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Prenatal nicotine exposure increases anxiety and modifies sensorimotor integration behaviors in adult female mice

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ABSTRACT

Prenatal exposure to nicotine (PNE) has been associated with a myriad of physiological, cognitive, and behavioral effects in the developing offspring. In this study, CD-1 dams were given injections of nicotine or control vehicle throughout gestation and their offspring were raised to 6 months of age. Adult mice were administered a battery of behavioral tests (the Suok test, the elevated platform test, and the elevated plus maze test) to assess anxiety and sensorimotor integration. PNE resulted in a decreased likelihood of jumping during the elevated platform test and decreased directed exploration in the Suok test, both indicative of increased anxiety. Also, PNE mice showed increased numbers of missteps while traversing an elevated rod in the Suok test, demonstrating altered sensorimotor integration. No significant differences were found in falls, segments traveled, latency to leave the central zone, vegetative responses, risk assessment behaviors, or autogroom behaviors. The elevated plus maze test revealed no significant differences between groups. No significant differences in body and brain weights, or cortical thickness within motor, somatosensory, and visual cortices were observed between PNE and control mice. Although neuroanatomical effects of PNE may be rescued as development progresses, defects in sensorimotor integration and increased anxiety persist into adulthood.

1. Introduction

Maternal smoking and the use of nicotine replacement therapies during pregnancy remain a concern, despite health advisories implicating nicotine as a dangerous neuroteratogen. Nicotine readily crosses the placental barrier during pregnancy and has been shown to result in numerous physiological, cognitive, and behavioral abnormalities (Luck et al., 1985; Ernst et al., 2001; Wickstrom, 2007). Maternal smoking has been associated with pregnancy complications as well as physical abnormalities, such as low birth weights and small head circumferences in the offspring (Ernst et al., 2001; George et al., 2006; Jaddoe et al., 2007; Ward et al., 2007).

Newborns born to smoking mothers are more excitable and show more signs of stress during the neonatal period (Law et al., 2003). Additionally, children whose mothers have smoked are more likely to have deficits in attention, memory, and overall cognitive function (Fried et al., 1992). Sensorimotor deficits have also been reported in infants exposed to tobacco, particularly in responsiveness to auditory stimuli (Saxton, 1978; Picone et al., 1982; Gusella and Fried, 1984), a finding that is mirrored in research with rodents (Ajarem and Ahmad, 1998). Increased incidences of attention-deficit/hyperactivity disorder (ADHD) have been documented in children whose mothers smoked during pregnancy, and smoking mothers are four times as likely to have a child with conduct disorder (Weissman et al., 1999; Thapar et al., 2003). These children also seem to be more at risk for nicotine dependence and other psychiatric disorders later on in life (Fergusson et al., 1998; Wickstrom, 2007). Studies in animal models have demonstrated physiological and behavioral effects that are consistent with human studies (Dwyer et al., 2009).

The developing brain is far more susceptible than the adult brain to the deleterious effects of toxins (Rodier, 1995; Rice and Barone, 2000). Nicotine activates and desensitizes nicotinic acetylcholine receptors (nAChRs) throughout the brain and is thought to exert its effects by targeting cholinergic systems during brain development (Navarro et al., 1989; Dani, 2001). We have reported previously that PNE impacts brain size at birth, resulting in a reduced cortical length (Santiago and Huffman, 2012). Because the neocortex is the part of the brain responsible for higher cognitive function, we hypothesized that PNE induced alterations in cortical development may underlie the behavioral phenotypes observed in children exposed to nicotine during the prenatal period. Based on this and our previous research, we hypothesized that prenatal nicotine exposure (PNE) impacts normal development of the sensory and motor cortices, leading to abnormal behavior and increased anxiety. We further predicted that subtle changes in neocortical thickness and

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anatomy would be detectable in adulthood and that these abnormalities would be correlated with deficits in sensorimotor behavior and increased anxiety.

The current study used a CD-1 mouse model to investigate the long-term anatomical and behavioral effects of prenatal nicotine expose (PNE) in 6-month old female mice. Control and experimental animals were subjected to behavioral assays of sensory and motor function as well as anxiety. Body weights, brain weights, and cortical sizes were recorded, and thicknesses of sensory and motor cortices were measured. Although much is known about the impact of PNE on early development and adolescent outcomes, little research has examined consequences of maternal smoking and PNE that persist well into adulthood. This study confirms the behavioral teratogenicity of nicotine in a mouse model.

2. Materials and methods

To test our hypotheses, profiles of anxiety and sensorimotor integration in adult PNE and control mice were assessed through the use of behavioral assays, namely, the Suok test, the elevated plus maze test, and the elevated platform test which gauge the animals' anxiety levels as well as their ability to integrate sensory inputs with motor outputs in order to maintain balance (Wang et al., 2002; Wozniak et al., 2004; Kalueff et al., 2008). After behavioral testing, animals were weighed and sacrificed, and their brains were removed. Neuroanatomical measures of brain weight, cortical width and length, and cortical thickness of motor, somatosensory, and visual cortices were measured.

2.1. Mouse colony

All breeding and experimental studies were conducted in strict accordance with protocol guidelines approved by the Institutional Animal Care and Use Committee (IACUC) at the University of California, Riverside. Experimental and control mouse pups were bred from timed pregnant mice dams from a CD1 colony originally purchased from Charles River. Mice were housed in a standard cage, with 12–12 h light–dark cycles and given ad libitum access to water and standard chow. After pairing and confirmation of pregnancy (noon on the day of cervical plug visualization was set as gestational day (GD) 0.5), each male was removed from the cage. All timed-pregnant female mice were housed individually and divided into experimental (nicotine treated) and control groups. For staging of pups, day of birth was considered P0. Control and experimental female pups were weaned at P21 and housed with same-sex litter mates until they reached 6 months of age, at which point they underwent behavioral tests and sacrifice.

2.2. Dosage and nicotine administration

Doses of 1.5–2 mg/kg/day of nicotine in rats are sufficient in producing neurochemical changes in the brain and have been shown to result in plasma nicotine levels similar to humans who smoke one pack of cigarettes a day (Murrin et al., 1987; Fung and Lau, 1989; Levin et al., 1996). Given the difference in human versus rodent pharmacodynamics, higher dosages in rodent models may be needed to mimic effects in humans (Barnes and Eltherington, 1973). Thus, previous studies have typically used dosages of 2–6 mg/kg/day to mimic the blood plasma levels of nicotine in moderate and heavy smokers (Lichtensteiger et al., 1988; Navarro et al., 1988; Levin et al., 1993; Shacka and Robinson, 1998; Eppolito et al., 2010).

Nicotine was administered via injection due to concerns about the palatability of nicotine, which has been shown to be aversive to rodents (Murrin et al., 1987; Le Houezec et al., 1989). 99% Free-base nicotine in a solution of 0.9% physiologic saline was administered to experimental dams via subcutaneous (SC) injection at a volume of 10 μL/g. These mice were injected twice daily with 2 mg/kg free base nicotine, yielding a total dose of 4 mg/kg/day administered throughout gestation from GD 0.5 until P0. Control dams were given sham SC injections of sterile 0.9% physiological saline twice daily, at a volume of 10 μL/g, to control for stress induced by handling and injection. Morning injections were given from 9:00 to 10:00 AM and afternoon injections were given from 4:00 to 5:00 PM. This schedule of administration is in accordance with a CD-1 PNE model used previously in our laboratory (Santiago and Huffman, 2012).

2.3. Behavioral tests

Behavioral tests in animal models are an invaluable tool to the diagnosis, understanding, and treatment of developmental deficits resulting from prenatal exposure to nicotine. At six months of age, animals underwent behavioral testing to investigate the impact of prenatal nicotine on sensory and motor function and anxiety. A sample size of 8–10 animals per group from four litters was used. All behavioral testing took place between 10:00 AM and 1:00 PM. After acclimating to the dimly lit behavioral testing room for 1 h, each mouse in turn was subjected first to the Suok test, then to the elevated plus maze, and the lastly to the elevated platform test.

2.3.1. The Suok test

The Souk apparatus was constructed in accordance with the specifications published in *Nature Protocols* (Kalueff et al., 2008) and consisted of an aluminum rod, 2-m in length by 2 in. in diameter, suspended between two vertical squares of plexiglass (50 cm × 50 cm). The rod is separated into two lengths of several 10-cm long segments on either side of a 20-cm long central zone (all marked with colored tape on the underside of the rod). The novel Suok test measures exploration, risk assessment behaviors, and sensorimotor integration; it was developed to target behavioral abnormalities that arise from pathways mediating anxiety and vestibular function. Behaviors are scored as the animal walks along a 2 m-long rod for 5 min. Horizontal beams have previously been used in behavioral tests profiling balance and vestibular proficiency (Paffenholz et al., 2004; Lalonde et al., 2005; Stanley et al., 2005). Similarly, the Souk makes use of a slippery, aluminum rod to pinpoint deficits in sensorimotor integration. Concurrent measurements of reduced exploration are indicative of increased anxiety (Pellow et al., 1985; Crawley, 1999; Walf and Frye, 2007). The Souk test is a novel test that targets stress or anxiety-evoked alterations in sensorimotor integration. Given that individuals with PNE are characterized by depression/anxiety disorders (Sobrian et al., 2003), the Suok presents itself as an appropriate behavioral assay to relate animal behavior correlates to human phenotypes.

At the start of each 5-min testing period, animals were placed on the central zone with their snouts facing either end of the rod. Mice that fell off the rod were quickly repositioned on the apparatus in the same position and location. Several measures of behavior were observed and scored: (1) sensorimotor ability, as measured by number of missteps and falls from the rod, (2) locomotor activity, as assessed by number of segments traveled, (3) exploration activity, which includes directed exploration, latency to leave the central zone, risk assessment behaviors (full-body sniffing stretches; RABs), (4) vegetative responses (number of urinations and defecations), and (5) autogrooming behaviors. Differences between the two groups were analyzed with the use of independent samples *t*-tests.

2.3.2. The elevated plus maze test

The elevated plus maze has been validated as a useful technique for assessing anxiety related behaviors, relying on the animals' unconditioned fear of heights and inclination toward dark enclosed