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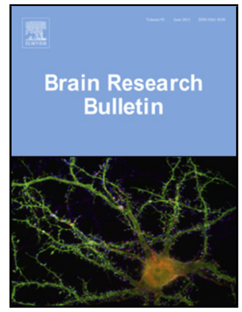

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APOE genetic background and sex confer different vulnerabilities to postnatal chlorpyrifos exposure and modulate the response to cholinergic drugs

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Highlights:

Postnatal exposure to CPF impairs recognition memory in apoE4 mice

Recognition memory is impaired in apoE4 females treated with rivastigmine

ApoE4 males exposed to CPF are improved in recognition memory by rivastigmine

The GABAergic agonist alprazolam decreases recognition memory in apoE4 females

ApoE4 mice show sex-dependent sensitivities to cholinergic and GABAergic drugs

Abstract

Chlorpyrifos (CPF) is an extensively used organophosphate pesticide. Exposure to CPF has been related to neurobehavioral disorders, particularly during neurodevelopment. Apolipoprotein E (apoE) is a lipid and cholesterol carrier and a susceptibility factor for cognitive impairment which can influence the response to toxic exposures. The study was aimed at assessing the effects of postnatal exposure to CPF on object recognition memory and its modulation by sex and apoE genotype. Human apoE3 and apoE4 targeted replacement mice and C57BL/6 mice were postnatally exposed to 0 or 1 mg/kg/day of CPF. Recognition memory was evaluated in an Object Recognition Test (ORT). In order to study the contribution of cholinergic and GABAergic neurotransmitter systems to recognition memory, a pharmacological challenge was included. Sex, genotype and postnatal exposure to CPF were key factors throughout the testing period. Specifically, CPF increased exploratory behavior and impaired discrimination performance. We observed that administering scopolamine, a cholinergic antagonist, was detrimental to recognition memory. However, discrimination in C57BL/6 and apoE4 males improved with the administration of the cholinergic agonist rivastigmine, but the same drug worsened retention in apoE4 females. Finally, the GABAergic agonist alprazolam altered performance in a sex- and genotype-dependent manner. Overall, these results suggest complex interactions between sex, *APOE* genotype and postnatal CPF exposure and indicate a different functioning of both the cholinergic and GABAergic neurotransmitter system between groups.

Abbreviations:

CPF, chlorpyrifos; ChE, cholinesterase; AChE, acetylcholinesterase; BChE, butyrylcholinesterase; PND, postnatal day; nAChR, nicotinic receptors; mAChR, muscarinic receptors; apoE, apolipoprotein E; AD, Alzheimer’s Disease; TR, targeted replacement; ORT, Object Recognition Test; GABA, γ-Aminobutyric acid; ANOVA, analysis of variance; MWM, Morris water maze; 5-CSRTT, five-choice serial reaction time task

Keywords:

Chlorpyrifos, Pesticide, APOE, Cholinergic system, Recognition memory, Brain development

1. Introduction

The impact of environmental toxic exposure during brain development has enormous consequences for the general population. As far as subclinical behavioral or cognitive deficits caused by toxic exposure are elusive in diagnoses, most of the neurotoxic agents affecting the brain lack specific environmental regulations. This is the case of the worldwide used pesticide chlorpyrifos (CPF), which targets the cholinergic neurotransmitter system. Despite the fact that its residential use was banned in 2001, CPF is still one of the most widespread compounds. The general population is permanently exposed to nontoxic low levels of the pesticide through the diet [1], being known that young subjects are more sensitive than adults to CPF toxicity [2,3]. The main detrimental effects of CPF are caused by the irreversible inhibition of cholinesterase enzymes (ChE) such as acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) [4]. To date, a number of studies have reported changes in the cholinergic system after exposure to CPF. For instance, Slotkin et al. [5] found a decreased binding in both α7 and α4β2 nicotinic receptors (nAChRs) in different brain regions of rats including the forebrain and cerebellum, following postnatal (postnatal days (PND) 11-14) exposure to the pesticide. In the same line, Richardson and Chambers (2005) observed a considerable dose-dependent decrease in muscarinic receptors (mAChRs) binding in the brain after postnatal (PND 1-21) exposure to CPF. The observed effects, which cover a wide spectrum, seem to be dependent on the sex and the period of exposure. For example, postnatal exposure to CPF has been related to increased aggressive behavior in adult males and an enhanced maternal response in females, while anxiety-like behaviors have been reduced in both sexes [7]. Likewise, postnatal exposure to CPF is known to induce changes in locomotor activity [8,9], as well as thyroid alterations [10].

To date, a number of studies have focused on the gene of apolipoprotein E (apoE) as another potential factor underlying cholinergic alterations. The apoE protein is implicated in lipid and cholesterol transport in the brain [11]. Three different isoforms have been described in humans: apoE2, apoE3 and apoE4. E4 is the isoform related to diminished functioning of the cholinergic system [12], being the major genetic risk factor for the development of Alzheimer's Disease (AD) [13,14]. Previous results from our group, using the targeted replacement (TR) mouse model that expresses the different human isoforms of apoE (apoE-TR), showed a wide variety of functional differences between genotypes as well as different vulnerabilities to the detrimental effects of CPF exposure. There are differences between genotypes, for example, in terms of spatial learning and memory [15], attention, impulsivity and compulsivity [16,17], and these influence the effects of CPF during adulthood [18] or throughout the developmental period [19]. Likewise, differences in the gene expression of cholinergic elements were observed between genotypes after postnatal exposure to the pesticide, together with developmental alterations [20].

Behavioral testing has played a crucial role in evaluating the effects of toxic exposures and in unraveling their interactions with environmental factors. The Object Recognition Test (ORT) is a behavioral task used to study recognition memory, which is based on the innate preference of rodents for novelty (i.e., to explore a new object over a familiar one). This innate novelty preference is ultimately used to test recognition memory because mice preferring a novel object would need to successfully recognize a familiar one [21]. In mice, the brain structures likely to be implicated in object recognition memory are the hippocampus and rhinal cortices. More specifically, the perirhinal cortex is a crucial structure in the acquisition, consolidation and retrieval of information [22–24]. The use of ORT has increased in recent years because of advantages over other well-established tasks: it does not require water or food deprivation, reinforcing stimuli or the learning of associations, being suitable for pharmacological screening [25,26]. The integrity of the cholinergic system appeared to be determinant for correct recognition memory. The use of the cholinergic antagonist scopolamine has become a widely established pharmacological model for cognitive impairments on object recognition [27,28]. On the other hand, the use of nAChR agonists and AChE inhibitors [29,30] has been associated with an improvement in the performance of the task. The GABAergic system has also been reported to play a role in recognition memory as the administration of γ-Aminobutyric acid (GABA) induced long-term improvements in recognition memory in rats [31]. Amnesic effects and subsequent impairments have also been described in recognition memory after administration of an allosteric modulator of GABAA receptor such as alprazolam [32,33].

The purpose of this investigation was to assess the effects of postnatal exposure to CPF on recognition memory and to investigate how individual intrinsic variables including sex and *APOE* genotype influence its toxicity. We also aimed to shed light on the neuropharmacological basis of these effects. To explore the role of both cholinergic and GABAergic systems on recognition memory and their interactions with the *APOE* genotype and CPF, a pharmacological challenge with agonist and antagonist drugs was used.

2. Materials and Methods

2.1. Animals and care

In the present study, we used male and female apoE3- and apoE4-TR mice from Taconic (Taconic Europe, Lille Skensved, Denmark) and C57BL/6 mice from Charles River (Charles River, Barcelona, Spain). The apoE-TR mouse model was originally created by Sullivan et al. [34]. These mice have a C57BL/6NTac background and their murine *apoE* gene was replaced by the human allele *APOE*. Females and males from the same genotype were subjected to mating sessions up to pregnancy. The day at birth was recorded as PND 0 and litters were left undisturbed until treatment. After weaning, animals were housed under standard conditions with

2-5 individuals per cage of the same genotype, sex and treatment group. Behavioral testing was conducted when mice were two months old. The animal room was maintained at a temperature of 22 ± 2 °C, a relative humidity of 50 ± 10%, and a 12 h light/dark automatic light cycle (light on 8:00-20:00). All animals were allowed free access to food and water and given a normal chow diet (Panlab, Barcelona, Spain). The use of animals and the experimental protocol were approved by the Animal Care and Use Committee of the Rovira i Virgili University (Tarragona, Spain) and were conducted in accordance with the Spanish Royal Decree 53/2013 on the protection of experimental animals, and the European Communities Council Directive (2010/63/EU).

2.2. Chemicals and treatment

CPF [0,0-diethyl O-(3,5,6-trichloropyridin-2-yl) phosphorothioate], purity 99.5%, was provided by Sigma-Aldrich Co. LLC. (Madrid, Spain). The compound was dissolved in corn oil and adjusted to administer 1 mg/kg in 1 μL/g of body weight by oral gavage, using a micropipette. The treatment was conducted from PND10 to PND15. Control groups were administered corn oil throughout the same period of time. Animals were periodically monitored and kept under standard conditions for two months prior to starting the behavioral assessment.

To study the influence of GABAergic and cholinergic systems on the behavioral task performance, the following drugs were used: Rivastigmine L-Tartrate (CAS Number: 129101-54-8, TCI Europe N.V., Zwijndrecht, Belgium), scopolamine hydrobromide (CAS Number: 6533-68-2, Sigma-Aldrich, Madrid, Spain) and alprazolam (Trankimazin 0.75mg/ml, CAS Number: 28981-97-7, Pfizer, S.A., Alcobendas, Spain).

2.3. Object Recognition Test

Recognition memory was tested in an ORT, a one-trial learning paradigm based on the natural tendency of mice to prefer a new object over a familiar one. The experimental protocol was designed based on the original setup first described by Ennaceur and Delacour (1988). The apparatus consisted of a square open field box (60 cm x 60 cm x 50 cm). During the pre-training period, mice were habituated to the testing area for 30 min. During habituation, the total distance traveled and the time spent in the center were measured to test general motor activity and anxiety-like behavior. A typical ORT session consisted of two parts: the acquisition and the retention phase. During acquisition, two identical objects were placed in the open field. Each individual was allowed to freely explore the objects for 15 min. The mouse was considered to be exploring an object when: (a) the head of the mouse was directed towards the object at a distance of 2 cm or less from it, or (b) when the animal was sniffing it. During acquisition, we studied the time spent exploring the two identical objects. The retention part was carried out 4 h after the acquisition session. During the retention session, one of the previous two objects was replaced with a new one, and each animal was allowed to explore the new setting for 5 min. A discrimination index (time spent exploring the new object/time spent exploring the familiar object) was used to test retention memory. All objects were white, being of similar sizes, but with perfectly distinguishable shapes. All objects and the open field box were carefully cleaned with 70% ethanol after each trial to reduce olfactory cues. All objects used were previously tested in another set of animals in order to discard any object preference bias (data not shown). Experiments were automatically recorded and analyzed using a video-tracking software (EthoVision®, Noldus Information Technologies, the Netherlands).

2.3.1. Inclusion criteria

A total of 146 animals were used in this study. As far as retention could be biased by exploration activity, we set an inclusion criteria (at least 2 seconds exploring one object and at least 10 seconds of total time exploring the objects) to gain access to the retention phase. Therefore, the number of animals in each group during the different parts was the following: habituation n=11-13, acquisition n=11-13 and retention n=8-13 (Table 1).

2.3.2. Basal condition and pharmacological challenge

Mice were habituated to the injection daily over a period of three days before the experiment using 0.9% saline. Animals in the basal conditions received intraperitoneal infusions of 0.9% saline 30 min. before the acquisition session started. During the pharmacological challenge, animals received the drugs in different sessions: the cholinergic agonist rivastigmine (0.25 mg/kg), the cholinergic antagonist scopolamine (1.6 mg/kg) and the GABAergic agonist alprazolam (0.12 mg/kg). Saline and the drugs were administered in a Latin square design 30 min. (scopolamine), 40 min. (rivastigmine) and 2 h (alprazolam) before starting the acquisition session. The dose selection was based on previous studies [16] and the time of administration was chosen according to each compound's average time to reach peak plasma concentration. Provided that we administered the drug before the acquisition part, we expected it to affect the encoding of the object information during acquisition. All drugs were intraperitoneally injected except rivastigmine, which was administered subcutaneously. Individual mice were subjected to each treatment on different days, separated by at least 48 h. To this end, a large set of objects was used in order to avoid repetition and bias.

2.4. Study of the estrous cycle

The stage of the reproductive cycle was assessed in females right after the retention part of each session in order to test possible influences on behavioral performance. The stage of the cycle was assessed on the basis of the type of cells observed in the vaginal smears as described elsewhere [35]. In short, nucleated epithelial cells indicate the proestrus stage, while cornified squamous epithelial cells indicate the estrous stage. A mix of cell types is characteristic of the metestrus, while leukocytes are characteristic of the diestrus stage. The four stages were divided into two groups: proestrus/estrus and metestrus/diestrus, as described in previous studies [36,37].

2.5. Statistical analysis

Data were analyzed with the SPSS 25.0 software (IBM Corp, Chicago, USA). A three-way analysis of variance (ANOVA) for repeated measures was used to study the general activity in the open field, while a three-way ANOVA for each drug condition was used in both the acquisition and retention parts. A *t*-test for independent samples was used to asess the specific contribution of the postnatal treatment and the effects of the different drugs in each genotype and sex. A one-sample *t*-test allowed to compare the recognition memory with a neutral chance level during retention. The variance homogeneity was assessed 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. General activity during habituation

General activity during habituation was analyzed by a three-way ANOVA (sex x genotype x treatment) for repeated measures. The 5-minute fractions of time were the within-subject factor and the distance traveled as well as the time spent in the center of the open field were studied as the dependent variables. A decrease over time in the total distance traveled [$F(5,129) = 69.268$, $p<0.001$] indicated habituation to the space (Fig. 1A and 1B). An interaction between time and genotype [$F(10,260) = 3.107$, $p=0.001$] indicated that the habituation pattern depended on the *APOE* genotype. A three-way ANOVA (sex x genotype x treatment) showed the general effects of sex [$F(1,133) = 11.569$, $p=0.001$] and genotype [$F(2,133) = 3.208$, $p=0.044$], as well as an interaction between genotype and treatment [$F(2,133) = 5.461$, $p=0.005$] on total distance traveled. While females traveled greater distances than males (Fig. 1C), the postnatal CPF treatment only affected apoE4 mice, which traveled a greater total distance than the respective controls (Fig. 1D). On the other hand, the time spent in the center of the open field, as a measure of anxiety-like behavior, was similar between groups (data not shown).

3.2. Acquisition

Exploration performance during the acquisition session was analyzed by a three-way ANOVA (sex x genotype x treatment) for basal (saline) and pharmacological conditions. Treatment effects were further analyzed within each sex and genotype group by a t-test for independent samples. A t-test for independent samples was also used to compare the mice's exploratory behavior between each drug and the basal conditions

3.2.1. Basal condition

The cumulative time exploring the two identical objects in basal conditions was influenced by genotype [F(2,132) = 4.072, *p*=0.019] and postnatal CPF treatment [F(1,132) = 10.619, *p*=0.001] (Fig. 2A and 2B). We found a triple interaction between sex, genotype and treatment [F(2,531) = 6.484, *p*=0.002]. Therefore, we split the groups up by genotype and sex to study the postnatal treatment effects. The results showed that the time exploring the objects in CPF-treated apoE4 females was significantly higher than their respective control group [t = 3.716; d.f.23; *p*=0.001] (Fig. 3B).

3.2.2. Pharmacological challenge

Exploratory activity during acquisition upon different pharmacological drugs, showed that the cholinergic antagonist scopolamine affected exploratory behavior, as the general effects of genotype and postnatal treatment observed in the basal conditions were no longer detected. Specifically C57BL/6 exposed mice and apoE3 either exposed or control significantly decreased their activity (Fig. 3C and 3D) compared to the basal conditions (Fig. 3A and 3B) while control C57BL/6 and apoE4 mice were not affected by scopolamine. Rivastigmine treatment (Fig. 3E and 3F) and alprazolam treatment (Fig. 3G and 3H) did not change basal conditions. A significant effect of the genotype [F(2,133) = 3.588, *p*=0.030] was still observed under the influence of rivastigmine. Similarly, postnatal treatment effects observed in basal conditions were also evident under the influence of rivastigmine [F(1,133) = 12.178, *p*=0.001] and of alprazolam [F(1,133) = 9.437, *p*=0.003]. In fact, apoE4 females postnatally exposed to CPF mantained high exploration levels on both rivastigmine [t = 5.014; d.f.24; *p*<0.001] and alprazolam [t = 2.365; d.f.24; *p*=0.026] conditions (Fig. 3F and 3H, respectively).

3.3. Retention

The discrimination ratio between familiar and novel objects during the retention session was analyzed by a three-way ANOVA (sex x genotype x treatment). We also used a one-sample *t*-test to analyze the discrimination ratio in comparison to the chance level (a theoretical value that assumes that mice spend the same time exploring familiar and novel objects). Finally, to establish the specific contribution of each drug, we compared the results of the different drugs in basal conditions using a *t*-test for independent samples.

3.3.1. Basal condition

The discrimination ratio in basal conditions was influenced by sex [$F(1,120) = 7.752$, $p=0.006$] and postnatal treatment [$F(1,120) = 6.328$, $p=0.013$]. Female mice performed better than males (Fig. 4A), with postnatal CPF exposure impairing retention (Fig. 4B). When the discrimination ratio was compared to chance level, control apoE4 males [$t = 2.860$; d.f.10; $p=0.017$], control apoE4 females [$t = 4.958$; d.f.11; $p<0.001$], and control [$t = 2.948$; d.f.10; $p=0.015$] and CPF-treated [$t = 2.493$; d.f.10; $p=0.032$] C57BL/6 females presented a significant preference for the novel object (Fig. 5A and 5B). It is worth noting that neither apoE3 males and females nor C57BL/6 males displayed any retention of the test in basal conditions.

3.3.2. Pharmacological challenge

Pharmacological testing showed an overall effect of the genotype [$F(2,462) = 8.874$, $p<0.001$], postnatal treatment [$F(1,462) = 4.695$, $p=0.031$] and an interaction between postnatal treatment and sex [$F(1,462) = 9.199$, $p=0.003$]. To better understand these effects, each drug condition was analyzed for each genotype, sex and treatment (Fig. 5).

None of the groups under the influence of scopolamine showed recognition memory. Although control apoE4 males explored the novel object more than the familiar one, the differences with chance level or basal conditions were not significant. It must be highlighted that compared to basal conditions, female mice showed impairments in those groups that had good retention in the basal condition: C57BL/6 control [t = 2.741; d.f.18; p=0.013] and CPF-treated [t = 2.150; d.f.19; p=0.045], and apoE4 control mice [t = 2.598; d.f.18; p=0.018] (Fig. 5 C and 5D).

A tendency towards an interaction between sex and treatment [F(1,118) = 3.684, p=0.057] was noted after administration of rivastigmine. This drug improved retention in control C57BL/6 male mice, which displayed a preference for the novel object compared to the chance level [t = 3.377; d.f.11; p=0.006]. This also occurred in both control [t = 3.068; d.f.10; p=0.012] and CPF-treated [t = 2.310; d.f.9; p=0.046] apoE4 males (Fig. 5E). Among the female groups, only the control C57BL/6 [t = 3.596; d.f.10; p=0.006] showed a preference for the novel object. In addition, control apoE4 females showed a decrease in the discrimination ratio after rivastigmine administration [t = 2.536; d.f.19; p=0.020] compared to basal conditions (Fig. 5F).

Lastly, the administration of alprazolam led to an effect of genotype [F(2,123) = 5.657, p=0.004] and an interaction between sex and treatment [F(1,123) = 3.993, p=0.048]. Control apoE4 was the only group with a significant preference for the novel object in both males [t = 3.682; d.f.10; p=0.004] and females [t = 2.397; d.f.10; p=0.037] compared with the chance level (Fig. 5G and 5H). No effects of alprazolam were observed in comparison to basal conditions in males (Fig. 5G). In females, discrimination in control apoE4 mice decreased with respect to basal conditions after alprazolam administration [t = 2.075; d.f.21; p=0.015] (Fig. 5H).

Taken together the results obtained during the retention sesions show long lasting effects produced by the postnatal treatment with CPF on apoE4 mice, a lack of retention in apoE3 mice, a lack of effects produced by scopolamine and alprazolam in males and the divergent effects produced by rivastigmine depending on *APOE* genotype and sex.

3.4. Influences of the estrous cycle

The influence that the stage of the reproductive cycle has on exploration during the various parts of the task was analyzed by a four-way ANOVA (estrous cycle stage x genotype x treatment x drug). Since an interaction between the drug and the cycle was found [$F(3,243) = 5.102$, $p=0.002$], we studied each drug separately. The stage of the cycle had an effect only in the saline group [$F(1,60) = 10.291$, $p=0.002$]. Female mice in the diestrus/metestrus phase of the cycle explored significantly less than the females in the pro-estrus/estrus (Fig. 6). This effect was no longer observed after the drugs were administered, suggesting that they can mask the differences related to the physiology of the reproductive cycle. Furthermore, this effect was not detected during the retention part of the test, suggesting that the phase of the cycle did not significantly affect recognition memory recall.

4. Discussion

The current investigation was aimed at studying in mice the effects of postnatal exposure to the pesticide CPF on recognition memory. An ORT was used to assess recognition memory, based on the premise that preference for a novel object means that the representation of the familiar object still exists in the memory of the animals. The potential implication of both the GABAergic and the cholinergic systems was assessed according to sex, *APOE* genotype and postnatal treatment using pharmacological challenges.

During acclimatization to the ORT working space, mice explored the new environment. All groups decreased the distance traveled over time, indicating habituation. Differences between sex and genotype were observed in terms of total distance traveled, with females having the highest locomotor activity. Previous studies with apoE3- and apoE4-TR mice [38,39] at different ages (4-5 months and 15 months of age, respectively), and with young C57BL/6 mice (2-3 months of age) [40] also found that females were more active than males during habituation to a novel environment. In the same line, other studies reported an increase in activity after exposure to CPF [7,8] while others did not find effects on locomotor activity

[41,42]. Likewise, CPF-treated apoE4 mice traveled greater distances in the open field than their controls, reaching levels of activity similar to those of C57BL/6 mice. Taking into account that behavioral deficits associated to apoE4 have been related to an impaired cholinergic system [12], and that CPF targets the AChE enzyme and produces long-term effects on AChE-S and AChE-R mRNA at low doses [43,44], exposure to CPF during development may help to counterbalance a possible basal deficiency in cholinergic neurotransmission inherent to this genotype. Recent studies have already reported an enhancement of the performance of apoE4 mice after exposure to CPF, and thus, a resemblance with other genotypes including *APOE3* or wild-type C57BL/6 mice [17,19,45].

During the acquisition session, the exploratory behavior of groups exposed to postnatal CPF was greater than that of controls in basal conditions. In general, this effect was reversed by the cholinergic antagonist scopolamine. However, apoE4 mice and C57BL/6 control mice proved to be less sensitive to this drug.

Retention of the task was affected by sex, with females showing better discrimination index scores. Although there is no consensus in this regard, most data report that females perform better, especially in long-lasting retrieval memory [46,47]. Enhanced object recognition memory in females has also been reported in humans [48]. Other studies suggest no differences between males and females [49], or go even further, demonstrating that males perform better than females [50].

Differences in genotype were observed in the saline group during retention. Firstly, apoE3 mice failed to recognize the novel object during this part of the experiment, and none of the pharmacological conditions led to any improvement. For this reason, it is not possible to draw any conclusion on this genotype. On the other hand, both male and female control apoE4, and C57BL/6 female mice showed a significant preference for the novel object. The fact that apoE4-TR mice presented better retention than apoE3-TR, or even C57BL/6 mice, is in contrast to most effects described in the scientific literature. Non-apoE4 carriers have been observed to perform better than apoE4 carriers in most human [51] and animal studies [52,53]. For instance, studies using ORT found a clear advantage of apoE3-TR over apoE4-TR mice at 4 [53] and 12 months of age [52]. It is important to consider that apart from the differences in age between studies, both used different protocols, including shorter inter-trial times, and different parameters for assessing retention. Consequently, they are difficult to compare. Nevertheless, this is not the first study to report that apoE4 perform better than their apoE3 counterparts. Siegel et al. [54] reported that during Morris water maze (MWM) acquisition and passive avoidance training, apoE4 females performed better than apoE2- and apoE3-TR mice at different ages. This better performance of apoE4 correlated with higher levels of anxiety-like behavior in the elevated zero maze and the elevated plus maze [54]. Likewise, in a previous study we also found that apoE4 needed fewer sessions than apoE3 mice to meet the learning criteria in the five-choice serial reaction time task (5-CSRTT) [16].

CPF exposure impaired retention in apoE4 mice, which lost their preference for the novel object during this part of the test. Slotkin et al. [55] also observed a detrimental effect of postnatal (PND 1-4) CPF exposure on recognition memory. They reported this effect only in females, which performed better in basal conditions. Other studies testing different doses of CPF during the gestational period or throughout aduthood in males showed no differences between treatments in this task [42,56,57]. Altogether, these results suggest that the detrimental effects on recognition memory are dependent on the exposure period to the pesticide and highly influenced by sex.

The cholinergic system and its neurotransmitter ACh play a key role in the central and peripheral nervous system. ACh synthesis from choline and acetyl coenzyme A takes place in the cytoplasm of cholinergic neurons, being ACh then stored in synaptic vesicles. Once the vesicles with the neurotransmitter are released into the synaptic cleft, ACh binds to mAChRs and nAChRs, located at the pre- or postsynaptic neurons. ACh is then inactivated by cholinesterase enzymes such as AChE and BChE, which share high homology, but not equal efficiency to break the ACh, which terminates the cholinergic neurotransmission [58–60]. In a previous study assessing the gene expression of the various elements involved in cholinergic neurotransmission in the brain of nine-month-old mice, we observed differences between C57BL/6 and apoE4-TR mice [45]. More specifically, apoE4 males expressed lower levels of M1 and M2 mAChRs in the frontal cortex and females expressed higher levels of BChE in the hippocampus compared with C57BL/6 mice [45]. These constitutive differences in the cholinergic system may underlie the effects observed under the influence of the cholinergic drugs scopolamine and rivastigmine.

Administration of scopolamine impaired retention in the groups displaying good retention in the basal condition. As a result, none of the groups showed a significant preference for novelty. However, the control apoE4 male group still spent more time exploring the novel object. In previous studies, we found that apoE4 females were less sensitive to scopolamine during attention assessment in the 5-CSRTT [16]. Scopolamine is a cholinergic antagonist targeting the muscarinic receptors. It has been reported that apoE4 males express lower levels of muscarinic receptor M1 and M2 in the hippocampus and frontal cortex than their C57BL/6 counterparts [45]. These differences have not been observed in females, which suggests constitutive cholinergic variances between sexes. Likewise, the effect was no longer observed in CPF-treated apoE4 females, which suggests a compensating effect induced by exposure to CPF. Further investigations are required to unravel the underlying mechanisms of this conceivable compensation.

The cholinergic agonist rivastigmine improved performance in control C57BL/6 mice and in both control and CPF-exposed apoE4 males. Interestingly, rivastigmine did not produce the same effect in apoE4 females which, in the case of the control group, showed decreased retention after rivastigmine administration. These results suggest a sex-dimorphic effect of rivastigmine on recognition memory. The discrepancy between males and females has important implications because rivastigmine is one of the drugs currently used in the treatment of AD [61]. Previous studies with rats have reported that inhibition levels of ChEs in different brain areas in females are higher than in males after rivastigmine administration [62]. In general terms, it seems that these differences can be attributed once more to constitutive differences in the cholinergic system between males and females. In recent studies we found that expression levels of BChE were lower in apoE4-TR mice than in C57BL/6 mice, but only in females [45]. Therefore, apoE4 females may be less sensitive to the effects of rivastigmine because they express lower levels of BChE, one of its targets. Animals with decreased levels of BChE would present higher levels of ACh, just as they do after rivastigmine administration [63]. Hence, it can be hypothesized that these females undergo an overstimulation of the cholinergic system, which leads to the observed impairments in recognition memory. The results of the present study raises the possibility that the effective dose in females should be reviewed and maybe adapted to the intrinsic requirements defined by sex and genotype. Other studies have reported memory improvements in males after dietary exposures to rivastigmine in the MWM test [64]. However, the effects of rivastigmine on recognition memory have received little attention. Still, several studies using donepezil, another drug used in the treatment of AD, have reported positive effects on task performance [65]. These two drugs share a similar mechanism of action, as donepezil also inhibits AChE, but it does not inhibit BChE [66].

Alprazolam had a detrimental effect on both control and CPF-treated C57BL/6 females. After its administration, these animals lost the preference for novelty observed in basal conditions. Interestingly, the retention of control apoE4 females was worse than that of the saline group. This detrimental effect is in line with the amnesic propeties of alprazolam and low doses of the drug can impair object recognition in mice [32]. It has been reported that the GABAergic system can be influenced by apoE4 isoform in a sex-dependent manner [67], giving a plausible explanation for the different responses of the groups. Further investigations are required to establish the exact mechanisms underlying these observations.

One of the major limitations of the data analysis is that the different ORT protocols make it difficult to compare studies. Nonetheless, Akkerman et al. [68] pointed out that some factors are crucial to the correct performance of the task (for example, the habituation to treatment procedures or the use of meeting criteria regarding minimum exploration times). Other important factors may be the inter-trial time, the kind of objects used or the experimental design. Furthermore, the phase of the estrous cycle may also be a variability factor. We found that females in pro-estrus/estrus showed an advantage during the acquisition part of the task, but this advantage was imperceptible during the retention part and after the drug had been administered. The better performance during pro-estrus/estrus matches previous findings on differences in rats [36] and mice [69] during acquisition. However, unlike these researchers, we did not find any differences during retention. Other researchers have found that the estrous cycle did not lead to differences in any of the test parts [47]. Overall, our results do not support the exclusion of females arguing variability caused by the estrous cycle.

In summary, the findings of the current research provide insights into the detrimental effects of postnatal exposure to CPF on recognition memory in apoE4 mice. The use of scopolamine revealed different sensitivities of apoE4 males, while the use of rivastigmine revealed differences between apoE4 males and females. Altogether, these results show that the cholinergic system is clearly involved in recognition memory and provide valuable information about its modulation. They also raise questions about the current use of rivastigmine for AD in females. Alprazolam also influenced recognition memory in a genotype- and sex-dependent manner with apoE4 females being the most impaired, thus raising questions about the use of GABA agonists in apoE4 females. In conclusion, sex differences observed in apoE4 mice in response to cholinergic and GABAergic drug challenges give rise to an important debate about therapeutic approaches, which can be personalized according to genotype and sex.

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References

[1] R. Reiss, E.T. Chang, R.J. Richardson, M. Goodman, A review of epidemiologic studies of low-level exposures to organophosphorus insecticides in non-occupational populations, Crit. Rev. Toxicol. 45 (2015) 531–641. doi:10.3109/10408444.2015.1043976.

[2] V.C. Moser, S. Padilla, Age- and gender-related differences in the time course of behavioral and biochemical effects produced by oral chlorpyrifos in rats, Toxicol. Appl. Pharmacol. 149 (1998) 107–119.

[3] C.N. Pope, T.K. Chakraborti, M.L. Chapman, J.D. Farrar, D. Arthun, Comparison of in vivo cholinesterase inhibition in neonatal and adult rats by three organophosphorothioate insecticides, Toxicology. 68 (1991) 51–61. doi:10.1016/0300-483X(91)90061-5.

[4] J.E. Casida, G.B. Quistad, Organophosphate toxicology: Safety aspects of nonacetylcholinesterase secondary targets, Chem. Res. Toxicol. 17 (2004) 983–998. doi:10.1021/tx0499259.

[5] T.A. Slotkin, M.C. Southard, S.J. Adam, M.M. Cousins, F.J. Seidler, α7 Nicotinic acetylcholine receptors targeted by cholinergic developmental neurotoxicants: nicotine and chlorpyrifos, Brain Res. Bull. 64 (2004) 227–235. doi:10.1016/j.brainresbull.2004.07.005.

[6] J.R. Richardson, J.E. Chambers, Effects of repeated oral postnatal exposure to chlorpyrifos on cholinergic neurochemistry in developing rats, Toxicol. Sci. 84 (2005) 352–359. doi:10.1093/toxsci/kfi081.

[7] L. Ricceri, A. Venerosi, F. Capone, M.F. Cometa, P. Lorenzini, S. Fortuna, G.

Calamandrei, Developmental neurotoxicity of organophosphorous pesticides: Fetal and neonatal exposure to chlorpyrifos alters sex-specific behaviors at adulthood in mice, Toxicol. Sci. 93 (2006) 105–113. doi:10.1093/toxsci/kfl032.

[8] L. Ricceri, N. Markina, A. Valanzano, S. Fortuna, M.F. Cometa, A. Meneguz, G. Calamandrei, Developmental exposure to chlorpyrifos alters reactivity to environmental and social cues in adolescent mice, Toxicol. Appl. Pharmacol. 191 (2003) 189–201. doi:10.1016/S0041-008X(03)00229-1.

[9] K. Dam, F.J. Seidler, T.A. Slotkin, Chlorpyrifos exposure during a critical neonatal period elicits gender-selective deficits in the development of coordination skills and locomotor activity, Dev. Brain Res. 121 (2000) 179–187. doi:10.1016/S0165-3806(00)00044-4.

[10] S. De Angelis, R. Tassinari, F. Maranghi, A. Eusepi, A. Di Virgilio, F. Chiarotti, L. Ricceri, A. Venerosi Pesciolini, E. Gilardi, G. Moracci, G. Calamandrei, A. Olivieri, A. Mantovani, Developmental exposure to chlorpyrifos induces alterations in thyroid and thyroid hormone levels without other toxicity signs in Cd-1 mice, Toxicol. Sci. 108 (2009) 311–319. doi:10.1093/toxsci/kfp017.

[11] G.S. Getz, C.A. Reardon, Apoprotein E as a lipid transport and signaling protein in the blood, liver, and artery wall, J. Lipid Res. 50 (2009) S156–S161. doi:10.1194/jlr.R800058-JLR200.

[12] S.J. Allen, S.H. MacGowan, S. Tyler, G.K. Wilcock, A.G.S. Robertson, P.H. Holden, S.K.F. Smith, D. Dawbarn, Reduced cholinergic function in normal and Alzheimer's disease brain is associated with apolipoprotein E4 genotype, Neurosci. Lett. 239 (1997) 33–36. doi:10.1016/S0304-3940(97)00872-0.

[13] A.D. Roses, Apolipoprotein E and Alzheimer's disease. A rapidly expanding field with medical and epidemiological consequences., Ann NY Acad Sci. 802 (1996) 50–57.

[14] K.R. Bales, Brain lipid metabolism, apolipoprotein E and the pathophysiology of Alzheimer's disease, Neuropharmacology. 59 (2010) 295–302. doi:10.1016/j.neuropharm.2010.01.005.

[15] I. Reverte, A.B. Klein, C. Ratner, J.L. Domingo, M.T. Colomina, Behavioral phenotype and BDNF differences related to apoE isoforms and sex in young transgenic mice, Exp. Neurol. 237 (2012) 116–125. doi:10.1016/j.expneurol.2012.06.015.

[16] I. Reverte, F. Peris-Sampedro, P. Basaure, L. Campa, C. Suñol, M. Moreno, J.L. Domingo, M.T. Colomina, Attentional performance, impulsivity, and related neurotransmitter systems in apoE2, apoE3, and apoE4 female transgenic mice, Psychopharmacology (Berl). 233 (2016) 295–308. doi:10.1007/s00213-015-4113-9.

[17] F. Peris-Sampedro, I. Reverte, P. Basaure, M. Cabré, J.L. Domingo, M.T. Colomina, Apolipoprotein E (APOE) genotype and the pesticide chlorpyrifos modulate attention, motivation and impulsivity in female mice in the 5-choice serial reaction time task, Food Chem. Toxicol. 92 (2016) 224–235. doi:10.1016/j.fct.2016.03.029.

[18] F. Peris-Sampedro, P. Basaure, I. Reverte, M. Cabré, J.L. Domingo, M.T. Colomina, 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 (2015) 37–45. doi:10.1016/j.physbeh.2015.03.006.

[19] P. Basaure, L. Guardia-Escote, M. Cabré, F. Peris-Sampedro, F. Sánchez-Santed, J.L. Domingo, M.T. Colomina, Learning, memory and the expression of cholinergic components in mice are modulated by the pesticide chlorpyrifos depending upon age at exposure and apolipoprotein E (APOE) genotype, Arch. Toxicol. 93 (2019) 693–707. doi:10.1007/s00204-019-02387-9.

[20] P. Basaure, L. Guardia-Escote, M. Cabré, F. Peris-Sampedro, F. Sánchez-Santed, J.L. Domingo, M.T. Colomina, Postnatal chlorpyrifos exposure and apolipoprotein E (APOE) genotype differentially affect cholinergic expression and developmental parameters in transgenic mice, Food Chem. Toxicol. 118 (2018) 42–52. doi:10.1016/j.fct.2018.04.065.

[21] A. Ennaceur, J. Delacour, A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data, Behav. Brain Res. 31 (1988) 47–59. doi:10.1016/0166-4328(88)90157-X.

[22] M.W. Brown, G.R.I. Barker, J.P. Aggleton, E.C. Warburton, What pharmacological interventions indicate concerning the role of the perirhinal cortex in recognition memory, Neuropsychologia. 50 (2012) 3122–3140. doi:10.1016/j.neuropsychologia.2012.07.034.

[23] N.J. Broadbent, S. Gaskin, L.R. Squire, R.E. Clark, Object recognition memory and the rodent hippocampus, Learn. Mem. 17 (2009) 5–11. doi:10.1101/lm.1650110.

[24] A.M. Melichercik, K.S. Elliott, C. Bianchi, S.M. Ernst, B.D. Winters, Nicotinic receptor activation in perirhinal cortex and hippocampus enhances object memory in rats, Neuropharmacology. 62 (2012) 2096–2105. doi:10.1016/j.neuropharm.2012.01.008.

[25] E. Dere, J.P. Huston, M.A. De Souza Silva, The pharmacology, neuroanatomy and neurogenetics of one-trial object recognition in rodents, Neurosci. Biobehav. Rev. 31 (2007) 673–704. doi:10.1016/j.neubiorev.2007.01.005.

[26] B. Grayson, M. Leger, C. Piercy, L. Adamson, M. Harte, J.C. Neill, Assessment of disease-related cognitive impairments using the novel object recognition (NOR) task in rodents, Behav. Brain Res. 285 (2015) 176–193. doi:10.1016/j.bbr.2014.10.025.

[27] J.C. Dodart, C. Mathis, A. Ungerer, Scopolamine-induced deficits in a two-trial object recognition task in mice, Neuroreport. 8 (1997) 1173–1178. doi:10.1097/00001756-199703240-00023.

[28] I. Klinkenberg, A. Blokland, The validity of scopolamine as a pharmacological model for cognitive impairment: A review of animal behavioral studies, Neurosci. Biobehav. Rev. 34 (2010) 1307–1350. doi:10.1016/j.neubiorev.2010.04.001.

[29] P. Pichat, O.E. Bergis, J.P. Terranova, A. Urani, C. Duarte, V. Santucci, C. Gueudet, C. Voltz, R. Steinberg, J. Stemmelin, F. Oury-Donat, P. Avenet, G. Griebel, B. Scatton, SSR180711, a novel selective α7 nicotinic receptor partial agonist: (II) efficacy in experimental models predictive of activity against cognitive symptoms of schizophrenia, Neuropsychopharmacology. 32 (2007) 17–34. doi:10.1038/sj.npp.1301188.

[30] S.L. McLean, B. Grayson, S. Marsh, S.H.O. Zarroug, M.K. Harte, J.C. Neill, Nicotinic α7 and α4β2 agonists enhance the formation and retrieval of recognition memory: Potential mechanisms for cognitive performance enhancement in neurological and

psychiatric disorders, Behav. Brain Res. 302 (2016) 73–80. doi:10.1016/j.bbr.2015.08.037.

[31] P. Thanapreedawat, H. Kobayashi, N. Inui, K. Sakamoto, M. Kim, A. Yoto, H. Yokogoshi, GABA Affects Novel Object Recognition Memory and Working Memory in Rats., J. Nutr. Sci. Vitaminol. (Tokyo). 59 (2013) 152–157. doi:10.3177/jnsv.59.152.

[32] V. Bertaina-Anglade, E. Enjuanes, D. Morillon, C. Drieu la Rochelle, The object recognition task in rats and mice: A simple and rapid model in safety pharmacology to detect amnesic properties of a new chemical entity, J. Pharmacol. Toxicol. Methods. 54 (2006) 99–105. doi:10.1016/j.vascn.2006.04.001.

[33] K. Soni, M. Parle, Trachyspermum ammi Seeds Supplementation Helps Reverse Scopolamine, Alprazolam and Electroshock Induced Amnesia, Neurochem. Res. 42 (2017) 1333–1344. doi:10.1007/s11064-017-2177-0.

[34] P.M. Sullivan, H. Mezdour, Y. Aratanti, C. Knouff, J. Najib, R.L. Reddick, S.H. Quarfordt, N. Maeda, Targeted Replacement of the Mouse Apolipoprotein E Gene with the Common Human APOE3 Allele Enhances Diet-induced Hypercholesterolemia and Atherosclerosis, J. Biol. Chem. 272 (1997) 17972–17980. doi:10.1074/jbc.272.29.17972.

[35] C. Caligioni, Assessing Reproductive Status/Stages in Mice, Curr Protoc Neurosci. (2009) Appendix–4I. doi:10.1002/0471142301.nsa04is48.

[36] N.P. van Goethem, K. Rutten, F.J. van der Staay, L. a W. Jans, S. Akkerman, H.W.M. Steinbusch, A. Blokland, J. van't Klooster, J. Prickaerts, Object recognition testing: Rodent species, strains, housing conditions, and estrous cycle, Behav. Brain Res. 232 (2012) 323–334. doi:10.1016/j.bbr.2012.03.023.

[37] C.M. Contreras, M. Molina, M. Saavedra, L. Martínez-Mota, Lateral septal neuronal firing rate increases during proestrus-estrus in the rat, Physiol. Behav. 68 (2000) 279–284. doi:10.1016/S0031-9384(99)00169-9.

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

174–182. doi:10.1016/j.bbr.2008.05.008.

[39] J. Grootendorst, A. Bour, E. Vogel, C. Kelche, P.M. Sullivan, J.-C. Dodart, K. Bales, C. Mathis, Human apoE targeted replacement mouse lines: h-apoE4 and h-apoE3 mice differ on spatial memory performance and avoidance behavior, Behav. Brain Res. 159 (2005) 1–14. doi:10.1016/j.bbr.2004.09.019.

[40] A.E.D. Van Swearingen, Q.D. Walker, C.M. Kuhn, Sex differences in novelty- and psychostimulant-induced behaviors of C57BL/6 mice, Psychopharmacology (Berl). 225 (2013) 707–718. doi:10.1007/s00213-012-2860-4.

[41] C. López-Granero, A.M. Ruiz-Muñoz, F.A. Nieto-Escámez, M.T. Colomina, M. Aschner, F. Sánchez-Santed, Chronic dietary chlorpyrifos causes long-term spatial memory impairment and thigmotaxic behavior, Neurotoxicology. 53 (2016) 85–92. doi:10.1016/j.neuro.2015.12.016.

[42] C.Y. Savy, A.E. Fitchett, R. McQuade, S.E. Gartside, C.M. Morris, P.G. Blain, S.J. Judge, Low-level repeated exposure to diazinon and chlorpyrifos decrease anxiety-like behaviour in adult male rats as assessed by marble burying behaviour, Neurotoxicology. 50 (2015) 149–156. doi:10.1016/j.neuro.2015.08.010.

[43] C. López-Granero, D. Cardona, E. Giménez, R. Lozano, J. Barril, F. Sánchez-Santed, F. Cañadas, Chronic dietary exposure to chlorpyrifos causes behavioral impairments, low activity of brain membrane-bound acetylcholinesterase, and increased brain acetylcholinesterase-R mRNA, Toxicology. 308 (2013) 41–49. doi:10.1016/j.tox.2013.03.009.

[44] C. López-Granero, D. Cardona, E. Giménez, R. Lozano, J. Barril, M. Aschner, F. Sánchez-Santed, F. Cañadas, Comparative study on short- and long-term behavioral consequences of organophosphate exposure: Relationship to AChE mRNA expression, Neurotoxicology. 40 (2014) 57–64. doi:10.1016/j.neuro.2013.11.004.

[45] L. Guardia-Escote, P. Basaure, J. Blanco, M. Cabré, C. Pérez-Fernández, F. Sánchez-Santed, J.L. Domingo, M.T. Colomina, Postnatal exposure to chlorpyrifos produces long-term effects on spatial memory and the cholinergic system in mice in a sex- and

APOE genotype-dependent manner, Food Chem. Toxicol. 122 (2018) 1–10. doi:10.1016/j.fct.2018.09.069.

[46] P. Ghi, M. Orsetti, S.R. Gamalero, C. Ferretti, Sex differences in memory performance in the object recognition test. Possible role of histamine receptors, Pharmacol. Biochem. Behav. 64 (1999) 761–766. doi:10.1016/S0091-3057(99)00143-4.

[47] J.S. Sutcliffe, K.M. Marshall, J.C. Neill, Influence of gender on working and spatial memory in the novel object recognition task in the rat, Behav. Brain Res. 177 (2007) 117–125. doi:10.1016/j.bbr.2006.10.029.

[48] L.J. Levy, R.S. Astur, K.M. Frick, Men and women differ in object memory but not performance of a virtual radial maze, Behav. Neurosci. 119 (2005) 853–862. doi:10.1037/0735-7044.119.4.853.

[49] T.S. Benice, A. Rizk, S. Kohama, T. Pfankuch, J. Raber, Sex-differences in age-related cognitive decline in C57BL/6J mice associated with increased brain microtubule-associated protein 2 and synaptophysin immunoreactivity, Neuroscience. 137 (2006) 413–423. doi:10.1016/j.neuroscience.2005.08.029.

[50] K.M. Frick, J.E. Gresack, Sex differences in the behavioral response to spatial and object novelty in adult C57BL/6 mice., Behav. Neurosci. 117 (2003) 1283–1291. doi:10.1037/0735-7044.117.6.1283.

[51] F. Berteau-Pavy, B. Park, J. Raber, Effects of sex and APOE ε4 on object recognition and spatial navigation in the elderly, Neuroscience. 147 (2007) 6–17. doi:10.1016/j.neuroscience.2007.03.005.

[52] R. Chouinard-Watkins, M. Vandal, P. Léveillé, A. Pinçon, F. Calon, M. Plourde, Docosahexaenoic acid prevents cognitive deficits in human apolipoprotein E epsilon 4-targeted replacement mice, Neurobiol. Aging. 57 (2017) 28–35. doi:10.1016/j.neurobiolaging.2017.05.003.

[53] S. Salomon-Zimri, A. Boehm-Cagan, O. Liraz, D.M. Michaelson, Hippocampus-Related Cognitive Impairments in Young apoE4 Targeted Replacement Mice, Neurodegener. Dis. 13 (2013) 86–92. doi:10.1159/000354777.

[54] J.A. Siegel, G.E. Haley, J. Raber, Apolipoprotein E isoform-dependent effects on anxiety and cognition in female TR mice, Neurobiol. Aging. 33 (2012) 345–358. doi:10.1016/j.neurobiolaging.2010.03.002.

[55] T. a. Slotkin, S. Skavicus, E.D. Levin, F.J. Seidler, Prenatal nicotine changes the response to postnatal chlorpyrifos: Interactions targeting serotonergic synaptic function and cognition, Brain Res. Bull. 111 (2015) 84–96. doi:10.1016/j.brainresbull.2015.01.003.

[56] A. Lan, M. Kalimian, B. Amram, O. Kofman, Prenatal chlorpyrifos leads to autism-like deficits in C57Bl6/J mice, Environ. Heal. 16 (2017) 43. doi:10.1186/s12940-017-0251-3.

[57] A. V. Terry, D.A. Gearhart, W.D. Beck, J.N. Truan, M.-L. Middlemore, L.N. Williamson, M.G. Bartlett, M.A. Prendergast, D.W. Sickles, J.J. Buccafusco, Chronic, Intermittent Exposure to Chlorpyrifos in Rats: Protracted Effects on Axonal Transport, Neurotrophin Receptors, Cholinergic Markers, and Information Processing, J. Pharmacol. Exp. Ther. 322 (2007) 1117–1128. doi:10.1124/jpet.107.125625.

[58] Y. Abreu-Villaça, C.C. Filgueiras, A.C. Manhães, Developmental aspects of the cholinergic system, Behav. Brain Res. 221 (2011) 367–378. doi:10.1016/j.bbr.2009.12.049.

[59] H. Soreq, S. Seidman, Acetylcholinesterase - new roles for an old actor, Nat. Rev. Neurosci. 2 (2001) 294–302. doi:10.1038/35067589.

[60] A. Hrabovska, E. Krejci, Reassessment of the Role of the Central Cholinergic System, J. Mol. Neurosci. 53 (2014) 352–358. doi:10.1007/s12031-013-0164-8.

[61] R. Anand, K.D. Gill, A.A. Mahdi, Therapeutics of Alzheimer’s disease: Past, present and future, Neuropharmacology. 76 (2014) 27–50. doi:10.1016/j.neuropharm.2013.07.004.

[62] R.H. Wang, C. Bejar, M. Weinstock, Gender differences in the effect of rivastigmine on brain cholinesterase activity and cognitive function in rats, Neuropharmacology. 39 (2000) 497–506. doi:10.1016/S0028-3908(99)00157-4.

[63] F. Cerbai, M.G. Giovannini, C. Melani, A. Enz, G. Pepeu, N1phenethyl-norcymserine, a selective butyrylcholinesterase inhibitor, increases acetylcholine release in rat cerebral

cortex: A comparison with donepezil and rivastigmine, Eur. J. Pharmacol. 572 (2007) 142–150. doi:10.1016/j.ejphar.2007.06.053.

[64] P. Basaure, F. Peris-Sampedro, M. Cabré, I. Reverte, M.T. Colomina, Two cholinesterase inhibitors trigger dissimilar effects on behavior and body weight in C57BL/6 mice: The case of chlorpyrifos and rivastigmine, Behav. Brain Res. 318 (2017) 1–11. doi:10.1016/j.bbr.2016.10.014.

[65] I. Kendall, H.A. Slotten, X. Codony, J. Burgueño, P.J. Pauwels, J.M. Vela, K.C.F. Fone, E-6801, a 5-HT6 receptor agonist, improves recognition memory by combined modulation of cholinergic and glutamatergic neurotransmission in the rat, Psychopharmacology (Berl). 213 (2011) 413–430. doi:10.1007/s00213-010-1854-3.

[66] R.M. Lane, M. Kivipelto, N.H. Greig, Acetylcholinesterase and its inhibition in Alzheimer disease, Clin. Neuropharmacol. 27 (2004) 141–149.

[67] Y. Li, H. Sun, Z. Chen, H. Xu, G. Bu, H. Zheng, Implications of GABAergic neurotransmission in Alzheimer's disease, Front. Aging Neurosci. 8 (2016) 1–12. doi:10.3389/fnagi.2016.00031.

[68] S. Akkerman, A. Blokland, O. Reneerkens, N.P. van Goethem, E. Bollen, H.J.M. Gijselaers, C.K.J. Lieben, H.W.M. Steinbusch, J. Prickaerts, Object recognition testing: Methodological considerations on exploration and discrimination measures, Behav. Brain Res. 232 (2012) 335–347. doi:10.1016/j.bbr.2012.03.022.

[69] J. Cordeira, S.S. Kolluru, H. Rosenblatt, J. Kry, R.E. Strecker, R.W. McCarley, Learning and memory are impaired in the object recognition task during metestrus/diestrus and after sleep deprivation, Behav. Brain Res. 339 (2018) 124–129. doi:10.1016/j.bbr.2017.11.033.

Figure 1| Distance traveled divided into fractions of 5 min. in an open field during the 30 min. of habituation to the working space of the ORT in: males (A) and females (B). Total distance traveled by sex (C) and by genotype and treatment (D). An asterisk indicates that performance is significantly different at $p<0.05$ from the respective control group. Groups with different letters (a,b) are significantly different ($p<0.05$).

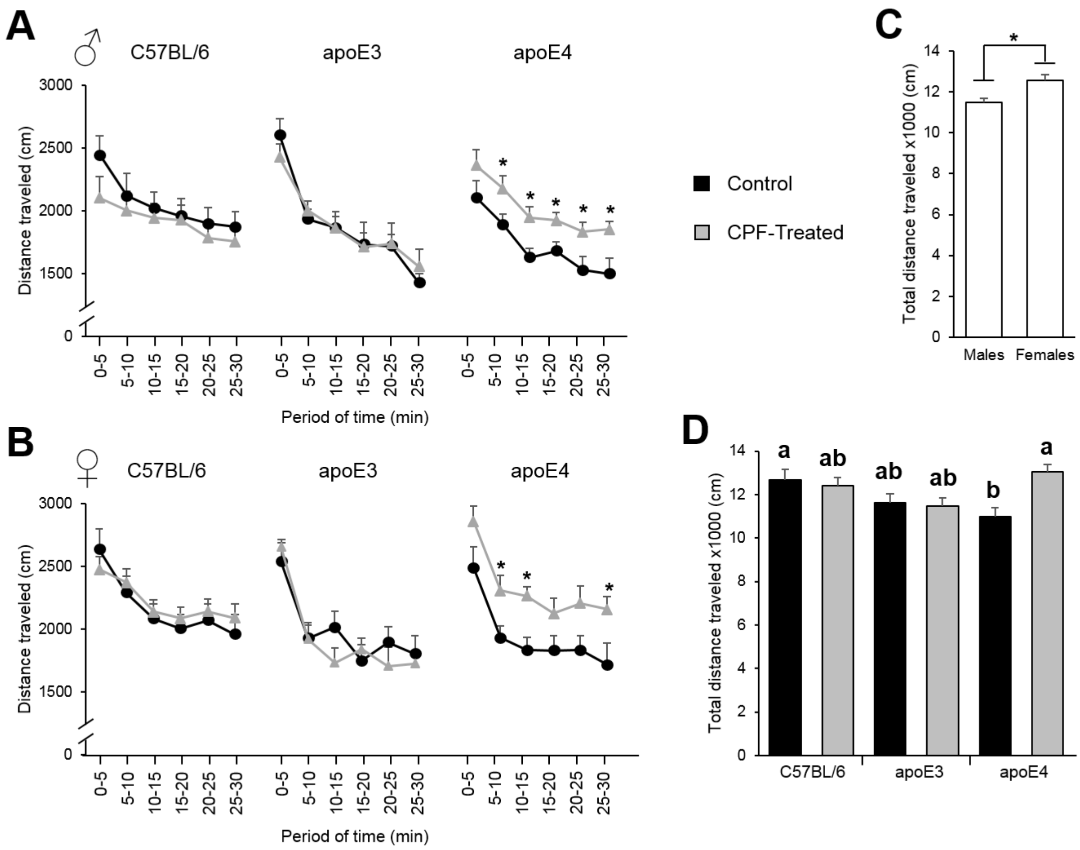


Figure 2| Cumulative time exploring two identical objects during the acquisition phase of the ORT. General effect of genotype (A) and treatment (B) in basal conditions after saline administration. An asterisk indicates that performance is significantly different at $p<0.05$.

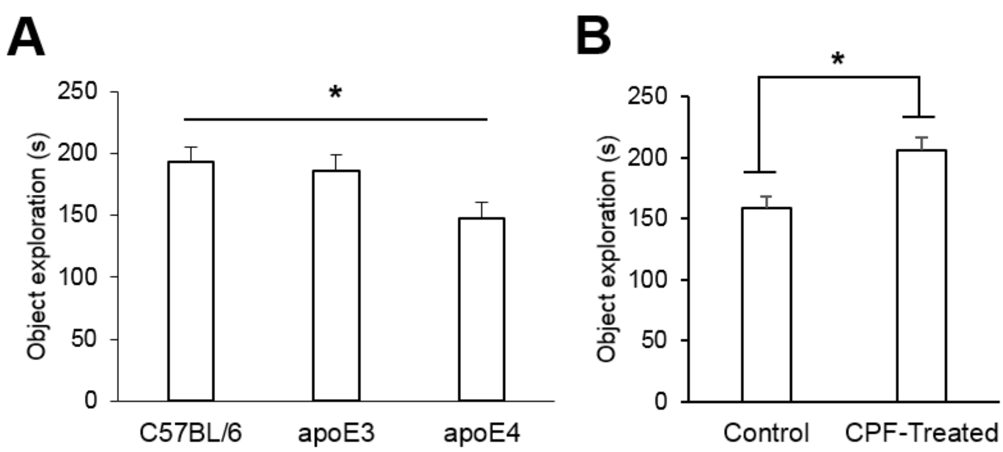


Figure 3| Time exploring two identical objects during the acquisition phase of the ORT after the administration of saline (A,B), scopolamine (C,D), rivastigmine (E,F) and alprazolam (G,H). The symbol (&) indicates that performance is significantly different to the respective control at $p<0.05$. The symbol (#) indicates significant differences with respect to the saline group ($p<0.05$).

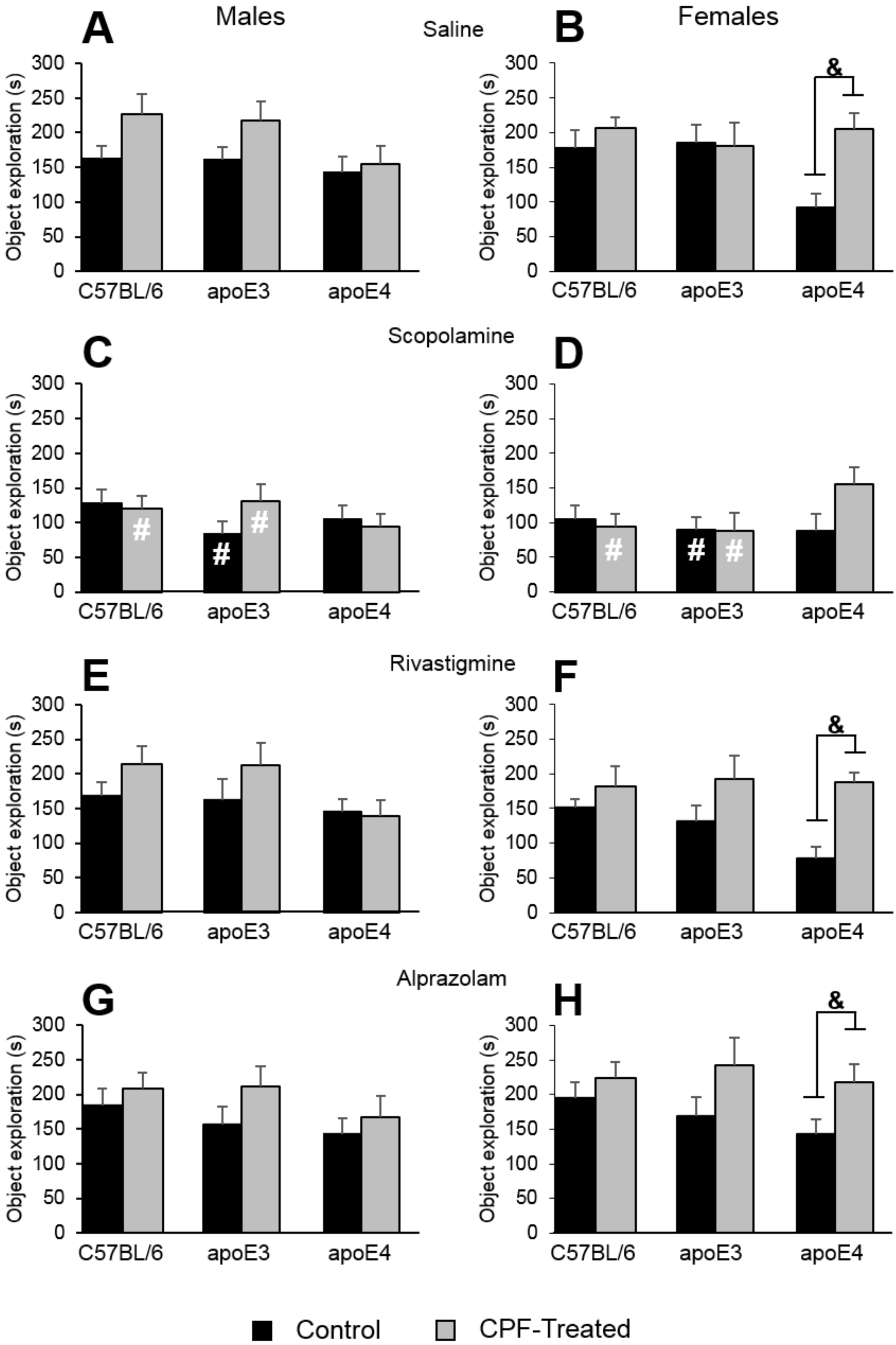


Figure 4| Discrimination ratio (time exploring the novel object/time exploring the familiar object) during the retention session. General effects of sex (B) and postnatal treatment (C) on basal conditions after saline administration. An asterisk indicates that performance is significantly different at $p<0.05$.

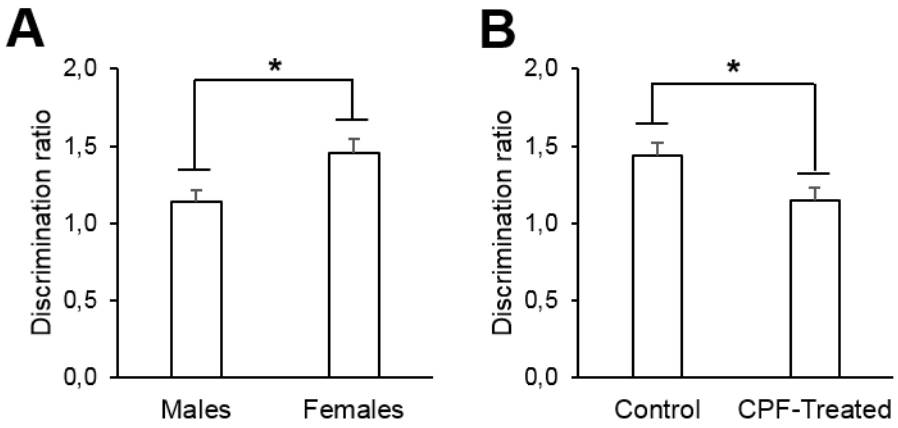


Figure 5| Discrimination ratio during the retention session after the administration of saline (A,B), scopolamine (C,D), rivastigmine (E,F) and alprazolam (G,H). The symbol (#) indicates significant differences with respect to the saline group ($p<0.05$) and the asterisk (*) indicates significant differences with the chance level (equal time exploring novel and familiar object) at $p<0.05$.

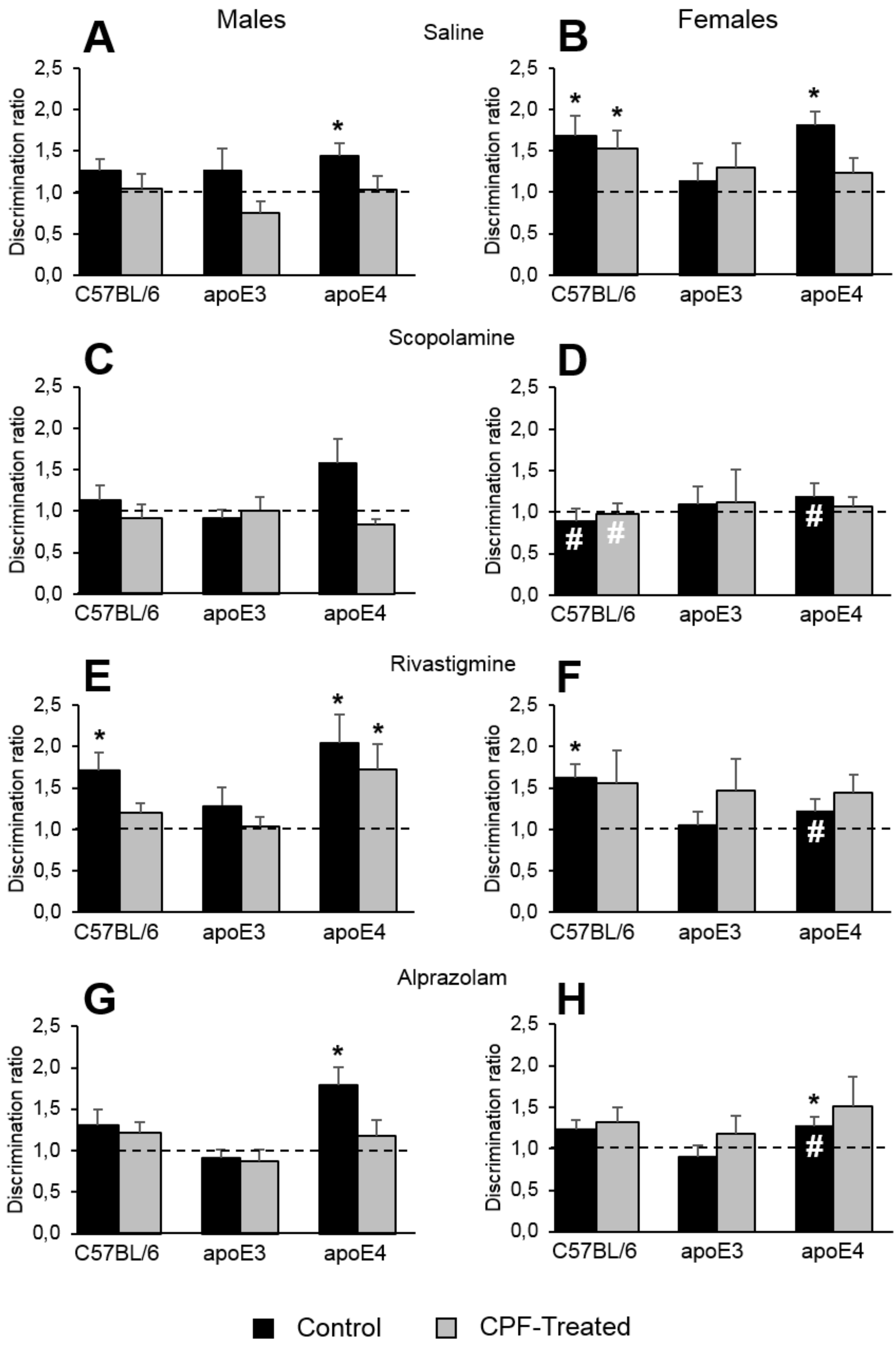
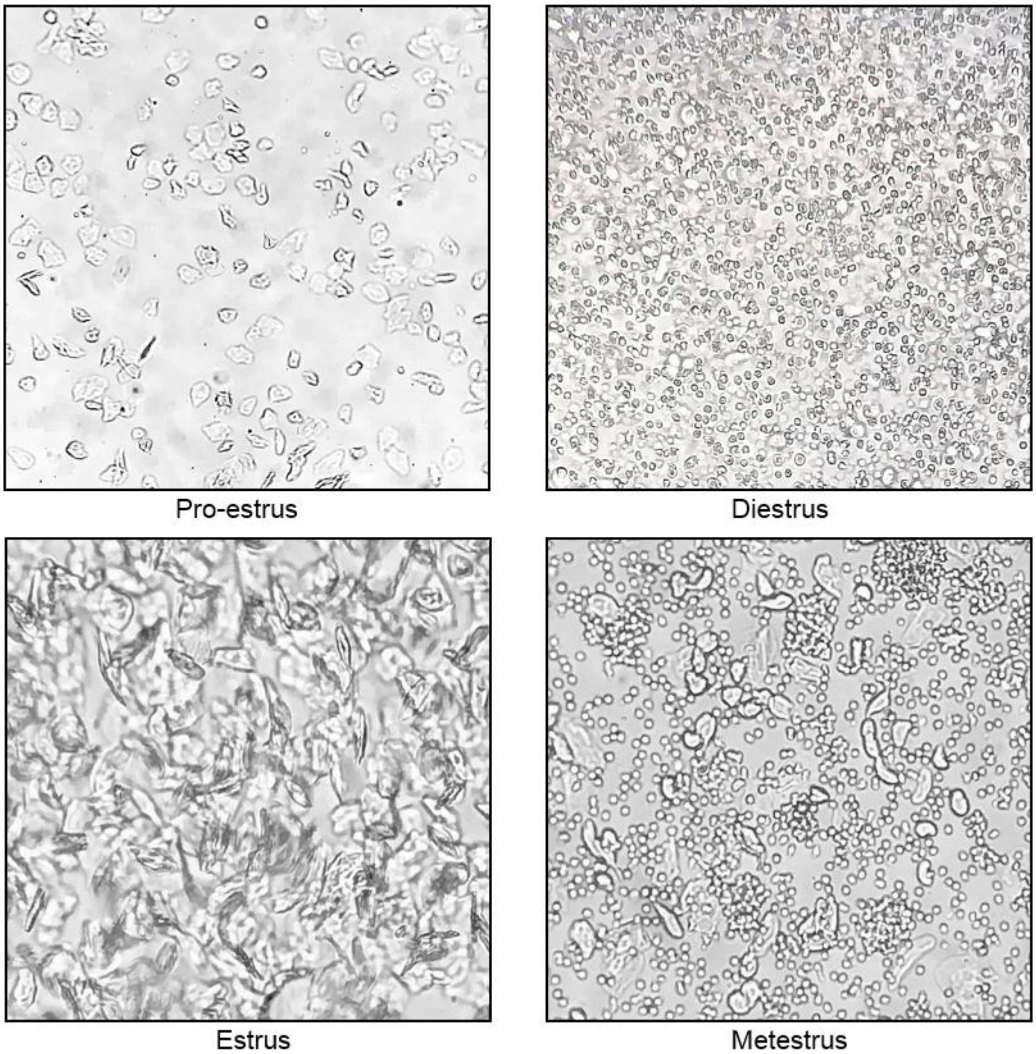


Figure 6| Time exploring two equal objects during the acquisition session. Involvement of the estrous cycle in the exploration activity. An asterisk indicates that performance is significantly different at $p<0.05$.



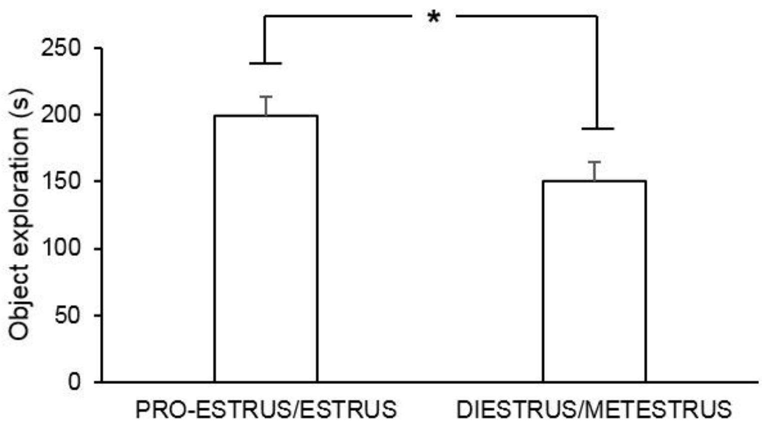


Table 1 | Number of animals in each experimental group

		Habituation	Acquisition	Retention			
Group				*Saline*	*Scopolamine*	*Rivastigmine*	*Alprazolam*
Males	CNT C57	12	12	10	10	11	9
	CPF C57	12	11	10	10	11	11
	CNT apoE3	11	12	10	8	10	11
	CPF apoE3	12	12	9	9	10	11
	CNT apoE4	12	12	11	9	11	11
	CPF apoE4	13	13	12	8	9	11
Females	CNT C57	12	12	11	9	10	10
	CPF C57	13	13	13	12	13	13
	CNT apoE3	12	12	12	10	12	12
	CPF apoE3	12	12	11	9	10	12
	CNT apoE4	12	12	12	9	12	12
	CPF apoE4	12	12	11	10	11	12

Abbreviations: CNT, Control; CPF, CPF-treated; C57, C57BL/6