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Sex-dimorphic effects of gestational exposure to the organophosphate insecticide chlorpyrifos on social investigation in mice

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ABSTRACT

Several pieces of evidence from animal and human studies indicate that the organophosphate insecticide chlorpyrifos (CPF) acts as a developmental neurotoxicant at environmentally relevant doses, and it is possibly endowed with endocrine-disrupting activity. Data collected in rodent models show that developmental exposure to CPF at sub-toxic doses induces long-lasting and sex-dimorphic changes in social and investigative responses in exposed offspring. The aim of this study was to evaluate the effects of gestational CPF treatment on social and olfactory discrimination in adult mice of both sexes. Pregnant CD1 out-bred mice were exposed to CPF per os on gestational days (GD) 14–17 at the sub-toxic dose of 6 mg/kg/bw. At adulthood, male and female offspring underwent the same experimental paradigms, namely i) a social discrimination test where mice were presented with a simultaneous binary choice between a novel conspecific and a familiar one, and ii) an olfactory habituation/dishabituation test to evaluate their capability to discriminate between odors with different eco-ethological salience (non-social vs. social odors).

Results showed that in the social discrimination test prenatal CPF primarily affected the female sex by raising the investigation time in females to the same levels as found in vehicle- and CPF-exposed males. The ability to discriminate between a familiar and a novel social mate was not affected by CPF in either sex. In the olfactory habituation/dishabituation test, mice of both sexes successfully discriminated non-social from social odors regardless of the prenatal treatment received.

These results confirm previous evidence indicating that developmental exposure to CPF causes long-lasting and sex-dimorphic changes in responsiveness to social cues, in the absence of significant impairment of social and olfactory discrimination capacity. These findings are discussed within the framework of recent data pointing to the limbic/hypothalamic circuitry and steroid hormonal regulations as possible targets for CPF neurotoxicity.

1. Introduction

Chlorpyrifos (CPF) is the most used non-persistent organophosphate (OP) pesticide worldwide in both agricultural and urban communities. While the acute neurotoxicity of this chemical is associated with systemic and brain acetylcholinesterase (AChE) inhibition, an increasing body of experimental data suggests that, at low doses, CPF also targets non-cholinergic mechanisms (Eaton et al., 2008; Slotkin and Seidler, 2012).

Several epidemiological studies to date support the hypothesis that at low, environmentally relevant doses, CPF acts as a developmental neurotoxicant. Analysis of longitudinal birth cohorts from either agricultural or urban communities found associations between prenatal exposure to CPF as measured in cord blood and a higher risk of mental and motor delay, pervasive developmental disorder (PDD), and hyperactive behaviors in exposed children (Rauh et al., 2006).

The mechanisms by which CPF affect neurobehavioral development in exposed children are unclear, as are the possible delayed effects of this chemical at older ages. Studies in rodents demonstrated that at doses devoid of systemic toxicity, developmental exposure to CPF interferes with DNA synthesis, neuronal differentiation, synaptogenesis, and affects the expression levels of critical genes involved in brain development (Betancourt et al., 2006; Crumpton et al., 2000; Dam et al., 1998). Furthermore, fetal exposure to subtoxic doses of CPF alters neural systems beyond cholinergic transmission, such as serotonergic and dopaminergic neurotransmission, in a sex-dimorphic fashion (Slotkin and

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Seidler, 2007). Numerous rat studies have shown long-term effects on behavior after developmental exposure to subtoxic doses of CPF (Aldridge et al., 2004; Aldridge et al., 2005; Dam et al., 1999; Raines et al., 2001; Slotkin et al., 2002). Specifically, prenatal CPF exposure causes multiple behavioral alterations in rats tested from adolescence to adulthood; locomotor activity habituation and working and reference memory are affected with significant damping of sex differences in CPF exposed females (Levin et al., 2002). Similarly in the mouse species, CPF doses below the threshold for adverse effects on fetal growth or viability and maternal toxicity induce sex-dimorphic and long-term changes in a number of behavioral end points. In particular, in addition to increasing motor activity in the males, CPF targets different items of the social repertoire of laboratory mice in both sexes, including social recognition and pup-directed responses in virgin females (Ricceri et al., 2006), aggressive interactions between males to achieve social rank (Ricceri et al., 2006), social recognition (Venerosi et al., 2006), and nest defense response in lactating females (Venerosi et al., 2009). We have also shown that CPF has long lasting effects on the neurohormones implicated in the modulation of social and affective responses such as oxytocin (OT) and vasopressin (AVP) (Tait et al., 2009). Notably, hypothalamic neuropeptides are considered components of a sex-dimorphic hypothalamic–limbic micronet modulating social responses in rodents (Choleris et al., 2003), and regulating social recognition at the level of the olfactory system in rodents (Bielsky and Young, 2004; Wacker and Ludwig, 2012).

On the basis of this experimental evidence, we hypothesized that one of the possible mechanisms underlying CPF behavioral toxicity may be the interference with endocrine factors and/or sexually dimorphic features of brain maturation (Venerosi et al., 2012). As social investigation and discrimination are markedly sex-dimorphic in rodents, we expect to find sex-dependent vulnerability to CPF effects by assessing adult mice of both sexes with the same experimental paradigm. Specifically in the present study, we evaluated the effects of gestational CPF exposure on social investigation and discrimination by applying a social discrimination test where two social stimuli are repeatedly presented to the same animal before the final simultaneous binary choice between a novel conspecific and a familiar one (Choleris et al., 2003). Additionally, in order to verify the possibility that CPF alters social responses by interfering with the detection and/or processing of olfactory cues, we assessed the same mice in a habituation/dishabituation olfactory test where non-social (water, vanilla, almond) or social (same-sex urine, opposite-sex urine) odors were presented sequentially to mice to evaluate their ability to distinguish between odors with different ethological value and to habituate to them (Yang and Crawley, 2009). CPF or its vehicle were administered at the sub-toxic dose of 6 mg/kg/bw by oral gavage from gestational day (GD) 14 to 17 to pregnant mice of the CD1 strain.

2. Materials and methods

2.1. Subjects and treatment

Forty male and female mice of the out bred Swiss-derived strain (CD1, Harlan, S. Pietro al Natisone, Italy), were housed in pairs in breeding cages (polycarbonate cages 33 × 13 × 14 cm) with a 12-h light–dark cycle (lights on at 8 pm) with free access to food (enrichment standard diet for mice, from Mucedola, Settimo Milanese, Italy) and water.

Females were inspected daily for the presence of the vaginal plug (gestational day 0). The stud was removed 10 days after the discovery of the vaginal plug. On GD14, 26 pregnant females were randomly assigned to one of the two prenatal treatments [vehicle (Veh), CPF]. CPF (Chem. Service, West Chester, PA) was dissolved in peanut oil (Veh) to provide rapid and complete absorption. CPF (in a volume of 0.1 ml/10 g at a dose of 6 mg/kg/bw) or its vehicle was administered to pregnant females from GD 14 to 17 by intraoral gavages. Extensive work by Slotkin's group in rats has shown a greater sensitivity of late gestational phases (for doses comprised between 1 and 5 mg/kg/bw) for the effects of prenatal CPF exposure on both neural systems and behavior (Aldridge et al., 2005; Qiao et al., 2003).

This same late gestation exposure window and the dose used in our present study was found to produce significant effects on adult social responses and hypothalamic neuropeptide levels in mice (Ricceri et al., 2006; Tait et al., 2009). Notably, the lowest observed adverse effect level (LOAEL) established by the EU for neurotoxicity in rats after chronic exposure is 10 mg/kg/bw/day, although most of the developmental neurotoxicity studies in rats and mice found delayed behavioral effects at doses comprised between 1 and 6 mg/kg/bw/day. Estimated CPF exposure in the general population including children and women of childbearing age today is primarily through diet and in the 10^{-1} to 10^{-3} μg/kg-d dose range (Li et al., 2012), although much higher exposure might occur in the fetus in areas with intensive pesticide use (Ostrea et al., 2002).

The 6 mg/kg dose is safe with respect to the reproductive performance of treated dams (pregnancy length, number of pups at delivery, sex ratio), and it does not induce overt toxic symptoms in dams or major effects on pups' health parameters such as weight at delivery and impairment of growth rate (Ricceri et al., 2006). Following CPF dose administration to pregnant mice after applying the same treatment schedule of the present study, we found no effects on brain AChE activity and a mild transient inhibition (20% of control values) in serum AChE activity in offspring when measured at birth 24 h after the last exposure (Ricceri et al., 2006). We cannot exclude that limited but significant brain AChE inhibition could have been present at earlier time points and recovery could have occurred by 24 h following exposure as previously indicated in the rat species by (Mattsson et al., 2000). Of the twenty-six pregnant females treated, four females (two Veh and two CPF treated) gave birth on GD 17 and were thus excluded from further study. A total of twenty-two litters (12 Vehicle-treated and 10 CPF-treated) were used. On the day of birth, number of pups delivered, overall weight of the litter, and sex ratio were recorded to verify the absence of CPF effects on reproductive performance by considering the body weight of each pregnant female before the beginning of treatment as a covariate. We assessed the sex of the pups by evaluation of ano-genital distance and litters were culled to a maximum of 10 pups while always trying to maintain a comparable number of pups of the two sexes within the litter ($0.6 \leq$ sex ratio ≤ 1.5). Offspring were weaned on postnatal day 23, housed in cages containing littermates of the same sex, and left undisturbed until the beginning of the behavioral assessment. At adulthood, one female and one male from each Veh (12) and CPF-treated (10) litter underwent the social discrimination test, while one–two females and one–two males from the same litters were assessed in the olfactory habituation/dishabituation test.

All experiments on animals were performed according to the European Community Council Directive 2010/63/EU and to Italian Legislation on Animal Experimentation (Legislative Decree 116/92).

2.2. Social discrimination paradigm

On postnatal day 70, both females (Veh, $n = 12$; CPF, $n = 9$; one female was excluded due to loss of video recorded data) and males (Veh, $n = 12$; CPF, $n = 10$) from each treatment underwent a social discrimination test as described by Choleris et al. (2006). The test was performed during the dark phase of the light/dark cycle in a novel polycarbonate cage (48 × 27 × 21 cm) where the experimental subject underwent a five trial social discrimination test between two social stimuli (age- and sex-matched CD1 mice).

In each trial (T1–T5), two wired cylinders (Galaxy Cup, Kitchen Plus, http://www.kitchen-plus.com; diameter 10 cm, height 10.5 cm), each containing a stimulus mouse from different cages, were placed in two opposite sides of the test cage. The use of wired cylinders allowed for passage of olfactory cues while preventing direct interactions between stimulus and experimental mice. This ensured that experimental mice