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Choline ameliorates adult learning deficits and reverses epigenetic modification of chromatin remodeling factors related to adolescent nicotine exposure

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ABSTRACT

Earlier initiation of smoking correlates with higher risk of nicotine dependence, mental health problems, and cognitive impairments. Additionally, exposure to nicotine and/or tobacco smoke during critical developmental periods is associated with lasting epigenetic modifications and altered gene expression. This study examined whether adolescent nicotine exposure alters adult hippocampus-dependent learning, involving persistent changes in hippocampal DNA methylation and if choline, a dietary methyl donor, would reverse and mitigate these alterations. Mice were chronically treated with nicotine (12.6 mg/kg/day) starting at post-natal day 23 (pre-adolescent), p38 (late adolescent), or p54 (adult) for 12 days followed by a 30-day period during which they consumed either standard chow or chow supplemented with choline (9 g/kg). Mice then were tested for fear-conditioning and dorsal hippocampi were dissected for whole genome methylation and selected gene expression analyses. Nicotine exposure starting at p21 or p38, but not p54, disrupted adult hippocampus-dependent fear conditioning. Choline supplementation ameliorated these deficits. 462 genes in adult dorsal hippocampus from mice exposed to nicotine as adolescents showed altered promoter methylation that was reversed by choline supplementation. Gene network analysis revealed that chromatin remodeling genes were the most enriched category whose methylation was altered by nicotine and reversed by choline dietary supplementation. Two key chromatin remodeling genes, *Smarca2* and *Bahcc1*, exhibited inversely correlated changes in methylation and expression due to nicotine exposure; this was reversed by choline. Our findings support a role for epigenetic modification of hippocampal chromatin remodeling genes in long-term learning deficits induced by adolescent nicotine and their amelioration by dietary choline supplementation.

1. Introduction

Changes in cognition contribute to nicotine addiction and conversely, nicotine may disrupt cognition (Gould, 2010; Patterson et al., 2010). Both human and animal studies have shown that chronic nicotine exposure during adolescence leads to long lasting cognitive and behavioral impairments, including effects on memory and attention and reduced prefrontal cortex activation (Jacobsen et al., 2005; Trauth, Seidler, & Slotkin, 2000). Additionally, younger onset of nicotine use has been directly linked to severity of smoking and greater severity of nicotine dependence in adulthood (Center for Disease Control, 2012; Chassin, Presson, Pitts, & Sherman, 2000).

Nicotine differentially affects the adult versus the adolescent brain and subsequently has age-dependent effects on learning. Acute nicotine

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exposure enhanced hippocampus-dependent learning in adult mice while withdrawal from chronic nicotine exposure produced transient impairments (Davis, James, Siegel, & Gould, 2005; Gould and Higgins, 2003; Gould and Lommock, 2003; Portugal and Gould, 2009). In contrast, adolescent mice exposed to chronic nicotine exhibited long-term deficits in hippocampal-dependent contextual fear manifesting in adulthood; mice exposed to nicotine in adulthood did not exhibit significant long-term contextual fear learning impairments (Holliday and Gould, 2017; Holliday et al., 2016; Portugal, Wilkinson, Turner, Blendy, & Gould, 2012). The developmentally sensitive effects of nicotine on behavior extend to altered morphology of pyramidal cells in the hippocampus with adolescent nicotine exposure leading to atrophy of apical dendrites of pyramidal cells in the CA1 region and adult nicotine exposure leading to atrophy of basilar CA3 dendrites (Holliday et al., 2016).

The molecular mechanisms by which nicotine elicits these long-lasting changes are still poorly understood. Recently, nicotine has been shown to promote epigenetic changes in a neonatal nicotine exposure model, inducing histone methylation changes previously linked predominantly to gene activation (Jung et al., 2016). Epigenome Wide Association Studies "EWAS" comparing methylation levels of peripheral blood from adult smokers and infants exposed to tobacco smoking *in-utero* identified a number of genes showing developmentally specific alterations in methylation patterns, with changes associated with neonatal exposure but not adult exposure (Pirini, Guida, Lawson, Mancinelli, & Guerrero-Preston, 2015; Rzehak et al., 2016; Zeilinger et al., 2013). Additionally, longitudinal analysis of methylation changes following *in utero* smoke exposure identified methylation changes that persisted into adolescence (Lee et al., 2015). This suggests a role for epigenetic modifications in the cognitive and behavior impairments observed after developmental chronic nicotine exposure, but the effects of adolescent exposure are unknown.

Choline, an essential nutrient, can modulate cognitive function, especially during development (Meck et al., 2008; Thomas and Tran, 2012). In animals, choline supplementation partially restored cognitive performance when administrated prenatally and even postnatally in models of prenatal alcohol exposure (Ryan, Williams, & Thomas, 2008; Schneider and Thomas, 2016). Choline may modulate neurocognitive processes via a number of different mechanisms still largely unexplored. First, choline is a precursor of acetylcholine (Murai et al., 1994) and a α7 nicotinic receptor agonist (Alkondon, Pereira, Cortes, Maelicke, & Albuquerque, 1997; Mike, Castro, & Albuquerque, 2000). Second, choline is also a precursor for phospholipids abundant in the cell membrane and myelin sheath, potentially affecting membrane potential and neuronal functions. Lastly, choline is the primary methyl donor for DNA methylation and can thereby influence gene expression across the transcriptome (Niculescu, Craciunescu, & Zeisel, 2006). In support of choline mediating epigenetic processes, changes in global DNA 5mC levels in both hippocampus and prefrontal cortex were observed in rats prenatally exposed to ethanol and then postnatally treated with a choline-supplemented diet compared with littermates with no choline supplementation (Otero, Thomas, Saski, Xia, & Kelly, 2012). Given that choline has positive restorative effects on cognition in prenatal and early postnatal alcohol exposed rats (Corriveau and Glenn, 2012; Velazquez et al., 2013; Wong-Goodrich et al., 2008) and that nicotine exposure during adolescence impairs adult cognition (Portugal et al., 2012; Holliday and Gould, 2017), we investigated the long-term effects of adolescent nicotine exposure on learning and changes in hippocampal epigenetic regulation of gene expression and whether choline could reverse epigenetic changes and restore hippocampus-dependent learning in adult mice exposed to nicotine during adolescence.

2. Material and methods

2.1. Subjects and experimental conditions

Male C57BL/6J mice were obtained from Jackson Laboratories (Bar Harbor, ME) at either post-natal day (p) p16, p31, or p47. P16 mice were shipped with dams and weaned at p21 into groups of four mice with a maximum of two littermates per group. Each age had four experimental groups consisting of two drug conditions (nicotine or saline) and two diet conditions (standard and choline supplemented). Thus, each age group had the following conditions: (1) Saline-Standard (SAL-CHOW; n = 12–16), (2) Saline-Choline (SAL-CHOL; n = 12–16), (3) Nicotine-Standard (NIC-CHOW; n = 12–16), and (4) Nicotine-Choline (NIC-CHOL; n = 12–16). Whole genome methylation and subsequent analyses were based on a subset of mice (n = 4/condition) from the late adolescent cohort.

2.2. Drug and diet methods

One week following arrival at the Temple University animal facility, mice were subcutaneously implanted with a mini osmotic pump to deliver saline or nicotine (Sigma, St. Louis, MO; freebase, 12.6 mg/kg/day) at p23 (pre-adolescent), p38 (late adolescent), and p54 (adulthood); 12.6 mg/kg/day produces plasma nicotine and cotinine levels in the range seen in human smokers (Benowitz, Hukkanen, & Jacob, 2009; Cole, Poole, Guzman, Gould, & Parikh, 2015; Davis et al., 2005). Nicotine or saline was delivered continuously for 12 days at which time the mini pump was removed via a second incision. All mice underwent a 30-day prolonged abstinence period during which they were given *ad libitum* access to either standard mouse chow (LabDiet Mouse Chow 5015) or a choline-supplemented diet (TestDiet; Richmond, IN). Standard mouse chow had 2000 ppm (2 g/kg) of choline and the TestDiet choline-supplemented diet had 9000 ppm (9 g/kg), which was 4.5× greater than the standard diet. This concentration of choline was chosen based on previously published observations indicating reversal of cognitive deficits associated with rat models of fetal alcohol syndrome and schizophrenia (Corriveau and Glenn, 2012; Velazquez et al., 2013; Wong-Goodrich et al., 2008). Following 30 days nicotine-free with continuous access to either standard diet or choline-supplemented diet, all mice were fear conditioned with access to respective diets in between sessions.

2.3. Fear conditioning

Training and testing procedures for fear conditioning have been described in detail previously (Gould and Higgins, 2003; Gould and Lommock, 2003; Gould, Feiro, & Moore, 2004). Briefly, mice were trained and tested in four identical chambers (17.78 cm × 19.05 cm × 38.10 cm) housed in sound attenuating boxes (Med-Associates, St. Albans, VT) for contextual fear conditioning. Mice were exposed to an auditory conditioned stimulus (CS, 85db white noise) lasting for 30 sec that co-terminated with a 2 s shock, serving as the unconditioned stimulus (US, 0.57 mA, minimum level necessary for learning) for a total of two CS-US presentations. On the second day, mice were placed in the same chamber and freezing behavior was scored for the next 5 min to measure contextual conditioning. One hour later, mice were placed in different chambers with an additional vanilla extract olfactory cue and allowed to explore for 180 s with freezing behavior assessed (preCS) before presented with the auditory stimulus (CS) for another 180 s. Experimenters were blind to treatment conditions. Both contextual and cued fear conditioning were assessed to examine hippocampus-dependent (contextual fear conditioning) and hippocampus-independent (cued fear conditioning) learning (Fanselow, 2000; Kim and Fanselow, 1992; Logue, Paylor, & Wehner, 1997; Phillips and LeDoux, 1992).