



Research report

Persistent behavioral alterations in rats neonatally exposed to low doses of the organophosphate pesticide, parathion

Olga A. Timofeeva^a, David Sanders^a, Kristen Seemann^a, Liwei Yang^a, Daniel Hermanson^a, Sam Regenbogen^a, Samantha Agoos^a, Anita Kallepalli^a, Anit Rastogi^a, David Braddy^a, Corinne Wells^a, Charles Perraut^a, Frederic J. Seidler^b, Theodore A. Slotkin^{a,b}, Edward D. Levin^{a,b,*}

^a Department of Psychiatry and Behavioral Sciences, Box #3412, Duke University Medical Center, Durham, NC 27710, USA

^b Department of Pharmacology and Cancer Biology, Box #3813, Duke University Medical Center, Durham, NC 27710, USA

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ABSTRACT

Although developmental exposures of rats to low levels of the organophosphate pesticides (OPs), chlorpyrifos (CPF) or diazinon (DZN), both cause persistent neurobehavioral effects, there are important differences in their neurotoxicity. The current study extended investigation to parathion (PTN), an OP that has higher systemic toxicity than either CPF or DZN. We gave PTN on postnatal days (PND) 1–4 at doses spanning the threshold for systemic toxicity (0, 0.1 or 0.2 mg/kg/day, s.c.) and performed a battery of emotional and cognitive behavioral tests in adolescence through adulthood. The higher PTN dose increased time spent on the open arms and the number of center crossings in the plus maze, indicating greater risk-taking and overall activity. This group also showed a decrease in tactile startle response without altering prepulse inhibition, indicating a blunted acute sensorimotor reaction without alteration in sensorimotor plasticity. T-maze spontaneous alternation, novelty-suppressed feeding, preference for sweetened chocolate milk, and locomotor activity were not significantly affected by neonatal PTN exposure. During radial-arm maze acquisition, rats given the lower PTN dose committed fewer errors compared to controls and displayed lower sensitivity to the amnesic effects of the NMDA receptor blocker, dizocilpine. No PTN effects were observed with regard to the sensitivity to blockade of muscarinic and nicotinic cholinergic receptors, or serotonin 5HT₂ receptors. This study shows that neonatal PTN exposure evokes long-term changes in behavior, but the effects are less severe, and in some incidences opposite in nature, to those seen earlier for CPF or DZN, findings consistent with our neurochemical studies showing different patterns of effects and less neurotoxic damage with PTN. Our results reinforce the conclusion that low dose exposure to different OPs can have quite different neurotoxic effects, obviously unconnected to their shared property as cholinesterase inhibitors.

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1. Introduction

The developmental neurotoxicity of organophosphate (OP) pesticides is of great concern because human exposures appear to be

nearly ubiquitous [8]. Although the acute, high dose toxic effects of OPs involve cholinergic hyperstimulation consequent to inhibition of acetylcholinesterase, the persisting adverse effects of low dose exposure on brain development arise from a variety of mechanisms that impact brain development, including impairments of neural cell proliferation, differentiation, axonogenesis and synaptogenesis [20,26–28]. Importantly, the neurodevelopmental effects occur at doses below the threshold for systemic toxicity or even for acetylcholinesterase inhibition, so that the outcomes can differ among the various OPs. In a series of studies, we compared the long-term neurochemical and behavioral effects after exposure of neonatal rats to chlorpyrifos (CPF) or diazinon (DZN) in doses spanning the threshold for barely detectable cholinesterase inhibition and found some similarities, but also major differences in outcome [2,13,16,17,19,21,23,33]. More recently, we have begun to examine parathion (PTN), a more systemically toxic OP that has been far less

* Corresponding author at: Department of Psychiatry and Behavioral Sciences, Box #3412, Duke University Medical Center, Durham, NC 27710, USA.

Tel.: +1 919 681 6273; fax: +1 919 681 3416.

E-mail addresses: timofeev@duke.edu (O.A. Timofeeva), david.j.sanders@duke.edu (D. Sanders), kristen.seemann@duke.edu (K. Seemann), liwei.yang@duke.edu (L. Yang), daniel.hermanson@duke.edu (D. Hermanson), sam.regenbogen@duke.edu (S. Regenbogen), samantha.agoos@duke.edu (S. Agoos), anita.kallepalli@duke.edu (A. Kallepalli), anit.rastogi@duke.edu (A. Rastogi), david.braddy@duke.edu (D. Braddy), corinne.wells@duke.edu (C. Wells), cmp11@duke.edu (C. Perraut), seidler@duke.edu (F.J. Seidler), t.slotkin@duke.edu (T.A. Slotkin), edlevin@duke.edu (E.D. Levin).

studied. Because the maximum tolerated dose of PTN is much lower than that of the other agents, we postulated that there might be less long-term neurotoxic damage from low doses, given the fact that systemic toxicity and developmental neurotoxicity involve separate mechanisms [23,28]. In particular, we found less initial neural damage and a smaller impact on developing acetylcholine and serotonin (5HT) systems.

In the current study, we evaluated the long-term cognitive and emotional effects in adolescent and adult rats exposed to PTN during the early postnatal period, using treatment paradigms and behavioral tests comparable to those in our earlier work with CPF and DZN. Our objectives were to find out whether early postnatal exposure to low doses of PTN can cause persistent behavioral impairments, and to compare the effects of PTN to those of CPF and DZN.

2. Methods

2.1. Animal subjects

All experiments were carried out in accordance with protocols approved by the Institutional Animal Care and Use Committee and in accordance with federal and state guidelines. Timed-pregnant Sprague–Dawley rats (Charles River, Raleigh, NC, USA) were housed in plastic breeding cages under a 12-h light–dark cycle and free access to food and water. On the day of birth, the postnatal day (PND) 0, all pups were randomized and redistributed to the dams with a litter size of 12 (6 males and 6 females) to maintain a standard nutritional status. Same-sexed rats from each litter were weighed in a group and given daily subcutaneous injections of 0, 0.1, or 0.2 mg/kg PTN (Chem Service, West Chester, PA, USA) on PND 1–4, a time period for peak sensitivity to OPs [17,23]. Because of its poor water solubility, PTN was dissolved in dimethylsulfoxide to provide consistent absorption [34] and was injected at a volume of 1 ml/kg once daily. Control rats received equivalent injections of the dimethylsulfoxide vehicle. There were 12 dams per treatment group. These doses span the threshold for minimally detectable cholinesterase inhibition (ca. 10%) and emergence of systemic toxicity as defined by decreased viability [23,28]. Rats were randomized within treatment groups at intervals of several days, and in addition, dams were rotated among litters every 2–3 days to distribute any maternal caretaking differences randomly across litters and treatment groups.

Pups were weaned on PND 21 and transferred to another room with a reversed dark–light cycle (lights off at 8:00 AM) and weighed at least weekly. Weaned pups were originally housed in same-sex and same-exposure groups of 6 per cage, but as they grew, they were subdivided into smaller groups in accordance with the federal guidelines. By the time of behavioral testing, the males were housed in pairs whereas the females were housed three to a cage. All behavioral testing was carried out during the dark phase, the active period for rats, but in lighted environments so that the rats could access the visual cues necessary to perform the tasks. To facilitate comparisons across OPs, the test battery was substantially the same as we have previously used to assess the persisting behavioral effects of chlorpyrifos and diazinon [2,13,16,17,19,33]. Detailed methods are given in those papers with brief descriptions given below. The tests were performed during adolescence through adulthood beginning with T-maze spontaneous alternation (PND 35–45), elevated plus maze (PND 50–53), Figure-8 locomotor activity (PND 58–61), novelty-suppressed feeding (PND 64–72), home cage feeding (PND 73–78), prepulse inhibition (PND 78–81), chocolate milk anhedonia test (PND 81–94), followed by radial-arm maze (RAM) training (PND 112–182) and drug challenges (PND 181–328). All rats were exposed to these tests in a sequential order, 31 of which (16 males and 15 females) served as controls, 36 (18 males and 18 females) were treated with low and 34 (16 males and 18 females) with high dose of PTN.

2.2. T-maze spontaneous alternation

A T-maze, constructed of black-painted wood, elevated 1 m from the floor was used. Alleys were 10 cm wide with a 65 cm long stem and 40 cm long choice arms. After 10 s in the start area the rat was permitted to roam about the maze, allowing 30 s to enter one of the arms. After a choice the rat was kept in the choice arm for 30 s and then placed back in the start area. Five trials were run during the single session. Percent of alternation between left and right arms and average response latency (time between the moment of raising the barrier in the start area and the moment when rat enters one of the arms) were calculated. The spontaneous alternation test is without explicit reward other than the motivation to explore a new area. Normally rats will alternate choices approximately 85% of the time in such a test.

2.3. Elevated plus maze

The maze was constructed of black-painted wood with arms 55 cm long and 10.2 cm wide, 50.8 cm above the floor. Two opposed arms had walls that were

15.2 cm high (closed arms), and the other two opposed arms, had railings 2 cm tall (open arms). Rats were placed in the center of the maze facing an open arm and allowed to roam freely for a total of 300 s. The time spent on the closed arms and the number of open and closed arm entries were the dependent measures.

2.4. Figure-8 locomotor activity

The maze consisted of a continuous enclosed alley 10 cm × 10 cm in the shape of Figure-8 and two blind alleys extending from either side. Eight infrared photobeams crossing the maze alleys were used to assess locomotor activity. The number of photobeam breaks was tallied in 5 min time blocks during a single 1-h session. The linear and quadratic trends across the twelve blocks were calculated to test the hypothesis that PTN exposure affects the rate of habituation. Four of such mazes were assigned for males only, and four for females.

2.5. Novelty-suppressed feeding

The methods for novelty-suppressed feeding were modified from [5–7]. Rats were food deprived for approximately 24 h prior to testing. A novel environment was created by placing a plastic rectangular cage that was slightly different from their home cage in the middle of the test room without any bedding or cage top. Each rat was tested in a clean novel cage. Twelve food pellets same as those used in their regular diet (Purina Rodent Chow Diet 5001; Ralston–Purina, St. Louis, MO, USA) and approximately 2.5 cm in length were weighed prior to testing and spread across the floor. Each rat was tested for 10 min in the novel cage, and the experimenter recorded the latency to begin eating, the number of eating bouts, the total time spent eating and the amount of food eaten.

Previous reports of novelty-suppressed feeding have evaluated home cage feeding to determine if the experimental condition alters appetite or motivation to eat [4,7]. Thus, home cage feeding was evaluated in rats 1 week following the novelty-suppressed feeding. Rats were food deprived for approximately 24 h and tested with their cage mates in their home cage within the cage bank. Twelve pellets per rat were weighed and placed in the home cage in rows for the 10 min test session, and the amount of food eaten in the home cage was determined.

2.6. Chocolate milk anhedonia test

Methods were modified from [2]. One hour after the start of the dark cycle, rats were placed in individual clean cages in the test room. The rats had *ad libitum* food and water prior to testing, but food was not available during the test session. Rats were presented with a choice of two bottles. One bottle contained tap water, and the other contained Hunter Farms Truly chocolate milk (High Point, NC, USA). The amount of fluid consumed from each bottle in the 2-h test was determined. The rats had no prior experience with drinking from bottle spouts as their home cages had an automatic watering system.

2.7. Prepulse inhibition (PPI)

Startle reflex and prepulse inhibition were measured using an adaptation of the classic acoustic method outlined by Swerdlow et al., [30–32] to assess sensorimotor gating. The currently used test had an acoustic prepulse and a tactile startle stimulus. The test apparatus was in a sound-attenuating box within which the rat was placed inside a clear Plexiglas tube on top of a force transducing response platform. The startle reflex amplitude and PPI were measured using San Diego Instruments Startle Reflex System (San Diego, CA, USA). To habituate the rats to the holding tube they were placed in the Plexiglas tube for 5–min 1–2 days before testing. There was a continuous 65 dB background white noise during the session. After a 5-min acclimation period inside the box testing began. The startle response was measured in three test blocks and was elicited by a 4 pounds per square inch (27.6 kPa) air puff to the back of the rat. The first block had 6 trials of startle alone. Block two had 12 trials of startle alone, and 36 prepulse plus startle trials, with prepulse/startle delay of 100 ms. The intertrial duration was 10–20 s with a null period of 100 ms. Trials were conducted in a random order with respect to startle and prepulse startle. There were three prepulse levels administered randomly in the prepulse startle trials (68, 71, and 77 dB pure tone). In the third block of trials, the rats were given additional trials of the startle response. The auditory and tactile stimuli had 2 ms rise/fall time.

2.8. Radial-arm maze (RAM)

During the RAM training and drug testing, rats had *ad libitum* access to water with daily feeding after testing, to maintain body weight at a lean health weight with a target of approximately 85% of free feeding level. This was determined of the weight growth in rats having free access to food and water, matching in age.

The black-painted wood maze was at an elevation of 30 cm. The central platform had a diameter of 50 cm and 16 arms (10 cm × 60 cm) projected radially outward. Each arm contained a food cup 2 cm from the distal end. The test areas contained numerous visual cues. Rats were given 2 shaping sessions in which they were placed individually in a large, opaque cylinder on the platform of the maze, given food reinforcement (halves of sugar coated cereal, Froot Loops®, Kellogg's, Battle Creek,

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