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Repeated exposures to diisopropylfluorophosphate result in impairments of sustained attention and persistent alterations of inhibitory response control in rats

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ABSTRACT

Organophosphate (OP)-based chemicals are used worldwide for many purposes and they have likely saved millions of people from starvation and disease. However, due to their toxicity they can also pose a significant environmental risk. While considerable research has focused on the acute symptoms and long-term consequences of overtly toxic exposures to OPs, less attention has been given to the subject of repeated exposures to levels that are not associated with acute symptoms (subthreshold exposures). There is clinical evidence indicating that this type of OP exposure can lead to prolonged deficits in cognition; however only a few studies have addressed this issue prospectively in animal models. In this study, repeated subthreshold exposures to the OP nerve agent diisopropylfluorophosphate (DFP) were evaluated in a 5-Choice Serial Reaction Time Task (5C-SRTT), an animal model of sustained attention. Adult rats were trained to stably perform the 5C-SRTT and then injected subcutaneously with vehicle or DFP of 0.5 mg/kg every other day for 30 days. Behavioral testing occurred daily during the DFP-exposure period and throughout a 45 day (OP-free) washout period. Compared to vehicle-treated controls, DFP-treated rats exhibited deficits in accuracy, increases in omissions and timeout responses during the OP exposure period, while no significant effects on premature responses, perseverative responses, or response latencies were noted. While the increase in timeout responses remained detectible during washout, all other DFP-related alterations in 5C-SRTT performance abated. When the demands of the task were increased by the presentation of variable intertrial intervals, premature responses were also elevated in DFP-treated rats during the washout period. These results indicate that repeated exposures to subthreshold doses of DFP lead to reversible impairments in sustained attention as well as persistent impairments of inhibitory response control in rats.

1. Introduction

The class of chemicals known as the "organophosphates" (OPs) is found in hundreds of compounds including pesticides, herbicides, anthelmintics, chemical warfare ("nerve") agents, ophthalmic agents, and industrial solvents (Katz and Brooks, 2010). In addition, the OP metrifonate has been documented to have moderate, but favorable effects on the cognitive symptoms in Alzheimer disease (Becker et al., 1998). The value of OPs as pesticides (their most common use) in optimizing agricultural productivity, the control of deadly vector-borne illnesses (e.g., malaria, yellow fever, typhus), and "nuisance" pests (e.g., flies, roaches, mosquitoes) is clear (reviewed, Cooper and Dobson, 2007) and they have likely saved millions of people from starvation and disease. Accordingly, OPs comprise the most widely used insecticides in the world and their use is increasing (see Ross et al., 2013). An unfortunate consequence of this widespread use, however, is the increasing number of OP poisonings worldwide, particularly in developing countries where adequate protective measures are often lacking (De Silva et al., 2006). While less likely for most people, the threat of OP exposure from intentional poisonings by rogue governments and terrorist organizations is also an ongoing concern. The Iraqi military (nerve agent) attacks on Kurdish civilians in the 1980s (Macilwain, 1993), the Tokyo Sarin attack in 1995 by domestic terrorists (Nagao et al., 1997), and the recent sarin attacks on civilians in Syria (United Nations Security Council Report, 2013) exemplify this concern.

The acute symptoms of OP toxicity have been studied extensively and are relatively-well characterized; however, the consequences of prolonged or repeated exposures to levels of OPs that produce no overt signs of acute toxicity are much less well understood. This type

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of exposure (referred to as "low level", "subacute", "subtoxic", "subclinical", and "subthreshold") has been associated with prolonged neurological deficits including impairments of cognition. For example, a recently published meta-analysis of 14 studies (selected from more than 600) which fulfilled strict statistical criteria for inclusion and data from more than 1600 participants, found an association between subthreshold OP exposures and impaired neurobehavioral function. The domains of cognition most affected included attention, working memory, executive function, visuospatial ability and visual memory (Ross et al., 2013). However, these authors commented in their discussion that it remains unclear whether the human health risks of exposure to some OPs have been underestimated or overestimated. Moreover, due to the retrospective nature of many of the relevant OP studies in humans, the variability of the testing methods used, the lack of knowledge on exposure levels, etc., the health outcomes in subjects chronically exposed to OPs, but never acutely poisoned, remain to be clearly elucidated (see reviews, Colosio et al., 2003, 2009). Prospective animal studies conducted in our laboratories and others appear to support the argument that chronic (or repeated) exposures to OPs at levels that are not associated with acute toxicity can indeed result in a variety of neurobehavioral effects, particularly cognitive deficits. For example, sustained deficits in delayed matching performance, sensorimotor gating, spatial learning and retention, recognition memory, and cognitive flexibility have been reported in association with subthreshold exposures to OPs (Bushnell et al., 1991; Terry et al., 2003, 2007, 2011, Yan et al., 2012; Terry et al., 2012). It is important to note, however, that in female rats gavaged 5 days a week for 4 weeks with the insecticide, chlorpyrifos (dose range 1–10 mg/kg/day). Maurissen et al. (2000) found no deficits in a matching-to-position task.

We (Middlemore-Risher et al., 2010) have also observed protracted impairments of sustained attention and an increase in impulsive behaviors in rats exposed to subthreshold doses of the insecticide OP, chlorpyrifos (CPF) in a 5-Choice Serial Reaction Time Task (5C-SRTT), an animal model of sustained attention (see Robbins, 2002). In the study described here, we determined the effects of repeated subthreshold exposures to the prototypical nerve agent OP diisopropylfluorophosphate (DFP), on sustained attention in rats using the 5C-SRTT. The subthreshold dose of DFP (0.5 mg/kg) was determined in a previous study (Terry et al., 2011) and operationally defined as a dose that does not produce overt signs of cholinergic toxicity (e.g., fasciculations, seizures, diarrhea, excessive urination, and salivation; see reviews, Rusyniak and Nañagas, 2004; Sungurtekin et al., 2006). DFP was originally developed by British researchers as a potential chemical warfare agent (see Saunders, 1957) and it possesses a great deal of structural homology with other highly toxic nerve agents such as sarin and soman, but is less potent (Hobbiger, 1972) and dangerous for laboratory personnel.

2. Materials and methods

2.1. Compound formulation

Rats (see Section 2.2 below) received subcutaneous injections of vehicle (Kroger® Pure Peanut Oil) or DFP, CAS 55-91-4 (Sigma-Aldrich D0879-1G Lot: 126K1306, St. Louis, MO) dissolved in vehicle over the course of a 30-day treatment period. All other chemicals were reagent grade or better and purchased from Fisher Scientific and Sigma-Aldrich.

2.2. Test subjects and drug administration

A total of 40 male albino Wistar rats (approximately 3 months old) were purchased from Harlan Sprague–Dawley, Inc., Indianapolis, IN, USA and housed individually in a temperature controlled room (25 °C), maintained on a 12:12 h (lights on at 7:00 am) with free access to water and food (Teklad Global Rodent Diet 2918, Harlan, Madison, WI, USA) during the first week. From week 2 until the end of the study animals were food restricted to approximately 85% of their age-dependent, free-feeding weights based upon Harlan Laboratories growth rate curves. This was accomplished by a daily ration of approximately 12 g of food administered per rat on weekdays in the afternoon after behavioral testing, with supplemental food administered on weekends (up to a total of approximately 20 g/day). When 24 subjects (N = 12 per test group) achieved the performance criteria (see Section 2.3.2 below) they received subcutaneous injections of vehicle or DFP, 0.5 mg/kg dissolved in vehicle in a volume of 0.7 ml/kg body weight every other day over a 30 day treatment period. The test subjects were weighed and monitored (in their home cages for a period of approximately 5 min each day) for visible cholinergic signs (diarrhea, excessive salivation or lacrimation, respiratory difficulties, muscle fasciculations) or other signs of distress throughout the study. This dosing procedure was selected based on previous studies by Terry et al. (2011), and was defined as subthreshold according to the definition provided above. Both the cholinergic sign assessments and the behavioral evaluations described below were conducted by technicians who were blinded to the treatment group.

All procedures employed during this study were reviewed and approved by the Georgia Regents University Institutional Animal Care and Use Committee and are consistent with AAALAC guidelines. Measures were taken to minimize pain and discomfort in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised 1996.

2.3. Behavioral experiments

The animals in each behavioral cohort were transferred (in their home cages) to the behavioral testing rooms each morning approximately 30 min before the beginning of experiments.

2.3.1. Test apparatus

DFP and vehicle-treated rats were evaluated using an automated 5C-SRTT described previously (Middlemore-Risher et al., 2010; Terry et al., 2012). Training and testing in the 5C-SRTT were conducted using eight ventilated, sound attenuated operant chambers (Med Associates, St. Albans, VT, USA). Each operant chamber consisted of nine nose pokes/apertures (2.5 cm wide, 4 cm deep), four of which were closed off with metal inserts thus every other nose poke was available. The apertures, arranged on a curved panel 2 cm above the floor of the chamber, were equipped with a photocell beam to detect nose pokes. Each aperture was equipped with a lamp (2.8 W) on the rear wall that could be illuminated randomly and for varying durations. Food pellets (45 mg chow pellet, BioServ, Frenchtown, NJ, USA) were delivered automatically to a magazine, located on the opposite wall to the nose pokes, that was also equipped with a light that turned on to indicate that a pellet had been dispensed. The food magazine was equidistant from all nose poke apertures. The house light remained on for the entire session unless an error or omission occurred. The apparatus was controlled using MedPC software (Med Associates, St. Albans, VT, USA).

2.3.2. 5C-SRTT training

Rats began 5C-SRTT training with the stimulus duration (SD) of 10 s, each session being 100 trials or 30 min in duration with intertrial intervals (ITI) of 5 s. An initial pellet was delivered to the magazine to facilitate the start of each session. One of the 5 nose poke apertures was illuminated randomly for 10 s after which the light was extinguished. The animal was then required to respond correctly by nose poking the previously illuminated aperture within 5 s of the light being extinguished. A correct response in the previously established time frame (5 s) resulted in a pellet being dispensed into the magazine that was simultaneously illuminated for a maximum of 5 s or until the animal retrieved the pellet. Collection of this pellet initiated the intertrial interval, a delay of 5 s, before the next trial began. An incorrect response, premature response, or failure to respond within 5 s of the light stimulus being extinguished (omission) resulted in a 5 s timeout, marked by