Apolipoprotein E *(APOE)* genotype and the pesticide chlorpyrifos modulate attention, motivation and impulsivity in female mice in the 5-choice serial reaction time task

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Abstract

Organophosphate pesticides - and chlorpyrifos (CPF) in particular - contribute to a wide range of neurobehavioural disorders. Most experimental research focuses on learning and memory processes, while other behaviours remains understudied. The isoforms of the human apolipoprotein E (apoE) confer different cognitive skills on their carriers, but data on this topic are still limited. The current study was performed to assess whether the APOE genotypic variability differently modulate the effects of CPF on attentional performance, inhibitory control and motivation. Human apoE targeted replacement adult female mice (apoE2, apoE3 and apoE4) were trained to stably perform the 5-choice serial reaction time task (5-CSRTT). Animals were then subjected to daily dietary CPF (3.75mg/kg body weight) for 4 weeks. After CPF exposure, we established a 4-week CPF-free period to assess recovery. All individuals acquired the task, apoE2 mice showed enhanced learning, while apoE4 mice displayed increased premature and perseverative responding. This genotype-dependent lack of inhibitory control was reversed by CPF. Overall, the pesticide induced protracted impairments in sustained attention and motivation, and it reduced anticipatory responding. ApoE3 mice exhibited delayed attentional disruptions throughout the wash-out period. Taken together, these findings provide notable evidence on the emergence of CPF-related attentional and motivational deficits.

Keywords: Chlorpyrifos, Attention, Impulsivity, Motivation, Apolipoprotein E, 5-CSRTT

1. Introduction

The onset of cognitive deficits and behavioural disorders after exposure to organophosphate (OP) pesticides – in particular to the widely-used chlorpyrifos (CPF) – has been reported in the scientific literature (Mackenzie Ross et al., 2010; Roldán-Tapia et al., 2005). In the last decade, environmental agencies have taken steps to reduce the non-agricultural uses of CPF. In 2006, however, its residues were still present in 78% of randomly-selected homes in the United States (US) (Stout et al., 2009), being also recently detected in both urban (Ccanccapa et al., 2015; Quijano et al., 2016) and rural areas (Page et al., 2014), so that implying a pervasive pattern of exposure. Although CPF may be absorbed by inhalation or through the skin, dietary intake appears to be the most common source of exposure for the general population (Boon et al., 2008; Lu et al., 2008). In this context, several epidemiological approaches have attempted to estimate typical dietary food consumption values for CPF in adult individuals (e.g., 0.46μg/day reported by MacIntosh et al., 2001) (Buck et al., 2001; Curl et al., 2015; MacIntosh et al., 2001; Melnyk et al., 2011). Nonetheless, the additive effect of all routes of exposure, as well as the variety of human behaviours and activities make it difficult to estimate the total daily exposure to the pesticide (Saunders et al., 2012).

A constellation of epidemiological investigations has demonstrated that OPs induce deficits in cognitive processes, such as sustained attention, memory, and processing speed (De Silva et al., 2006; Mackenzie Ross et al., 2010; Miyaki et al., 2005; Roldán-Tapia et al., 2005). Consistently, data from animal models of acute or repeated CPF exposure highlighted learning and memory impairments (López-Granero et al., 2014; Peris-Sampedro et al., 2015a, 2014; Salazar et al., 2011), deficits in sustained attention (Middlemore-Risher et al., 2010; Samsam et al., 2005), destabilized inhibitory control (Middlemore-Risher et al., 2010; Montes de Oca et al., 2013), and anhedonia (Aldridge et al., 2005).

Once CPF has entered the body, it undergoes an oxidative desulfuration to its active metabolite CPF-oxon, which expresses a potent anticholinesterase activity. The inhibition of cholinesterases (ChE) elicits the accumulation of acetylcholine (ACh) at the synapses of both

the central and peripheral nervous systems (CNS, PNS), leading ultimately to acute cholinergic neurotoxicity. In addition, an increasing number of reports have endorsed the involvement of other neurotransmitter systems, such as the GABAergic system, in the neurotoxicity of CPF (Cardona et al., 2006; Montes de Oca et al., 2013). Thus, Cardona et al. (2006) reported that the administration of diazepam – a GABAergic agonist – potentiates the long-term CPF-related effects observed in a schedule-induced polydipsia paradigm in rats. Interestingly, recent data have claimed for a neglected role of GABA in impulsivity (Hayes et al., 2014).

In 1983, Robbins and co-workers designed a test to assess attentional processes in rats, which was based on the continuous performance task used for the same purpose in humans (Robbins, 2002). Nowadays, the 5-choice serial reaction time task (5-CSRTT) enables various aspects of performance to be assessed simultaneously (Bari et al., 2008; Sanchez-Roige et al., 2012). To date, only two studies have used this paradigm to evaluate the detrimental effects of CPF on cognition (Middlemore-Risher et al., 2010; Montes de Oca et al., 2013). Both studies, carried out in male rats, found disturbed inhibitory control in the short (Middlemore-Risher et al., 2010) and the long-term (Montes de Oca et al., 2013) after relatively high doses of CPF. Moreover, Middlemore-Risher et al. (2010) also reported impairments in sustained attention with no signs of altered motivation that were still evident one month after the exposure.

In addition to the well-characterized role of apolipoprotein E (apoE) in maintaining lipid homeostasis, this glycoprotein also contributes to several neurological functions in the CNS (Hauser et al., 2011), and its three major isoforms (apoE2, apoE3 and apoE4) confer different neurobehavioural attributes on their carriers. Although other mammals express apoE, allelic variation (*ε2*, *ε3* and *ε4*) is unique to humans. Sullivan et al., (1997) designed the apoE targeted replacement (TR) mouse model by replacing the murine *apoE* gene by one of the three human *APOE* allelic variants, thus allowing them to systemically express functional human apoE isoforms.

Learning and memory processes have widely been studied in apoE TR mice (Bour et al., 2008; Grootendorst et al., 2005; Peris-Sampedro et al., 2015a; Reverte et al., 2013, 2012). However, there is still considerable uncertainty about the extent to which *APOE* genotype contributes to

other cognitive and behavioural processes, such as sustained attention, inhibitory control and motivation. Most studies have focused on deciphering the behavioural attributes inherent to the *APOE4* genotype, since it is the largest genetic risk for Alzheimer's disease (AD) (Raber et al., 2004). Particularly, the *APOE4* genotype has traditionally been associated with poor cognitive outcome (Peris-Sampedro et al., 2015a; Reverte et al., 2012; Siegel et al., 2012), which has sometimes been attributed to a hypothetical cholinergic dysfunction (Yun et al., 2005). Furthermore, recent experimental evidence has revealed that only the *APOE4* genotype confers on its carriers deficient inhibitory control and impaired attentional accuracy on the 5-CSRTT (Reverte et al., 2016). On the other hand, the most common isoform in humans, apoE3, has recently been linked to an increased risk of developing obesity and a diabetic profile upon exposure to CPF (Peris-Sampedro et al., 2015a, 2015b). In this regard, a growing body of evidence has considered both impulsivity and compulsivity as potential feeding behaviour disruptors contributing to the obesity epidemics (Schag et al., 2013; Smith and Robbins, 2013).
To the best of our knowledge, no information is available on the use of the 5-CSRTT to assess the impact of dietary exposure to CPF on attention, inhibitory control and motivation in apoE TR mice. Hence, this investigation seeks (a) to determine whether CPF alter the 5-CSRTT baseline performance of apoE TR female mice previously trained, (b) to investigate whether such CPF-related effects persist over time, and (c) to assess the extent to which human *APOE* genetic variations modulate the effects of both CPF and alprazolam.

2. Material and methods

2.1 Animals and care

Adult apoE TR female mice, homozygous for the human *ε2*, *ε3* or *ε4* alleles, were purchased from Taconic (Taconic Europe, Lille Skensved, Denmark). They were housed in pairs under a 12-h light-dark cycle (lights off at 8 pm) in an environmentally controlled room held at 22±2°C and at a relative humidity of 50%±10%. Food (Panlab standard rodent chow, Barcelona, Spain) and water were available *ad libitum*. Before the behavioural task started, mice were gradually

food deprived (2g/mouse/day) to approximately 80-85% of their free feeding weight (i.e., 20g). These feeding conditions were maintained until the end of the study. All experiments took place five days a week and were carried out during the light phase (Reverte et al., 2016). Five animals failed to reach criterion performance and were excluded from the 5-CSRTT training (apoE2 = 1, apoE3 = 3, apoE4 = 1).

Experimental procedures were conducted in accordance with the Animal Care and Use Committee of the Rovira i Virgili University (Tarragona, Spain). Likewise, in conformity with the Spanish Royal Decree 53/2013 and the European Communities Council Directive (86/609/EEC) efforts were made to alleviate animal suffering.

2.2 Drugs

During the manufacture process, standard rodent chow was supplemented with 37.5mg CPF/kg chow (CPF purity 99.5%, Sigma-Aldrich, Seelze, Germany). Given the feeding conditions and body weight stability over time, mice were subjected to 3.75mg/kg body weight/day dietary CPF. The dose of CPF was chosen on the basis of earlier work (Peris-Sampedro et al., 2015a, 2015b), and was expected to induce a moderate inhibition of plasma cholinesterase without signs of acute toxicity. The GABAergic agonist alprazolam was supplied by Pfizer (Pfizer, S.A., Alcobendas, Spain) and was used for the pharmacological challenge at a dose of 0.12mg/kg (Reverte et al., 2016).

2.3 Five-choice serial reaction time task (5-CSRTT)

2.3.1 Apparatus

The behavioural training was carried out in two identical acrylic operant chambers (24x20x15cm) (Med Associates Inc., St. Albans VT, USA), provided with steel grid floors and enclosed in ventilated wooden sound-attenuating boxes. Each chamber consisted of a curved aluminium wall containing nine equally-spaced holes. Four of the initial round apertures were

closed off with metal inserts. Thus, only five evenly-spaced 2.5cm holes were operative and equipped with infrared detectors and a bright yellow led (1.7W) at the rear. The magazine, located centrally in the opposite metallic wall, was equipped with an infrared detector and automatically delivered 0.01ml of grape juice (commercially available grape juice containing 15.13% sugar, López Morenas, SL, Spain) via a liquid dispenser. The record of the behavioural task was controlled by a Fader Control interface and MED-PC software (Med Associates Inc., St. Albans VT, USA).

2.3.2 5-CSRTT training

Pre-training and training procedures in the 5-CSRTT were performed as previously described (Reverte et al., 2016). Briefly, mice were first habituated to the liquid reinforcer and 5-CSRTT chambers. Then, two 20-min pre-training stages were established to gradually introduce the mice to the task. In these phases, animals were required to learn to poke their noses into an illuminated hole in order to trigger a reward in the magazine entry. Subsequently, they were progressively trained to detect a brief visual stimulus presented pseudo-randomly in one of the five operating holes. The stimulus duration (SD) was reduced from 30 to 1s throughout 10 acquisition stages. A nose-poke into the illuminated magazine initiated each trial, which consisted of a fixed 5-s inter-trial interval (ITI) set prior the random presentation of the visual stimulus. If the mice responded in the illuminated hole within the SD or before the end of the limited hold (LH) (5s), 0.01ml of grape juice was delivered in the magazine dispenser and a *correct response* was recorded. On the contrary, they were not rewarded when *incorrect responses* (nose-pokes made within a non-illuminated aperture), *omissions* (failure to respond within the SD and/or the LH) or *premature responses* (responses made in any of the five holes during the ITI) were recorded. Furthermore, these responses were all punished with a 5-s brightness period (time-out, TO), in which no new trials could be started. Additional responses in a hole after a correct response and before the reinforcer collection (*perseverative responses*) were also recorded, but were not punished. In either case, each training session lasted for 20min

or a maximum of 70 discrete trials. Once the mice reached a stable baseline performance for 5 consecutive days (final parameters: SD = 1s, ITI = 5s, LH = 5s, TO = 5s; criteria: > 50% of the total trials, > 80% accuracy, < 25% omissions), they were tested under experimental conditions involving behavioural manipulations and pharmacological challenges.

2.3.3 Behavioural manipulations

The behavioural testing was initiated after successful and stable acquisition of the task and lasted for 4 weeks. In brief, impulsivity and attentional performance were assessed once a week (i.e., Wednesday) for two consecutive weeks, by increasing the ITI from 5 to 7s, and shortening the SD from 1 to 0.5s, respectively (Reverte et al., 2016; Sanchez-Roige et al., 2012). Every other day of the week, mice were trained with standard baseline parameters (for more details, please refer to Reverte et al., 2016).

2.3.4 Pharmacological challenge

Following behavioural manipulations, we tested the effects of the GABAergic agonist alprazolam on the 5-CSRTT performance. Twice a week, typically on Wednesday and Friday, alprazolam was *i.p.* injected 30min before the 5-CSRTT training. To provide a control injection condition, 0.9% saline was *i.p.* injected on Tuesday and Thursday 30min before the training started. In either case, mice were trained with standard baseline parameters.

2.4 Treatment procedures

After both behavioural and pharmacological challenges, mice were trained in standard conditions in order to restore their baseline level. A total of 20 female mice were exposed to CPF (apoE2 = 8, apoE3 = 6, apoE4 = 6). At the beginning of the treatment, the mean ages of apoE2, apoE3 and apoE4 mice were 11.19±0.53, 12.25±0.25 and 13.17±1.01 months, respectively.

2.4.1 Effects of repeated exposure to CPF on 5-CSRTT performance, impulsive condition, sustained attention and GABAergic system functioning

To investigate the effects of repeated exposure to CPF, mice were fed CPF incorporated into standard rodent chow for 4 consecutive weeks. When administration of CPF began, animals were assessed daily for signs of acute cholinergic toxicity. Throughout the treatment period, mice continued to perform the task daily with standard baseline parameters, allowing the assessment of the CPF impact on their basal 5-CSRTT performance.

To determine whether CPF exposure elicited impulsive responding or impaired sustained attention, behavioural manipulations were carried out once a week during the last two weeks of treatment, typically on Wednesday. In particular, the session in which the ITI was increased (7s) took place in the third week, while the SD was shortened (0.5s) in the fourth week.

In order to provide further insight into the interaction between the GABAergic and cholinergic systems, mice were subjected to a single *i.p.* dose of alprazolam, administered 30min before the last session of the CPF exposure period started (i.e., Friday).

2.4.2 Assessment of the recovery period

A 4-week wash-out period followed CPF exposure. Mice were again fed standard rodent chow and subjected to daily 5-CSRTT training with standard baseline parameters. The ability of the mice to overcome the treatment with CPF was then assessed by analysing their task performance. We used the same procedure above described (see *2.4.1)* to investigate whether both impulsive and attentional statuses returned to their previous state by manipulating both the ITI and the SD.

2.4.3 Cholinesterase (ChE) activity assessment

ChE activity was tested in plasma and frontal cortex in a second cohort of naïve females. Mice (n = 18) were distributed into three groups, according to the experimental conditions of the study: controls (n = 2/genotype), CPF exposure (n = 2/genotype), and wash-out (n = 2/genotype). Brain ChE activity was determined in all these individuals, while plasma ChE activity was randomly assessed in 6 mice as an indicator of acute systemic CPF effect (controls = 3, CPF-exposed = 3) (Eaton et al., 2008; Peris-Sampedro et al., 2015a). Enzymatic assays procedures, as well as detailed description of sample processing can be found elsewhere (Montes de Oca et al., 2013; Peris-Sampedro et al., 2015a, 2015b; Salazar et al., 2011). Briefly, at the end of each treatment condition, mice were anesthetized with carbon dioxide before being euthanized. Blood was obtained by cardiac puncture and immediately centrifuged to obtain plasma, which was stored at -80ºC until use. After the blood draw, mice were rapidly decapitated and the whole brains were removed, dissected, homogenized and ultimately centrifuged. In both cases, enzyme activity was determined spectrophotometrically using the Ellman method (Ellman et al., 1961), and was calculated relative to protein concentration contained in the sample using the Bradford method (Bradford, 1976). Finally, ChE activity of exposed animals was estimated on the basis of the activity value of the control mice, and represented as a percentage.

2.5 Data collection

The following variables were recorded throughout the experimental procedures. Attentional performance was assessed by the *percentage of accuracy* (number of correct responses divided by the sum of correct and incorrect responses x 100), *the percentage of omissions* (number of omissions divided by the total number of trials completed x 100) and by a measure of processing speed (*correct latency*: time required to respond correctly after the onset of the stimulus) (Sanchez-Roige et al., 2012). To evaluate inhibitory control, both impulsivity and compulsivity were recorded in terms of *percentage of premature responses* (premature responses divided by the total number of trials completed x 100) and *perseverative responses*

(number of reiterative responses made into the holes after a correct response), respectively (Sanchez-Roige et al., 2012). The *total number of trials* (the sum of correct, incorrect and omitted responses), and the *reward latency* (time needed to retrieve the reward after a correct response) were recorded as motivational parameters (Dalley et al., 2007). The *total number of trials* was not analysed for the sessions in which the ITI was lengthened because the session duration was increased to 25 min to ensure mice had enough time to perform at least 50% of the trials.

2.6 Statistical analyses

Data processing was performed using the SPSS statistical package (version 20.0). We used one-way analysis of variance (ANOVA) (genotype) to determine the number of sessions required at each stage of the training. The animals performance on the 5-CSRTT during baseline, CPF exposure, and wash-out period, as well as data from the behavioural and pharmacological challenges in each treatment condition were all analysed by one-way repeated-measures (RMANOVA). The genotype was used as the between-subject factor, while weeks or days were used as the within-subject factor. A two-way ANOVA was performed to establish the contribution of both CPF exposure and *APOE* genetic background to brain ChE activity. Plasma ChE activity was analysed by means of one-way ANOVA (CPF treatment). When appropriate, Tukey's *post-hoc* comparisons were used. Statistical significance was set at $p < 0.05$, and results are reported as mean values ±SE.

3. Results

3.1 Acquisition of the 5-CSRTT

During acquisition, all mice progressed at similar rates during the first four training levels. However, when attentional demands were higher, some differences between genotypes emerged. We observed that the genotype affected the average number of sessions required to

achieve the performance criterion on training stages 5 [$F_{2, 19} = 13.778$, $p < 0.001$], 6 [$F_{2, 19} = 6.966$, $p = 0.006$], 7 [$F_{2, 19} = 12.242$, $p = 0.001$], 8 [$F_{2, 19} = 8.440$, $p = 0.003$], and 9 [$F_{2, 19} = 7.105$, $p = 0.006$] (Fig. 1). ApoE2 mice were generally faster learners than both apoE3 (stages 5 to 9, $p < 0.05$) and apoE4 (stages 7 to 9, $p < 0.05$). Ultimately, mice fully acquired the task, and there were no differences between genotypes in the total number of sessions they took (Fig. 1). Moreover, once the baseline state was reached, the animals attained and maintained high levels of accuracy (Fig. 2A).

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3.2 5-CSRTT performance under standard conditions

3.2.1 Baseline

The baseline performance on the 5-CSRTT is illustrated in Figure 2 and Table 1. No differences between genotypes were noted for the percentages of accuracy (Fig. 2A), omissions (Fig. 2B) and premature responses (Fig. 2C), nor for the total number of trials and correct latency (data not shown). In contrast, the genotype did affect perseverative responding [$F_{2, 19} = 5.833$, $p = 0.012$]. ApoE4 mice persevered more than apoE2 ($p = 0.011$) and apoE3 mice ($p = 0.053$) (Fig. 2D). The apoE4 group also showed an upward trend in latency to collect the reward [genotype: $F_{2, 19} = 3.435$, $p = 0.056$], which might be partly explained in terms of increased perseverative responding. *Post-hoc* analyses revealed that apoE2 mice were faster at retrieving the reward than apoE4 ($p = 0.054$) (Table 1).

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3.2.2 CPF exposure

The exposure to CPF led to a progressive reduction in both the percentage of premature responses made [weeks: $F_{3, 19} = 3.571$, $p = 0.040$] and the total number of trials completed [weeks: $F_{3, 19} = 8.631$, $p = 0.001$] (data not shown). No differences between genotypes were observed in any of the 5-CSRTT performance variables measured. Therefore, the effect of the genotype previously reported on perseverative responses during baseline was not noted during the exposure period.

3.2.3 CPF-free wash-out period

During the 4-week wash-out period there was a gradual recovery of both affected parameters: the percentage of premature responses [weeks: $F_{3, 19} = 11.919$, $p < 0.001$] and the total number of trials [weeks: $F_{3, 19} = 3.960$, $p = 0.029$] (data not shown). As previously found during CPF exposure, the genotype did not affect any of the performance variables measured.

3.3 Longitudinal characterization of the intoxication and detoxification periods

In order to assess the potential impact of exposure to dietary CPF on the 5-CSRTT baseline performance, we looked at the progression in mice performance over the three treatment conditions (Fig. 3). The genotype did not show any significant effect on the variables analysed. Considering the three experimental phases as a longitudinal study, we found that five variables varied: omissions [$F_{2, 19} = 11.158$, $p = 0.001$] (Fig. 3B), correct latency [$F_{2, 19} = 6.876$, $p = 0.007$] (Fig. 3F), premature responses [$F_{2, 19} = 4.738$, $p = 0.024$] (Fig. 3C), total number of trials [$F_{2, 19} = 11.460$, $p = 0.001$] (Fig.3E), and reward latency [$F_{2, 19} = 16.091$, $p < 0.001$] (Fig. 3G). In agreement with the results presented in the above subsections (see *3.2.2* and *3.2.3*), an interaction time x period was found for the percentage of premature responses [$F_{6, 19} = 5.700$, $p = 0.005$], and the total number of trials [$F_{6, 19} = 4.670$, $p = 0.011$].

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As for attentional performance, *post-hoc* analyses revealed that CPF exposure resulted in a significant increase in omissions compared to the baseline period ($p < 0.001$) (Fig. 3B). Moreover, CPF also worsened the processing speed, manifested by higher correct latencies ($p = 0.009$) (Fig. 3F). Both parameters were not recovered during the wash-out period (Fig. 3B, 3F).

With regard to inhibitory control, the exposure to CPF significantly reduced the percentage of premature responses compared to baseline ($p = 0.005$) (Fig. 3C).

In terms of motivation, mice exposed to CPF appeared to be less likely to perform the 5-CSRTT. In particular, CPF-fed mice completed fewer trials than at baseline ($p < 0.001$) (Fig. 3E), and were slower to retrieve the subsequent reward ($p = 0.001$) (Fig. 3G). These two parameters not only remained unchanged during wash-out, but also continued to increase in the case of reward latency (CPF *vs* wash-out, $p = 0.031$) (Fig. 3E, 3G).

3.4 Behavioural manipulations

Figure 4, Figure 5 and Table 2 provide an overview of the behavioural effects on the 5-CSRTT described for the three genotypes throughout the challenge sessions.

3.4.1 Inter-trial interval challenge

Baseline: Significant increases in both omissions [ITI: $F_{1, 16} = 5.292$, $p = 0.037$] (Fig. 4B) and premature responses [ITI: $F_{1, 16} = 19.308$, $p = 0.001$] (Fig. 4C) were observed when the ITI was increased from 5- to 7-s. We also found that the genotype considerably affected premature responses [$F_{2, 16} = 6.943$, $p = 0.008$], with a trend towards a significant ITI x genotype interaction [$F_{2, 16} = 3.474$, $p = 0.060$]. Further analyses revealed that apoE4 mice generally showed higher premature responding relative to apoE2 ($p = 0.009$) and apoE3 mice ($p = 0.042$) (Fig. 4C). Specifically, after lengthening the ITI, apoE4 mice continued to display more premature responses than apoE2 mice ($p = 0.012$) (Fig. 4C).

CPF exposure: CPF counteracted the increase in omissions caused by lengthening the ITI during the baseline period (Fig. 4B). Although significant increases in premature responding were again found as a result of lengthening the ITI [ITI: $F_{1, 19} = 14.041$, $p = 0.002$], the exposure to CPF neutralized the effect of the genotype noted at baseline (Fig. 4C). Furthermore, the correct latency decreased during the ITI challenge in the treatment period [ITI: $F_{1, 19} = 9.741$, $p = 0.006$] (Table 2).

Wash-out: Just as during CPF treatment, manipulating the ITI had no effect on the percentage of omissions during wash-out (Fig. 4B). As was the case in the other two periods, significant increases in premature responding were again found after lengthening the ITI [ITI: $F_{1, 19} = 7.705$, $p = 0.013$] (Fig. 4C). Similarly to CPF exposure, mice subjected to a challenged ITI showed reduced correct latencies during wash-out [ITI: $F_{1, 19} = 12.199$, $p = 0.003$] (Table 2). We observed a main effect of the genotype on accuracy [$F_{2, 19} = 11.492$, $p = 0.001$], which was not found for the other two periods (Fig. 4A). A *post-hoc* analysis indicated that accuracy in apoE4 mice decreased more steeply than in apoE2 ($p = 0.001$) and apoE3 ($p = 0.010$).

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3.4.2 Stimulus duration challenge

Baseline: A significant decrease in accuracy [SD: $F_{1, 14} = 25.544$, $p < 0.001$] (Fig. 5A) and an increase in omissions [SD: $F_{1, 14} = 22.125$, $p = 0.001$] (Fig. 5B) were observed when the SD was reduced from 1- to 0.5-s. Moreover, correct latencies fell throughout the SD challenge [SD: $F_{1, 14} = 13.757$, $p = 0.003$] (Table 2). We also found that the genotype mainly affected accuracy [$F_{2, 14} = 4.688$, $p = 0.031$] and the total number of trials [$F_{2, 14} = 6.707$, $p = 0.011$]. Further analyses revealed a more pronounced drop in accuracy in apoE4 mice than in apoE2 ($p = 0.026$) (Fig.

5A), and generally fewer completed trials in the apoE4 group relative to the apoE2 group (p = 0.010) (data not shown).

CPF exposure: Overall, manipulating the SD during CPF exposure period did not affect accuracy (Fig. 5A), omissions (Fig. 5B), or latency to respond correctly (Table 2). Furthermore, CPF neutralized the effect of the genotype found at baseline on both accuracy (Fig. 5A) and the total number of trials (data not shown). The SD challenge during the exposure to CPF, however, led to a decline in perseverative responses [$F_{1, 19}$ = 6.172, p = 0.024] (Fig. 5D).

Wash-out: As at baseline, a significant decrease in accuracy [SD: $F_{1, 19}$ = 11.211, p = 0.004] (Fig. 5A) and an increase of omissions [SD: $F_{1, 19}$ = 8.354, p = 0.010] (Fig. 5B) were again found when the SD was reduced from 1- to 0.5-s. The genotype significantly influenced the total number of trials performed [$F_{2, 19}$ = 6.756, p = 0.007], and tended to do so for percentage of omissions [$F_{2, 19}$ = 2.944, p = 0.080]. Overall, further *post-hoc* analyses pointed to a markedly deterioration in performance in apoE3 mice: they completed fewer trials (p = 0.005) (data not shown) and made more omissions (p = 0.041) than apoE2 mice (Fig. 5B).

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3.5 Pharmacological challenge

Figure 6 and Table 2 summarize the behavioural effects on the 5-CSRTT described for the three genotypes throughout the administration of the GABAergic agonist alprazolam.

Baseline: Alprazolam improved overall performance. Specifically, it increased the number of trials completed [day: $F_{1, 12}$ = 14.980, p = 0.003] (Table 2), decreased omissions [drug: $F_{1, 12}$ = 68.273, p < 0.001] (Fig. 6B) and decreased both correct [drug: $F_{1, 12}$ = 3.876, p = 0.077] and reward latencies [drug: $F_{1, 12}$ = 6.280, p = 0.031] (Table 2). However, it increased premature

responding [drug: $F_{1, 12} = 13.427$, $p = 0.004$] (Fig. 5C). The genotype was also observed to have an effect on perseverative responding [$F_{2, 12} = 6.436$, $p = 0.016$]: apoE4 mice persevered more than apoE2 ($p = 0.038$) and apoE3 ($p = 0.020$) mice, as already observed under standard conditions at baseline (Fig. 6D, 2D). However, no interaction genotype x alprazolam was observed.

CPF exposure: During the treatment period, alprazolam-associated improvements were more discreet. Although omissions continued to decrease [drug: $F_{1, 19} = 6.486$, $p = 0.021$] (Fig. 6B), no effect was observed on either correct or reward latencies, or on the total number of trials (Table 2). Alprazolam continued to increase premature responding in CPF-exposed mice [drug: $F_{1, 19} = 9.668$, $p = 0.006$]. As above noted, no interaction genotype x alprazolam was observed.

-- Insert Figure 6 over here –

3.6 ChE activity

During the course of CPF exposure, we noticed no apparent signs of cholinergic toxicity in any group. Relative to controls, plasma ChE activity of CPF-exposed animals dropped to 22.06%. On the other hand, ChE activity in brain homogenates assessed immediately after the 4-week exposure to CPF was decreased to 76.54% of controls, and was totally recovered after the incorporation of the 4-week wash-out period. No differences between genotypes were observed in brain ChE activities.

4. Discussion

The aim of the current study was primarily to characterize the immediate and delayed effects caused by the exposure to CPF on attention, inhibitory control and motivation in pre-trained human apoE TR adult female mice. Although all the individuals eventually acquired the task, ε*2* carriers learned it more efficiently than their peers. The increases in premature and perseverative

responses found in the baseline performance analysis were genotype-dependent, pointing to deficient inhibitory control in apoE4 mice. Strikingly, this impulsive- and compulsive-like trait was no longer found during the CPF treatment, suggesting a specific interaction between the ChE inhibitor agent and the *APOE4* genotype. Overall, the 4-week dietary administration of CPF, devoid of signs of cholinergic toxicity, gradually compromised attentional accuracy and motivation, while it reduced premature responding. These effects persisted over time, as they were mostly maintained throughout the 4-week wash-out period even after brain ChE activities were totally recovered. Furthermore, and contrary to expectations, apoE3 mice showed attentional disruptions due to motivational factors one month after CPF exposure. The pharmacological challenges with the GABAergic agonist alprazolam, covering both baseline and CPF exposure periods, improved overall performance and generally increased impulsivity. However, interactions with the *APOE* genotype were not observed.

In this study, all the mice were able to cope with the 5-CSRTT. Moreover, as in previous investigations (Reverte et al., 2016; Siegel et al., 2010), there were no distinguishable differences in accuracy between genotypes after they reached the required level. Notwithstanding, throughout the acquisition process the rates of learning were different, with the *APOE2* genotype being the most gifted learner. These results match those recently reported by Reverte et al. (2016), and seem to further support the idea of an enhanced learning process among apoE2 female mice in the 5-CSRTT. Nevertheless, acquisition in apoE2 male mice was also faster than in the two other human apoE TR groups in a Barnes maze spatial task (Peris-Sampedro et al., 2015a). Thus, *ε2* carriers were the most frequent users of direct or serial pathways to the target hole, which implied they made a reflective choice (Peris-Sampedro et al., 2015a). In addition, apoE2 female mice exhibited the lowest reward latencies during the baseline period in the 5-CSRTT, indicating increased motivation. In a recent study carried out in our laboratory (Reverte et al., 2012), apoE2 female mice showed sustained exploratory behaviour in an open-field task. Despite the paucity of epidemiological data, the aforementioned results suggest an advantageous cognitive outcome of the *APOE2* genotype. Learning to associate specific actions with rewards and being able to remember them are higher-order

executive functions mostly dependent on prefrontal cortex (PFC) areas (Puig et al., 2014), which are highly innervated by dopaminergic neurons. Indeed, dopamine (DA) is involved in motor and reward systems and it contributes to adaptive behaviours, such as attention, learning and motivation (Nieoullon, 2002; Tye et al., 2012). It has been further shown that its depletion from PFC triggers poor attentional outcome and impulsivity (Puig et al., 2014; Puumala and Sirviö, 1998). Recently, Reverte et al. (2016) found that DA levels in the frontal cortex were higher in apoE2 than in apoE4 mice. Hence, increased basal levels of DA in certain brain areas intrinsic to the *APOE2* genotype may account for its privileged cognitive condition.

In agreement with our recent study (Reverte et al., 2016), the *APOE* genotype strongly influenced the baseline period in the 5-CSRTT. ApoE4 mice displayed impaired inhibitory control, manifested by higher levels of premature and perseverative responses, and also a decrease in accuracy when attention was challenged. In recent years, and despite some controversy, the hypothesis that a cholinergic dysfunction explains some of the cognitive deficits associated with the *APOE4* genotype has gained strength. In relation to this, higher levels of AChE (Eggers et al., 2006), a greater number of muscarinic receptors (mAChRs) (Cohen et al., 2003) and a reduced activity of cholinergic neurons (Salehi et al., 1998) have been considered as potential contributors to their cognitive failure. The current results suggest that CPF was able to match the attentional ability of the three genotypes, thereby mildly improving the deteriorated attentional condition of the *APOE4* genotype. Besides, existing research recognizes the critical role played by the cholinergic system in impulsivity (Cardona et al., 2006; Middlemore-Risher et al., 2010; Montes de Oca et al., 2013), but the underlying mechanisms are not yet fully understood. In the present investigation, apparently unprecedented, we found that CPF was surprisingly able to restore the impaired inhibitory control inherent to apoE4 mice. Similarly, being carrier of the *ε4* allele has been shown to condition the response to other cholinergic agents. Thus, ChE inhibitors used in the treatment of AD seem to be less effective in *APOE4* patients (Braga et al., 2014), and CPF itself impaired memory in apoE3 but not in apoE4 mice (Peris-Sampedro et al., 2015a). In our recent study (Reverte et al., 2016), apoE4 mice were the least affected by scopolamine - a muscarinic

antagonist -, while *ε3* carriers showed increased premature responding upon its administration. Recently, Potter et al., (2012) pointed out that the inhibitory behaviour of highly impulsive human subjects was improved by the interaction of nicotine with nAChRs. Interestingly, carriers of the apoE4 isoform benefit more from the improving effect of nicotine on cognition (Evans et al., 2013). Based on the above, it could be hypothesized that CPF improve the poor inhibitory control of apoE4 mice upon interacting with nAChRs.

The anxiogenic character of the *APOE4* genotype has extensively been addressed (Reverte et al., 2014; Siegel et al., 2012), and may partly explain its impulsive condition in the 5-CSRTT (Loos et al., 2009). Several studies have used GABAergic pharmacological approaches to test the hypothesis that impulsivity is associated with anxiety (Sanchez-Roige et al., 2012). However and just as previously reported (Reverte et al., 2016; Stonnington et al., 2009), *ε4* carriers did not show higher response to benzodiazepines (e.g., alprazolam and lorazepam).

A review of the literature shows that the 5-CSRTT is a useful tool for assessing not only attentional and motivational processes but also inhibitory behaviours (Robbins, 2002; Sanchez-Roige et al., 2012). A considerable amount of epidemiological data has confirmed that CPF contributes to boosting neuropsychological and psychiatric impairments (Mackenzie Ross et al., 2010; Roldán-Tapia et al., 2005). However, experimental studies on attention, motivation and inhibitory control using the 5-CSRTT are scant and rather conflicting. As for attention, while Montes de Oca et al. (2013) found no variations seven months after a single dose of 250mg/kg CPF, Middlemore-Risher and co-workers (2010) revealed deficits during and after prolonged treatment with CPF. In the present investigation, repeated dietary exposure to CPF produced protracted attentional disturbances in apoE TR female mice that had stable baseline performance, as revealed by increased number of omissions and deteriorated processing speed. Despite differences in experimental protocols, our results partially agree with those reported by Middlemore-Risher et al. (2010) who found decreased accuracy and increased omissions throughout both 14-day and 30-day every other day exposures to 18mg/kg CPF. In line with our results, the effects they observed were maintained over a 30-day period of detoxification. The mechanisms by which CPF exerts its detrimental effect on attention may be multifactorial. For

instance, several studies have attributed these effects to the ability of CPF to inhibit AChE (Middlemore-Risher et al., 2010; Samsam et al., 2005), but they also emphasized a possible role of nAChRs (Hoyle et al., 2006; Middlemore-Risher et al., 2010). With regard to anticipatory behaviour, an array of research has demonstrated that CPF increases premature responding (Cardona et al., 2011, 2006; López-Granero et al., 2013; Middlemore-Risher et al., 2010). The current findings though, suggest that it has the opposite effect: the exposure to CPF gradually reduced premature responses in apoE TR female mice. It is worth noting that basal premature responses were notably low in these individuals. Accordingly, the level of premature responding in mice has been suggested to be lower than that seen commonly in rats (Humby et al., 1999). Nonetheless, the reasons for these discrepancies are not clear and deserve further investigation. One explanation may be the substantial differences between protocols. Indeed, all these studies were performed using male rats, while we used female mice. In addition, all but one (López-Granero et al., 2013) are based on single high (Cardona et al., 2011, 2006) or repeated relatively high (Middlemore-Risher et al., 2010) CPF doses. Furthermore, there are some differences between our dosing schedule and the others: CPF-related effects on the impulsive behaviour of the mice were evaluated once they had reached a stable baseline performance and they were followed up in the 5-CSRTT during exposure.

To date, little research has attempted to clarify the role of OPs in inducing motivational deficits. Epidemiological data on the subject reported emotional impairments (i.e., anxiety, depression, and irritability) as a consequence of occupational exposure to OPs (Bazylewicz-Walczak et al., 1999; Mackenzie Ross et al., 2010). In the present study, although food was restricted and a liquid reinforcer was used to prevent mice from becoming satiated, the reduction in the number of trials completed, as well as the increase in reward latencies here found indicate a loss of motivation in mice treated with CPF.

According to Middlemore-Risher et al. (2010), almost all the behavioural features described during the exposure to CPF in the 5-CSRTT were still apparent throughout the 4-week wash-out period. Strikingly, the *APOE3* genotype developed delayed attentional impairments, probably due to motivational factors, during the course of the detoxification. In previous studies, we

found that being carrier of the *ε3* allele increases vulnerability to developing obesity and related metabolic dysfunctions after CPF exposure (Peris-Sampedro et al., 2015a, 2015b). Furthermore, apoE TR mice that had been exposed to CPF ate more than their control counterparts (Peris-Sampedro et al., 2015a, 2015b). It is difficult to establish whether there are any links between the present and the previous results, and therefore they must be examined more closely in further investigations.

In recent years, there has been renewed interest in ascertaining the role of GABA in impulsivity (Hayes et al., 2014). Pharmacological interventions that target GABA receptors (e.g., benzodiazepines) have been shown to increase impulsive behaviour (Oliver et al., 2009). Moreover, alprazolam improves cognitive function in human volunteers (Bentué-Ferrer et al., 2001). The current results show that alprazolam improved the overall mice performance in the 5-CSRTT, notably by increasing the total number of trials and decreasing not only omissions but also both correct and reward latencies. Furthermore, the GABAergic agonist increased the percentage of premature responses. However, administering the drug during CPF treatment did not have any relevant effect.

In summary, the present study demonstrates that the *APOE* genotype affects attentional performance and inhibitory behaviour in the 5-CSRTT. Together with recent data (Peris-Sampedro et al., 2015a, 2015b), the current findings attest that the three apoE isoforms respond differently to a CPF challenge, highlighting the fact that genetics of the population must be taken into account in epidemiological studies. According to the current results, a fruitful area for further research should be to assess whether the three genotypes differ in terms of the brain expression and distribution of mAChRs and nAChRs. It would also be interesting to assess whether such genotype-related differences could elicit an array of therapeutic responses upon cholinergic treatment. The results of the present investigation provide some support for the conceptual premise of potential close links between the cholinergic system and the *APOE4* genotype. Overall, the results described strengthen existing evidence, and point to protracted detrimental effects on sustained attention following repeated exposure to CPF. Furthermore, this

research provides a framework for the exploration of motivational deficits that emerge after the administration of CPF, which could subsequently lead to appetite and emotional disturbances.

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References

Aldridge, J.E., Meyer, A., Seidler, F.J., Slotkin, T. a., 2005. Alterations in central nervous system serotonergic and dopaminergic synaptic activity in adulthood after prenatal or neonatal chlorpyrifos exposure. Environ. Health Perspect. 113, 1027–1031.

Bari, A., Dalley, J.W., Robbins, T.W., 2008. The application of the 5-choice serial reaction time task for the assessment of visual attentional processes and impulse control in rats. Nat. Protoc. 3, 759–67.

Bazylewicz-Walczak, B., Majczakowa, W., Szymczak, M., 1999. Behavioral effects of occupational exposure to organophosphorous pesticides in female greenhouse planting workers. Neurotoxicology 20, 819–26.

Bentué-Ferrer, D., Reymann, J.M., Tribut, O., Allain, H., Vasar, E., Bourin, M., 2001. Role of dopaminergic and serotonergic systems on behavioral stimulatory effects of low-dose alprazolam and lorazepam. Eur. Neuropsychopharmacol. 11, 41–50.

Boon, P.E., Van der Voet, H., Van Raaij, M.T.M., Van Klaveren, J.D., 2008. Cumulative risk assessment of the exposure to organophosphorus and carbamate insecticides in the Dutch diet. Food Chem. Toxicol. 46, 3090–8.

Bour, A., Grootendorst, J., Vogel, E., Kelche, C., Dodart, J.-C., Bales, K., Moreau, P.-H., Sullivan, P.M., Mathis, C., 2008. Middle-aged human apoE4 targeted-replacement mice show retention deficits on a wide range of spatial memory tasks. Behav. Brain Res. 193, 174–82.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–54.

Braga, I.L.S., Silva, P.N., Furuya, T.K., Santos, L.C., Pires, B.C., Mazzotti, D.R., Bertolucci, P.H., Cendoroglo, M.S., Smith, M.C., 2014. Effect of APOE and CHRNA7 genotypes on the cognitive response to cholinesterase inhibitor treatment at different stages of Alzheimer's disease. Am. J. Alzheimers. Dis. Other Demen. 30, 139–144.

Buck, R.J., Ozkaynak, H., Xue, J., Zartarian, V.G., Hammerstrom, K., 2001. Modeled estimates of chlorpyrifos exposure and dose for the Minnesota and Arizona NHEXAS populations. J. Expo. Anal. Environ. Epidemiol. 11, 253–68.

Cardona, D., López-Crespo, G., Sánchez-Amate, M.C., Flores, P., Sánchez-Santed, F., 2011. Impulsivity as long-term sequelae after chlorpyrifos intoxication: time course and individual differences. Neurotox. Res. 19, 128–37.

Cardona, D., López-Grancha, M., López-Crespo, G., Nieto-Escamez, F., Sánchez-Santed, F., Flores, P., 2006. Vulnerability of long-term neurotoxicity of chlorpyrifos: effect on schedule-induced polydipsia and a delay discounting task. Psychopharmacology (Berl). 189, 47–57.

Ccanccapa, A., Masiá, A., Andreu, V., Picó, Y., 2015. Spatio-temporal patterns of pesticide residues in the Turia and Júcar Rivers (Spain). Sci. Total Environ. doi:10.1016/j.scitotenv.2015.06.063

Cohen, R.M., Podruchny, T.A., Bokde, A.L.W., Carson, R.E., Herscovitch, P., Kiesewetter, D.O., Eckelman, W.C., Sunderland, T., 2003. Higher in vivo muscarinic-2 receptor distribution volumes in aging subjects with an apolipoprotein E-epsilon4 allele. Synapse 49, 150–6.

Curl, C.L., Beresford, S.A.A., Fenske, R.A., Fitzpatrick, A.L., Lu, C., Nettleton, J.A., Kaufman, J.D., 2015. Estimating pesticide exposure from dietary intake and organic food choices: the Multi-Ethnic Study of Atherosclerosis (MESA). Environ. Health Perspect. 123, 475–83.

Dalley, J.W., Lääne, K., Theobald, D.E.H., Peña, Y., Bruce, C.C., Huszar, A.C., Wojcieszek, M., Everitt, B.J., Robbins, T.W., 2007. Enduring deficits in sustained visual attention during withdrawal of intravenous methylenedioxymethamphetamine self-administration in rats: results from a comparative study with d-amphetamine and methamphetamine. Neuropsychopharmacology 32, 1195–206.

De Silva, H.J., Samarawickrema, N.A., Wickremasinghe, A.R., 2006. Toxicity due to organophosphorus compounds: what about chronic exposure? Trans. R. Soc. Trop. Med. Hyg. 100, 803–806.

Eaton, D.L., Daroff, R.B., Autrup, H., Bridges, J., Buffler, P., Costa, L.G., Coyle, J., McKhann, G., Mobley, W.C., Nadel, L., Neubert, D., Schulte-Hermann, R., Spencer, P.S., 2008. Review of the toxicology of chlorpyrifos with an emphasis on human exposure and neurodevelopment. Crit. Rev. Toxicol. 38 Suppl 2, 1–125.

Eggers, C., Herholz, K., Kalbe, E., Heiss, W.-D., 2006. Cortical acetylcholine esterase activity and ApoE4-genotype in Alzheimer disease. Neurosci. Lett. 408, 46–50.

Ellman, G.L., Courtney, K.D., Andres, V., Feather-Stone, R.M., 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 7, 88–95.

Evans, S., Dowell, N.G., Tabet, N., Tofts, P.S., King, S.L., Gray, M., Rusted, J.M., 2013. Nicotine effects on attentional reorienting in mid-age adults, and interactions with apolipoprotein E status. J. Psychopharmacol. 27, 1007–1014.

Grootendorst, J., Bour, A., Vogel, E., Kelche, C., Sullivan, P.M., Dodart, J.-C., Bales, K., Mathis, C., 2005. Human apoE targeted replacement mouse lines: h-apoE4 and h-apoE3 mice differ on spatial memory performance and avoidance behavior. Behav. Brain Res. 159, 1–14.

Hauser, P.S., Narayanaswami, V., Ryan, R.O., 2011. Apolipoprotein E: from lipid transport to neurobiology. Prog. Lipid Res. 50, 62–74.

Hayes, D.J., Jupp, B., Sawiak, S.J., Merlo, E., Caprioli, D., Dalley, J.W., 2014. Brain γ-aminobutyric acid: a neglected role in impulsivity. Eur. J. Neurosci. 39, 1921–32.

Hoyle, E., Genn, R.F., Fernandes, C., Stolerman, I.P., 2006. Impaired performance of alpha7 nicotinic receptor knockout mice in the five-choice serial reaction time task. Psychopharmacology (Berl). 189, 211–223.

Humby, T., Laird, F.M., Davies, W., Wilkinson, L.S., 1999. Visuospatial attentional functioning in mice: interactions between cholinergic manipulations and genotype. Eur. J. Neurosci. 11, 2813–23.

Loos, M., van der Sluis, S., Bochdanovits, Z., van Zutphen, I.J., Pattij, T., Stiedl, O., Smit, A.B., Spijker, S., 2009. Activity and impulsive action are controlled by different genetic and environmental factors. Genes. Brain. Behav. 8, 817–28.

López-Granero, C., Cardona, D., Giménez, E., Lozano, R., Barril, J., Aschner, M., Sánchez-Santed, F., Cañadas, F., 2014. Comparative study on short- and long-term behavioral consequences of organophosphate exposure: relationship to AChE mRNA expression. Neurotoxicology 40, 57–64.

López-Granero, C., Cardona, D., Giménez, E., Lozano, R., Barril, J., Sánchez-Santed, F., Cañadas, F., 2013. Chronic dietary exposure to chlorpyrifos causes behavioral impairments, low activity of brain membrane-bound acetylcholinesterase, and increased brain acetylcholinesterase-R mRNA. Toxicology 308, 41–9.

Lu, C., Barr, D.B., Pearson, M.A., Waller, L.A., 2008. Dietary intake and its contribution to longitudinal organophosphorus pesticide exposure in urban/suburban children. Environ. Health Perspect. 116, 537–42.

MacIntosh, D.L., Kabiru, C., Echols, S.L., Ryan, P.B., 2001. Dietary exposure to chlorpyrifos and levels of 3,5,6-trichloro-2-pyridinol in urine. J. Expo. Anal. Environ. Epidemiol. 11, 279–85.

Mackenzie Ross, S.J., Brewin, C.R., Curran, H.V., Furlong, C.E., Abraham-Smith, K.M., Harrison, V., 2010. Neuropsychological and psychiatric functioning in sheep farmers exposed to low levels of organophosphate pesticides. Neurotoxicol. Teratol. 32, 452–9.

Melnyk, L.J., Byron, M.Z., Brown, G.G., Clayton, C.A., Michael, L.C., 2011. Pesticides on household surfaces may influence dietary intake of children. Environ. Sci. Technol. 45, 4594–601.

Middlemore-Risher, M.L., Buccafusco, J.J., Terry, A. V, 2010. Repeated exposures to low-level chlorpyrifos results in impairments in sustained attention and increased impulsivity in rats. Neurotoxicol. Teratol. 32, 415–24.

Miyaki, K., Nishiwaki, Y., Maekawa, K., Ogawa, Y., Asukai, N., Yoshimura, K., Etoh, N., Matsumoto, Y., Kikuchi, Y., Kumagai, N., Omae, K., 2005. Effects of sarin on the nervous system of subway workers seven years after the Tokyo subway sarin attack. J. Occup. Health 47, 299–304.

Montes de Oca, L., Moreno, M., Cardona, D., Campa, L., Suñol, C., Galofré, M., Flores, P., Sánchez-Santed, F., 2013. Long term compulsivity on the 5-choice serial reaction time task after acute Chlorpyrifos exposure. Toxicol. Lett. 216, 73–85.

Nieoullon, A., 2002. Dopamine and the regulation of cognition and attention. Prog. Neurobiol. 67, 53–83.

Oliver, Y.P., Ripley, T.L., Stephens, D.N., 2009. Ethanol effects on impulsivity in two mouse strains: similarities to diazepam and ketamine. Psychopharmacology (Berl). 204, 679–92.

Page, D., Miotliński, K., Gonzalez, D., Barry, K., Dillon, P., Gallen, C., 2014. Environmental monitoring of selected pesticides and organic chemicals in urban stormwater recycling systems using passive sampling techniques. J. Contam. Hydrol. 158, 65–77.

Peris-Sampedro, F., Basaure, P., Reverte, I., Cabré, M., Domingo, J.L., Colomina, M.T., 2015a. Chronic exposure to chlorpyrifos triggered body weight increase and memory impairment depending on human apoE polymorphisms in a targeted replacement mouse model. Physiol. Behav. 144, 37–45.

Peris-Sampedro, F., Cabré, M., Basaure, P., Reverte, I., Domingo, J.L., Teresa Colomina, M., 2015b. Adulthood dietary exposure to a common pesticide leads to an obese-like phenotype and a diabetic profile in apoE3 mice. Environ. Res. 142, 169–176.

Peris-Sampedro, F., Salazar, J.G., Cabré, M., Reverte, I., Domingo, J.L., Sánchez-Santed, F., Colomina, M.T., 2014. Impaired retention in AβPP Swedish mice six months after oral exposure to chlorpyrifos. Food Chem. Toxicol. 72, 289–94.

Potter, A.S., Bucci, D.J., Newhouse, P.A., 2012. Manipulation of nicotinic acetylcholine receptors differentially affects behavioral inhibition in human subjects with and without disordered baseline impulsivity. Psychopharmacology (Berl). 220, 331–40.

Puig, M. V, Antzoulatos, E.G., Miller, E.K., 2014. Prefrontal dopamine in associative learning and memory. Neuroscience 282C, 217–229.

Puumala, T., Sirviö, J., 1998. Changes in activities of dopamine and serotonin systems in the frontal cortex underlie poor choice accuracy and impulsivity of rats in an attention task. Neuroscience 83, 489–99.

Quijano, L., Yusà, V., Font, G., Pardo, O., 2016. Chronic cumulative risk assessment of the exposure to organophosphorus, carbamate and pyrethroid and pyrethrin pesticides through fruit and vegetables consumption in the region of Valencia (Spain). Food Chem. Toxicol. doi:10.1016/j.fct.2016.01.004

Raber, J., Huang, Y., Ashford, J.W., 2004. ApoE genotype accounts for the vast majority of AD risk and AD pathology. Neurobiol. Aging 25, 641–50.

Reverte, I., Klein, A.B., Domingo, J.L., Colomina, M.T., 2013. Long term effects of murine postnatal exposure to decabromodiphenyl ether (BDE-209) on learning and memory are dependent upon APOE polymorphism and age. Neurotoxicol. Teratol. 40, 17–27.

Reverte, I., Klein, A.B., Ratner, C., Domingo, J.L., Colomina, M.T., 2012. Behavioral phenotype and BDNF differences related to apoE isoforms and sex in young transgenic mice. Exp. Neurol. 237, 116–25.

Reverte, I., Peris-Sampedro, F., Basaure, P., Campa, L., Suñol, C., Moreno, M., Domingo, J.L., Colomina, M.T., 2016. Attentional performance, impulsivity, and related neurotransmitter systems in apoE2, apoE3, and apoE4 female transgenic mice. Psychopharmacology (Berl). 233, 295-308.

Reverte, I., Pujol, A., Domingo, J.L., Colomina, M.T., 2014. Thyroid hormones and fear learning but not anxiety are affected in adult apoE transgenic mice exposed postnatally to decabromodiphenyl ether (BDE-209). Physiol. Behav. 133, 81–91.

Robbins, T.W., 2002. The 5-choice serial reaction time task: behavioural pharmacology and functional neurochemistry. Psychopharmacology (Berl). 163, 362–80.

Roldán-Tapia, L., Parrón, T., Sánchez-Santed, F., 2005. Neuropsychological effects of long-term exposure to organophosphate pesticides. Neurotoxicol. Teratol. 27, 259–66.

Salazar, J.G., Ribes, D., Cabré, M., Domingo, J.L., Sanchez-Santed, F., Colomina, M.T., 2011. Amyloid β peptide levels increase in brain of AβPP Swedish mice after exposure to chlorpyrifos. Curr. Alzheimer Res. 8, 732–40.

Salehi, A., Dubelaar, E.J., Mulder, M., Swaab, D.F., 1998. Aggravated decrease in the activity of nucleus basalis neurons in Alzheimer's disease is apolipoprotein E-type dependent. Proc. Natl. Acad. Sci. U. S. A. 95, 11445–9.

Samsam, T.E., Hunter, D.L., Bushnell, P.J., 2005. Effects of chronic dietary and repeated acute exposure to chlorpyrifos on learning and sustained attention in rats. Toxicol. Sci. 87, 460–8.

Sanchez-Roige, S., Peña-Oliver, Y., Stephens, D.N., 2012. Measuring impulsivity in mice: the five-choice serial reaction time task. Psychopharmacology (Berl). 219, 253–70.

Saunders, M., Magnanti, B.L., Correia Carreira, S., Yang, A., Alamo-Hernández, U., Riojas-Rodriguez, H., Calamandrei, G., Koppe, J.G., Krayer von Krauss, M., Keune, H., Bartonova, A., 2012. Chlorpyrifos and neurodevelopmental effects: a literature review and expert elicitation on research and policy. Environ. Heal. 11 Suppl 1, S5.

Schag, K., Schönleber, J., Teufel, M., Zipfel, S., Giel, K.E., 2013. Food-related impulsivity in obesity and Binge Eating Disorder - a systematic review. Obes. Rev. 14, 477–495.

Siegel, J.A., Craytor, M.J., Raber, J., 2010. Long-term effects of methamphetamine exposure on cognitive function and muscarinic acetylcholine receptor levels in mice. Behav. Pharmacol. 21, 602–614.

Siegel, J.A., Haley, G.E., Raber, J., 2012. Apolipoprotein E isoform-dependent effects on anxiety and cognition in female TR mice. Neurobiol. Aging 33, 345–58.

Smith, D.G., Robbins, T.W., 2013. The neurobiological underpinnings of obesity and binge eating: a rationale for adopting the food addiction model. Biol. Psychiatry 73, 804–10.

Stonnington, C.M., Snyder, P.J., Hentz, J.G., Reiman, E.M., Caselli, R.J., 2009. Double-blind crossover study of the cognitive effects of lorazepam in healthy apolipoprotein E (APOE)-epsilon4 carriers. J. Clin. Psychiatry 70, 1379–84.

Stout, D.M., Bradham, K.D., Egeghy, P.P., Jones, P.A., Croghan, C.W., Ashley, P.A., Pinzer, E., Friedman, W., Brinkman, M.C., Nishioka, M.G., Cox, D.C., 2009. American Healthy Homes Survey: a national study of residential pesticides measured from floor wipes. Environ. Sci. Technol. 43, 4294–300.

Sullivan, P.M., Mezdour, H., Aratani, Y., Knouff, C., Najib, J., Reddick, R.L., Quarfordt, S.H., Maeda, N., 1997. Targeted replacement of the mouse apolipoprotein E gene with the common human APOE3 allele enhances diet-induced hypercholesterolemia and atherosclerosis. J. Biol. Chem. 272, 17972–80.

Tye, K.M., Mirzabekov, J.J., Warden, M.R., Ferenczi, E.A., Tsai, H.-C., Finkelstein, J., Kim, S.-Y., Adhikari, A., Thompson, K.R., Andalman, A.S., Gunaydin, L.A., Witten, I.B., Deisseroth, K., 2012. Dopamine neurons modulate neural encoding and expression of depression-related behaviour. Nature 493, 537–541.

Yun, S.H., Park, K.A., Sullivan, P., Pasternak, J.F., Ladu, M.J., Trommer, B.L., 2005. Blockade of nicotinic acetylcholine receptors suppresses hippocampal long-term potentiation in wild-type but not ApoE4 targeted replacement mice. J. Neurosci. Res. 82, 771–7.

Figure captions

Figure 1

Cumulative representation of the total number of sessions required to successfully learn the 5-CSRTT throughout the 10 acquisition stages for each *APOE* genotype. Symbols indicate: apoE2 differs from apoE3 mice (*), and apoE2 differs from apoE3 and apoE4 mice (&) at $p < 0.05$.

Figure 2

Baseline performance of apoE TR female mice on the 5-CSRTT under standard conditions. Percentage of accuracy (A), percentage of omissions (B), percentage of premature responses (C), and number of perseverative responses (D) are depicted. The symbol º indicates differences between apoE2 and apoE4 mice at $p < 0.05$.

Figure 3

Performance progression of apoE TR female mice on the 5-CSRTT over the three experimental periods: baseline, CPF exposure, and wash-out. Percentage of accuracy (A), percentage of omissions (B), percentage of premature responses (C), number of perseverative responses (D), total number of trials (E), and both correct (F) and reward (G) latencies are illustrated.

Figure 4

Effects of inter-trial interval (ITI) manipulation on the 5-CSRTT performance of apoE TR female mice for the three experimental periods: baseline, CPF exposure, and wash-out. Percentage of accuracy (A), percentage of omissions (B), percentage of premature responses (C), and number of perseverative responses (D) are depicted. Symbols indicate: differences between genotypes (º), and effects of the ITI increase (*) within each period at $p < 0.05$.

Figure 5

Effects of stimulus duration (SD) manipulation on the 5-CSRTT performance of apoE TR female mice for the three experimental periods: baseline, CPF exposure, and wash-out. Percentage of accuracy (A), percentage of omissions (B), percentage of premature responses (C), and number of perseverative responses (D) are depicted. Symbols indicate: differences between genotypes (º), and effects of the SD reduction (*) within each period at $p < 0.05$.

Figure 6

Effects of alprazolam administration on the 5-CSRTT performance of apoE TR female mice for the two periods considered: baseline and CPF exposure. Percentage of accuracy (A), percentage of omissions (B), percentage of premature responses (C), and number of perseverative responses (D) are illustrated. Symbols indicate: differences between genotypes (º), and effects of the GABAergic agonist (*) within each period at $p < 0.05$.

Table 1. Total mean number of trials and both correct and reward mean latencies in apoE TR female mice on the 5-CSRTT throughout the baseline period

	apoE2	apoE3	apoE4
total number of trials	67.47 ± 1.11	65.10 ± 1.62	66.13 ± 0.66
correct latency (s)	0.88 ± 0.02	0.89 ± 0.05	0.91 ± 0.05
reward latency (s)	1.24 ± 0.05	1.28 ± 0.07	1.45 ± 0.07†

† $p = 0.054$, indicating a tendency *vs.* the apoE2 group in reward latency.

Table 2. Motivational status and processing speed during behavioural and pharmacological challenges on the 5-CSRTT for the three experimental periods

		total number of trials			correct latency (s)			reward latency (s)		
		baseline	exposure	wash-out	baseline	exposure	wash-out	baseline	exposure	wash-out
ITI (s)										
	5	-	-	-	0.90 ± 0.03	**1.02 ± 0.06**	**1.04 ± 0.04**	1.37 ± 0.06	1.53 ± 0.10	1.65 ± 0.12
	7	-	-	-	0.85 ± 0.03†	**0.91 ± 0.04***	**0.91 ± 0.04***	1.32 ± 0.05	1.53 ± 0.12	1.50 ± 0.07
SD (s)										
	1	67.6 ± 0.8	55.3 ± 3.1	65.1 ± 1.5	**0.93 ± 0.03**	1.12 ± 0.13	1.03 ± 0.09	1.33 ± 0.04	1.58 ± 0.11	1.56 ± 0.11
	0.5	66.3 ± 1.2	51.4 ± 2.5	62.8 ± 2.3	**0.82 ± 0.04***	0.91 ± 0.05	1.08 ± 0.11	1.36 ± 0.05	1.40 ± 0.05	1.51 ± 0.06
APZ (mg/kg)										
	sal	**64.3 ± 1.9**	55.3 ± 3.1	-	1.09 ± 0.05	1.12 ± 0.13	-	**1.45 ± 0.07**	1.58 ± 0.11	-
	0.12	**68.2 ± 0.8***	60.5 ± 3.6	-	0.98 ± 0.04‡	0.98 ± 0.05	-	**1.30 ± 0.06***	1.50 ± 0.13	-

Statistically different changes (* $p < 0.05$) or tendencies († $p = 0.054$, ‡ $p = 0.077$) within the same exposure condition (i.e., baseline, exposure, wash-out) for ITI challenges (5 *vs.* 7s), SD challenges (1 *vs.* 0.5s) and alprazolam (APZ) challenges (saline *vs.* 0.12mg/kg).

Figure 1

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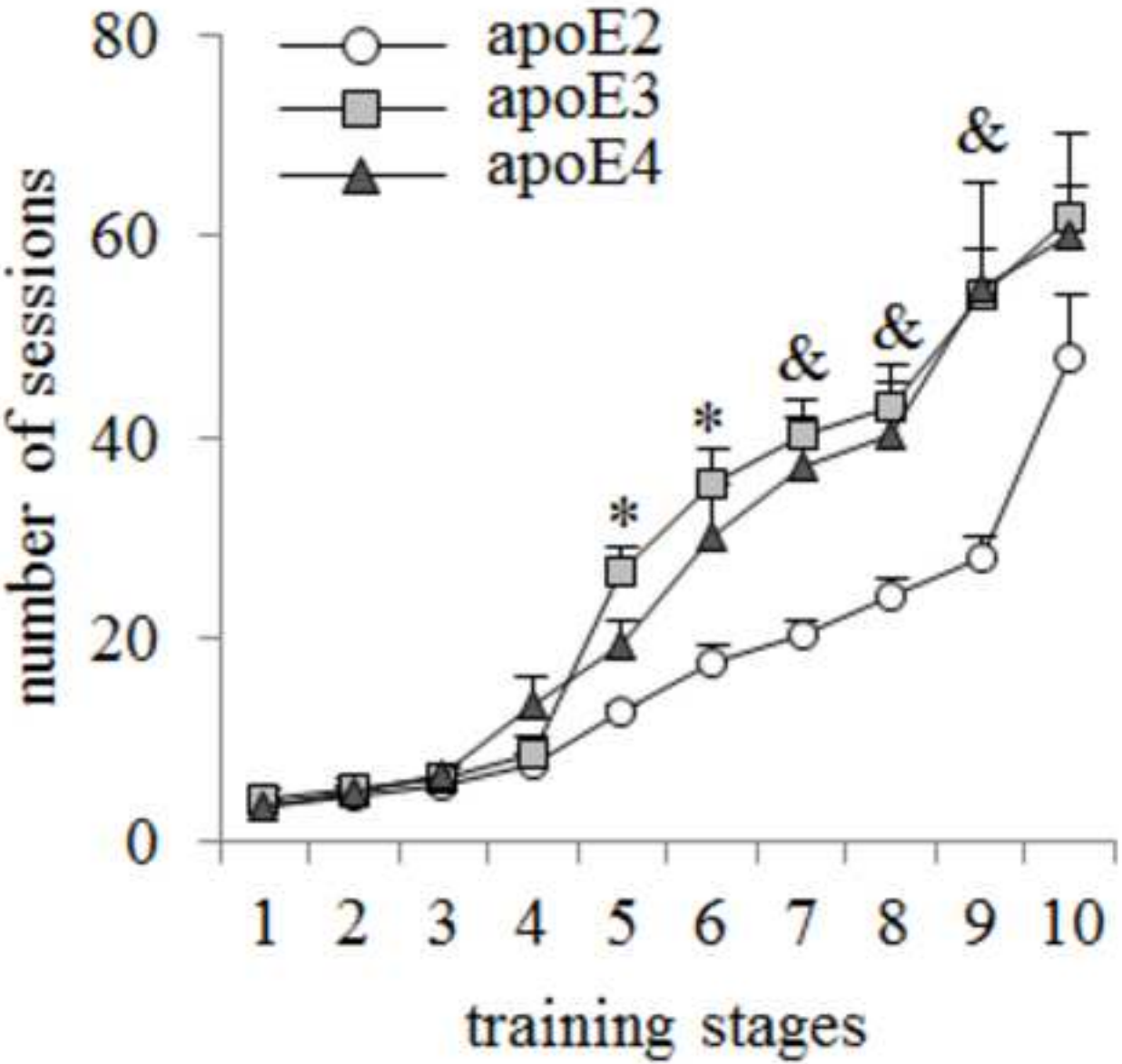

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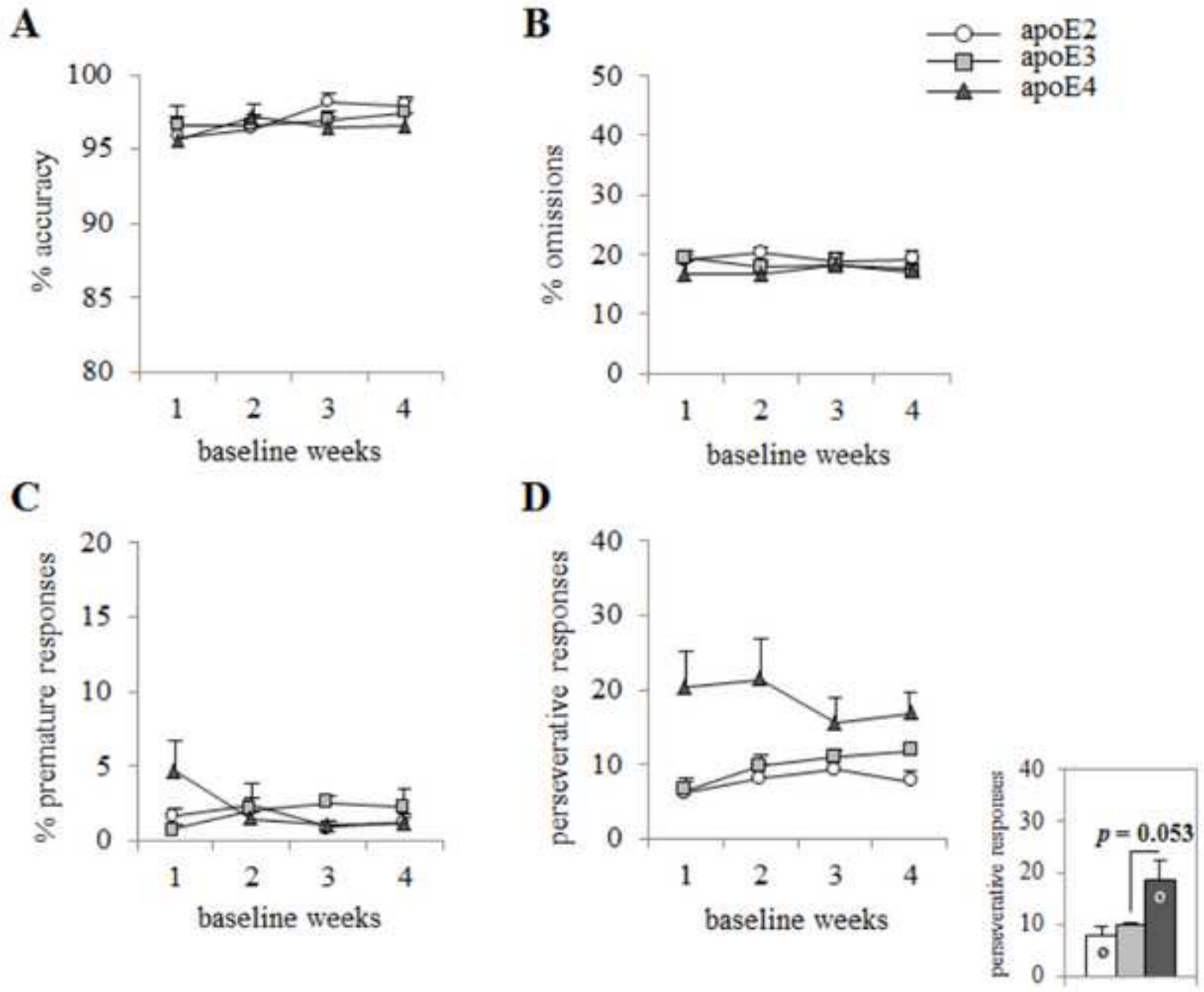

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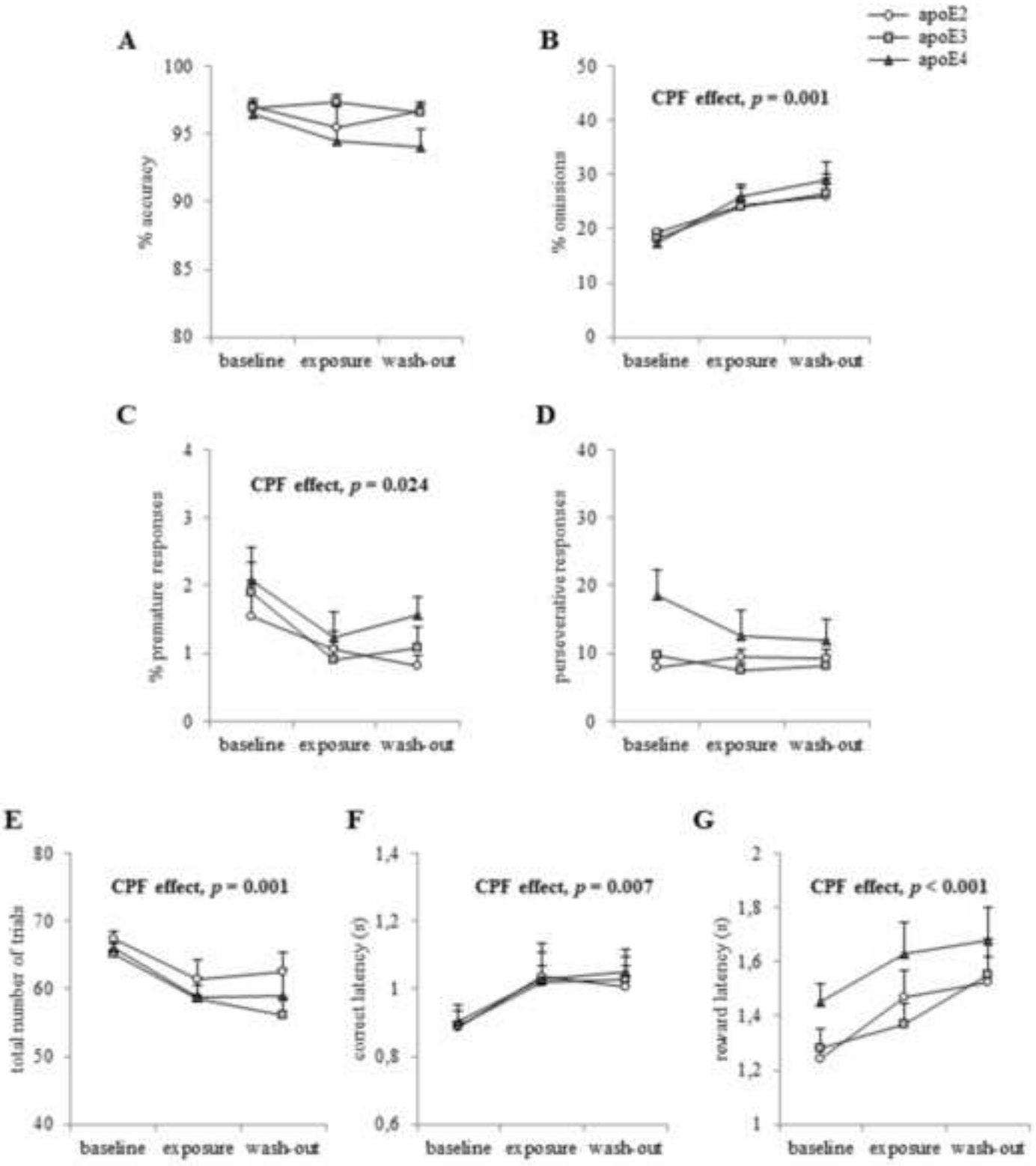

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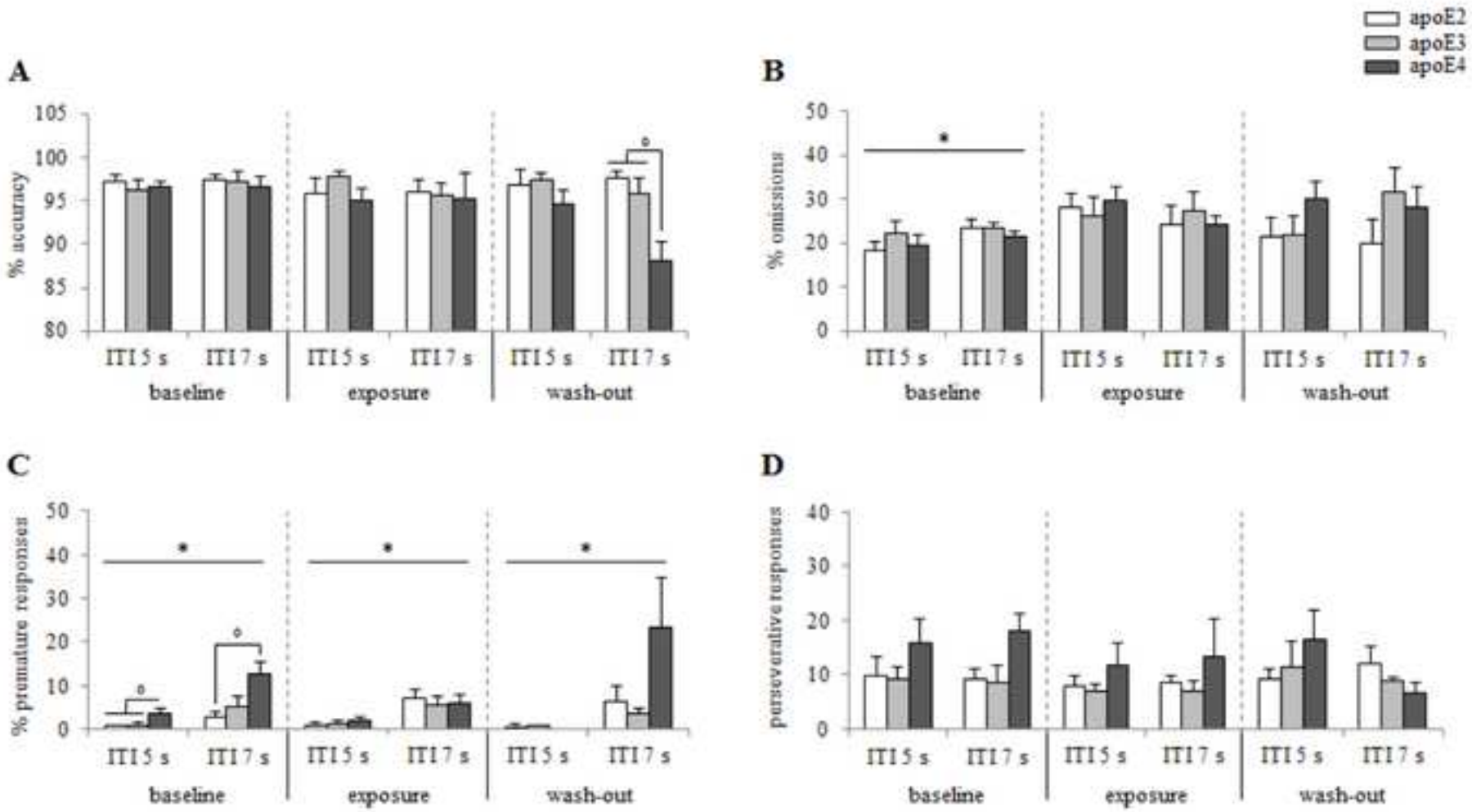

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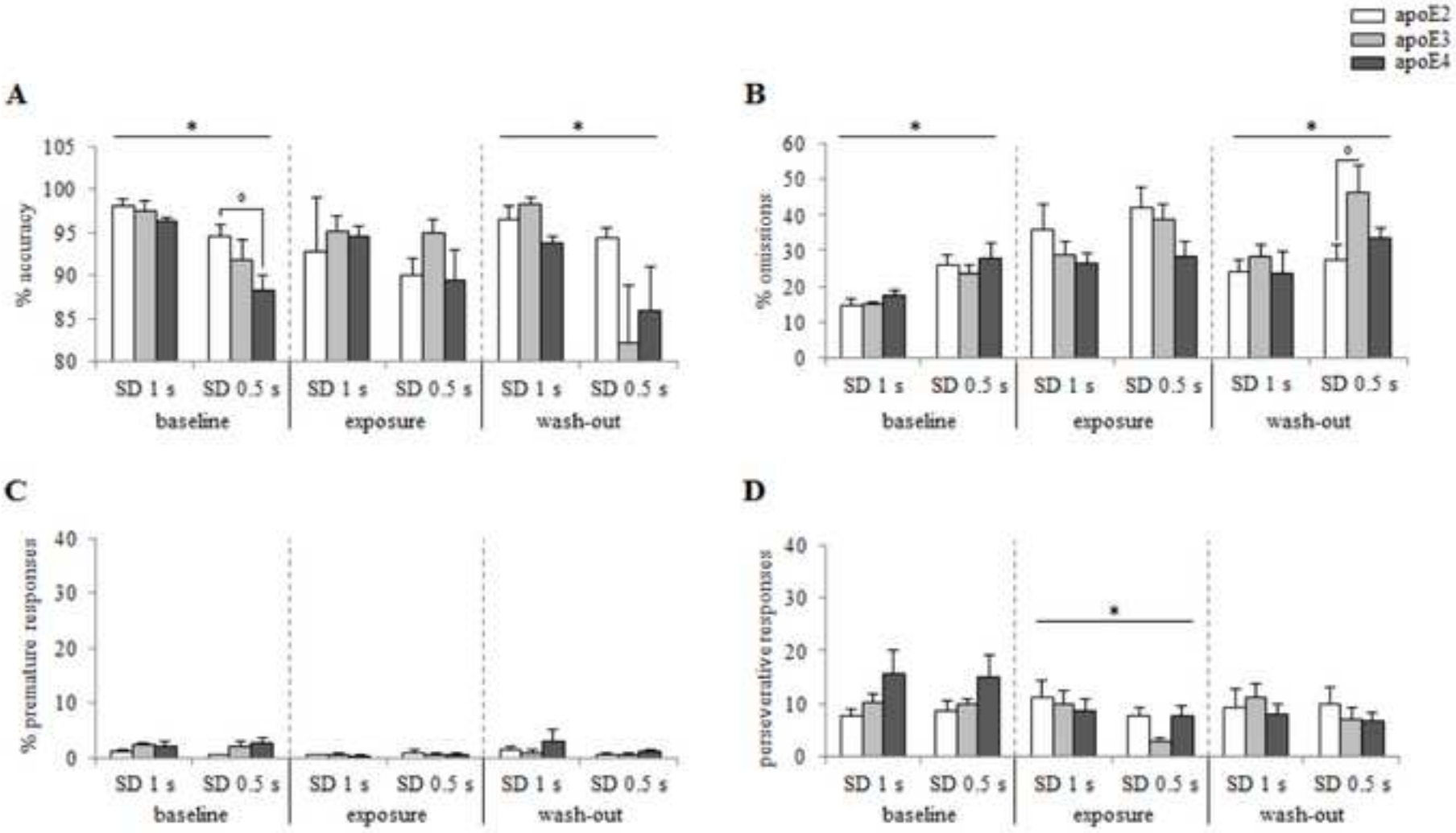

Figure 6

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