Neuroscience Research 73 (2012) 282–291

Contents lists available at SciVerse ScienceDirect

Neuroscience Research

journal homepage: www.elsevier.com/locate/neures

Postnatal effects of prenatal nicotine exposure on body weight, brain size and cortical connectivity in mice

Sarah E. Santiago, Kelly J. Huffman*

Department of Psychology and Interdepartmental Neuroscience Program, University of California, Riverside, 900 University Ave., Riverside, CA 92521, USA

ARTICLE INFO

Article history:
Received 7 November 2011
Received in revised form 13 April 2012
Accepted 14 May 2012
Available online 22 May 2012

Keywords:
Intraneocortical connections
Thalamocortical connections
Neocortical development
Birth weight
Brain weight
Brain length
CD-1 mice

ABSTRACT

Maternal smoking results in myriad physical, cognitive, and behavioral effects in offspring due to prenatal exposure to nicotine. As the mammalian neocortex coordinates sensory integration and higher-order processes including cognition and behavioral regulation, it follows that cognitive and behavioral phenotypes of prenatal nicotine exposure (PNE) may correlate with, or stem from changes in anatomy and physiology of the neocortex. The current study uses a prenatal nicotine mouse model to determine effects of PNE on body weight, brain weight, brain length and development of neocortical circuitry, including thalamocortical afferents (TCAs) and intraneocortical connections (INCs). Although dam nutrition, dam weight gain and litter size were not significantly affected by nicotine treatment, PNE resulted in lower newborn birth weight, brain weight and length. Interestingly, the reduction of body weight, brain weight, and brain length observed in newborn PNE mice compared to control mice was no longer present at postnatal day (P) 10. A morphological study of somatosensory and visual TCAs and INCs shows no major defects in areal patterning of these connections. These data add to a growing body of literature on the neurobiological effects of PNE by providing new information on the time course of PNE-related change in the postnatal brain.

1. Introduction

Despite widespread warnings concerning the teratogenic effects of tobacco use during pregnancy, an estimated 15.1% of all pregnant women in the US smoke, with nearly 2% smoking more than two packs a day (Perreira and Cortes, 2006). Smoking or using tobacco products while pregnant results in embryonic and fetal exposure to nicotine which has been demonstrated to result in pregnancy complications and physical abnormalities in offspring (Luck et al., 1985; Ernst et al., 2001; George et al., 2006b; Jaddoe et al., 2007; Ward et al., 2007), such as spontaneous abortions, smaller head circumferences, and lower birth weights in a dose dependent manner (Kline et al., 1977; Naeye, 1981; Bassi et al., 1984; Day et al., 1992; George et al., 2006a; Rivkin et al., 2008; Salmasi et al., 2010). Additionally, prenatal nicotine and tobacco exposure has been associated with cognitive and behavioral problems in childhood (Luck et al., 1985; Ernst et al., 2001; Linnet et al., 2003; Thapar et al., 2003; Maughan et al., 2004). Children whose mothers smoked during pregnancy have dose-dependent deficits in sustained attention, memory, response inhibition, receptive listening, and overall cognitive function (Fried et al., 1992). Maternal smoking is associated with increased incidence of attention-deficit hyperactivity disorder (ADHD) and sensori-motor deficits, and smoking mothers are four times more likely to have a child with conduct disorder (Saxton, 1978; Gusella and Fried, 1984; Weissman et al., 1999; Thapar et al., 2003). Similarly, rodents prenatally exposed to nicotine display deficits in attention and memory function (Peters and Ngan, 1982; Sorenson et al., 1991; Levin et al., 1993).

Nicotine acts on nicotinic acetylcholine receptors (nAChRs) that are distributed throughout the brain and the nervous system. Subtypes of these receptors can be seen early in the gestational period and are differentially distributed across the course of development, suggesting a putative role of nAChRs in brain development (Zhang et al., 1990; Nordberg et al., 1991; Hellstrom-Lindahl et al., 1998; Whiteaker et al., 1999; Atluri et al., 2001). Prenatal nicotine exposure has been shown to disrupt neuronal maturation during development, even at a dose that does not affect maternal health or neonatal growth (Navarro et al., 1989). Nicotine is thought to have a generally inhibitory effect in the development of the central nervous system (Torrao and Britto, 2002), perhaps through the modulation of neurotransmitter systems which have been shown to play a role in developmental growth by promoting or blocking neurite outgrowth (Lipton and Kater, 1989; Navarro et al., 1990; Seidler et al., 1992). Despite our understanding of mechanisms that underlie disruption of neural development, details of specific

* Corresponding author. Tel.: +1 951 827 4805; fax: +1 951 827 3985.
E-mail address: kelly.huffman@ucr.edu (K.J. Huffman).

0168-0102/$ – see front matter © 2012 Elsevier Ireland Ltd and the Japan Neuroscience Society. All rights reserved.
http://dx.doi.org/10.1016/j.neures.2012.05.005

anatomical changes that result from prenatal nicotine exposure are not completely known.

The neocortex is organized into anatomically and functionally distinct areas that are intricately connected via intraneocortical connections, or INCs. These connections, along with thalamocortical afferents (TCAs), form a complex circuit that is critical for normal sensory processing, sensori-motor integration, and proper cognitive and behavioral development. Disruptions in INC formation may underlie phenotypic aspects of certain developmental disorders. For example, our laboratory has found that prenatal ethanol exposure leads to disruptions in INC patterning in a murine model of FASD (El Shawa and Huffman, 2011). Given the ubiquity of cognitive–behavioral deficits observed in children who were exposed to nicotine during gestation, similar disruptions in the developing cortex might be present in mice prenatally exposed to nicotine. Specifically, we hypothesized that prenatal exposure to nicotine would generate an overall reduction in size of the developing brain and cause aberrant development of INCs, and possibly TCAs, resulting from exogenous activation of nAChRs during early development.

To test our hypotheses, a prenatal nicotine exposure (PNE) mouse model was created by injecting experimental timed-pregnant CD1 mouse dams with nicotine throughout the gestational period. Our laboratory has recently completed a thorough lifespan analysis of INC development in CD1 mice, and thus chose this strain for our model (Dye et al., 2011a,b). In order to assess anatomical changes induced by PNE, we compared body weights, brain weights and brain lengths as well as patterning of thalamocortical (TCA) and intraneocortical (INC) sensory connections of control and PNE mice on the day of birth, termed postnatal day (P) 0. We compared body weight, brain weight, and brain length measures at additional time points throughout life (P10, 20, and 50) across groups to determine whether changes observed at birth persisted through adulthood. In all, our study contributes to a growing body of literature of the postnatal effects of prenatal nicotine exposure on brain and neocortical development.

2. Materials and methods

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. All mice were housed in a standard cage, with 12 h–12 h light–dark cycle and given ad libitum access to standard chow and water. 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, weight-matched and divided into 2 groups, experimental (nicotine treated) and control. For staging of pups, day of birth was considered P0.

2.2. Dam nicotine administration

Previous studies have described changes in offspring birth weight as a result of prenatal nicotine exposure, to free base nicotine, tobacco, and cigarette smoke, via dam treatment (Kleinman and Madans, 1985; Roy and Sabherwal, 1994; Cutler et al., 1996; Windham et al., 2000; LeSage et al., 2006; Jaddoe et al., 2007). In our current study, by isolating the addictive component of nicotine from the wide spectrum of additives found in tobacco, we sought to examine some specific neurobiological effects of nicotine exposure on murine development.

2.2.1. Nicotine dosage and injection methods

In previous experiments with rats, a dose of 2 mg/kg/day was used to mimic the blood plasma levels of nicotine in smokers (Levin et al., 1993, 1996; Shacka and Robinson, 1998; Eppolito et al., 2010). Specifically, previous data in rats indicate that a dose of 1.5 mg/kg/day results in plasma nicotine levels comparable to those of humans who smoke one pack of cigarettes per day. This dose has been found to be sufficient in producing neurochemical changes in the brain (Fung and Lau, 1989; Levin et al., 1993).

We administered nicotine via injection, as oral administration of nicotine has been shown to be aversive (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. In order to eliminate the confounding effect of stress induced by handling and injection, control dams were given sham SC injections of sterile 0.9% physiological saline twice daily, at a volume of 10 μL/g. All morning injections were given from 9:00 to 10:00 AM and afternoon injections were given from 4:00 to 5:00 PM. Nicotine injection is one method of dam administration that has been used successfully by other researchers (Slotkin et al., 1987a; Roy and Sabherwal, 1994, 1998; Ajarem and Ahmad, 1998; Bruin et al., 2007; Feng et al., 2010).

2.3. Dam measurement techniques

2.3.1. Daily weight gain, food, and water intake

Average daily maternal weight gain was calculated from weight measures recorded each morning throughout gestation. For each dam, an average of her daily maternal weight gain values from GD 0.5 to GD 17.5 was calculated. The mean of all *average daily maternal weight gain values* of injected-control dams was compared to the mean of all *average daily maternal weight gain values* of nicotine-treated dams by way of an independent samples *t*-test.

Food weights were recorded daily from GD 0.5 until birth. For each dam, an average of her daily food intake values from days GD 0.5 to 17.5 was calculated. The mean of all *average daily food intake values* of injected-control dams was compared to the mean of all *average daily food intake values* of nicotine-treated dams by way of an independent samples *t*-test.

On day GD 0.5, calibrated water bottles containing 400 mL of water were placed in cages of experimental and control dams. Daily water levels were assessed via graduated marks on the bottles. For each dam, and average of her daily water intake values from days GD 0.5 to 17.5 was calculated. The mean of all *average daily water intake values* of injected-control dams was compared to the mean of all *average daily water intake values* of nicotine-treated dams by way of an independent samples *t*-test.

2.3.2. Litter size

The number of pups in a litter for each experimental and control dam were counted and recorded on P0. Average litter size born to experimental and control dams was analyzed with an independent samples *t*-test.

2.4. Offspring tissue preparation

2.4.1. P0 mice

Newborn PNE (from nicotine-treated dams) and control (from injected-control dams) pups were first weighed, euthanized with pentobarbitol (100 mg/kg, intraperitoneal (IP)) and transcardially perfused with 4% paraformaldehyde in 0.1 M phosphate buffer (PFA), pH 7.4. All P0 brains were removed from the skulls, and