Modeling gene x environmental effects in the mouse: the multigenerational phenotypes of the interaction of the rs16969968 SNP with developmental nicotine exposure

Heidi C. O'Neill and Jerry A. Stizel

The nicotinic acetylcholine system in the brain is involved in numerous developmental functions including promotion of cell migration and initiation/termination of axonogenesis and synaptogenesis. Mechanisms underlying deleterious phenotypic outcomes in infants and adolescents exposed to nicotine developmentally may be set in motion by premature exposure to nicotine acting as an acetylcholine agonist, perturbing normal developmental trajectories. Recent genome-wide association studies uncovered a non-synonymous single nucleotide polymorphism in the intracellular loop of the alpha 5 nicotinic receptor linked to increased risk for nicotine dependence. Our lab developed a mouse with the rs16969968 SNP (hereafter D397 for wild type; N397 for risk variant) and current studies are utilizing this mouse to evaluate the interaction of the SNP and developmental nicotine exposure. Female D397 and N397 mice received either nicotine or vehicle (0.2% saccharin) in their drinking solution from 30 days prior to breeding (for acclimation) through weaning (to prevent maternal withdrawal). Offspring (F1 generation: D397 veh, N397 veh, D397 nic, N397 nic) were not exposed to nicotine after weaning (unless in preference studies). To investigate potential multigenerational changes, we then crossed developmental nicotine exposed females of both genotypes (D397 and N397) to nicotine-naïve males to produce an F2 generation. To date, F1 mice have tested for nicotine consumption, conditioned place preference (CPP), homecage activity and pre-pulse inhibition of the startle reflex (PPI) and F2 mice have been tested for homecage activity and PPI. Nicotine preference studies in F1 generation offspring demonstrated a clear gene x environment interaction as nicotine exposure enhanced nicotine intake in N397 nic mice while dramatically decreasing intake in D397 nic mice compared to veh controls. Similarly, preliminary conditioned place preference (CPP) results showed greater preference in N397 nic exposed mice while D397 mice exhibited a conditioned place AVERSION. For homecage activity in F1 offspring, we found that N397 veh mice were more active than D397 veh mice. Developmental exposure led to an increase in activity for both D397 and N397 nic F1 mice relative to their respective veh controls. Both D397 and N397 nic F2 mice had dramatically increased activity in homecage relative to controls, suggesting multigenerational transmission of hyperactivity resulting from developmental exposure to nicotine. Lastly, PPI in D397 veh F1 mice was consistent with normal adult D397 mice. However, preliminary data shows that following developmental nic exposure, there is an impairment in D397 nic PPI; F2 generation D397 mice exhibited further impairment of PPI. In contrast, the N397 veh F1 mice displayed no PPI and in some dB levels we saw facilitation. N397 developmental nicotine exposed offspring saw improved PPI, and very preliminary data suggests N397 F2 mice are further improved suggesting that depending upon genotype, developmental nic exposure can either impair or improve PPI. As

methylation is required for normal development, we investigated global changes using a 5-methylcytosine ELISA; preliminary data using C57 mice indicated a significant decrease (compared to veh controls) in global methylation in both the striatum of C57 F2 generation mice (mother had developmental exposure) and in the frontal cortex of C57 nic mice. Combined, these data suggest epigenetic modifications may contribute to phenotypic outcomes. Our goal moving forward is to investigate candidate targets for epigenetic modifications that underlie these outcomes.