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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 remain 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 modulates 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 (3.75 mg/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.

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, 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., 0.46 μg/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.,

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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 homoeostasis, 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 (2 g/mouse/day) to approximately 80–85% of their free feeding weight (i.e., 20 g). 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 37.5 mg 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 3.75 mg/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 0.12 mg/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 (24 × 20 × 15 cm) (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 2.5 cm holes were