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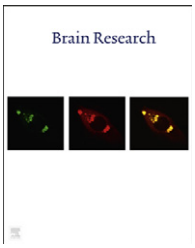

Research Report

Developmental exposure to chlorpyrifos and diazinon differentially affect passive avoidance performance and nitric oxide synthase-containing neurons in the basolateral complex of the amygdala

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ABSTRACT

Chronic exposure to low doses of organophosphates during brain development can induce persistent neurochemical and behavioral effects. This study sought to determine the long-lasting effects of developmental exposure to chlorpyrifos (CPF) and diazinon (DZN) on passive avoidance (PA) performance and neuronal nitric oxide synthase (nNOS)-containing neurons in the subnuclei within basolateral complex of amygdala (BLC). Developing rats were exposed to daily dose (1 mg/kg) of CPF or DZN during gestational days 15–18 and postnatal days (PND) 1–4. PA performance was assessed in young adulthood (PND 60). Brain sections were also processed by NADPH-diaphorase (NADPH-d) and nNOS immunohistochemistry. Gestational exposure to CPF increased NADPH-d$^+$/nNOS-immunoreactive (IR) neurons within the basolateral nucleus (BL) and medial paracapsular intercalated cluster, which was along with PA retention impairment in both male and female rats. Prenatal exposure to DZN did not significantly change the number of NADPH-d$^+$/nNOS-IR neurons in the BLC while impaired PA retention in females. Postnatal exposure to CPF decreased NADPH-d$^+$/NOS-IR neurons in the BL without affecting PA performance. Exposure to DZN during early postnatal period impaired PA retention in both sexes, albeit to a lesser extent in females, and was along with a considerable sex independent reduction of NADPH-d$^+$/NOS-IR neurons in all BLC subnuclei. Our data suggest that developmental exposure to apparently subtoxic dose of CPF and DZN elicit long-lasting impairment in PA retention that are associated, but not necessarily correlated with effects on NADPH-d$^+$/NOS-IR neurons in BLC of the amygdala.

Abbreviations: AChE, Acetylcholinesterase; BL, Basolateral amygdala; BM, Basomedial amygdala; CPF, Chlorpyrifos; DMSO, Dimethylsulfoxide; DZN, Diazinon; GD, Gestational day; Imp, Medial paracapsular intercalated; LA, Lateral amygdala; NADPH-d, Nicotinamide adenine dinucleotide phosphate–diaphorase; NO, Nitric oxide; NOS, Nitric oxide synthase; nNOS-IR, Neuronal NOS-immunoreactive; OP, Organophosphate; PA, Passive avoidance; PND, Postnatal day; STL, Step-through latency; TDC, Time spent in the dark compartment

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1. Introduction

Developmental exposure to low doses of some organophosphate (OP) compounds induces long-lasting neurobehavioral abnormalities. These effects seem to be mostly unrelated to the inhibition of acetylcholinesterase (AChE) and consequent cholinergic hyperexcitability, the common mechanism of OPs acute toxicity. Different developmental processes including neuronal differentiation, intracellular signaling cascades, axonogenesis and synaptogenesis are affected by chronic exposure to OPs (Qiao et al., 2002; Slotkin et al., 2001, 2007; Slotkin and Seidler 2007; Timofeeva et al., 2008). Evidences about the persisting adverse effects of OPs on the brain reveals three major principles: first, the developing brain is more susceptible to the alterations induced by chronic exposure to low doses of OPs than the adult brain (Eskenazi et al., 2008). Second, a variety of targets other than AChE are involved in the neurotoxic effects induced by OPs and they show substantial differences in their effects unrelated to AChE inhibition, which can underlie differences in the consequent behavioral abnormalities in terms of type and magnitude (Aldridge et al., 2005). Finally, many studies have reported dependency of biochemical mechanisms underlying the neurotoxic effects induced by low dose of OPs to be gender selective and dependent on time of exposure, which may also contribute to discrete behavioral outcomes (Johnson et al., 2009; Slotkin et al., 2001; Slotkin and Seidler 2007; Timofeeva et al., 2008). It has been frequently reported that developmental exposure to insecticides chlorpyrifos (CPF) and diazinon (DZN) induces persisting behavioral abnormalities and cognitive impairments. While home application of these OPs has been curtailed in some countries, they continue to be widely used throughout the commercial agriculture and even household (Aldridge et al., 2005; Timofeeva et al., 2008). CPF and DZN show disparities in their effect on different cognitive tasks and also on neurochemical markers functionally associated with different neurotransmitter systems. Both neurobehavioral and neurobehavioral effects of these OPs also show considerable dependencies to sex and time of exposure (Johnson et al., 2009; Levin et al., 2001).

The fear memory is a major biological adaptation that enables animal to recognize and behave properly when encounter to a danger. The PA, as a fear-motivated task, involves instrumental conditioning and indirectly measures Pavlovian contextual conditioning. In this task animal learns to avoid a specific place (for example dark compartment) associated with an aversive stimulus (typically foot-shock) (Wilensky et al., 2000). The amygdala, especially its basolateral complex (BLC) composed of lateral amygdala (LA), basolateral (BL) and basomedial (BM, known also as accessory basal) nuclei, is believed to be the central site of plasticity to learn fear-motivated tasks including PA (Lange et al., 2012; Wilensky et al., 2000). Furthermore, the BLC indirectly contributes to modulation of memory consolidation through its projections to hippocampus and cerebral cortex, which are also involved in PA performance (Izquierdo et al., 1997; McGaugh et al., 2002).

Nitric oxide (NO) is a neurotransmitter with multiple physiological and pathophysiological functions in both developing and adult brain. It is produced by enzyme nitric oxide synthase (NOS), which has three isoforms: neuronal (nNOS), endothelial (eNOS) and inducible (iNOS). NO has a well known role in long term potentiation (LTP), a form of synaptic plasticity considered as a cellular basis of memory consolidation, and lack or inhibition of nNOS induces learning and memory impairment (Lange et al., 2012; Yan et al., 2012; Zhou and Zhu 2009). Highly diffusible and instable nature of NO makes it a hard molecule to follow and measure but NOS-containing neurons could be detected in brain sections immunohistochemically with specific antibody for nNOS or by using nicotinamide adenine dinucleotide phosphate–diaphorase (NADPH-d) method. The recent method reliably stains nNOS containing neuron in many brain structures (Bredt et al., 1990; Dawson et al., 1991; Valtschanoff et al., 1993). The morphological features and pattern of distribution of NADPH-d^+/nNOS-containing neurons have been reported for developing and adult brain (Leigh et al., 1990; Olmos et al., 2005; Samama et al., 1995; Valtschanoff et al., 1993). Interestingly, many memory disorders are associated with changes in the number, distribution and morphological features of NADPH-d^+/NOS-containing neurons in the brain structures involved in memory formation and consolidation (Hamani et al., 1999; Rodella et al., 2006; Selvín-Testa et al., 1997). Different nuclei of BLC contain nNOS expressing neurons with their own specific features and pattern of distribution (McDonald et al., 1993; Olmos et al., 2005; Usunoff et al., 2006; Utkan et al., 2012). Some studies have implicated NO in LTP in BLC (Abe et al., 1996; Lange et al., 2012) and formation of PA memory, evidenced by an impaired PA performance after nNOS inhibition (Utkan et al., 2012; Yildirim and Marangoz, 2004).

It has been shown that neonatal exposure of mice to low dose CPF does not alter adult performance in passive avoidance task (Ricceri et al., 2003). This study was designed to characterize the effects of prenatal and early postnatal exposure to low dose of CPF and DZN on PA acquisition and retention in rat; and to evaluate the effects of these treatments on nNOS expressing neurons in the BLC of amygdala. Either of these OPs were administered during two windows of vulnerability; gestational days (GDs) 15–18 and postnatal days (PNDs) 1–4. The PA performance and NADPH-d positive (NADPH-d^+)/nNOS-immunoreactive (nNOS-IR) neurons within amygdaloid BLC were assessed at young adulthood (PND 60) in both sexes.

2. Results

2.1. *Locomotor activity in open field*

Neither the prenatal nor the postnatal administration of CPF or DZN induced a significant effect on locomotor activity as evaluated in the open field on PND 60 (Fig. 1).

2.2. *PA performance in prenatally treated animals*

Statistical analysis revealed no significant difference between three groups in the first STL (before the acquisition trial) (Fig. 2A). This result shows that prenatal exposures to low doses of CPF and DZN have no effect on the native preference