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Research report

# Prenatal dexamethasone, as used in preterm labor, worsens the impact of postnatal chlorpyrifos exposure on serotonergic pathways

Theodore A. Slotkin*, Jennifer Card, Frederic J. Seidler

*Department of Pharmacology & Cancer Biology, Duke University Medical Center, Durham, NC, USA*

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ABSTRACT

This study explores how glucocorticoids sensitize the developing brain to the organophosphate pesticide, chlorpyrifos. Pregnant rats received a standard therapeutic dose (0.2 mg/kg) of dexamethasone on gestational days 17–19; pups were given subtoxic doses of chlorpyrifos on postnatal days 1–4 (1 mg/kg, <10% cholinesterase inhibition). We evaluated serotonin (5HT) synaptic function from postnatal day 30 to day 150, assessing the expression of 5HT receptors and the 5HT transporter, along with 5HT turnover (index of presynaptic impulse activity) in brain regions encompassing all the 5HT projections and cell bodies. These parameters are known targets for neurodevelopmental effects of dexamethasone and chlorpyrifos individually. In males, chlorpyrifos evoked overall elevations in the expression of 5HT synaptic proteins, with a progressive increase from adolescence to adulthood; this effect was attenuated by prenatal dexamethasone treatment. The chlorpyrifos-induced upregulation was preceded by deficits in 5HT turnover, indicating that the receptor upregulation was an adaptive response to deficient presynaptic activity. Turnover deficiencies were magnified by dexamethasone pretreatment, worsening the functional impairment caused by chlorpyrifos. In females, chlorpyrifos-induced receptor changes reflected relative sparing of adverse effects compared to males. Nevertheless, prenatal dexamethasone still worsened the 5HT turnover deficits and reduced 5HT receptor expression in females, demonstrating the same adverse interaction. Glucocorticoids are used in 10% of U.S. pregnancies, and are also elevated in maternal stress; accordingly, our results indicate that this group represents a large subpopulation that may have heightened vulnerability to developmental neurotoxicants such as the organophosphates.



## 1. Introduction

One of the most important issues in developmental neurotoxicity is the existence of subpopulations that are especially vulnerable to environmental agents. Given the widespread exposures of the human population to thousands of neurotoxic chemicals and their likely contribution to the explosive increase in neurodevelopmental disorders (Grandjean and Landrigan, 2006), the identification of factors that render individuals more sensitive is particularly important in setting safety limits as well as in informing targeted populations to avoid exposure. The organophosphate pesticides provide a prime example: polymorphisms of the enzyme that breaks down these neurotoxicants clearly delineate a sensitive subpopulation (Povey, 2010). In contrast to the intense focus of many researchers on genetic factors that determine vulnerability, relatively little attention has been paid to the issue of how an individual's "chemical history" might create differential susceptibility, namely, whether prenatal exposures to neuroactive drugs or chemicals sensitize an individual to neurotoxicants encountered later in life. We recently conducted studies both in vitro (Slotkin et al., 2012) and in vivo (Slotkin et al., 2013), exploring the possibility of such an interaction between prenatal dexamethasone treatment, as used in the management of preterm labor (Gilstrap et al., 1995) and postnatal exposure to chlorpyrifos, one of the most commonly used organophosphates. This particular combination encompasses a relatively large subpopulation. Because glucocorticoids are the consensus treatment to prevent neonatal respiratory distress syndrome in preterm labor (Gilstrap et al., 1995), approximately 10% of all live births in the U.S. involve this treatment (Matthews et al., 2002); an even larger population is exposed to prenatal glucocorticoids endogenously from maternal stress. Superimposed on this subgroup, organophosphate exposures are virtually ubiquitous (Casida and Quistad, 2004).

In our earlier study, we showed that prenatal dexamethasone treatment sensitized developing rats to the subsequent effects of postnatal chlorpyrifos exposure on development of cholinergic neurotransmitter systems (Slotkin et al., 2013), including effects that were unique to the dual exposure (i.e. not seen with either agent alone); this did not reflect simply a change in chlorpyrifos pharmacokinetics or pharmacodynamics. Indeed, modeling the

*Abbreviations:* 5HT, 5-hydroxytryptamine, serotonin; ANOVA, analysis of variance; GD, gestational day; PN, postnatal day.

* Corresponding author. Tel.: +1 919 681 8015.
*E-mail address:* t.slotkin@duke.edu (T.A. Slotkin).

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same treatments in neuronotypic cell cultures (Slotkin et al., 2012), we found many of the same interactions, indicating that the adverse effects of the combined exposure largely reflected their convergence on common pathways that interfere with neuronal cell replication and differentiation. In the current work, we extended our observations to serotonin (5HT) systems; 5HT pathways are targeted both by chlorpyrifos and dexamethasone individually (Aldridge et al., 2003, 2004, 2005a,b; Slotkin et al., 1996, 2006a; Slotkin and Seidler, 2005, 2007b, 2008, 2010) and their effects on this neurotransmitter likely contributes to an adverse impact on emotional behaviors (Aldridge et al., 2005a; Mauro and Zhang, 2007; Nagano et al., 2008, 2012; Ricceri et al., 2003, 2006).

Our treatment models have been described earlier (Slotkin et al., 2013). First, we gave dexamethasone on gestational days (GD) 17–19, corresponding to the stage of brain development in which glucocorticoid therapy is typically given in preterm labor, using a dose (0.2 mg/kg) in the low therapeutic range (Gilstrap et al., 1995). The three-day regimen corresponds to multiple glucocorticoid courses, as used in approximately 85% of all cases (Dammann and Matthews, 2001), and the dose was chosen to produce submaximal effects to allow detection of interactions with chlorpyrifos (Kreider et al., 2005, 2006; Slotkin et al., 1996, 2006a). Chlorpyrifos was given daily on postnatal days (PN) 1–4 at a dose of 1 mg/kg, a regimen that is not systemically toxic, producing only barely-detectable inhibition of brain cholinesterase, but that nevertheless disrupts brain development (Slotkin, 1999, 2004, 2005; Song et al., 1997). This exposure model successfully predicts both the neurobehavioral deficits and abnormalities of brain structure seen in children exposed prenatally to supposedly "safe" levels of chlorpyrifos (Bouchard et al., 2011; Engel et al., 2011; Rauh et al., 2006, 2011, 2012). Our study thus encompassed four treatment paradigms: control, dexamethasone alone, chlorpyrifos alone, and dexamethasone followed by chlorpyrifos.

We conducted longitudinal evaluations of the effects on 5HT systems spanning adolescence, young adulthood and full adulthood, so as to focus on persistent alterations that are most likely to influence behavioral performance, in keeping with earlier observations that the impacts of both dexamethasone and chlorpyrifos emerge over these stages (Aldridge et al., 2003, 2004, 2005a,b; Slotkin et al., 1996, 2006a; Slotkin and Seidler, 2005, 2007b, 2008, 2010). We assessed multiple indices of 5HT synaptic function in all the brain regions comprising the major 5HT projections (frontal/parietal cortex, temporal/occipital cortex, hippocampus, striatum) as well as those containing 5HT cell bodies (midbrain, brainstem). We measured three 5HT synaptic proteins known to be highly affected by developmental exposure to both dexamethasone and chlorpyrifos (Aldridge et al., 2003, 2004, 2005a,b; Slotkin et al., 1996, 2006a; Slotkin and Seidler, 2005, 2007b, 2008, 2010), the $5HT_{1A}$ and $5HT_2$ receptors, and the presynaptic 5HT transporter. The two receptors play major roles in 5HT-related mental disorders, including depression (Arango et al., 2001; Fujita et al., 2000; Yatham et al., 1999, 2000), and the transporter, which regulates the synaptic concentration of 5HT, is the primary target for antidepressant drugs (Maes and Meltzer, 1995; Nemeroff, 1998; Nutt, 2002). Then, as an index of presynaptic impulse activity, we assessed the turnover of 5HT by measuring concentrations of 5HT and its principal metabolite, 5-hydroxyindoleacetic acid.

## 2. Materials and methods

### 2.1. Animal treatments

All experiments were carried out humanely and with regard for alleviation of suffering, with protocols approved by the Institutional Animal Care and Use Committee and in accordance with all federal and state guidelines. Timed-pregnant Sprague-Dawley rats were shipped by climate-controlled truck (total transit time < 1 h), housed individually and allowed free access to food and water; shipping occurred on GD11, so that animals had nearly a week in-house before treatments commenced. There were four treatment groups, each comprising 12–14 dams: controls (prenatal saline + postnatal dimethylsulfoxide vehicle), dexamethasone treatment alone (prenatal dexamethasone + postnatal vehicle), chlorpyrifos treatment alone (prenatal saline + postnatal chlorpyrifos), and those receiving the combined treatment (prenatal dexamethasone + postnatal chlorpyrifos). On GD17, 18 and 19, dams received subcutaneous injections of either saline vehicle or 0.2 mg/kg dexamethasone sodium phosphate, a dose at the lower range recommended for therapeutic use in preterm labor (Gilstrap et al., 1995). Parturition occurred during GD22, which was also taken as PN0. After birth, pups were randomized within treatment groups and litter sizes were culled to 10 (5 males and 5 females) to ensure standard nutrition. Control and dexamethasone-treated litters were then assigned to either the vehicle or chlorpyrifos postnatal treatment groups. Chlorpyrifos was dissolved in dimethylsulfoxide to provide consistent absorption (Whitney et al., 1995) and pups were injected subcutaneously at a dose of 1 mg/kg in a volume of 1 ml/kg once daily on postnatal days 1–4; control animals received equivalent injections of the dimethylsulfoxide vehicle. This regimen has been shown previously to produce developmental neurotoxicity, including robust effects on serotonergic systems, without eliciting growth retardation or any other signs of systemic toxicity (Aldridge et al., 2003, 2004, 2005a,b; Slotkin and Seidler, 2005). Pups were weighed, litters were re-randomized within treatment groups and dams were rotated among litters every few days to distribute differential effects of maternal caretaking equally among all litters, making sure that all the pups in a given litter were from the same treatment group to avoid the possibility that the dams might distinguish among pups with different treatments; cross-fostering, by itself, has no impact on neurochemical or behavioral effects of these treatments (Nyirenda et al., 2001). Animals were weaned on PN21.

On PN30, 60, 100 and 150, animals were decapitated and brain regions were dissected for determination of 5HT receptor and transporter binding, involving regions containing 5HT neuronal cell bodies (midbrain, brainstem) as well as synaptic projections (frontal/parietal cortex, temporal/occipital cortex, hippocampus, striatum). The two cortical regions were sectioned at the midline and the left half used for the binding determinations, whereas the right half was used for measurement of 5HT concentrations and turnover. The cerebellum, which is sparse in 5HT projections, was reserved for future studies. Tissues were frozen in liquid nitrogen and stored at −45 °C until assayed. Each treatment group comprised 12 animals at each age point, equally divided into males and females, with each final litter assignment contributing no more than one male and one female to any of the treatment groups. Assays were conducted on each individual tissue, so that each determination represented a value from the corresponding brain region of one animal.

### 2.2. 5HT receptors and transporter

All of the ligand binding methodologies used in this study have appeared in previous papers (Aldridge et al., 2004; Slotkin et al., 2006b; Slotkin and Seidler, 2005, 2007a), so only brief descriptions will be provided here. Tissues were thawed and homogenized (Polytron, Brinkmann Instruments, Westbury, NY) in ice-cold 50 mM Tris (pH 7.4), and the homogenates were sedimented at 40,000 × *g* for 15 min. The pellets were washed by resuspension (Polytron) in homogenization buffer followed by resedimentation, and were then dispersed with a homogenizer (smooth glass fitted