



Review Article

Cigarettes and the developing brain: Picturing nicotine as a neuroteratogen using clinical and preclinical studies

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ABSTRACT

Prenatal cigarette smoking exposure is not an uncommon phenomenon despite adverse publicity emphasizing its dangers. As nicotine stimulates nicotinic acetylcholine receptors, it is believed that this may disrupt the maturation of the developing brain. Several lines of evidence have accumulated to indicate that tobacco-related neurobehavioral impacts are by no means negligible. From human studies, various neuropsychiatric domains, including infant temperament, attention, externalizing behaviors, and higher cortical functions, have been examined. Although most studies have come out against smoking during pregnancy, a few studies have pointed to the fact that the epiphenomenon of smoking, rather than nicotine itself, is actually responsible for the neurobehavioral deficits or problems. Different genotypes among various candidate genes, including DAT1, DRD4, MAOA, COMT and GSTM-1, have been reported to interact with smoking to cause an adverse behavioral profile. Epigenetic approaches have also been initiated that carry us beyond the realm of genotype associations. Finally, animal studies have identified various direct neuroteratogenic effects in different regions of the developing animal brain, including neuron loss, acetylcholine receptor upregulation, diminished acetylcholinergic tone, dysregulated catecholaminergic tone, and altered intracellular signaling pathways. Notwithstanding the fact that the toxic effect that prenatal cigarette smoking exposure appears to have on neurodevelopment, there remains much to learn. Further and improved studies across all fields are encouraged in order to form a complete picture of nicotine as a teratogen, and it is hoped that this will emerge in the near future.

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1. Introduction

Tobacco is the most abused drug in the world [1], but its impact on prenatal, perinatal and postnatal health is not yet fully understood [2]. Some reports have indicated that more young pregnant women are engaging in smoking in western society [3]. In fact, at least 12–15% of women do smoke during their gestational period [4,5]. Nicotine crosses the placenta readily and is concentrated in

the fetal blood, where it has potentially disruptive effects on the developing brain via nicotinic acetylcholine receptor (nAChR) activation [2,6]. Among the known postnatal outcomes, one of the most highlighted domains is neurobehavioral [7,8]. For example, the team from Brown Medical School used NICU Network Neurobehavioral Scale (NNNS) to test nicotine-exposed neonates ($n = 27$) and unexposed controls ($n = 29$) [9]. After adjusting for socioeconomic background and other substance use, the exposed group of neonates showed more excitability, required more handling, and presented with more neurological and visual symptoms. Using a similar methodology, they tested infants aged 10–27 days [10]. Again the nicotine-exposed infants ($n = 28$) needed more handling and had poorer self-regulation than their controls ($n = 28$). As infants grow into their childhood and adolescence, the subtle

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neurobehavioral deficits seem to turn into overt psychopathology that manifests as learning difficulties, social deficits, violence and aggression [11–16].

Despite the growing evidence concerning smoking and neurobehavioral symptoms, it is still difficult to attribute the behavioral phenotypes to smoking itself [5,17–19]. As a maladaptive behavior, smoking is linked with lower social status, poverty, malnutrition, presence of toxins and stress, maternal personality/psychopathology, neighborhood environment, and other substance use. In other words, smoking is correlational to externalizing behaviors rather than being absolutely causal. As such, the so-called smoking effect may be spurious and the other factors related to smoking actually cause the adverse neurobehavioral outcomes. By contrast, some research has explored gene–environment interactions in this area by focusing on statistical interactions between smoking and specific genotypes. Several major findings in this area are reviewed below.

Theoretically, research concerning the impact of prenatal substance exposure on fetal development is largely based upon the premise of prenatal programming effect; this posits that prenatal exposure leads to long-term and pervasive negative outcomes across the whole lifespan [20]. Under such a premise, animal models are perfect for inspecting potential adverse outcomes [7,21,22]. Experimental animals can be exposed to simulated doses of a teratogen. Given that all environmental variables are well controlled in a laboratory setting, any observed neurobehavioral effects should be the direct consequence of the toxic effect of the substance. Using an animal model, it is also feasible to explore the underlying molecular or receptor mechanisms responsible for any nicotine-related developmental defects [23–34]. In this review, we summarize some interesting observations elicited in this type of preclinical study.

2. Smoking and its effect on human neurobehavioral outcomes

Human studies are mostly epidemiological in nature because the participants cannot be administered with nicotine, unlike experimental animals [5,18,19]. In retrospective studies such as case–control studies, recall bias can have a tremendous effect, especially when the offspring have some perinatal problems. Therefore, the best approach when carrying out prenatal cigarette smoke exposure (PCSE) (or prenatal nicotine exposure) studies is to follow at-risk pregnant women prospectively [8]. To validate maternal reporting, biological sampling of urine cotinine levels is helpful [8]. When the behavioral testing is virtually real-time with the biological sampling, some sort of dose–response relationship (between PCSE and behavior symptom severity) may be revealed [35]. However, for an epidemiological report to be valid, numerous covariates have to be controlled in order to disentangle the compound effects statistically from the demographic background, maternal personality, maternal psychological status, paternal characteristics, other substance use, perinatal complications, and environmental factors [5,18,19,36].

From neonates to infants, children and finally adolescents, different outcomes have been used to delineate PCSE neurobehavioral effects [35–39]. In infant populations, virtually no complex assessments are used because of potential harm and the limited responses available to many tests. Except for event-related potentials or electroencephalography measurements, basically only crude behavioral phenotypes can be explored [35,40]. As infants grow and develop with time, they can be tested using not only behavior check lists but also brain imaging [41,42], as well as complex cognitive functions like intelligence quotient (IQ) [43,44]. In summary, in most studies, PCSE infants, children and adolescents

appear inferior to their nonexposed controls. For instance, greater irritability and hypertonicity [45], a less easy temperament [37], less focused attention and auditory responses [46], more externalizing and internalizing symptoms [47], lower language comprehension [48], lower IQ [43,44], greater reductions in cortical gray matter, total parenchymal volume and head circumference [41], and thinner orbitofrontal and middle frontal cortices [42] have been found in PCSE offspring.

Harmful as smoking may seem, it is hard to conclude that nicotine is the culprit causing all the adverse outcomes because many of these studies involved volunteer participants who had not had their backgrounds controlled in a detailed manner [5,17–19]. Even in well-controlled or statistically adjusted studies, residual confounding factors remain. For instance, in a cohort of over 18,000 9-month-old infants, the mothers who quit smoking during pregnancy tended to have offspring who scored higher on the easy temperament scale [37]. This observation still held true after many factors related to maternal smoking status were adjusted. At first glance, if quitters were psychosocially comparable to smoking mothers, and quitters delivered healthier (in this case, easier temperament) babies, then nicotine would certainly seem to have direct teratogenic effects on the developing fetuses. However, the real story may not be so straightforward. Mothers who quit smoking during pregnancy may be inherently different from mothers who do not quit [39]. This could be due to underlying maternal personality differences or to genetic differences between the two groups, and it is these that contribute to both the mother's success in quitting smoking and to the baby's easy temperament [49]. Furthermore, a 9-month-old infant may have been exposed to significant amounts of environmental tobacco smoke (ETS) after birth, especially from a smoking mother or father [50,51]. Along with ETS, postnatal environmental factors are extremely difficult to control. Even though researchers are enthusiastic about proving the long-term effects of PCSE on children, adolescents and even adults, it is important to bear in mind that environmental factors are bound to come into play that are able to confound the effects of PCSE in an insidious way.

3. Human genetics and environmental interactions

To bypass the innumerable phenotypic or psychosocial covariates associated with humans, many of which are highly unstable, some investigators have targeted the relationship between various genetic polymorphisms and the effects of PCSE. Intuitively, nAChRs ought to play some sort of role in fetal neuroteratogenicity. However, nAChRs that are ubiquitously expressed in the brain consist of various different subunits and serve many functions during the brain maturation process [2,52]. Thus, it is by no means easy to target any specific behavioral phenotype and its relationship with a certain nAChR genotype [53].

Conventionally, researchers have chosen specific candidate genes that are believed to be related to externalizing problems. For instance, dopamine and serotonergic pathways are believed to be involved in motivation and inhibitory control [54–56]. Dopamine transporter gene DAT1 and dopamine receptor gene DRD4 have been found to be correlated with attention-deficit hyperactivity disorder (ADHD); however, the results concerning DAT1 and DRD4 interacting with smoking and ADHD remain inconclusive. Monoamine oxidases (MAOs) are a group of metabolic enzymes acting on monoamines that are coded by genes on the X chromosome [52]. Functionally, MAOA is associated with the modulation of serotonergic tone and morphogenesis. Phenotypically, MAOA knockout mice display aggressive behaviors that mimic human externalizing disorders. Interestingly in this context, some of the more than 4000 non-nicotinic components in tobacco smoke are suspected of being

able to inhibit MAOs. One study has shown that male infants with PCS exposure with low activity of MAOA 5' have an increased risk of conduct disorder symptoms [57]. The polymorphisms affecting the genes encoding another important metabolic enzyme, catechol-*O*-methyl transferase, have also been extensively studied. According to a recent study, the catechol-*O*-methyl transferase Val108/158Met polymorphism (rs4680) interacts with maternal smoking during pregnancy, and this predicts aggressive behavior in offspring aged 15–20 years old [58].

In contrast to the above, other investigators have focused on enzymes involved in metabolizing tobacco smoke and have tried linking polymorphism in these genes to externalizing behaviors or cognitive functioning [59–63]. For instance, Morales et al have found that glutathione-*S*-transferase Mu 1 deficiency increases the cognitive deficits of preschoolers who have been subjected to PCSE, indicating that inadequate detoxification in fetuses may cause harm [63].

There are epigenetic models for fetal alcohol spectrum disorder and therefore there should be similar models for PCSE teratogenesis [64]. Epigenetic reprogramming occurs during peri-implantation (genome-wide demethylation), gastrulation (*de novo* methylation) and at later stages (genome-wide *de novo* methylation and demethylation at both imprinted and nonimprinted loci) [65]. Toledo-Rodriguez has proposed that mechanisms associated with PCSE may include DNA methylation in genes important for brain development [66]. Their research findings have suggested that PCSE may cause long-term downregulation of brain-derived neurotrophic factor expression through DNA methylation of its promoter region. Other epigenetic mechanisms, such as noncoding RNAs, may also contribute to nicotine-related neuroteratogenicity [67].

4. Basic science research for nicotine as a neuroteratogen

Slotkin has developed an animal model to study nicotine neuroteratogenicity [26–32,68]. Although birds, rodents, or monkeys will never be able to capture fully all aspects of human smoking, the pure effect of nicotine on a fetus can never be elicited without using an animal model. Nicotine, on the one hand, may cause intermittent hypoxia–ischemia episodes in the mother and fetus. On the other hand, the dietary restrictions caused by the anorexic effects of nicotine on the mother may also confound the direct effects of nicotine, not to mention the plethora of epiphenomena that circumscribe smoking behaviors.

There are several lines of research that have targeted the prenatal or perinatal effects of nicotine on the developing brain. First, brain cell loss (or suppression of DNA synthesis) has been measured using regional DNA content. In this situation, neurons usually continue to decline postpartum beyond the schedule of nicotine administration. Even though DNA content eventually recovers in some brain regions, neurogenesis usually stops before this, meaning that neurons are being substantially replaced by glial tissue [2,27,32]. Second, cholinergic synaptic function has been measured. This is a trophic factor in the brain and therefore cholinergic function is of prime importance to brain morphogenesis. Fetal nicotine exposure mostly upregulates nAChRs, but the peak of developmental cholinergic tone seems to be blunted [28,65]. Lasting deficits in choline transporter expression have also been observed [69]. Third, catecholaminergic function has been measured because of its dual relationship with both nAChRs and behavioral problems. Although temporary regulation of receptor subtypes might differ, persistent deficits in noradrenergic and dopaminergic functions have been consistently reported [65,68]. Ironically, prenatal nicotine exposure does not seem to be able to stimulate catecholamine release after birth [25]. Other than acetylcholinergic or catecholaminergic mechanisms, upregulation of adenylate cyclase expression [2], increased excitatory postsynaptic

potentials (EPSPs) mediated by *N*-methyl-*D*-aspartic acid receptors in auditory cortex [23], decreased EPSPs mediated by 2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl)propanoic acid (AMPA) receptors in hippocampus [33], and decreased lipid peroxidation [30] have all been reported in the context of prenatal or perinatal nicotine exposure.

Basic scientists generally agree that prenatal nicotine has a harmful effect on the developing animal brain. However strong this evidence is, it is not completely transferable to human studies because of the innate limitations of animal studies. These include the facts that the trimester stages in human gestation are different to the various stages of fetal rat development; rats and humans are pharmacodynamically different, with the former generally requiring a proportionately higher nicotine dose; and nicotine is administered to rats via an infusion minipump rather than by active inhalation [2].

5. Summary and future research

Human observational studies have immediate relevance to decisions on policy, but the contextual and individual confounders that usually affect their conclusions make them hard to accept. With so many psychosocial variables to be adjusted for, the sample size needs to be sufficient, which is very large even in a prospective cohort. In this context, researchers try their best to collect covariate information; nevertheless, no single study is able to cover all aspects of this problem given the sample size limitation. As such, only the most important variables are collected and adjusted for. One way to consider the compound impact of all the relevant covariates is to use propensity score analysis. The technique is especially useful because it increases the statistical efficiency of a not-so-large cohort. Although most published papers seem to embrace the theory that nicotine is a neuroteratogen, some researchers have obtained contrary findings [4,70,71]. Statistical adjustment can never be exhaustive, therefore, it can always be argued that the association between PCSE and neurobehavioral symptoms is spurious. In these circumstances, trimester-specific smoking data are need because they could help to document the presence of any precise temporal effects. By contrast, the simple causality theory needs to be modified to accommodate controversial findings. For instance, Boutwell has proposed that PCSE is related to childhood externalizing problems only in the mothers who smoked more than one pack a day during pregnancy.

When using experimental designs that investigate gene–environment interactions, only selective candidate genes have been tested for any association. To the best of our knowledge, no genome-wide scan of human genes has been applied in this field. Epigenetic hypotheses need to be tested more extensively because these will deepen our understanding of tobacco-induced epigenetic changes. To theorize boldly, smoking *per se* (not necessarily in conjunction with pregnancy, breastfeeding, or ETS) may be hazardous enough to alter the epigenetic information in gametes.

Animal studies are indispensable and are able to illuminate theories on the effect of PCSE on neurobehavior. Their use is limited by inaccurate quantitative assessments of exposure and timing [7,21,22,72,73]. According to the work series by Slotkin et al [26–32,68], studies are moving from the areas of DNA content, AChR responsiveness, acetylcholinergic tone, catecholaminergic responses, and intracellular secondary messengers towards the intricate interconnections of all these items. Impressive as their work is, it is merely the tip of the iceberg. For example, just in terms of neurotransmitter theory, it is important to realize that the glutaminergic and GABAergic pathways are also important.

At this point, even though we cannot be certain enough to announce that smoking during pregnancy is definitely detrimental

to fetal neurodevelopment as a whole (depending on the timing and severity), nobody should be opposed to the notion that tobacco is a potential neuroteratogen. Given the large amounts of evidence from observational, clinical and preclinical studies, it is important that the long-lasting neurobehavioral impacts of prenatal tobacco exposure are not overlooked. However, it is important to realize that there is much about which we are still uncertain and even totally ignorant. Well-designed as well as novel studies in all fields will help to increase dramatically our knowledge base related to tobacco-related neuroteratogenicity.

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