago, USA). Body weight gain was analyzed with a four-way analysis of variance (ANOVA), with sex, genotype, treatment, and diet as the main factors. A one-way ANOVA was used to examine differences between groups. Repeated measures ANOVA was applied to study the acquisition of the MWM task. A one-sample t-test was used to analyze the retention. Post-hoc Tukey's test was used to analyze differences between groups. Homogeneity of variance was tested by a Levene test. Statistical significance was set at $p < 0.05$. Results are reported as mean values ± S.E.M.

3. Results

3.1. Dietary intervention: high fat diet

In the same cohort of animals, we have previously examined the effects of postnatal CPF treatment and dietary manipulation on body weight during the postnatal period and the subsequent eight-week HFD challenge (Guardia-Escote et al., 2020b). Here, we focus on body weight gain, providing complementary insights into weight regulation.

Body weight gain after an eight-week dietary challenge was assessed by a four-way ANOVA (sex x genotype x treatment x diet). A significant main effect was observed for sex [$F(1177) = 25.105$, $p < 0.001$], genotype [$F(2177) = 4.673$, $p = 0.011$] and diet [$F(1177) = 180.317$, $p < 0.001$]. A significant interaction between sex and diet was also found [$F(1177) = 13.989$, $p < 0.001$], with HFD-fed males showing the highest body weight gain. As expected, males gained more body weight than females during this period (Fig. 2A), and a clear effect of the diet was observed, with HFD-fed animals showing the highest body weight gain (Fig. 2B). ApoE3-TR mice showed the greatest increase compared to the other genotypes (Fig. 2C).

Then, we separately analyzed the differences for each genotype and sex. A two-way ANOVA (treatment x diet) showed significant effects of the diet ($p < 0.001$) in all groups. A one-way ANOVA (group) and further *post hoc* analysis showed significant differences between the HFD-fed groups and their respective controls at $p < 0.05$ (Fig. 2D-F). Only the control C57BL/6 females fed with the HFD (Control – High-fat diet) were not significantly different from the control group (Control – Control diet) (Fig. 2D).

3.2. Morris water maze

3.2.1. Acquisition

The performance during the five days of acquisition in the MWM was analyzed for each sex and genotype by a two-way ANOVA (treatment x diet) for repeated measures. The session was the within-subject factor while the dependent variables were the escape latency to the platform (s), the distance traveled (cm) and the swim velocity (cm/s). Results for distance traveled are provided in the Supplementary Material (Figure S1).

An overall improvement was observed in all groups during the acquisition part by a decrease in the latency to reach the platform ($p < 0.001$) (Fig. 3A-C). Furthermore, C57BL/6 males presented a significant interaction between session and postnatal treatment [$F(9,26) = 2.710$, $p = 0.023$] and between session and diet [$F(9,26) = 3.325$, $p = 0.045$], while C57BL/6 females showed a significant interaction between session, treatment and diet [$F(9,19) = 3.001$, $p = 0.021$]. These interactions suggest that CPF exposure and HFD differentially

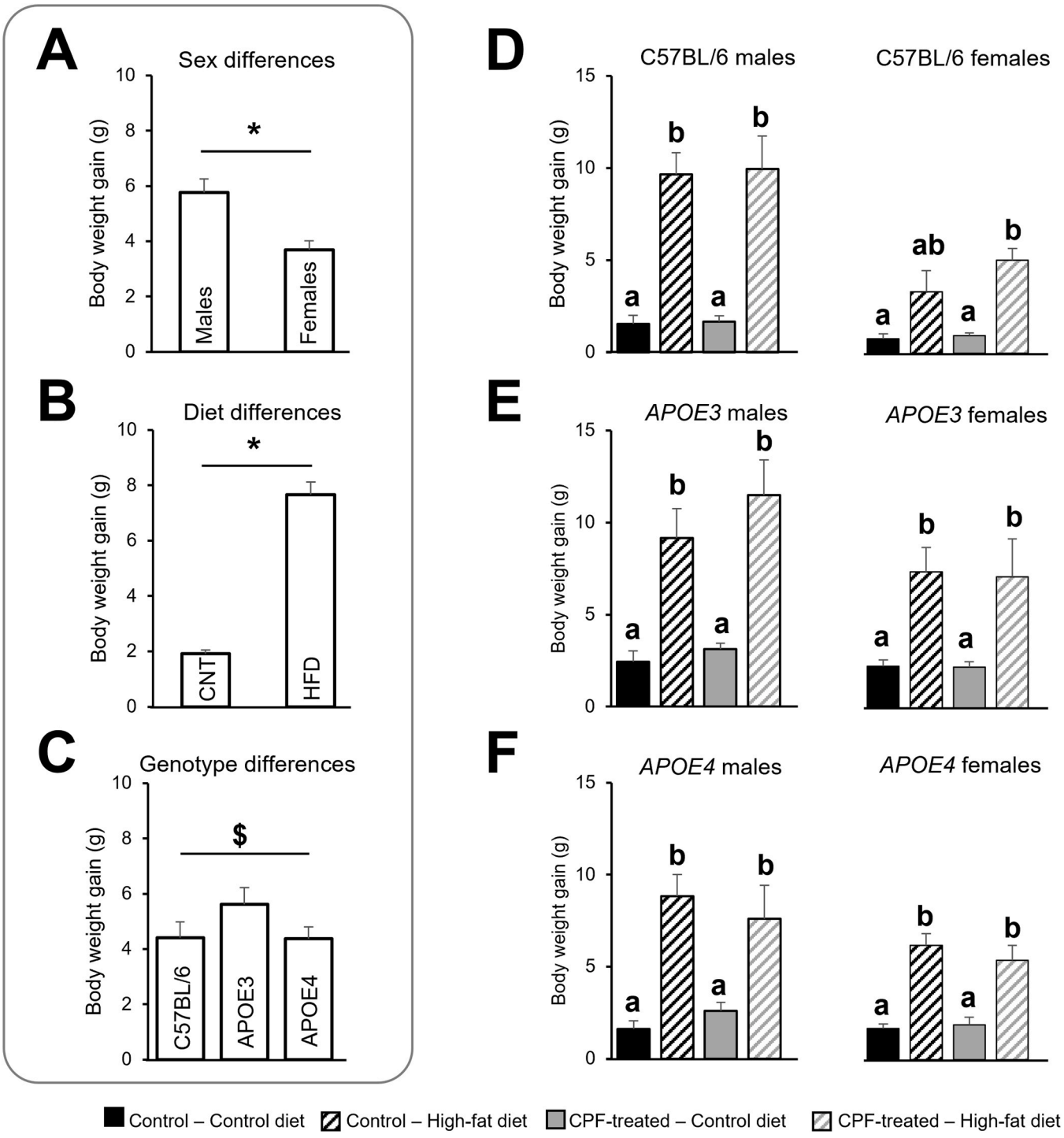

Fig. 2. Body weight gain during the eight-week dietary challenge. General effects regarding sex (A), diet (B), and genotype (C). Differences in body weight gain for each genotype and sex: C57BL/6 (D), *APOE3* (E) and *APOE4* (F) males and females. The asterisk (*) indicates significant differences between groups and the symbol $ indicates significant differences between genotypes at $p < 0.05$. Different letters (a, b) represent significant differences between groups at $p < 0.05$. Abbreviations: CNT, control; CPF, chlorpyrifos-treated; HFD, high-fat diet.

modulated learning performance depending on genotype and sex.

The cumulative time to reach the platform revealed a significant effect of the postnatal treatment in apoE3-TR males [$F(1,28) = 4.487$, $p = 0.043$] and females [$F(1,27) = 5.995$, $p = 0.021$], and apoE4-TR females [$F(1,29) = 5.240$, $p = 0.030$] (Fig. 3D-F). CPF exposure led to improved performance in apoE3-TR males and apoE4-TR females, as shown by reduced cumulative times, while in apoE3-TR females it had a detrimental effect, increasing the time needed to find the platform.

Overall changes in swim velocity were observed in all groups during the acquisition part ($p < 0.001$) (Fig. 4A-F). Moreover, C57BL/6 males presented a significant interaction between session and postnatal treatment [$F(9,26) = 2.303$, $p = 0.047$] while C57BL/6 females showed a significant interaction session and postnatal treatment [$F(9,19) = 4.761$, $p = 0.002$] and between session, treatment and diet [$F(9,19) = 2.996$, $p = 0.021$]. ApoE4-TR females showed an interaction between session and treatment [$F(9,21) = 2.486$, $p = 0.041$].

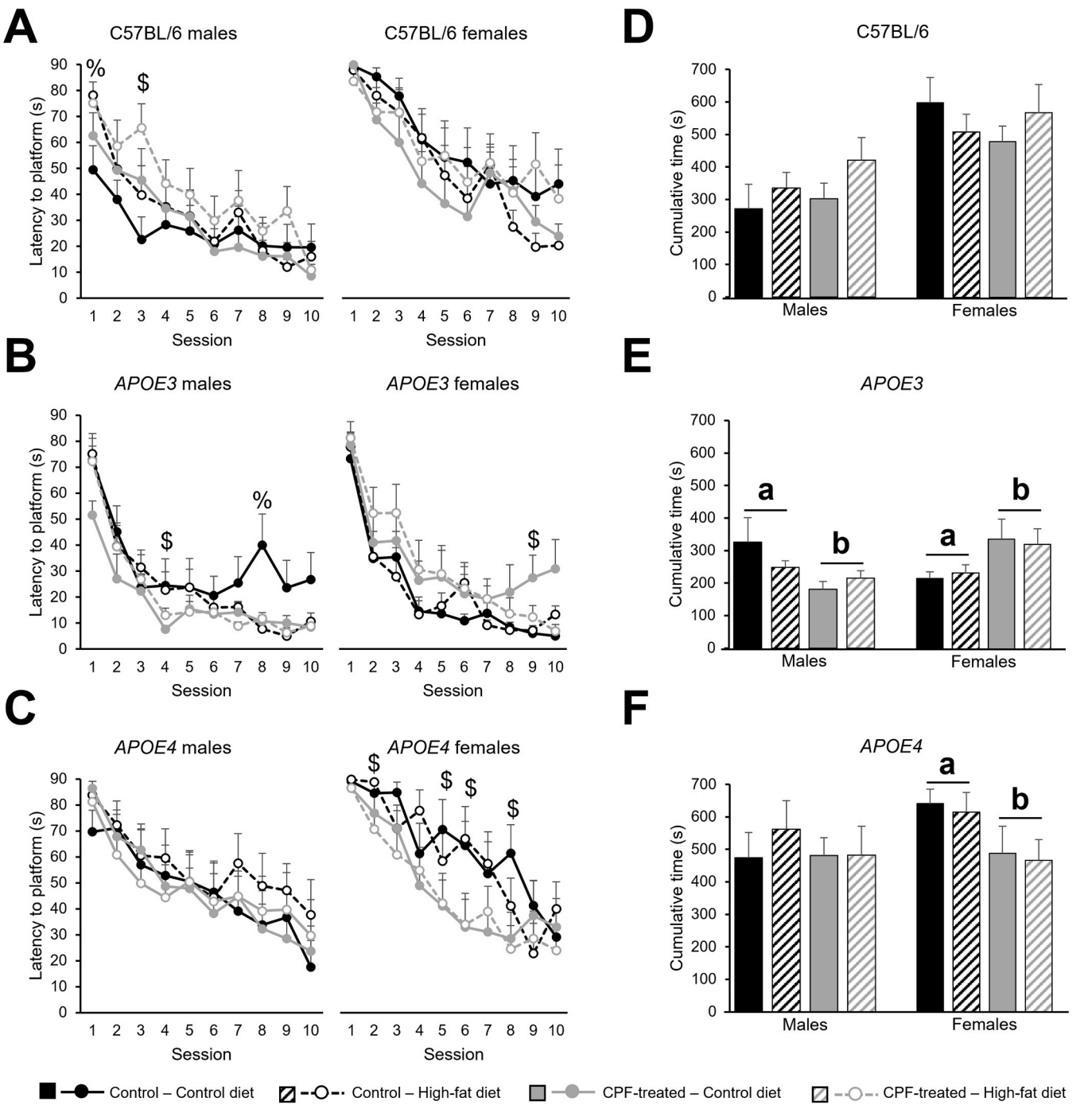

Fig. 3. Acquisition part of the MWM. Latency to the escape platform for both males and females C57BL/6 (A), *APOE3* (B) and *APOE4* (C), as well as cumulative time for C57BL/6 (D), *APOE3* (E) and *APOE4* (F) males and females. The ($) indicates significant differences depending on the postnatal treatment while (%) indicates significant differences based on the diet. Different letters (a, b) represent significant differences between groups at $p < 0.05$. Abbreviations: CNT, control; CPF, chlorpyrifos-treated; HFD, high-fat diet.

The study of the mean swim velocity throughout the acquisition showed an overall effect of the genotype ($p < 0.001$). In fact, swim velocity was higher in apoE3-TR mice and lower in apoE4-TR mice compared to C57BL/6 mice (Fig. 4G).

3.2.2. *Retention*

Retention was assessed by means of two retention sessions throughout the MWM (probe 1 and 2) and one retention session 72 h after the end of the task (probe 3). We performed a one-sample *t*-test to analyze the time spent in the TQ where the platform was formerly allocated, compared to the chance level of 15 s. C57BL/6 males exhibited a significant preference for the TQ in all retention sessions except for the CPF-treated group in probe 1 (Fig. 5A). On the other hand, C57BL/6 females showed poor performance in all retention sessions, except for the CPF-treated group at probe 2 [$t = 2.513$, d.f.$= 7$, $p = 0.040$] and the HFD-fed control group at probe 3 [$t = 2.662$, d.f.$= 7$, $p = 0.032$], both of which showed a significant preference for the TQ (Fig. 5D). These results suggest that CPF exposure may improve retention in C57BL/6 females, but the feeding of a HFD may reverse this effect. Furthermore, this advantage in CPF-exposed females may be lost in

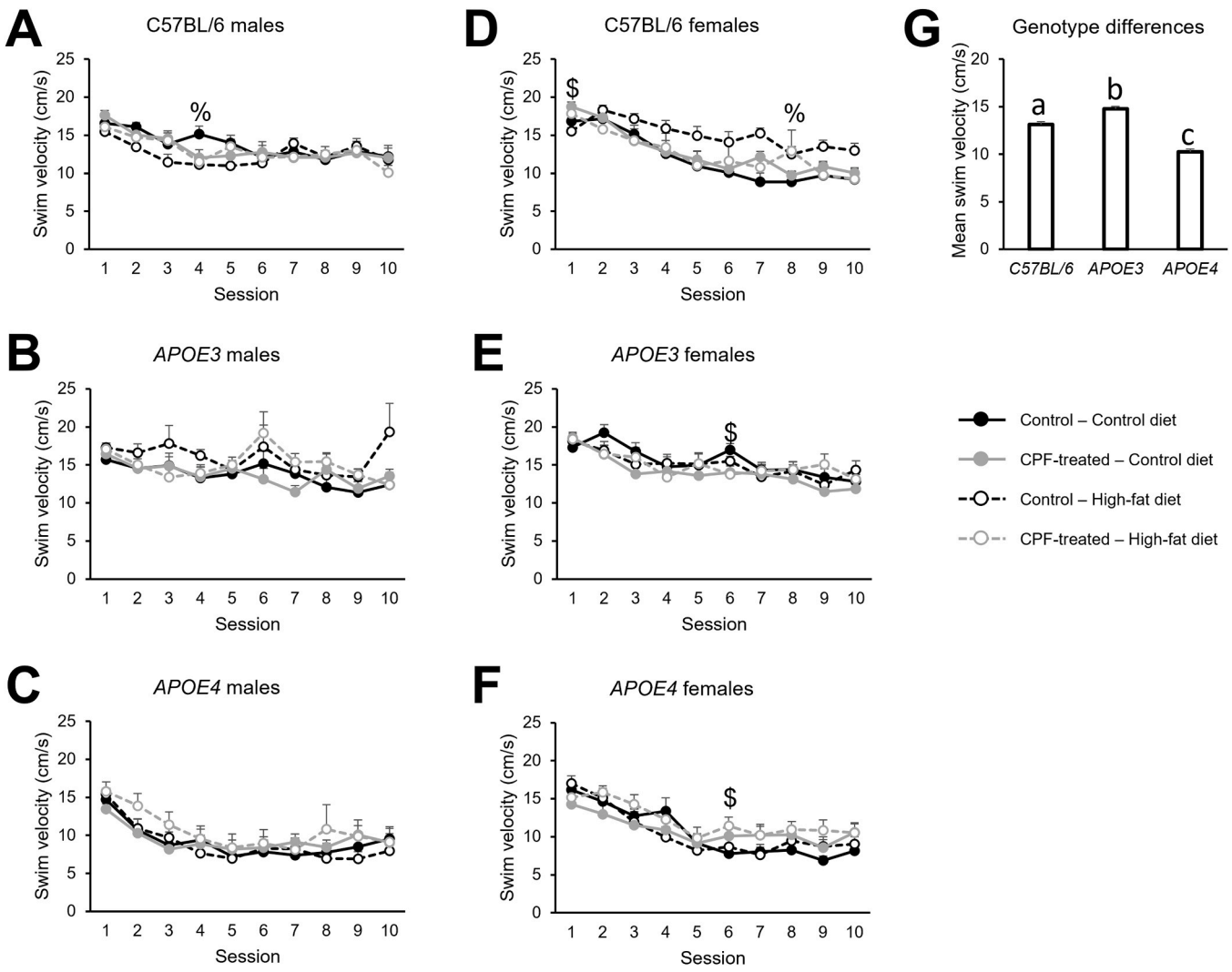

Fig. 4. Acquisition part of the MWM. Swim velocity for C57BL/6 (A), *APOE3* (B) and *APOE4* (C) males, as well as swim velocity for C57BL/6 (D), *APOE3* (E) and *APOE4* (F) females. Mean swim velocity (G) during the acquisition part of the task. The ($) indicates significant differences depending on the postnatal treatment while (%) indicates significant differences based on the diet. Different letters (a, b, c) represent significant differences at $p < 0.05$. Abbreviations: CNT, control; CPF, chlorpyrifos-treated; HFD, high-fat diet.

the long-term retention, where only a beneficial effect of the HFD would be observed in the control group.

ApoE3-TR males presented a significant preference for the TQ in all retention sessions except for the control group in probe 1, although they showed a good retention throughout the task (Fig. 5B). For their part, apoE3-TR females treated with CPF lost their preference for the TQ in the probe 3, showing a detrimental effect of the postnatal treatment on long-term retention, regardless of the diet (Fig. 5E).

ApoE4 males did not show any preference during probe 1, but retention was improved in subsequent sessions. However, control apoE4-TR fed with a HFD lost this preference in the long-term retention, suggesting a detrimental effect of the diet only in this group (Fig. 5C). On their side, apoE4-TR females exhibited a variable response. Control females fed with control diet did not show a preference for the TQ in any of the retention sessions. The administration of a HFD improved the performance in probe 1 and probe 3, whereas it altered the long-term retention in the CPF-treated mice, which showed preference for the TQ in all the probes.

3.3. Visual reversal

The performance during the visual reversal was analyzed for each sex and genotype by a two-way ANOVA (treatment x diet) for repeated measures. The trial was the within-subject factor while the dependent variable was the escape latency to the platform (s). An overall improvement was observed in all groups during the acquisition part by a decrease in the latency to reach the platform ($p < 0.001$) (Fig. 6A-F).

Mean swim velocity throughout the trials showed a general effect of the genotype ($p < 0.001$). Further *post-hoc* analysis found that swim velocity was higher in apoE3-TR and C57BL/6 mice compared to apoE4-TR mice ($p < 0.001$) (Fig. 6G). In the same line, the cumulative time spent to reach the platform was also higher in apoE4-TR mice compared to their counterparts ($p < 0.001$) (Fig. 6H).

4. Discussion

The primary objective of this study was to assess the impact of *APOE* genotype, sex, postnatal exposure to CPF and HFD in adulthood on spatial learning and memory. The results show distinct responses influenced by these factors and their interactions, contributing to a deeper understanding of gene-environment interactions in cognitive functions. As expected, animals fed a HFD for 8 weeks showed a significant increase in body weight compared to those on a regular chow diet, reinforcing the well-established link between HFD and weight gain

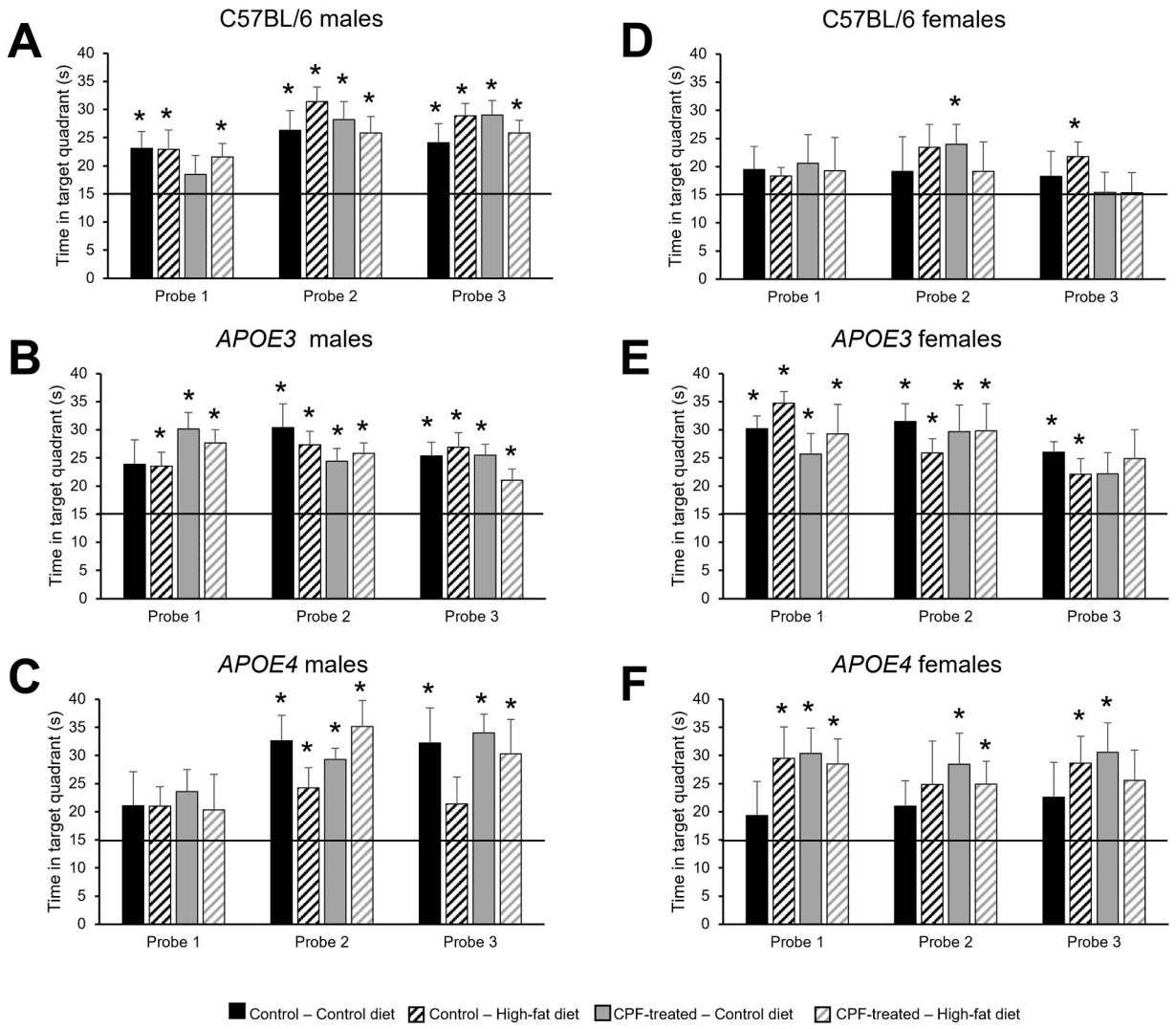

Fig. 5. Retention part of the MWM. Time spent in the TQ in C57BL/6 (A), *APOE3* (B) and *APOE4* (C) males, as well as C57BL/6 (D), *APOE3* (E) and *APOE4* (F) females during the two retention sessions (probe 1 and 2), as well as during the long-term retention session at 72 h (probe 3). The asterisk (*) indicates significant differences from the chance level of 15 s at $p < 0.05$. Abbreviations: CNT, control; CPF, chlorpyrifos-treated.

(Choi et al., 2015). Although there are differences depending on diet composition, most studies using HFDs report similar body weight increases (Casimiro et al., 2021; Haleem and Mahmood, 2019; Jones et al., 2019; Yoshizaki et al., 2020). A clear sex-dependent response to the diet was observed, consistent with previous findings that males typically gain more weight than females under similar feeding conditions (Chowen et al., 2019; Jones et al., 2019). For instance, Jones et al. (2019) found that six-month-old male apoE3-TR and apoE4-TR mice fed a HFD (45 % fat) for 12 weeks exhibited greater body weight than females, regardless of the genotype. These differences have been attributed to variations in reproductive hormone levels and sex-specific differences in the hypothalamic circuits that regulate feeding behavior and metabolism (Asarian and Geary, 2013; Chowen et al., 2019). In this context, we have previously identified sex-specific differences in DNA methylation patterns in hypothalamic genes associated with feeding control, including proopiomelanocortin, neuropeptide Y, leptin receptor, and insulin-like growth factor 2 (Guardia-Escote et al., 2020b). Genotype also played a significant role in body weight gain, with apoE3-TR mice exhibiting the highest increase. Previous research has described that apoE3-TR females, whether on a low-fat diet or a HFD, gained more body weight than their apoE4-TR counterparts (Huebbe et al., 2015), suggesting a link between the *APOE* genotype, lipid metabolism, and body weight regulation. Notably, Huebbe et al. (2015) found that apoE3-TR mice tend to use dietary energy more efficiently and favor fat storage in adipose tissue, whereas apoE4-TR mice are more likely to expend it.

All groups demonstrated successful task acquisition during learning on the MWM; however, differences in learning patterns emerged depending on the treatment and diet. Among apoE3-TR males, all groups demonstrated learning, but the CPF-treated group had the shortest cumulative time to reach the platform, suggesting enhanced performance. These results indicate that apoE3-TR mice are particularly sensitive to the effects of CPF on learning. Paradoxically, CPF exposure appeared to improve their performance. Although apoE3-TR mice being the most affected by CPF aligns with previous literature, this effect is typically detrimental. For instance, postnatal CPF exposure has been shown to disrupt spatial search strategies in the Barnes Maze test (Basaure et al., 2019b) and impair sociability in the three-chamber test (Basaure et al., 2019a) in apoE3-TR mice. However, in the latter study, adult CPF exposure enhanced social and novelty indices in apoE3-TR, highlighting the importance of the exposure timing (Basaure et al., 2019a). These results suggest that while long-term effects of CPF are often negative, short-term or acute exposure may transiently enhance certain cognitive functions, potentially through cholinergic stimulation. This hypothesis is supported by our finding that CPF-treated apoE4-TR females also showed improved performance compared to controls. It has been

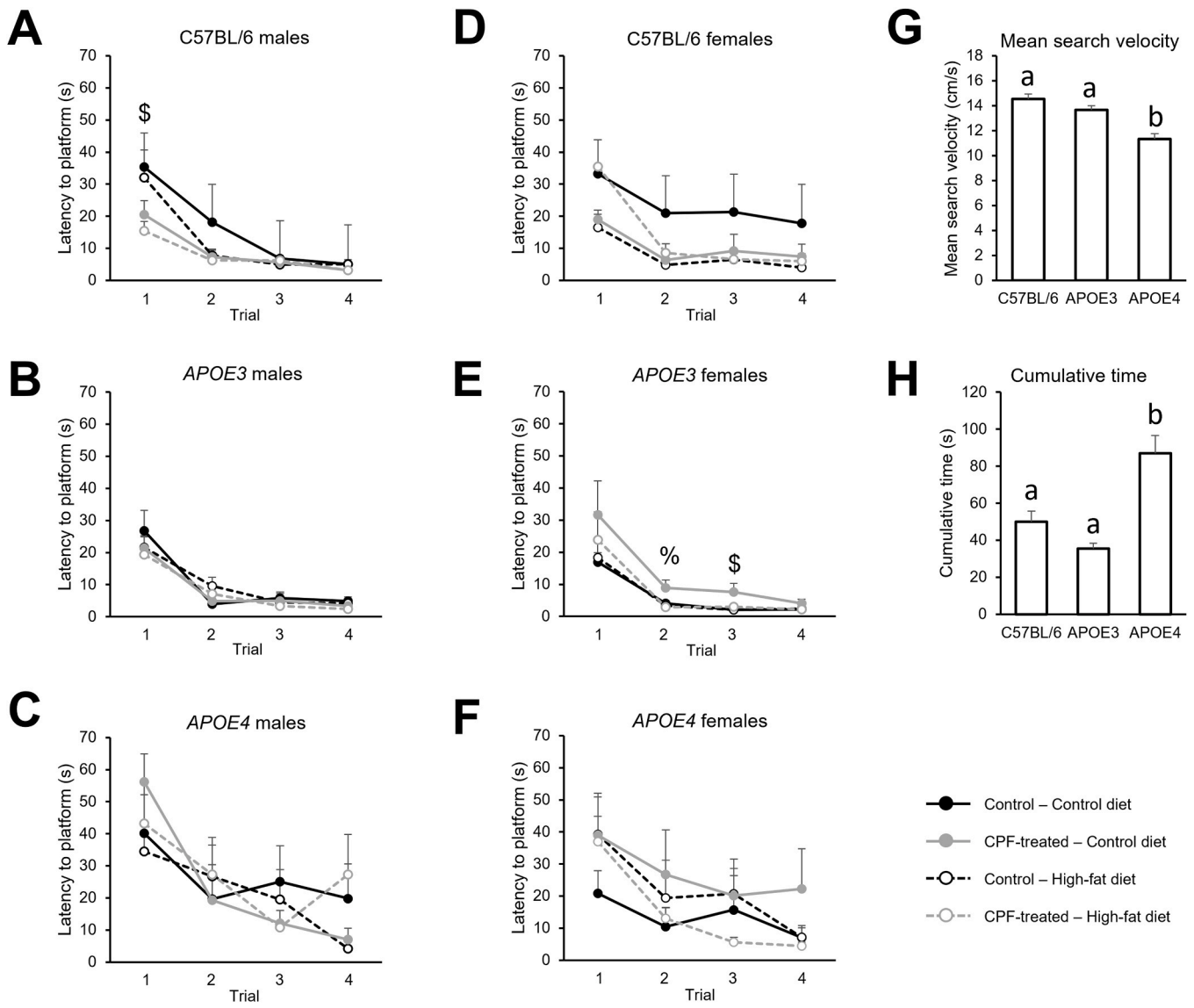

Fig. 6. Visual reverse part of the MWM. Latency to the escape platform for C57BL/6 (A), *APOE3* (B) and *APOE4* (C) males, as well as C57BL/6 (D), *APOE3* (E) and *APOE4* (F) females during the four trial sessions. General effects of mean search velocity (G) and cumulative time to the platform (H). The ($) indicates significant differences depending on the postnatal treatment while (%) indicates significant differences based on the diet. Different letters (a, b, c) represent significant differences at $p < 0.05$. Abbreviations: CNT, control; CPF, chlorpyrifos-treated.

proposed that postnatal CPF exposure may compensate for the cholinergic deficits frequently associated with this genotype, thereby enhancing long-term learning and memory performance (Basaure et al., 2019; Guardia-Escote et al., 2018). *APOE4* is known to be linked to reduced cholinergic tone and synaptic acetylcholine availability (Poirier et al., 1995), a deficit that could be transiently alleviated by CPF-mediated inhibition of acetylcholinesterase. This compensatory cholinergic modulation may restore or enhance cholinergic signaling in a genotype-specific manner, temporarily improving cognitive outcomes in apoE4-TR females.

Differences in mean swim velocity were also observed between genotypes: apoE4-TR mice swam the slowest, while apoE3-TR had the highest speed. Swim speed is an important factor to consider, as slower movement may increase search time and potentially confound the interpretation of cognitive outcomes in the MWM. Our observations are in line with the findings of Kundu et al. (2022), who reported that apoE3-TR and WT mice swam faster than their apoE4-TR counterparts, although this did not impact task performance. The authors found a link between the swimming speed and the glucose transporter levels in the brain, with higher levels in the hippocampus and lower levels in the cortex in apoE4-TR compared to WT and apoE3-TR mice. Notably, abnormalities in brain glucose transport have also been observed in AD patients (Chen and Zhong, 2013).

In general, males outperformed females during retention, particularly in the C57BL/6, where males exhibited good recall of the platform location, whereas females showed impaired retention. This result was unexpected, as previous studies have reported comparable cognitive performance in C57BL/6 females at different ages (Guardia-Escote et al., 2018; Melgar-Locatelli et al., 2024). However, it is important to consider that the experimental settings of the MWM test differed between studies. The protocols used in these studies can be considered as more intensive learning paradigms, which may have led to different outcomes in this group. In contrast, apoE3-TR males and females both showed good retention, except for CPF-exposed females, who demonstrated impaired recall 72 h after the last trial, regardless of the diet. Similar deficits were previously observed in apoE3-TR mice after postnatal CPF exposure using the Barnes Maze (Basaure et al., 2019b). ApoE4-TR mice presented a clear sexually dimorphic response. Males initially showed low

retention in the first probe trial, but improved over time, with strong recall on later trials. However, apoE4-TR control males exhibited specific long-term retention deficits when subjected to a HFD. These results indicate that although they are capable of learning, memory consolidation requires more time and is particularly vulnerable to metabolic challenges. Notably, CPF exposure during development did not affect retention in apoE4-TR males. On the other side, apoE4-TR control females consistently failed to recall the platform's location across all trials. Interestingly, those fed a HFD improvement performance in probe trials 1 and 3, while CPF exposure enhanced retention throughout all the trials. This paradoxical effect of CPF in apoE4-TR females supports the previously mentioned cholinergic hypothesis, suggesting that CPF-induced modulation of cholinergic neurotransmission may enhance cognitive function in this genotype.

In addition to cholinergic modulation, the potential impact of sex hormones signaling in the observed cognitive phenotype should also be considered. Some environmental chemicals, including CPF, have been shown to bind to estrogen receptor alpha (ER-α), thereby disrupting estrogen signaling pathways (Hazarika et al., 2020). ER-α is expressed in various brain regions, including the hippocampus, where it plays a critical role in cognitive processes (Bean et al., 2014; Frick et al., 2015). Moreover, ER-α activation has been reported to differentially affect spatial learning performance in males and females in the MWM task (Fugger et al., 1998). Although estrogen-mediated mechanisms were not directly assessed in the present study, they may contribute to the sexually dimorphic responses observed, particularly in apoE4-TR females. Another factor to consider is anxiety-like behavior, which could have influenced performance in the MWM by promoting increased thigmotaxis. Supporting this notion, chronic CPF exposure at 5 mg/kg/day for six months in adult rats has been shown to alter search strategies in the MWM, including increased thigmotaxic behavior (López-Granero et al., 2016). In the present study, we did not collect specific data on the time or distance spent in the peripheral area of the maze, which represents a limitation when interpreting whether the observed deficits reflect genuine cognitive impairments or are confounded by anxiety-related behaviors. In addition to these factors, neuroinflammatory mechanisms may also contribute to the observed cognitive deficits. The expression of apoE4 is associated with a distinct baseline inflammatory profile compared to apoE2 and apoE3 (Dias et al., 2025), and both CPF and HFD have been shown to enhance inflammatory responses and upregulate proinflammatory cytokines (AlKahtane et al., 2020; Buckman et al., 2014; Butler, 2021; Küçükler et al., 2024). These processes may act synergistically to exacerbate cognitive dysfunction, particularly in vulnerable genotypes such as *APOE4*.

In our study, females showed a more complex interaction between genetic background and environmental exposures than males. HFD improved long-term performance in CNT females, especially in groups with impaired retention, namely C57BL/6 and apoE4-TR. This effect may be linked to the timing of HFD exposure, as previous studies using the Tg6799 model demonstrated that initiating HFD at or before three months of age had protective effects, reducing Aβ pathology and enhancing cognitive function. Moreover, HFD-fed animals exhibited reduced fibrinogen extravasation –another hallmark associated with AD– which may serve as a potential mechanism underlying these protective effects (Amelianchik et al., 2021). Similarly, in another AD mouse model (Tg2576), animals fed a HFD for 12 months presented better learning in the MWM, which was attributed to improvements of the blood-brain barrier function (Goldman et al., 2018). Although there is no consensus on the effects of HFD on cognitive outcomes, it is frequently associated with neurological impairment (Wang et al., 2021; Yuan et al., 2019). Nevertheless, some evidence suggests that hypercaloric diets may improve learning and memory under specific conditions. One proposed mechanism underlying these effects is the reduction of anxiety (Haleem and Mahmood, 2019; Yoshizaki et al., 2020). For example, a study using male Wistar rats fed a normal diet or a HFD (60 % fat) for 12 weeks found that long-term HFD intake enhanced exploratory behavior in the Open Field test and reduced anxiety in the Elevated Plus Maze (EPM) (Haleem and Mahmood, 2019). Additionally, an improvement in learning and memory was observed, although reference memory was impaired, as assessed by the MWM. Interestingly, circulating leptin levels were higher in HFD-fed rats compared to controls, and serotonin (5-HT) levels were elevated in the hippocampus but lower in the hypothalamus of HFD animals, suggesting a possible role of metabolic and neurotransmitter modulation in these effects (Haleem and Mahmood, 2019). Similarly, a seven-week HFD (60 % fat) in 8-week-old male C57BL/6 J mice improved working memory and reduced anxiety, as measured by the Y-Maze and EPM, respectively (Yoshizaki et al., 2020). Our findings suggest that HFD exposure exert differential effects depending on sex and genotype, with a potentially more favorable outcome in apoE4-TR females under the specific conditions tested. In contrast, other groups, such as apoE4-TR males, may be more vulnerable to its adverse effects. While these observations are preliminary, they underscore the need for further research into sex- and genotype-specific responses to dietary interventions, moving toward more personalized approaches rather than universal recommendations.

The combination of postnatal CPF exposure and HFD impaired performance across all female groups, suggesting a synergistic interaction leading to neurobehavioral dysfunction. Early-life exposure to CPF is known to predispose individuals to long-term metabolic and cognitive alterations, and when coupled with HFD later in life, these effects may be exacerbated. Similar findings have been reported in animals exposed to the organophosphate parathion during PND1–4 and later fed a HFD (58 % fat) for 8 weeks in the adulthood (Slotkin et al., 2009). The current study suggests that males may be less susceptible to these environmental stressors, as the sex-specific responses observed in females were largely absent in males. Therefore, this observation leads us to speculate that while males may be more physically affected by the diet, females may experience more pronounced cognitive effects, particularly in long-term retention. This supports previous findings by Mattar et al. (2022), which showed that while apoE4-TR males experienced metabolic disruptions leading to cognitive impairments, females exhibited cognitive deficits without accompanying metabolic alterations, indicating the involvement of alternative pathways.

Although all groups successfully reached the platform during the visual reversal task, apoE4-TR mice took the longest time and had the lowest search velocity compared to the other genotypes. These observations suggest that apoE4-TR mice have difficulty adapting to the changed position of the platform, indicating lower cognitive flexibility. We understand behavioral flexibility as the adaptive change in the behavior of an animal in response to internal or external changes (Brown and Tait, 2010). In a previous study, Schmitt et al. (2021) assessed flexibility as a marker of cognitive decline in apoE3-, apoE4-TR, and WT mice, reporting a flexibility deficit in young adult apoE4-TR mice tested at 6 months of age compared to the other groups. In contrast, apoE3-TR mice only exhibited a decline at middle age (14 months), performing similar to their apoE4 counterparts in the aquatic Y-maze, where apoE4-TR mice exhibited no age-related differences. Altogether, these results suggest that apoE4 may accelerate the onset of flexibility impairments that otherwise occur during normal aging in mice. Additionally, the study found no correlation between deficits in cognitive flexibility and spatial memory acquisition, indicating that these two functions are independent (Schmitt et al., 2021).

Overall, this study contributes to our understanding of how diet can modulate not only body weight gain but, more importantly, influence cognitive performance. These observations may pave the way for targeted interventions and personalized treatment strategies. However, further investigations are needed to elucidate the molecular mechanisms underlying these effects.

CRediT authorship contribution statement

Judit Biosca-Brull: Methodology. **Laia Guardia-Escote:** Writing –

original draft, Methodology, Data curation, Conceptualization. **Maria Cabré:** Writing – review & editing, Conceptualization. **Jordi Blanco:** Methodology. **Cristian Pérez-Fernández:** Methodology. **Pia Basaure:** Methodology. **Domingo José Luis:** Writing – review & editing. **Fernando Sánchez-Santed:** Funding acquisition. **Colomina M.Teresa:** Writing – review & editing, Funding acquisition, Conceptualization.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used ChatGPT to improve the clarity and readability of the manuscript. The tool was employed exclusively for language refinement and rephrasing, not for content generation. All content was subsequently reviewed and edited by the authors, who take full responsibility for the content of the publication.

Declaration of Competing Interest

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

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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.neuro.2025.07.004.

Data availability

Data will be made available on request.

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