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Sex-dependent effects of developmental exposure to different pesticides on spatial learning. The role of induced neuroinflammation in the hippocampus

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ABSTRACT

The use of pesticides has been associated with impaired neurodevelopment in children. The aims of this work were to assess: 1) the effects on spatial learning of developmental exposure to pesticides 2) if the effects are sex-dependent and 3) if hippocampal neuroinflammation is associated with the impairment of spatial learning. We analyzed the effects of developmental exposure to four pesticides: chlorpyrifos, carbaryl, endosulfan and cypermethrin. Exposure was from gestational day 7 to post-natal day 21 and spatial learning and memory was assessed when the rats were young adults. The effects of pesticides on spatial learning were pesticide and gender-dependent. Carbaryl did not affect spatial learning in males or females. Endosulfan and chlorpyrifos impaired learning in males but not in females. Cypermethrin improved spatial learning in the Morris water maze both in males and females while impaired learning in the radial maze only in males. Spatial learning ability was lower in control female rats than in males. All pesticides induced neuroinflammation, increasing IL-1b content in the hippocampus and there is a negative correlation between IL-1b levels in the hippocampus and spatial learning. Neuroinflammation would contribute to the effects of pesticides on spatial learning.

1. Introduction

The use of pesticides is critical for the modern agricultural industry. However, some commonly used pesticides have been associated with impaired neurodevelopment in children (Bouchard et al., 2011; Grandjean et al., 2006; Guillette et al., 1998). The association between exposure to low levels of pesticides and human health outcomes, including neurodevelopmental disorders, has been recently reviewed (Androutsopoulos et al., 2013; Koureas et al., 2012). Moreover, different recent studies show an association between occupational exposure to pesticides in agricultural workers and increased risk for neurodegenerative diseases, such as Alzheimer or Parkinson disease (Baldi et al., 2003; Pezzoli and Cereda, 2013).

Four main chemical families of pesticides are organophosphates, carbamates, organochlorines and pyrethroids. Representative pesticides of each one of these families are chlorpyrifos, carbaryl, endosulfan and cypermethrin, respectively. Endosulfan is persistent while the others are biodegradable.

A number of studies have analyzed the developmental effects of organophosphates or pyrethroid pesticides on learning and memory while few studies reported developmental effects of carbamates or endosulfan. Exposure to chlorpyrifos induces developmental neurotoxicity in humans (Shelton et al., 2014; Grandjean and Landrigan, 2014) and rats (Slotkin, 1999; Levin et al., 2001), suggesting more severe effects in males than in females (Levin et al., 2001).

Most pesticides are neurotoxic and operate through one of three main mechanisms, inhibiting: 1) acetylcholinesterase, 2) voltage-gated sodium channel disruption, and/or 3) GABA receptors (Casida, 2009).

Although acute neurotoxicity of chlorpyrifos is mediated by inhibition of acetylcholine esterase, prenatal and neonatal exposure to chlorpiryfos doses lower than those that produce acute toxicity, induce cognitive impairment which would be mediated by mechanisms other than inhibition of acetylcholinesterase (Terry,

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2012). Some of these mechanisms could be covalent modification of proteins by chlorpyrifos through binding to tyrosine and lysine residues (Grigoryan et al., 2008), lipid peroxidation in the developing brain (Slotkin et al., 2005), altered expression of neurotrophic factors (Slotkin et al., 2007) or induction of inflammatory responses with increased levels of cytokines (Rohlman et al., 2011; Singh and Jiang, 2003).

Acute administration of carbaryl inhibits acetylcholinesterase while chronic administration did not (Sachana et al., 2001). Other mechanisms by which carbaryl may be neurotoxic are: antagonism of estrogen receptors (Lemaire et al., 2006); inhibition of neurite outgrowth (Sachana et al., 2003) or alteration of immune function (Jorsaraei et al., 2014).

Cypermethrin prolongs the opening of sodium channels and affects the function of chloride, voltage-gated calcium and potassium channels and of glutamate and acetylcholine receptors. Only at high doses cypermethrin inhibits acetylcholinsterase activity (Singh et al., 2012). Cypermethrin may also induce astrogliosis and neuroinflammation (Maurya et al., 2015).

Endosulfan is a persistent pesticide. Endosulfan is an endocrine disruptor, antagonizing estrogen receptor β and alters estradiol metabolism (Lemaire et al., 2006; Mrema et al., 2013). Endosulfan is also an antagonist at the GABA-A receptor (Mrema et al., 2013) and may also increase pro-inflammatory cytokines (TNF-α and IL-1β) in plasma (Omurtag et al., 2008).

The above studies indicate that different pesticides may have more than one mechanism of neurotoxicity and that most of them induce inflammation. Exposure to pesticides has been shown to induce systemic inflammation associated with oxidative stress (Tsitsimpikou et al., 2012; Zafiropoulos et al., 2014). For example, Cypermethrin induces systemic oxidative stress associated with systemic inflammation (Vardavas et al., 2016a,b). Systemic inflammation and oxidative stress often induce neuroinflammation, which, in turn, may contribute to cognitive impairment (Cornejo and von Bernhardi, 2016; Rodrigo et al., 2010; Felipo et al., 2012; Montoliu et al., 2015; Mossakowski et al., 2015).

Inflammatory cytokines impair spatial memory (Wenk et al., 2003), but no clear link has been established between inflammatory responses and organophosphates-related neurobehavioral deficits (reviewed by Rohlman et al., 2011).

Some pesticides induce sex-dimorphic effects, for example, developmental exposure to chlorpyrifos increases estrogen receptor beta in hypothalamus and decreases oxytocin in amygdala in males but not in females (Venerosi et al., 2015) and seems to induce more severe cognitive deficits in males than in females (Levin et al., 2001).

The aims of this work were to assess:

1) the effects on spatial learning of developmental exposure to pesticides representative of four different chemical families;
2) if the effects are gender dependant and
3) if hippocampal neuroinflammation is associated with the impairment of spatial learning induced by developmental exposure to the pesticides.

We analyzed the effects of developmental exposure to chlorpyrifos, carbaryl, endosulfan and cypermethrin. Developmental exposure was from gestational day 7 to post-natal day 21 and spatial learning and memory was assessed using the Morris water maze and radial maze tasks.

2. Materials and methods

2.1. Animals and exposure to pesticides

Pregnant Wistar rats (Charles River) were treated with pesticides or vehicle (corn oil, controls) from gestational day 7 to postnatal day 21. Pesticides were dissolved in corn oil and administered daily mixed in a sweet jelly (the volume of corn oil in the sweet jelly was between 50 and 200 μL every day. We confirmed that all rats eat all the sweet jelly and, therefore, all the dose of pesticide). The rest of the diet was standard laboratory diet. Pesticides were purchased from Sigma-Aldrich (Refs.: endosulfan (alpha + beta = 2 + 1) PESTANAL® 32015; alpha-Cypermethrin PESTANAL®, 45806; Chlorpyrifos PESTANAL®, 45395 and Carbaryl PESTANAL®, 32055). Seven groups of rats were used, summarized in Table 1. Pups were weaned at 21 days. Spatial learning and memory tests were performed when the pups were 2–3 months-old. None of the pesticides, at the doses used, significantly affects the number of offspring, survival or growth curve and body weight of animals. The offspring from 6 dams per group was used. Litter effects were controlled by using pups from different litters per treatment group in each experiment. Animal procedures were approved by the Center and met the guidelines of the European Union for care and management of experimental animals (Directive, 2010/63/EU).

2.2. Spatial learning in the Morris water maze

The test was carried out as described by Monfort et al. (2007). After pre-training, the rats were trained to learn the fixed location of the invisible platform during a 4 days time period. The time needed to find the hidden platform was recorded with the video-camera and specific software from View Point (Lyon, France) and used as a measure of learning of the task. Twenty four hours after the last training trial, the platform was removed from the pool, the rats were allowed to swim for 90 s in the pool and the time spent in the quadrant where the platform was during training was recorded as a measure of spatial memory.

2.3. Spatial learning in the eight arm radial maze

The distal extreme of each arm had a cup for the food. Rats were allowed to explore the maze for 10 min on two consecutive days in the presence of distal cues (posters and objects of different sizes), which remained in place throughout training. Training in the radial maze was composed of five trials per day performed on five consecutive days. The task involved locating four pellets, each placed at the end of a different arm according to a random configuration as in Johansson et al. (2015). The number of spatial reference errors (first visits to unbaited arms) and working errors (visits to arms already visited in the same trial) were calculated. A learning index is calculated as number of right choice-number of errors for the first entry into each arm.

2.4. Analysis of protein content in hipocampues by western blott

Rats were sacrificed by decapitation 5–7 days after the end of the behavioral testing and the hippocampus was dissected and

Table 1
Rat groups and dose of the treatments.

Group	DOSE (mg/kg/day)
Control	Sweet jelly with 100 μl of corn oil
Cypermethrin	1.5
Endosulfan	0.5
Carbaryl	15
Chlorpyrifos	0.1
Chlorpyrifos	0.3
Chlorpyrifos	1