

Long-term consequences of maternal smoking and developmental chronic nicotine exposure

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1. ABSTRACT

Every year, a large number of children are exposed to smoking during pregnancy which increases the risk of decreased birth weight, fetal morbidity and behavioral abnormalities. Therefore, nicotine replacement therapy (NRT) is often considered as a treatment option. Despite a large number of epidemiological studies, there are conflicting reports about the long-term consequences of maternal smoking on cognitive function, attention deficit hyperactivity disorder (ADHD) and other behavioral abnormalities. Animal studies are also often contradicting with respect to the effects of developmental nicotine, the psychoactive ingredient in tobacco. After a critical review of the literature, it appears that 1) maternal smoking causes low birth weight and nicotine, seems play a significant role in reducing body weight; 2) maternal smoking and developmental nicotine exposure have only minor effects on cognitive functions in children or animals, respectively; 3) maternal smoking is a risk factor for ADHD, but a causal link between nicotine and hyperactivity is not well established; 4) developmental nicotine increases anxiety-like behavior in animals but it remains to be seen if maternal smoking or NRT, would have similar long-term effects in children. Future studies should address if nicotine is involved in the increased risk to develop ADHD and how developmental nicotine leads to increased anxiety.

2. INTRODUCTION

Smoking during pregnancy affects a large number of people in the US and worldwide. The proportion of pregnant women who smoke is estimated to be 20 to 25 %, resulting in about 800,000 babies prenatally exposed to tobacco smoke born annually in the US (1-2). *In utero* exposure to tobacco smoke results in low oxygen and high carbonmonoxid blood levels, reduced placental function, increased vasoconstriction and placental constriction which deprives the fetus periodically of oxygen, and exposure to thousands of chemical compounds that are in tobacco smoke and reach the fetus (3). Maternal smoking is associated with an increased risk of perinatal morbidity such as low birth weight, perinatal mortality and Sudden Infant Death Syndrome (SIDS) (4-6). However, despite numerous epidemiological studies and the large number of children exposed to gestational smoke, there is not a clear clinical picture emerging of the long-term consequences of maternal smoking.

Nicotine is the major psychoactive component in tobacco and believed to be the driving force for tobacco consumption. Thus, given the major medical, social and fetal consequences of smoking, nicotine replacement therapy (NRT) for pregnant women is considered as a pharmacological intervention to curb smoking (7-8). This

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would reduce the number of toxic compounds to which the mother and the fetus are exposed to only nicotine. At this point it is important to understand the long-term consequences of smoking and in particular of nicotine, to better inform pregnant mothers of the risks involved and to evaluate the risks and benefits of NRT.

This review will critically evaluate the results from human epidemiological studies and compare such findings to those obtained with animal models for developmental nicotine exposure. It is an attempt to understand the long-term consequences of prenatal tobacco smoke exposure so that future clinical and preclinical studies can focus their attention on the most likely effects of maternal smoking and prenatal nicotine exposure.

3. CONSEQUENCES OF MATERNAL SMOKING AND DEVELOPMENTAL NICOTINE EXPOSURE

3.1. Clinical studies evaluating the long-term effects of smoking

Countless epidemiological studies have looked into the relationship between maternal smoking during pregnancy, and the long-term behavioral problems associated with it (for reviews, see 3, 10). Most studies have focused on cognitive performance, hyperactivity and attention deficits and increased risk of drug abuse in school-age children, although the risk of other behavioral abnormalities has also been investigated.

As with all epidemiological studies, it is often difficult to establish a causal relationship between maternal smoking during pregnancy and the observed effect in the offspring due to a number of contributing factors that may influence maternal smoking behavior as well as children's behavior later in life. Women, who smoke during pregnancy tend to be younger, less educated, of lower socio-economic status and have lower IQs (11). These factors can affect the home environment and parenting style. Mothers who continue smoking despite the strong social pressure and medical advice could try to self-medicate their own psychological and behavioral problems and represent a certain psychological phenotype which could have a genetic base that could be inherited by their children (12). This is supported by the finding that increased anxiety and depression are more often seen in smoking than non-smoking mothers (13-14). Furthermore, illicit drug use and alcohol consumption during pregnancy is more likely in mothers who continue to smoke which could also influence the long-term behavioral characteristics of the children (11). Thus, studies evaluating the effects of prenatal tobacco smoke exposure need to take these confounding factors into account when evaluating a causal link between prenatal smoking and the long-term behavioral effects in the offspring.

3.1.1. Effects of smoking on birth weight

The most clearly established consequence of maternal smoking during pregnancy is decreased birth weight (15). There is a strong dose dependent negative correlation between the number of cigarettes consumed per

day and fetal growth. In general, full-term babies born to mothers who smoked during pregnancy weight 170 to 250 g less than the average newborn (16-18), with those born to heavy smokers weighting about 377 g less at birth (19). In 1979, the US Surgeon General concluded that maternal smoking affects birth weight independently of other determinants (20). Thus, smoking is most likely causally related to decreased birth weight.

Long-term consequences on physical growth and body weight have been reported by some, but not other studies (10). There seems to be no further impairment of body growth later in life, but the initial differences in height might extend into adolescence. However, low birth weight increases the risk of adverse outcomes and could therefore contribute to health and behavioral problems associated with maternal smoking.

3.1.2. Effects on cognitive functions

Most epidemiological studies investigating a link between maternal smoking and cognitive performance in the children are in agreement that maternal smoking is a predictor of lower cognitive function in the offspring, whether measured as IQ scores or academic achievement (10-11, 21-33). However, when data are corrected for confounding factors such as age of the mother, socioeconomic status, mother's education and others, the relationship between prenatal exposure to tobacco smoking and cognitive performance in children is drastically reduced. The conclusions vary from a clearly statistically significant to no effect on cognitive function (10).

For example, in a study that enrolled 30,000 subjects, Nichols and Chen (34) reported that when demographic and socio-environmental factors were controlled, learning difficulties were no longer associated with maternal smoking. Whereas Naeye and Peters (31) reported that maternal smoking during pregnancy was significantly related to lower test scores in reading and writing, even after adjusting for confounding factors.

Most recent studies have concluded that the correlation between maternal smoking during pregnancy and cognitive performance in the offspring has been overestimated (11, 22, 27). Strong supporting evidence for this conclusion comes from a large population-based Swedish cohort study involving 400,000 subjects (27). This study found that children from mothers who smoked during their pregnancy had an increased risk of poor academic achievement, even when adjusted for known confounding factors. However, because of the large sample size, this study was able to compare the risks of poor cognitive performance in siblings not exposed to maternal smoking after the mother had stopped smoking. If smoking during pregnancy is causal for decreased school performance, cessation of smoking before a second pregnancy should eliminate that risk. However, the findings show that if the mother smoked during one pregnancy the risk of poor academic achievement was also increased in the unexposed sibling, suggesting a genetic component that impacts both, smoking behavior in the mother and cognitive performance in the children.

It is known that there is a substantial genetic contribution to cognitive abilities (35-36); and lower cognitive performance increases the risk of engaging in smoking behavior. Thus, maternal IQ could be a key factor in learning and memory performance as well as IQ scores, and an important confounder in studies linking maternal smoking to decreased cognitive ability in the offspring. However, maternal IQ is often not taken into account because the data are not readily available. One study that included mothers' IQ scores (22) found that unadjusted for covariates, children of mothers who smoked during pregnancy scored significantly lower than those of mothers who are non-smokers. However, children of mothers who smoked but not during pregnancy also scored significantly lower than the reference group. When adjusted for mother's IQ and education, the correlation between maternal smoking during pregnancy and decreased offspring IQ was eliminated. In contrast, the same study looked at low birth-weight-related deficits in IQ scores and found that adjusting for maternal education and IQ did not eliminate the effects of low birth-weight on cognitive deficits.

This finding is supported by another recent study which included 5578 children (data are derived from the US National Longitudinal Survey of Youth 1979), Batty *et al.* (11) found a strong dose dependent ($1 \leq$ pack per day or $1 \geq$ pack per day) inverse association between maternal smoking during pregnancy and offspring IQ test scores (PIAT-based) in the unadjusted analysis. However, when corrected for the mother's IQ and education, the association no longer existed without further adjustment for other covariates, suggesting a strong link between maternal IQ and education, and cognitive performance in the offspring, but only a small or insignificant link for smoking during pregnancy.

Thus, review of the current literature suggests that the effects of maternal smoking during pregnancy on cognitive function in exposed children later in life might be very limited and that smoking might be an indicator, but not the cause, of decreased cognitive performance later in life.

3.1.3. Effects of smoking on attention deficits and hyperactivity disorder

Attention deficit hyperactivity disorder (ADHD) is one of the most common behavioral disorders in children today with a prevalence rate of 3 to 5% (37). Children with ADHD are characterized by symptoms of hyperactivity, impulsivity and poor sustained attention. Boys are disproportionately affected and show more disruptive behavior than girls. Although ADHD lessens with age, a milder form of ADHD often persist into adulthood (38). ADHD has a strong genetic component and many genes associated with ADHD code for proteins involved in catecholamine signaling, such as the dopamine receptors D4 and D5, dopamine transporter, dopamine beta-hydroxylase, serotonin transporter, the serotonin 5HT1B receptor, alpha2A adrenergic receptor and the synaptosomal-associated protein 25 gene (SNAP25) (39-40). In addition, environmental factors contribute to the development of ADHD. Over the years, several studies

have evaluated the association between smoking during pregnancy and ADHD or ADHD symptoms (for a review, see 41). However, the establishment of a causal link between gestational exposure to tobacco smoke and ADHD has been difficult because of a number of confounding factors including socioeconomic status, poly-drug use during pregnancy, and the finding that people with ADHD during childhood or as adults have significantly higher smoking rates than the rest of the population (42). Thus, women who continue to smoke during pregnancy may have personality traits associated with smoking and ADHD, which can result in an increased risk for ADHD in the offspring, unrelated to maternal smoking during pregnancy.

However, in the review by Linnet *et al.* (41) of the six case-controlled and 18 cohort studies published between 1973 and 2002, all but four come to the conclusion that, even after adjusting for confounding factors, children of mothers who smoked during pregnancy are at greater risk for ADHD or conduct disorders. In addition, a large population-based study revealed a three-fold higher risk for ADHD which was still significant when controlled for socioeconomic factors, history of mental disorders, parental age, low birth-weight, preterm delivery and low Apgar scores (28).

Of the four studies that did not report a significant association between prenatal exposure to smoking and ADHD, Weissman *et al.* (43) found a trend to increased risk for ADHD but it did not reach significance. A cohort study by Hill and coworkers (44) concluded that alcohol dependence represented a familial risk factor for ADHD and that smoking during pregnancy did not increase the risk further. Wakschlag *et al.* (45) found that maternal smoking during pregnancy was a significant independent risk factor for conduct disorder in male offspring. Conduct disorder is often associated with ADHD (46). Only one cohort study contrasts with the others, Cornelius *et al.* (23) reported that smoking was significantly associated with deficits in learning and memory, but not with ADHD.

Altogether, the vast majority of epidemiological studies report an increased risk of ADHD, conduct disorder, or behavioral problems in children after exposure to prenatal maternal smoking. Although strong genetic and socioeconomic components contribute to these findings, the consistencies of the studies including those well-controlled for confounding factors, lead to the conclusion that maternal smoking during pregnancy is an independent risk factor for ADHD, and an environmental factor which increases the risk of developing ADHD in those with a genetic predisposition.

3.2. Animal studies evaluating the long-term effects of developmental nicotine exposure

3.2.1. Pre- and postnatal animal models investigating the developmental effects of nicotine

Since nicotine is the psychoactive substance in tobacco, animal studies have focused on nicotine in an attempt to link nicotine with the observed adverse effects found in babies whose mothers smoked during pregnancy. Nicotine penetrates the placental barrier and can be

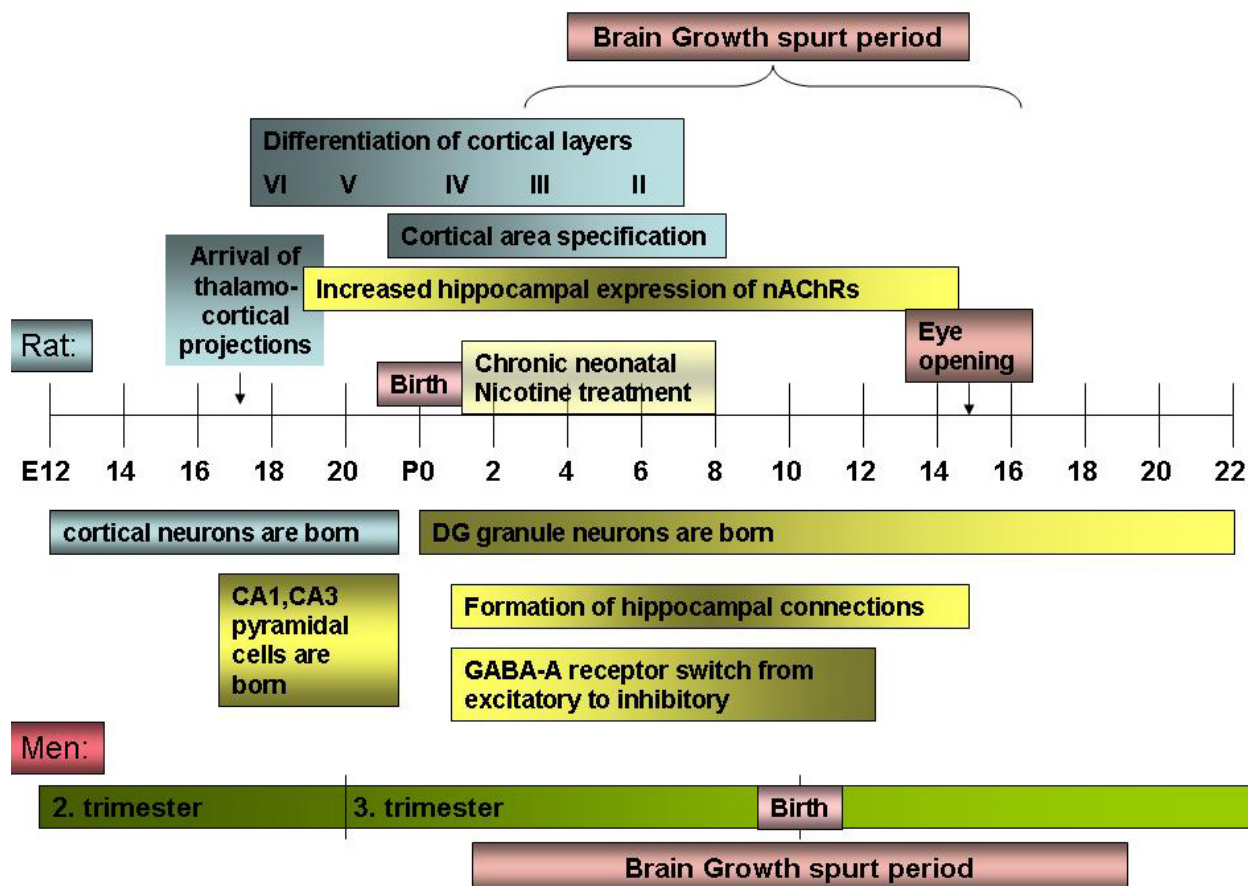


Figure 1. Schematic representation of major developmental events during rat cortical and hippocampal development in rat and an estimation how it compares to human.

detected in fetal tissue at equal or higher levels than in the mother where it is then metabolized to the inactive compound cotinine (47-48). Animal models have been developed to study the short- and long-term consequences of developmental nicotine exposure, using mostly rodent, but also non-human primate and avian species.

Due to the immaturity of the rodent brain at birth, several neuro-developmental processes take place during the first postnatal weeks (Figure 1). In particular, in several late developing structures, such as the olfactory bulb, hippocampus and the cerebellum, neurogenesis continues after birth in rodents but is mostly completed during the second trimester in humans (49). In areas involved in cognitive functions such as cortex and hippocampus, several developmental processes take place during the first two postnatal weeks such as neuronal maturation, area specification and synapse formation. In humans, these developmental processes are mostly completed *in utero* during the second and third trimester, although formation of synaptic connections and activity driven rearrangement of connections also occur after birth (50). Because some processes are prolonged in humans and others are shortened in rats, it is difficult to exactly align the time scales of brain development between rats and humans. In an attempt to compare developmental periods, Dobbing and Sands (51)

proposed to use periods of increased brain growth, based on brain weight gain per day (brain growth spurt periods) between species. Acknowledging the difficulty in correlating temporal sequences of brain growth in different species, they proposed to divide species based on their brain growth spurt period into prenatal, perinatal and postnatal brain developers. According to this classification, humans are perinatal developers with their growth spurt period taking place during the third trimester and after birth, although the brain weight does not reach adult levels for 10 years. Rats are early postnatal developers with tremendous brain growth occurring during the first two to three weeks after birth, and brain weight reaching adult levels around postnatal day 35, the onset of puberty. Thus, the first two weeks after birth in rats are comparable to the third trimester in humans with regard to the growth spurt period.

Most studies investigating the effects of developmental nicotine exposure have used prenatal treatment models covering a developmental period roughly equivalent to the first and second trimester in humans. Nicotine is administered either continuously by infusion via minipumps implanted into the dam, or intermittently via repeated injections or via drinking water. Each administration route has its own advantages and disadvantages. During continuous infusion, blood nicotine

levels are constant, similar to people using nicotine patches for NRT. The constant nicotine levels result in desensitization of nAChR, rendering them unresponsive to acetylcholine and nicotine (52-53). Repeated injections of nicotine mimics the highs and lows in blood nicotine levels found in smokers, but daily repeated injections are stressful to the dam, triggering the release of stress hormones, which can influence fetal development. Nicotine administration via drinking water avoids the stress associated with injections and also results in fluctuations of nicotine blood levels, but due to the bitter taste of nicotine, the dams tend to reduce their water intake which could affect fetal development, in addition, delivery of exact nicotine doses becomes problematic.

A few investigators have used early postnatal exposure models employing different strategies of drug delivery. Nicotine can be delivered indirectly to the pups by suckling nicotine containing milk. The dams are either implanted with a minipump or exposed to nicotine via drinking water (54-55). This strategy allows for the pups to remain undisturbed, but exact dosing of the pups with nicotine is not possible and littermates cannot be used as controls. Nicotine can also be delivered directly to the pups via repeated injections (56-57), gastric intubation (58), or via gastrostomy as used in an artificial rearing model (pup-in-the cup) (59-60). Direct drug delivery allows for controlled dosing based on body weight and littermates can be used as controls. However, repeated injections of tiny pups run the risk of injury and infections in addition to being stressful. The 'pup-in-the-cup' model, originally described by West and coworker (61) to investigate the effects of postnatal alcohol exposure, removes the pups from the dam during the treatment period and delivers controlled amounts of drug and nutrients through a gastrostomy implanted tube. This approach controls caloric intake and eliminates differential maternal behavior towards drug exposed pups, which could interfere with the results. However, the lack of maternal care can introduce long-lasting behavioral consequences, and suboptimal nutritional support (milk formula instead of rat milk) could mask the effects of nicotine. We use a modification of the pup-in-the-cup model, using gastric intubation to deliver individualized doses of nicotine to neonatal pups three times per day without removing the pups from the dam except for the treatments (58). The risk of injury and stress to the animals is lower than with repeated injections and maternal separation is kept to a minimum. Unlike alcohol, nicotine does not appear to interfere with the suckling behavior of the pups; thus, milk intake should not be compromised, and it is assumed that caloric intake is similar to controls. In studies with pre- and postnatal exposure, dams were implanted with minipumps which were either not removed after birth, or implanted with a fresh minipump, extending the period of nicotine exposure (62).

The dosing range varies widely between studies from very low doses (less than 1 mg/kg/day) to very high doses (9 mg/kg/day). Most studies did not test blood or brain nicotine levels but instead rely on estimations based on the findings by others (52, 63). However, nicotine blood

levels are not only influenced by the dose but also by the route of administration, making it difficult to compare nicotine levels between studies, and there is evidence suggesting that nicotine doses used in animal studies often result in blood nicotine levels far exceeding those seen in smokers (64).

3.2.2. Molecular changes of nAChRs

An indication for the successful delivery of nicotine to the brain is increased expression of nAChR binding sites, a hallmark for chronic nicotine exposure in adult and developing animals, human smokers and human fetuses exposed to smoking *in utero* (54, 65-72). In particular, heteromeric $\alpha 4/\beta 2$ -containing nAChRs, which form most of the high affinity nicotine binding sites in the brain, are upregulated (73-75). Other nAChR subtypes such as the $\alpha 7$ homomeric receptor have rarely been reported to change in response to chronic nicotine and might only be affected by very high levels (76-77).

In postnatal rat pups, upregulation of heteromeric nAChR binding sites after chronic neonatal nicotine treatment with a high dose (6 mg/kg/day) is short lived, with epibatidine binding returning to control levels after the end of treatment (69). Similarly, postnatal chronic nicotine exposure to a low dose (0.1 mg/kg/day) transiently increased high affinity nAChR binding sites and returned to control levels after withdrawal from nicotine (57). Thus, an increase in heteromeric nAChR binding might not have long lasting consequences on receptor numbers. However, one study reported long-lasting down-regulation of epibatidine binding to heteromeric nAChRs after chronic gestational/postnatal nicotine exposure (78). It is possible that the extended duration of nicotine exposure is responsible for this discrepancy.

Most studies agree that upregulation of nAChRs is not associated with increased mRNA expression for nAChR subunits (57, 69, 79-80), but due to posttranscriptional regulation and increased receptor stabilization (81-84). However, long-term effects of chronic neonatal nicotine exposure could include altered expression of nAChR subunits (78, 85).

3.2.3 Morphological changes

3.2.3.1 Nicotine's effects on cell morphology

Several studies have reported that chronic developmental nicotine exposure can alter hippocampal and cortical morphology, resulting in decreased neuronal area and increased packing densities in hippocampal principal cells and cortical neurons (86-89). Roy and coworkers (86-88) treated rat dams prenatally with nicotine (2.5 mg/kg/day i.p. injections, or 0.7 mg/kg/day via minipump infusion) and measured neuronal size and density in adolescent animals in the pyramidal and granule neurons of the hippocampus and in layer V somatosensory cortex, whereas Huang *et al.* (89) treated neonates during the first postnatal week with nicotine (6 mg/kg/day, orally) and measured the effects on morphology in neurons of the hippocampus (pyramidal and granule cells) and germinal, granule and Purkinje cells in the cerebellum of eight day old rat pups. Thus, it seems that the effects of nicotine on

neuronal morphology are a direct consequence of developmental nicotine exposure and not an indirect effect resulting from nicotine-induced placental dysfunctions or hypoxia because similar results are found after pre- and postnatal chronic treatment.

In addition, it seems that nicotine has a rapid and long-lasting effect on hippocampal neuronal morphology with a large window of vulnerability from prenatal to postnatal ages. However, the effects are area specific, because the late developing cerebellum did not show altered neuronal morphology (89). It would be interesting to see if chronic nicotine consumption causes similar anatomical changes in the adolescent or adult brain or if this effect is restricted to prenatal and early postnatal development.

The mechanisms underlying these anatomical changes are not well understood. However, one potential candidate for a developmental role is the homomeric $\alpha 7$ nAChR (90). Activation of $\alpha 7$ receptors results in an increase in intracellular calcium concentrations (91-92). Calcium signaling is vital for normal neural development, and rapid changes in neurite extension in response to $\alpha 7$ activation have been reported for cultured primary neurons (93). Furthermore, there is evidence for an involvement of $\alpha 7$ homomeric nAChRs in the developmental switch of GABA from being an excitatory to inhibitory neurotransmitter which could influence neuronal morphology (94-95). In addition, nicotine via $\alpha 7$ homomeric receptors enhance glutamatergic transmission and prematurely converts presynaptically silent synapses into active ones (96). Thus, the strong expression of $\alpha 7$ during the critical period of perinatal development (97-98) could contribute to nicotine's effect on cell morphology.

3.2.3.2. Nicotine's effects on cell death

Nicotinic receptors are often viewed as being neuroprotective in situations that challenge neuronal survival (for review, see 99). In particular, the heteromeric $\alpha 4/\beta 2$ nAChR has been linked to increased neuronal survival, whereas the homomeric $\alpha 7$ receptor can mediate increased cell death in immature neurons (100-101). Nicotinic receptors often exhibit area specific, transient expression in the developing brain (90, 102-104). Thus, depending on nAChR subtype expression, neuronal vulnerability could either be decreased or increased in response to nicotine, and immature neurons might be particularly sensitive to nicotine's neurotoxic effects. Two *in vitro* studies found that nicotine exposure alone is sufficient to induce increased numbers of apoptotic cells in immature hippocampal progenitor cells or embryonic brain slices (100, Roy 105). However, in animal studies chronic developmental nicotine treatment did not increase cell death in either mature or immature cells, even in brain areas of increased $\alpha 7$ expression (89, 106) and did not result in decreased cell numbers in the hippocampus or cerebellum (107). Thus, developmental chronic nicotine alone does not seem to be neurotoxic to the immature brain.

On the other hand, there is evidence that chronic developmental nicotine decreases neuronal death. Huang *et*

al. (89) found decreased expression of cell death markers in CA3 radiatum and CA3 oriens of the hippocampus and in the granule cell layer in the cerebellum after chronic neonatal nicotine treatment. Others have also documented a cell-sparing effect of developmental nicotine in specific neuronal populations (108-109). Thus, nicotine could perhaps interfere with developmental cell death, which might not necessarily be beneficial. For example, increased numbers of GABAergic neurons in the hippocampus could result in increased GABAergic tone, which could interfere with cognitive functions.

3.3. Long-term effects of developmental nicotine exposure

3.3.1. Effects of nicotine on body weight in pups

Low birth weight is a hallmark for maternal smoking during pregnancy. The mechanism for intrauterine growth restriction have been largely attributed to the indirect effects of smoking such as decreased oxygen availability and reduced blood flow to the fetus (3). In addition, in heavy smokers, placental villi are atrophic and placental weight is reduced, compromising placental function and contributing to fetal growth retardation (110-112). However, in adult smokers there is an inverse relationship between the number of cigarettes smoked and body weight (113-114), and nicotine acts as an anorexic drug suppressing appetite and increasing energy expenditure in adults (115-117). Therefore, nicotine could also directly affect body weight and body growth in the developing fetus by altering energy expenditure, contributing to the low birth weight seen in children whose mothers smoked.

Animal studies have shown surprisingly variable results. Chronic nicotine exposure in prenatal models has been found to either decrease birth weight in both genders (118), affect females only (119), males only (120), or to result in no change in birth weight (62, 121-124). In humans, the third trimester is most strongly correlated with decreased birth weight, which is a period when fat depots are formed (125). Thus, prenatal rodent models might not adequately reflect nicotine's anorexic effects because an increase in body weight due to fat deposits occurs after birth.

A few reports on nicotine's postnatal effects on body weight have been published. Although most postnatal studies did not report a significant change in body weight in response to nicotine (54, 126), our studies in neonates have shown a rapid and dose-dependent effect on weight in male and female rat pups, with doses as low as 0.5 mg/kg/day resulting in significant reduction in weight gain and consequently reduced body weight (58, 127). The effect is immediately reversed after withdrawal from nicotine, and blocked by the heteromeric $\alpha 4/\beta 2^*$ nAChR antagonist dihydro-beta-erythroidine (DH β E). This strongly points to a direct role for nicotine in body weight regulation via increased energy expenditure involving a nicotinic cholinergic mechanism which seems to be already functional in neonates. Thus, nicotine could be directly responsible for the reduced birth weight in babies whose

mother smoked. This needs to be taken into consideration when considering NRT for pregnant women.

The mechanism by which nicotine regulates body weight seems to be independent of nicotine-stimulated leptin release because of the complete lack of correlation between blood leptin levels and nicotine-induced reduction in body weight gain in postnatal animals. Our studies point to a central mechanism that involves the hypothalamic arcuate nucleus and the regulation of feeding peptide expression, in particular proopiomelanocortin (POMC) (127). Chronic neonatal nicotine exposure increased the expression of POMC, a precursor for the anorexic peptide alpha-melanocortin stimulating hormone (alpha-MSH). Correlating with the loss of the anorexic effect, the up-regulation is blocked by DH β E, implicating heteromeric $\alpha 4/\beta 2^*$ nAChR in regulating peptide expression and energy homeostasis.

3.3.2. Effects of nicotine on learning and memory tests

Because of the initial reports that smoking during pregnancy decreases cognitive performance in the offspring (31, 128), numerous animal studies have set out to determine if developmental exposure to nicotine can be correlated with decreased cognitive functions later in life. Similar to the epidemiological studies, the results are mixed, with some reporting decreased cognitive performance and others finding only minor impairment or no effect after developmental nicotine exposure.

Sorenson *et al.* (129) first reported an effect of prenatal chronic nicotine exposure on learning and memory. Nicotine delivered via drinking water (6 mg/kg/day) throughout gestation resulted in a significant treatment effect during the acquisition phase of correct arm choices in the radial-arm maze in adolescent male and female rats when analyzed as litter means. However, a closer review of the data shows that the largest differences were found during the initial testing trials in female rats, and that at the end of the acquisition phase difference between treatment groups in males and females had disappeared.

In 1992, Yanai and coworkers (130) reported that chronic prenatal exposure via daily nicotine injections of 3 mg/kg from gestational day 9 to 18 resulted in significantly decreased performance in the radial-arm maze and in the water maze in nicotine exposed young adult mice.

Levin *et al.* (121) found that chronic prenatal nicotine (2 mg/kg/day) from gestational day 4 to 21 via continuous infusion had no effect on acquisition of choice accuracy performance in the radial-arm maze in 60 day old animals. Only during the first three training sessions did males and females show significant differences. In a follow-up study, chronic prenatal nicotine exposure again had no significant effect on choice accuracy in the T-maze alteration test with 10, 20 or 40 seconds delay; only at 0 second delay did 50 day old prenatally nicotine-treated male rats exhibited a deficit. The same study reported that there was no significant treatment effect with regard to latency during the acquisition phase in the radial-arm maze (131).

Cutler and Levin *et al.* (118) further reported that continuous nicotine infusion with a higher dose (4 mg/kg/day) from gestational day 4 to 20 had no significant main effect of nicotine on reference or working memory in the water maze and no significant effect of nicotine on choice accuracy acquisition in the radial-arm maze in either male or female offspring when tested as per-pubertal or pubertal rats.

Postnatal chronic exposure models reported similarly inconsistent results. In the study by Yanai *et al.* (130) mentioned above, young adult mice postnatally treated with nicotine by daily injections (1.5 mg/kg from postnatal day 2 to 21) exhibited significantly decreased performance in the radial-arm maze and the water maze. Whereas another study with 4 month old male mice treated twice daily with low doses of nicotine (6.6, 66, 132 μ g/kg/day) from postnatal day 5 to 15 did not show a significant difference in escape latency compared to controls when tested in the water maze (132).

Using higher doses and the artificial rearing model, 6mg/kg/day nicotine from postnatal day 5 to 9 had no effect on the performance of young adults in the water maze compared to either suckle or treatment controls (133).

In our studies, young adult male and female rats treated neonatally with nicotine (6 mg/kg/day via gastric intubation from postnatal day 1 to 7) showed no difference in escape latency during the learning phase and no difference in the probe trial in the water maze. In the T-maze test, there was a gender but not a treatment effect with regard to number of trials to criterion and no effect of nicotine on reversal learning (135).

In a combing pre- and postnatal nicotine exposure study (0.96 mg/kg/day via continuous infusion through the dam from gestational day 4 to postnatal day 11) young adult female but not male rats exhibited significantly decreased performance in the water maze when analyzed by repeated measures nested ANOVA (55). However, at the end of the training period (days 4 and 5) performance between control and nicotine treated females (and males) did not seem to be significantly different nor was there a difference in performance in the probe trial.

Thus, after a critical review of the published reports, the evidence presented for a detrimental effect of developmental nicotine exposure on cognitive function as claimed by some (135-136) is not a sure thing. At best, the effects seem to be minor and are often most noticeable during the initial training phase. However, the findings are in agreement with the epidemiological studies on maternal smoking during pregnancy (see section 3.1.2), in that the most recent studies do not describe a major effect on cognitive functions when corrected for confounding factors.

3.3.3. Effects of nicotine on hyperactivity

From the beginning, reports on the effects of prenatal nicotine exposure using animal models were inconsistent. Martin and Becker (137) reported no

difference in locomotor activity in 52 to 57 day old rats prenatally exposed to nicotine (3 mg/kg injected twice daily, for 21 days), whereas Peters *et al.* (138) found that the offspring of rat dams given nicotine in the drinking water during gestation showed increased spontaneous motor activity.

Two decades later, Ajarem and Ahmad (139) used adolescent mice from dams treated daily with repeated nicotine injections (4.5 to 5 mg/kg/day) during gestation. At 36 days of age, the offspring exhibited significantly increased locomotor activity. This result was supported by another study in mice, where prenatal nicotine exposure (3.5 mg/kg/day, via drinking water throughout gestation) also resulted in significantly increased spontaneous locomotor activity in young adult male and female offspring suggesting hyperactivity (140).

In rats the results are less consistent. Tizabi *et al.* (76) using prenatal continuous infusion (9 mg/kg/day) throughout gestation, reported that prenatal nicotine did not change horizontal activity but increased vertical activity (rearing) in male rat pups tested at postnatal day 20 to 24. Another study reported that continuous prenatal nicotine (6mg/kg/day) resulted in elevated locomotor activity in 25 day old male rats, whereas females showed decreased activity, but increased vertical activity compared to controls (119). In contrast, Romero and Chen (141), using continuous release pellets (35 mg over 21 days) implanted into dams on gestational day 8, reported no change in locomotor activity between control and nicotine-treated males, but young adult nicotine-treated females failed to increase ambulatory activity observed in control females over the three day testing period in the open-field.

However, LeSage *et al.* (142) reported on the effects of nicotine (2 mg/kg/day via i.v. bolus injections from gestational day 4 to birth) exposure on locomotor activity in pre-weanling rats tested from postnatal day 19 to 21. They found significantly decreased activity in nicotine treated animals upon initial exposure to a novel open-field testing apparatus, but the effect diminished in the following testing days.

In postnatal exposure models, lower doses of nicotine did not significantly affect spontaneous activity. Five days of nicotine treatment (0.132 mg/kg/day) starting at postnatal day 3, 10 or 19 did not alter spontaneous behavior in 4 month old mice (Eriksson *et al.*, 2000). However, when challenged with nicotine as adults, nicotine induced hypoactivity in mice treated from postnatal day 10 to 15 but not postnatal day 19 to 24; a response that seems to also depend on the initial dose of nicotine during treatment (132).

Higher doses of nicotine were used by Thomas *et al.* (126) in a postnatal artificial rearing protocol with nicotine (6 mg/kg/day) treatment from postnatal day 4 to 9. Nicotine treated pups had significantly increased ambulatory activity, compared to treatment and suckling controls when tested at postnatal day 18/19, indicating hyperactivity. However, results from our studies using

adolescent and young adult male and female rats treated from postnatal day 1 to 7 orally with 6 mg/kg/day nicotine showed no difference in distance traveled in the open field indicating no change in activity with treatment (134).

Taken together, prenatal nicotine exposure in mice seems to result in increased activity (hyperactivity) in the offspring, whereas in rats the outcome is more variable from hyper- to hypoactivity, to no effect at all. The reported differences in outcome could be a reflection of the different species, but could also result from different treatment and testing protocols, doses of nicotine and age at testing. Postnatal nicotine exposure seems to have no effect on spontaneous activity, except in the artificial rearing model, where the treatment procedure seems to result in suboptimal growing conditions based on reduced weight gain in control and nicotine gastrotomy groups compared to suckle controls (126). In this model, nicotine could perhaps influence activity pattern later in life by exacerbating stress induced by the procedure and maternal separation. Taken together, 30 years after the initial reports, the question of whether developmental nicotine has an effect on spontaneous locomotor behavior and induces hyperactivity remains unanswered.

3.3.4. Effects of nicotine on anxiety

Epidemiological studies on anxiety-related behavior in children or adults exposed to maternal smoking during pregnancy are sparse. However, there are indications that developmental nicotine exposure affects the development of central dopaminergic, noradrenergic and serotonergic systems in rat (144-145). Dysfunctions in these systems have been implicated in affective disorders such as anxiety and depression (146).

In animal studies, anxiety-like behavior is often measured with the elevated plus maze or the open-field test (147-148). Both tests are based on the natural aversion of rodents to be exposed to open, or elevated spaces, and have been used by numerous studies as generalized tests of anxiety.

An initial preclinical report suggested that prenatal exposure to nicotine (repeated nicotine injections) produces an anxiolytic effect. Nicotine treated mice exhibited significantly more entries into and longer stays in the open arm in the elevated plus maze compared to controls (139).

In rats however, developmental nicotine exposure seems to increase anxiety-like behavior in young adults. Vaglenova *et al.* (119) reported that after continuous prenatal nicotine (6mg/kg/day) exposure, nicotine-treated adolescent rats spent significantly less time in the open arm and had reduced number of entries compared to saline-treated controls.

Results from our studies support this finding. Neonatally nicotine (6mg/kg/day, postnatal day 1 to 7) treated adolescent and young adult male and female rats exhibited increased anxiety-like behavior (134). In the elevated plus maze, animals spend significantly less time in

the open arm and had fewer number of entries compared to controls. Consistent with this result, nicotine treated animals spend less time in the center and more time in corners in the open field test.

The anxiety-inducing effect of nicotine does not seem to be restricted to either pre- or postnatal age of exposure. Nicotine treatment during adolescence also results in increased anxiety (149-150).

Together, the results suggest that developmental nicotine exposure has a long lasting effect on anxiety in rats similar to those observed with other drugs such as alcohol, opioids or serotonin reuptake inhibitors (151-153). If confirmed, this could explain some of the findings from other studies where significant differences were found during the initial phase of training. In addition, increased anxiety in novel situations could significantly influence cognitive performance (154) and lead to the impression that developmental nicotine affects learning and memory when in fact it increases anxiety.

4. PERSPECTIVE

After reviewing a large number of studies on the short-term and long-term effects of maternal smoking during pregnancy and developmental nicotine exposure, only a few findings seem to be certain. Smoking causes low birth weight and nicotine is at least partially responsible for the growth retardation. Smoking seems to have only a minor effect on cognitive functions in exposed children and similarly nicotine's effects on learning and memory in animals studies seems to be minor. Smoking seems to be a risk factor to develop ADHD, but a causal effect of developmental nicotine exposure on hyperactivity is not well established and other factors in tobacco smoke might contribute to the development of ADHD in exposed children. In contrast, developmental nicotine seems to result in long-lasting increases in anxiety, but it remains to be seen if maternal smoking or nicotine exposure during NRT has a similar effect in children. Thus, future studies should address the question if nicotine is involved in the increased risk to develop ADHD and how nicotine affects anxiety-like behavior. In particular, animal studies are needed with adjusted doses of nicotine which better reflect those found in smokers. This knowledge will be especially helpful when discussing the pros and cons of NRT in pregnancy, breastfeeding and adolescent and adult smokers.

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