Chronic oral exposure to low doses of Chlorpyrifos differentially affects physical and behavioral endpoints in ApoE2, ApoE3 and ApoE4 transgenic mice

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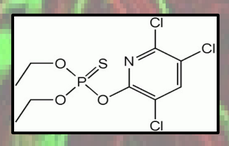
Introduction

Chlorpyrifos (CPF) is an organophosphate pesticide widely used over the world in intensive agriculture and livestock. Various studies have demonstrated neurotoxic effects in adult mammals after chronic and acute CPF exposure such as cognitive impairments (1), oxidative stress (2) and neuronal damage (3), which suggest a possible relationship between CPF exposure and Alzheimer 's disease (AD) or cognitive impairment in aged population (4). Genetics, gender or age provide distinct protection or vulnerability to AD. According to this, being carrier of the ε4 allele of the apolipoprotein E (ApoE) gene is a well-established risk factor to develop AD. In addition to the neurotoxic effects, recently, several studies have begun to describe metabolic effects resulting from exposure to chlorpyrifos (5). The present study aims to evaluate physical and behavioral effects in ApoE transgenic male mice carrying different polymorphisms of human ApoE (ε2, ε3, ε4) after a chronic oral exposure to low doses of CPF.



Materials and Methods

Animals Adult (3/6 months) ApoE (ε2, ε3, ε4) transgenic male mice

Treatment Diet exposure using a CPF supplemented food (2mg/kg), or its respective control, throughout thirteen weeks

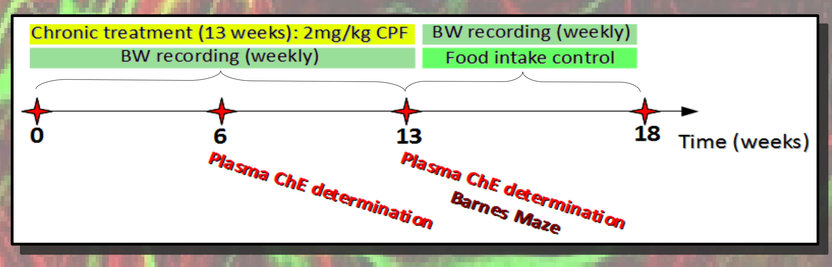
Weekly body weight control over the whole treatment period. After it, five additional weekly records were made.

Food intake control during the five post-treatment weeks.

Barnes maze Spatial reference memory task during the last week of CPF treatment (3 months after the CPF treatment started)

- ***Acquisition***: 5 training days, 2 trials/day
  Maximum time allowed to find the escape box = 180 s
  Time in escape box = 30 s
  Time inter-trial = 30-60 min
- ***Retention***: 1 probe trial, 24 h after the last acquisition session
  Without escape box
  Time of free movement = 120 s

Cholinesterase (ChE) activity Plasma ChE levels assessed by the Ellman method using *Cobas Mira* analyzer in two stages: half of treatment (1,5 months) and the end of it (3 months)



Results and Discussion

Body weight changes

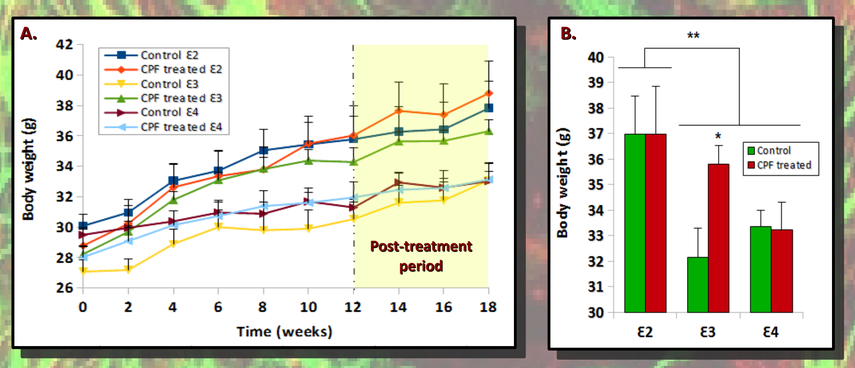


Fig. 1. A. Temporary changes in body weight during both treatment and post-treatment periods. Values are expressed as mean ± SEM.
Fig. 1. B. Cumulative representation of both treatment and post-treatment period body weights. Values are expressed as mean ± SEM. *p<0.05 indicates significant differences between CPF treated and control ApoE ε3 mice. **p<0.05 indicates significant differences between ApoE ε2 and ApoE ε3, ApoE ε4 groups.

ChE activity

Time of determination	Inhibition mean (%)	Minimum Maximum
1,5 months	83,99	77,10 – 90,90
3 months	77,82	69,01 – 84,44

Fig. 2. Plasma ChE inhibition levels assessed by *Cobas Mira* analyzer in two stages: half of treatment (1,5 months) and the end of it (3 months)

Food intake control

There were no significant differences in food intake among CPF treated subjects and their respective controls, nor between the three different genotypes.

Barnes Maze

Acquisition

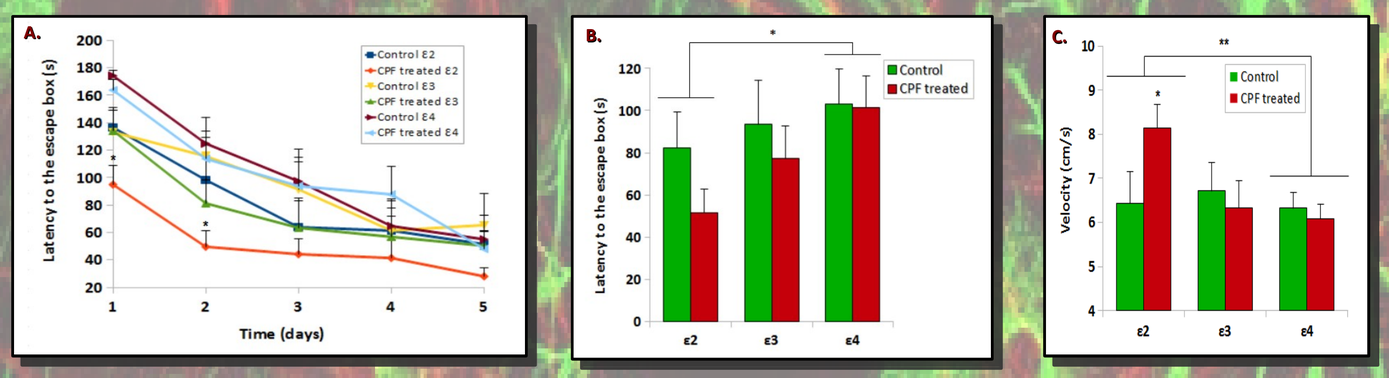


Fig. 3. A. Escape latency to the target hole, during the 5 days of acquisition period in the BM, made the last chronic CPF treatment week. Values are expressed as mean ± SEM. *p<0.05 indicates significant differences in the latency of scape to the target hole on day 1 and 2 of ApoE ε2 treated subjects compared to their respective controls.
Fig. 3. B. Cumulative representation of the escape latency to the target hole, during the 5 days of acquisition period in the BM, made the last chronic CPF treatment week. Values are expressed as mean ± SEM. *p<0.05 indicates significant differences in the latency of scape to the target hole between ApoE ε2 and ApoE ε4 genotypes.
Fig. 3. C. Cumulative representation of the total velocity in the BM arena, during the 5 days of acquisition period in the BM, made the last chronic CPF treatment week. Values are expressed as mean ± SEM. *p<0.05 indicates significant differences in the total velocity of treated ApoE ε2 subjects compared to their respective controls. **p<0.05 indicates differences in the total velocity between ApoE ε2 mice and the two oder genotypes, ApoE ε3 and ApoE ε4.

Retention

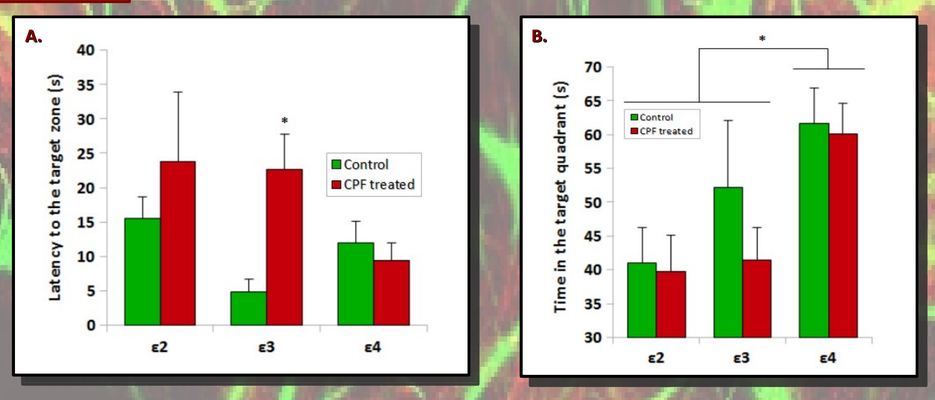


Fig. 4. A. Escape latency to the target zone of the BM, during the retention session made 24h after the last acquisition session. Values are expressed as mean ± SEM. *p<0.05 indicates significant differences in the latency of scape to the target zone of ApoE ε3 treated subjects compared to their respective controls.
Fig. 4. B. Total time spent in the target quadrant of the BM during the retention session made 24h after the last acquisition session. Values are expressed as mean ± SEM. *p<0.05 indicates significant differences in the total time ApoE ε4 mice spent in the target quadrant compared to the total time spent by ApoE ε2 and ApoE ε3 subjects.

Conclusions

Body Weight	Activity/Acquisition	Learning and Memory
Significant body weight increase in CPF treated ApoE ε3 subjects compared to their respective controls, **over the whole experiment** (18 weeks)	In the acquisition period, **CPF treated ApoE ε2 mice were more motivated to escape to the target hole** compared to the two oder genotypes. Regarding this, **CPF increase activity and alertness in ApoE ε2 subjects.**	In the retention trial, **ApoE ε4 subjects appeared to have better retention than the other genotypes.** Furthermore, **CPF impairs long term retention in ApoE ε3 subjects.**

References

1. Chen XP, Chen WZ, Wang FS, Liu JX. Selective cognitive impairments are related to selective hippocampus and prefrontal cortex deficits after prenatal chlorpyrifos exposure. Brain Res., 2012, 1474:19-28.
Ruiz-Muñoz AM, Nieto-Escamez FA, Aznar S, Colomina MT, Sanchez-Santed F. Cognitive and histological disturbances after chlorpyrifos exposure and chronic Aβ(1-42) infusions in Wistar rats. Neurotoxicology, 2011, 32(6):836-44.
2. Elsharkawy EE, Yahia D, El-Nisr NA. Sub-chronic exposure to chlorpyrifos induces hematological, metabolic disorders and oxidative stress in rat: attenuation by glutathione. Environ. Toxicol. Pharmacol., 2013, 35(2):218-27.
López-Granero C, Cañadas F, Cardona D, Yu Y, Giménez E, Lozano R, Avila DS, Aschner M, Sánchez-Santed F. Chlorpyrifos-, diisopropylphosphorofluoridate-, and parathion-induced behavioral and oxidative stress effects: are they mediated by analogous mechanisms of action? Toxicol Sci., 2013, 131(1):206-16.
3. Roy TS, Sharma V, Seidler FJ, Slotkin TA. Quantitative morphological assessment reveals neuronal and glial deficits in hippocampus after a brief subtoxic exposure to chlorpyrifos in neonatal rats. Brain Res Dev Brain Res., 2005, 155(1):71-80.
4. Hayden KM, Norton MC, Darcey D, Ostbye T, Zandi PP, Breitner JC, Welsh-Bohmer KA; Cache County Study Investigators. Occupational exposure to pesticides increases the risk of incident AD: the Cache County study. Neurology., 2010, 74(19):1524-30.
5. Lassiter TL, Brimijoin S. Rats gain excess weight after developmental exposure to the organophosphorothionate pesticide, chlorpyrifos. Neurotoxicol Teratol. 2008, 30(2):125-30.
Slotkin TA. Does early-life exposure to organophosphate insecticides lead to prediabetes and obesity? Reprod Toxicol., 2011, 31(3):297-301.

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