

Nicotine-induced DNA methylation in spermatozoa: Consequences for offspring's behavior and gene expression in the brain

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Cigarette smoking and other forms of nicotine use remain a leading cause of disease, disability and death in the United States. Nicotine's effects on epigenetic modification of somatic cell DNA are becoming known. However, little is known about nicotine's effects on the germ line or the potential consequences of any such effects on the offspring's brain or behavior. With this in mind, we exposed adult male mice to nicotine (200µg/ml) in drinking water for 12 weeks. While the nicotine exposure was ongoing, the mice were bred with drug naïve females. To evaluate the hypothesis that nicotine can induce epigenetic modification of male germ cell DNA, we collected spermatozoa from the nicotine-exposed males and examined methylation of spermatozal DNA using DNA immunoprecipitation combined with qPCR. We found significant changes in genome-wide DNA methylation as well as DNA methylation at dopamine receptor promoter regions in the nicotine-exposed fathers' spermatozoa. The offspring of the nicotine-exposed males displayed hyperactivity and inattention, phenotypes commonly associated with ADHD. Interestingly, the nicotine-exposed mice (fathers) did not display either of these behavioral phenotypes. We also examined dopamine receptor mRNA expression in the offspring's brain using quantitative real-time PCR. Dopamine D1, D2, D4 and D5 receptor mRNA showed sex- and brain region-specific changes in the offspring, although the offspring were not exposed to nicotine during their life cycle. These data suggest that nicotine-induced epigenetic modification of the father's germ line is associated with behavioral phenotypes and molecular changes in the offspring's brain. Our findings call for revision of the current education, research and public health efforts, which focus primarily on nicotine exposure of women, so that nicotine's effects on men, especially their germ line, can receive equal attention.

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