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Central myelin gene expression during postnatal development in rats exposed to nicotine gestationally

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HIGHLIGHTS

- Postnatal myelin gene expression was examined in rats exposed to gestational nicotine (GN).
- Myelin gene expression in the brain was altered in GN-treated juveniles, adolescents, and adults.
- Age, brain region, and sex differences were observed in GN's effect on myelin gene expression.
- The myelin gene expression response to GN in adolescence is unique.
- Long-term effects on myelin gene expression were observed in female but not male adults.

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ABSTRACT

Abnormal myelin gene expression in the central nervous system (CNS) is associated with many mental illnesses, including psychiatric disorders and drug addiction. We have previously shown that prenatal exposure to nicotine, the major psychoactive component in cigarette smoke, alters myelin gene expression in the CNS of adolescent rats. To examine whether this effect is specific for adolescents, we examined myelin gene expression in the CNS of juveniles and adults. Pregnant Sprague-Dawley rats were treated with nicotine (3 mg/kg/day; GN) or saline (GS) via osmotic mini pumps from gestational days 4–18. Both male and female offspring were sacrificed at postnatal day P20–21 (juveniles), P35–36 (adolescents), or P59–60 (adults). Three limbic brain regions, the prefrontal cortex (PFC), caudate putamen (CPu), and nucleus accumbens (NAc), were dissected. The expression of genes encoding major myelin components was evaluated using quantitative RT-PCR. We found that GN altered myelin gene expression in juveniles with brain region and sex differences. The pattern of alteration was different from that observed in adolescents. Although these genes were expressed normally in male adults, we observed decreased expression in GN-treated female adults, especially in the CPu. Thus, GN altered myelin gene expression throughout postnatal development and adulthood. The effect on adolescents was quite different from that at other ages, which correlated with the unique symptoms of many psychiatric disorders during adolescence.

1. Introduction

Maternal smoking during pregnancy (MSDP) has been associated with many neurobehavioral problems in the offspring. Those whose mothers smoke during pregnancy are more likely to show reduced cognitive abilities [10] and to develop neuropsychiatric disorders such as attention deficit hyperactivity disorder, conduct disorder, depression, autism, and drug addiction [24].

To evaluate the underlying mechanisms, we established a rat model of gestational exposure to a moderate dose of nicotine (GN) [35], the major psychoactive component of tobacco. Our previous studies focusing on adolescents showed that GN altered behavioral responses to addictive substances [16,17], cell death/survival pathways [45], and expression of cell adhesion molecules in the central nervous system (CNS) [7]. Recently, we found that central myelin gene expression was also changed during adolescence by GN treatment in a brain region- and sex-dependent manner [8]. These studies suggest that nicotine replacement therapy during pregnancy may carry many of the same risks to the offspring as maternal smoking.

Myelin is a membrane structure produced by oligodendrocytes (OLGs) in the CNS and consists of many specific proteins and

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large amounts of glycolipids and cholesterol [3,12]. Myelin basic protein (MBP) and proteolipid protein (PLP) contribute approximately 85% of the protein content of myelin. The remaining 15% includes 2′,3′-cyclic nucleotide 3′-phosphodiesterase (CNP), myelin oligodendrocyte glycoprotein (MOG), myelin-associated oligodendrocytic basic protein (Mobp), and T-cell differentiation protein (Mal) [3]. Deficits in these major myelin components lead not only to abnormal myelin structure but also to axonal degeneration [3].

The functional acknowledgment of the OLG–myelin complex has greatly advanced in the past decades. Myelin structure not only increases the conduction velocity of action potentials [22], but also interacts with axons to support neuronal survival and modulate neurotransmission [13,34,39]. OLGs also synthesize neurotrophic factors to promote neuronal survival and axonal growth [46]. Recently, OLG–myelin complex has attracted new interest because of its apparent involvement in drug addiction and various psychiatric disorders such as schizophrenia, bipolar disorder, and major depression [6,15].

The process of myelination involves OLG-precursor migration, proliferation, and differentiation into OLGs followed by maturation and formation of a myelin sheath around axons [3]. Myelination initiates during embryonic development, continues into adolescence, and still exhibits great plasticity in the adult nervous system [2,4,31]. As the major resource for cholesterol production in the brain and high energy demands for producing and maintaining the massive membrane structure, the process of myelination is vulnerable to environmental challenges [2]. Our recent study suggested that prenatal nicotine exposure affects myelination in the adolescent brain. However, it is not clear whether this effect is specific to adolescents. Therefore, we examined major myelin gene expression in GN-treated rats at different ages, including juveniles and young adults.

2. Materials and methods

2.1. Animals and tissue collection

Sprague-Dawley rats were maintained in a temperature (21 °C)- and humidity (50%)-controlled room on a 12-h light–dark cycle (lights on 0700–1900) with unlimited access to food and water. Pregnant rats (Harlan, San Diego, CA) were treated with either nicotine at a concentration of 3 mg/kg/day (calculated free base) or saline via osmotic minipumps (Alzet Model 2002) as described previously [35]. The minipump has a 14-day delivery period and was implanted subcutaneously on the back of each dam on gestational day 4. Blood concentrations resulting from this dose of nicotine are equivalent to those found in humans who smoke about 1½ packs of cigarettes per day [29]. After birth, pups were cross-fostered on drug-naive dams to minimize the effects of abnormal maternal behaviors or milk output attributable to nicotine treatment. As previously reported [16], GN treatment did not influence dam weight gain, litter size, or pup weight gain during postnatal development. Pups were weaned on postnatal day (P) 21, and for each sex at each age, non-sibling animals were used. Brain tissues from the prefrontal cortex (PFC), dorsal caudate putamen (CPu), and nucleus accumbens (NAc) were collected from pups at P20–21 (juveniles), P35–36 (adolescents), or P59–60 (adults) and stored at −80 °C until being used for quantitative real-time polymerase chain reaction (qRT-PCR) assay ($N = 5$ or 6). The tissues were excised using a brain punch tissue set (Stoelting, WI) and rat brain matrices (Kent Scientific, CT) according to coordinates from Paxinos and Watson [37]. All experiments were carried out in accordance with the Institutional Animal Care and Use Committees at the University of California, Irvine, and University of Virginia and were consistent with Federal guidelines.

2.2. Quantitative real-time PCR array

We examined eight genes encoding major myelin components, namely, myelin basic protein (Mbp), proteolipid protein 1 (Plp1), 2′,3′-cyclic nucleotide 3′ phosphodiesterase (Cnp), T-cell differentiation protein (Mal), gap junction protein, gamma 3 (Gjc3), myelin-associated oligodendrocytic basic protein (Mobp), myelin oligodendrocyte glycoprotein (Mog), and aspartoacylase (Aspa) using qRT-PCR. The primers were designed using Primer Express (v. 3.0) software (Applied Biosystems) and spanned introns to avoid amplifying genomic DNA. The amplicon sequences were subjected to a BLAST search to ensure the specificity of the primers for the target genes and synthesized by Fisher Scientific (Pittsburgh, PA). All the primers were tested for their specificity by checking cycle number and the dissociation curve prior to inclusion in the qRT-PCR array. The primer sequences are listed in Supplementary Table 1.

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.neulet.2013.08.012.

The RNA was isolated from each brain region using TRIZol reagent (Invitrogen) according to the manufacturer's instructions and amplified as described previously for adequate cDNA [7]. The qRT-PCR was conducted as described previously [21,27]. Briefly, the RT product was amplified in a volume of 10 μl containing 5 μl of 2× *Power* SYBR® Green PCR Master Mix (Applied Biosystems) and combined sense and antisense primers (3 μl; final concentration 250 nM) in a 384-well plate using the 7900HT Sequence Detection System (Applied Biosystems). Expressions of all genes were normalized to the expression of actin and GAPDH and then analyzed using a comparative C_t method [47]. Because data normalized to GAPDH yielded results similar to those normalized by actin, only the results normalized by actin are provided in this report.

2.3. Data analysis

Instead of comparing gene expression directly across ages and sexes, we calculated the ratios of gene expression in the GN group versus the corresponding GS group at each age and for each sex. This eliminates the potential impact of age and sex differences on brain structure and tissue collection.

Data were analyzed by mixed-design ANOVA with between-subjects factors (Age, Sex, Brain Region, and Drug) and within-subject factor (Gene). Significant main effects and interactions were further analyzed by appropriate ANOVA and post hoc analysis with Bonferroni correction for multiple comparisons. Significant alteration in mRNA expression was defined as a fold change >20% with a p value < 0.05.

3. Results

ANOVA analysis revealed the expression levels of myelin genes were altered by GN treatment with a significant interaction of treatment with brain region, sex, and age ($F_{4, 122} = 6.665$, $p < 0.0001$).

3.1. Prefrontal cortex

In the PFC, myelin gene expression was altered by GN treatment with a significant interaction of treatment with Sex and Age ($F_{2, 37} = 7.487$; $p = 0.002$). All examined genes showed similar patterns of alteration within each age and sex, as indicated by an insignificant Gene effect and insignificant interactions of Gene with other factors (Fig. 1). In juveniles, these genes generally showed decreased expression in GN-treated animals compared with GS controls in both males ($p < 0.05$ for *Mbp*, *Plp1*, *Mal*, *Gjc3*, *Mobp*, *Mog*, and *Aspa*) and females ($p < 0.05$ for *Mbp*, *Mobp*, *Mog*, and *Aspa*;