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Critical developmental periods for effects of low-level tobacco smoke exposure on behavioral performance

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ABSTRACT

Tobacco exposure during development leads to neurobehavioral dysfunction in children, even when exposure is limited to secondhand smoke. We have previously shown in rats that developmental exposure to tobacco smoke extract (TSE), at levels mimicking secondhand smoke, starting preconception and extending throughout gestation, evoked subsequent locomotor hyperactivity and cognitive impairment. These effects were greater than those caused by equivalent exposures to nicotine alone, implying that other agents in tobacco smoke contributed to the adverse behavioral effects. In the present study, we examined the critical developmental windows of vulnerability for these effects, restricting TSE administration (0.2 mg/kg/day nicotine equivalent, or DMSO vehicle, delivered by subcutaneously-implanted pumps) to three distinct 10 day periods: the 10 days preceding mating, the first 10 days of gestation (early gestation), or the second 10 days of gestation (late gestation). The principal behavioral effects revealed a critical developmental window of vulnerability, as well as sex selectivity. Late gestational TSE exposure significantly increased errors in the initial training on the radial-arm maze in female offspring, whereas no effects were seen in males exposed during late gestation, or with either sex in the other exposure windows. In attentional testing with the visual signal detection test, male offspring exposed to TSE during early or late gestation showed hypervigilance during low-motivating conditions. These results demonstrate that gestational TSE exposure causes persistent behavioral effects that are dependent on the developmental window in which exposure occurs. The fact that effects were seen at TSE levels modeling secondhand smoke, emphasizes the need for decreasing involuntary tobacco smoke exposure, particularly during pregnancy.

1. Introduction

Active maternal smoking during pregnancy is a clear contributor to increased risk of neurodevelopmental disorders (Pauly and Slotkin, 2008; Gaysina et al., 2013). Indeed, even lower-level exposures associated with secondhand tobacco smoke can cause persistent neurobehavioral effects (DiFranza et al., 2004; Yolton et al., 2005; Herrmann et al., 2008). These include increased externalizing behavior (Liu et al., 2013), emotional dysfunction (Bandiera et al., 2011) and impaired neuromotor development (Evlampidou et al., 2015; Yeramaneni et al., 2015). In previous studies, we showed that tobacco smoke extract (TSE) administered to rat dams at levels simulating secondhand smoke exposure, commenced in the premating period and continued throughout gestation, leads to persistent anomalies of both synaptic and behavioral function (Slotkin et al., 2015; Hall et al., 2016). Importantly, the effects of TSE were greater than those elicited by equivalent exposures to nicotine alone, indicating that other components of tobacco smoke including polycyclic aromatic hydrocarbons such as benzo[*a*]pyrene or heavy metals such as cadmium contribute significantly to the adverse outcomes.

Subsequently, we developed models to explore the impact of TSE exposure during restricted periods during the reproductive cycle: for ten days before mating; ten days during early gestation or ten days during late gestation. Rats are born at an immature state relative to humans. The first and second halves of the rat gestational period correspond to the first two trimesters of human gestation. In a neurochemical study that was the companion of the current neurobehavioral study, we found that late gestational TSE exposure caused the most

Abbreviations: ANOVA, analysis of variance; PND, postnatal day; TSE, tobacco smoke extract

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pronounced effects on synaptic development in cholinergic and serotonergic systems (Slotkin et al., 2016). More modest neural effects were seen with early gestational exposure. There were also some significant neurochemical anomalies evoked by exposure prior to mating, which may be due to a persistence of TSE chemical components well past the end of direct exposure, or alternatively, epigenetic changes in the ovum that ultimately affect brain development even when exposure terminates prior to brain formation. In the current study, we assessed the long-term behavioral effects of low-level TSE exposure during the same critical periods, focusing on tests of locomotor activity, emotional behaviors and cognitive function, and distinguishing between males and females.

2. Methods

2.1. Tobacco smoke extract

Tobacco Smoke Extract (TSE) was prepared by Arista Laboratories (Richmond, VA, USA) from Kentucky Reference cigarettes (KY3R4F) on a Rotary Smoke Machine under International Organization for Standardization (ISO) mechanical smoking conditions. The smoke condensate was collected on 92 mm filter pads, which were then extracted by shaking with undiluted dimethyl sulfoxide (DMSO) for 20 min, to obtain a solution extract of 20 mg of TSE per ml. Condensate aliquots were stored in amber vials at −80 °C until used. Two cigarettes were smoked to produce each ml of extract and the final product contained 0.8 mg/ml nicotine as determined by Arista Laboratories (Richmond, VA, USA).

2.2. Animal exposure

The study was conducted humanely with the protocols approved by the Duke University Animal Care and Use Committee and were in accordance with the federal and state guidelines. Sprague-Dawley rats were purchased from Charles River Laboratories (Raleigh, NC, USA) and were shipped by climate-controlled truck (transportation time < 1 h). They were allowed to adjust to the laboratory housing facility for at least two weeks before the study commenced and were kept on a reverse 12:12 h light/dark schedule. The rats had free access to water and food except when food was restricted for food-motivated tests (see below). Behavioral testing took place during the dark (active) part of the cycle.

Treatments were given via iPrecio® microinfusion pumps (Primetech, Inc., Tokyo, Japan), implanted subcutaneously. These pumps are refillable via a percutaneous septum, so that a single surgery and pump implant could be used for delivery of different treatments in sequence. Animals were anesthetized (60 mg/kg ketamine + 0.15-0.50 mg/kg dexmedetomidine given i.p; followed post-implant by 0.15 mg/kg atipamezole + 5 mg/kg ketoprofen given s.c. and topical bupivacaine). There were four treatment groups, each comprising 12–13 dams: (1) controls received DMSO vehicle throughout the entire 30-day treatment period; (2) the TSE premating group received TSE for the first 10 days, followed by DMSO for the remaining 20 days; (3) the TSE early gestation group received DMSO for the first 10 days, then TSE for the next 10 days, followed by DMSO for the last 10 days; and (4) the TSE late gestation group received DMSO for the first 20 days, then TSE for 10 days. To maintain a constant dose level equivalent to 0.2 mg/kg/day of nicotine, the pump flow rate was adjusted upward to compensate for weight gain during pregnancy. At the end of the first 10-day infusion period, mating was initiated by including a male rat in the cage for 4 days, after which the pregnant dams were placed in breeding cages. Because conception could have taken place at any point during the mating period, the actual gestational exposure periods have an uncertainty of a few days; using parturition dates as a benchmark, the values ranged as follows: TSE premating group, begun 10–13 days prior to fertilization and terminating between 0–3 days pre-fertilization; TSE early gestation group, begun between 1–4 days pre-fertilization and terminating between gestational days 6–9; TSE late gestation group, begun between 7–9 days of gestation and terminating between days 17–19 of gestation. We previously established the bioequivalence of nicotine delivered in TSE as compared to nicotine alone (Slotkin et al., 2015).

The birth date was designated postnatal day (PN) 0, at which point litters were culled to 8–10 pups to ensure standard nutrition. The culled litters were as nearly sex balanced as possible. Weaning occurred on PN21. Animals underwent behavioral testing, in seven separate cohorts, with each treatment group represented within each cohort.

2.3. Behavioral test battery

At weaning (PN21), one male and one female were chosen from each litter in each exposure group to undergo behavioral tests. There were 11–13 litters in each condition with one male and one female tested from each litter. Males and females were housed in same-sex cages with three to four animals in each cage and had free access to food until testing began for the radial-arm maze and the operant visual signal detection task. The rats were also briefly food-deprived for 24 h before novelty suppressed feeding tests. Behavioral testing began when the rats were four weeks of age and continued on a week-to-week basis into full adulthood as described below.

The behavioral test battery included tests of locomotor activity, emotional function and cognition. This is a similar test battery to the one we used in our previous study of TSE and nicotine exposure throughout gestation (Hall et al., 2016). Tests were administered with at least three days between successive tests to minimize carryover effects. All rats from all treatment groups went through the same battery in the same test order.

2.4. Week 4: elevated plus maze

Animals were tested on the elevated plus maze (Med Associates, St Albans, VT, USA) to assess their anxiety-like behavior vs. risk-taking behavior. The maze measured 142 cm x 104 cm x 76 cm high and consisted of two arms with 15 cm high enclosed walls and two open arms with low 2 cm railings. Each rat was assessed individually on the elevated plus maze for a single five-min session. The percent time the rat spent in the open vs. enclosed arms of the maze was calculated, as well as the number of crossings across the center. Arm entries were defined as all four paws crossing the arm threshold of the maze. The dependent measures were percent of time in the open arms to index anxiety-like behavior and the number of center crossings to measure locomotion in this five-min test.

2.5. Week 5: Figure-8 apparatus test of locomotor activity

Locomotor activity was assessed in an enclosed maze in the shape of a figure-8. The Figure-8 apparatus consisted of a continuous alley that measured 10 cm x 10 cm, with the entire maze measuring 70 cm x 42 cm. Animals were allowed to freely explore the apparatus, and locomotor activity was assessed by the crossing of eight photo-beams located at equal points in the alley. Each locomotor test session lasted 1 h, and photo-beam breaks were tallied in 5 min blocks across the one-hour test session. The mean number of photobeam breaks per five-minute time block within the session indexed locomotor activity, while the linear trend of decreasing beam breaks over the twelve sequential time blocks within the session indexed the habituation of activity with experience in the apparatus over the one-hour test session.

2.6. Week 6: novelty suppressed feeding

To assess fear responsivity, the offspring rats were tested for suppression of feeding in a novel environment. The rats had food restricted