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Prenatal cigarette smoke exposure causes hyperactivity and aggressive behavior: Role of altered catecholamines and BDNF

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ABSTRACT

Smoking during pregnancy is associated with a variety of untoward effects on the offspring. However, recent epidemiological studies have brought into question whether the association between neurobehavioral deficits and maternal smoking is causal. We utilized an animal model of maternal smoking to determine the effects of prenatal cigarette smoke (CS) exposure on neurobehavioral development. Pregnant mice were exposed to either filtered air or mainstream CS from gestation day (GD) 4 to parturition for 4 h/d and 5 d/wk, with each exposure producing maternal plasma concentration of cotinine equivalent to smoking <1 pack of cigarettes per day (25 ng/ml plasma cotinine level). Pups were weaned at postnatal day (PND) 21 and behavior was assessed at 4 weeks of age and again at 4–6 months of age. Male, but not female, offspring of CS-exposed dams demonstrated a significant increase in locomotor activity during adolescence and adulthood that was ameliorated by methylphenidate treatment. Additionally, male offspring exhibited increased aggression, as evidenced by decreased latency to attack and number of attacks in a resident–intruder task. These behavioral abnormalities were accompanied by a significant decrease in striatal and cortical dopamine and serotonin and a significant reduction in brain-derived neurotrophic factor (BDNF) mRNA and protein. Taken in concert, these data demonstrate that prenatal exposure to CS produces behavioral alterations in mice that are similar to those observed in epidemiological studies linking maternal smoking to neurodevelopmental disorders. Further, these data also suggest a role for monaminergic and BDNF alterations in these effects.

Introduction

Despite many public health programs promoting the risks of smoking while pregnant, only 25% of women who smoke quit smoking cigarettes during pregnancy. Several reports established that maternal smoking during pregnancy adversely affects pre- and post-natal growth and increases the risk of fetal mortality, pre-term birth, low birth weight, and altered cognitive development (Wigle et al., 2008). Importantly, the adverse effects of maternal smoking on neurodevelopment persist through at least the adolescent period and may extend into adulthood (Keyes et al., 2011).

Although a large number of epidemiological studies reporting increased neurobehavioral deficits following maternal smoking exist, recent reports questioned their validity. The most notable regards the association between maternal smoking and attention-deficit hyperactivity disorder (ADHD). Several groups linked maternal smoking to an increased risk of ADHD (Banerjee et al., 2007; Langley et al., 2005; Linnet et al., 2003). In one of the first studies to directly assess the relationship between maternal smoking and ADHD, Milberger et al. (1998) found a 2.7-fold increased risk for ADHD associated with maternal smoking, a finding that has been replicated by several groups in a variety of cohort studies (Leech et al., 1999; Linnet et al., 2005; Obel et al., 2009; Thapar et al., 2003; Weissman et al., 1999). Using a cross-sectional study design from the National Health and Nutrition Examination Survey, Braun and co-workers found that pre- but not post-natal exposure to tobacco smoke led to an increased risk of ADHD diagnosis and the authors calculated that maternal smoking led to 270,000 excess cases of ADHD (Braun et al., 2006). This finding was confirmed even when more stringent criteria were applied (Froehlich et al., 2009).

However, several recent papers concluded that there is no causal relationship between maternal smoking and behavioral dysfunction, or that the effect sizes in previous studies were over-estimated (Ball et al., 2010; Langley et al., 2012; Lindblad and Hjern, 2010; Obel et al., 2011; Thapar et al., 2009). Rather, these studies argued that the

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association between maternal smoking and these effects is likely the result of a variety of confounders including genetic factors, other environmental factors, and/or existing maternal psychopathology that were not satisfactorily adjusted for in the previous studies.

To address this controversy and to gain insight into the mechanism(s) responsible for potential neurobehavioral effects of maternal smoking, we employed a pregnant mouse model of maternal smoking. Data demonstrate that *in utero* exposure to cigarette smoke (CS; at a concentration reflective of smoking <1 pack of cigarettes per day) produces behavioral alterations in the offspring (specifically, the males) that are similar to those observed in epidemiological studies of maternal smoking, including hyperactivity and increased aggression. Mechanistically, these behavioral deficits are associated with decreased monoamine levels and brain-derived neurotrophic factor mRNA and protein. These data provide mechanistic support for the reported link between *in utero* CS exposure and behavioral deficits in the epidemiological literature.

Materials and methods

Animals

B6C3F1 male and female mice were purchased from Jackson Laboratory (Bar Harbor, ME). These mice were chosen based on previous experiments characterizing developmental outcomes of CS exposure (Ng et al., 2006). Mice were housed in pairs (females) or individually (males) in polycarbonate cages (with corncob bedding) in temperature-controlled (20 °C–23 °C) and humidity-controlled (~55% RH) rooms. Food (purified AIN-98) and tap water were available *ad libitum*. The light/dark cycle was maintained on 12 h intervals. Mice were acclimated for at least 1 week prior to use. For breeding, a single male mouse was paired with two females for four days, with the 4th day of coupling designated gestation day (GD) 4. All animal procedures were conducted under an animal protocol approved by the New York University and Robert Wood Johnson Medical School's (RWJMS) Institutional Animal Care and Use Committee (IACUC).

Smoke generation

Mainstream cigarette smoke was generated from the burning of filtered 1R3F cigarettes (Kentucky Tobacco Research & Development Center, Lexington, KY) using an automated cigarette generation system (Baumgartner-Jaeger CSM 2070, CH Technologies [USA] Inc., Westwood, NJ). Reference cigarettes were stored long-term at 4 °C–7 °C (55% RH); 24 h prior to use, cigarettes were relocated to a humidor and stored at 20 °C–23 °C (55% RH). The continuous smoking machine was adjusted to load and light 4–5 cigarettes simultaneously, each of which produced 2-s puffs of 35 ml volume/puff under the control of an automatically regulated piston pump that cycled once per minute (Ng et al., 2006). Smoke was diluted 90% prior to introduction into the exposure chamber. Filtered dilution air entered the bottom of the generation chamber, and the output was introduced into the top of the chamber.

Cigarette smoke exposure

Males were removed and 2 females per cage were exposed whole-body to either mainstream cigarette smoke (CS) or filtered air in polycarbonate cages with wire mesh tops. Cages were rotated among three racks in the chamber to assure even smoke distribution to all animals. Chamber levels of carbon monoxide (CO) and total suspended particulates (TSP) were monitored throughout the exposure. Particle samples were collected from the exposure chambers every hour (for the entire duration of exposure) on Pallflex EMfab filters (Pall Corporation, East Hills, NY); mean TSP levels were determined gravimetrically from filters weighed before and after sampling. Chamber CO levels were measured continually over the entire 4-h exposure period suing a 48C CO analyzer (Thermo Environmental Instruments, Inc., Franklin, MA). This exposure paradigm produces plasma cotinine levels in the dams of approximately 25–28 ng/ml (Ng et al., 2006), equivalent to smoking 1–7 cigarettes per day, or less than 1 pack (Peacock et al., 1998).

On GD18, dams were separated and housed individually and each mother/offspring set was maintained in clean filtered air following parturition. Pups were weaned at 3 weeks of age and group housed based on sex with no more than 5 mice per cage. Early behavioral parameters (social interaction, play, and initial motor activity determinations) were determined at 4 weeks of age, as described below. Following these determinations, mice were shipped to RWJMS and allowed to acclimate for 2 months before additional behavioral testing was conducted. All subsequent behavioral analysis and sacrifices for neurochemical determinations were conducted during the light phase.

Social interaction and play behaviors

Pups were individually housed 4–5 days prior to the social behavioral test session. Pairs of non-sibling, same-sex and same-treatment condition pups were placed in a standard large cage crossed by infrared beams and observed for social interactions at 4 weeks of age for one 30 min session and scored by two trained observers for the number of times that a member of the pair engaged in a behavior. During testing, the behaviors observed were: ano-genital sniffs, face sniffs, crawl-under/over behaviors, self-grooming, fighting behaviors, paired motor activity, and allogrooming. Allogroom behaviors are defined as one mouse rising up on its hind legs to touch paws and snout to the other mouse to perform grooming motions. For more extensive information on testing conditions please refer to Yochum et al. (2008).

Locomotor activity

For studies in 4-week old mice, the activity chamber consisted of a standard large cage crossed by infrared beams (Opto-Varimex, Columbus OH), as described previously (Sheleg et al., 2013). The number of horizontal motor movements was calculated by infrared beam breaks for a 30 min session. For studies in adults, 4-month old mice were placed in sound-attenuated boxes equipped with photobeams (Med Associates, St. Albans, VT). Locomotor activity was quantified over 1–2 h, with the first 30 min considered a habituation period, and calculated over 5 min blocks and summed across the session. For experiments with methylphenidate, mice were habituated to the open field by 4 daily 60 min sessions. On the fifth day, mice were gavaged with saline after a 30 min habituation period in the locomotor box, and locomotor activity was monitored for an additional 60 min. On the sixth day, mice were gavaged with methylphenidate (1 mg/kg) after the 30 min habituation period and locomotor activity was monitored for an additional 60 min. Locomotor activity was calculated as total distance traveled in cm.

Resident–intruder task

Residents were adult male mice (4 months old) that were individually housed two weeks prior to testing. Intruders were age-matched adult males of the same treatment as the resident. An intruder was introduced into the home cage of the resident for a 10-min session. The latency for the first attack, the number of attacks and which mouse attacked (either the resident or the intruder) were recorded.

HPLC analysis

HPLC analysis was performed as described previously (Bradner et al., 2013; Schuh et al., 2009). Briefly, samples were sonicated in