

## **Paternal nicotine exposure trans-generationally alters fear response and cholinergic function: potential epigenetic mechanisms**

Kutlu<sup>1</sup> M.G., Cole<sup>2</sup> R., Tumolo<sup>2</sup> J.M., Parikh<sup>2</sup> V., Gould<sup>1</sup> T.J.

<sup>1</sup>Penn State University, Department of Biobehavioral Health, University Park, PA

<sup>2</sup>Temple University, Department of Psychology, Neuroscience Concentration  
Philadelphia, PA

Tobacco use has declined in the US from 42% in the 1960's to 20% in 2004. However, even though these rates have remained relatively stable since then, smoking is still the leading cause of preventable death in the US, responsible for over 440,000 deaths per year in the US alone, which consists of 20% of all deaths in the country. In addition, numerous studies have indicated a relationship between smoking and mental health problems including but not limited to anxiety and stress disorders, ADHD, depression, and schizophrenia. Recent studies have also revealed that effects of substance of abuse are not confined within the same generation. Importantly, the effects of exposure to addictive drugs such as alcohol, opiates, cocaine, and nicotine are trans-generationally transmitted through epigenetics mechanisms. While trans-generational effects of nicotine have been documented, influence of nicotine exposure on pathogenesis of fear and anxiety-related disorders in subsequent generations are virtually unknown. Therefore, in order to investigate the trans-generational effects of paternal nicotine exposure on fear learning and anxiety, we conducted a series of experiments where male adult C57BL6/J mice received either chronic nicotine (28 days, 12.6 mg/kg/d) or chronic saline exposure. The offspring of nicotine (Nic-Sired) and saline (Sal-Sired) exposed mice were tested in contextual and cued fear conditioning in order to understand paternal nicotine's effects on fear processing. Our results demonstrated that paternal nicotine exposure resulted in augmented cued and contextual fear learning in the subsequent (F1) generation compared to Sal-Sired mice. This effect was reversed when F1 generation mice received acute nicotine injections at the 0.09 and 0.18 mg/kg doses. The paternal nicotine exposure-induced augmentation of fear learning also persisted in the F2 generation mice bred from the F1 male mice. However, acute nicotine had no effects on paternal nicotine exposure-induced enhancement of fear learning in F2 generation. Moreover, although extinction of contextual fear conditioning was normal in the F1 generation Nic-Sired mice, they showed more pronounced spontaneous recovery of fear following extinction, indicating altered fear reactivity. Importantly, Nic-Sired mice showed normal memory function in the non-affective Novel Object Recognition paradigm, which suggests that the effect of paternal nicotine does not generalize to other types of learning. In follow-up experiments, we did not find any effect of paternal nicotine exposure in the elevated plus maze or open field tasks, which excluded the possible confounding effects of anxiety and locomotor activity. In addition to the behavioral testing, we also measured potassium and nicotine-evoked acetylcholine release as well as nicotinic acetylcholine receptor (nAChR) binding in the hippocampus using an electrochemical recording technique and a receptor binding assay, respectively. Our results showed reduced ventral, but not dorsal, hippocampal cholinergic function in the Nic-Sired mice. Furthermore, we found upregulated nAChR binding overall in the hippocampus. In parallel, we also found increased DNA methylation in the ventral hippocampi of the Nic-Sired mice whereas this effect was absent in the dorsal hippocampus. We also have RNASeq preliminary data showing that Nic-Sired mice show significant gene expression alterations in genes involved in glutamatergic and GABAergic transmission. Together, our results suggest that paternal nicotine exposure may result in alterations in the epigenome, which, in turn, leads to exaggerated fear learning and abnormal cholinergic function in subsequent generations.

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