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Chronic fluoxetine ameliorates adolescent chronic nicotine exposure-induced long-term adult deficits in trace conditioning

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ABSTRACT

Development of the brain, including the prefrontal cortex and hippocampus, continues through adolescence. Chronic nicotine exposure during adolescence may contribute to long-term deficits in forebrain-dependent learning. It is unclear if these deficits emerge immediately after exposure and if they can be ameliorated. In this study, C57BL/6J mice were treated with chronic nicotine (6.3 or 12.6 mg/kg/day) over 12 days beginning at adolescence, postnatal day (PND) 38, or adulthood, PND 56–63 ± 3. We investigated the effects of short-term (24 h) abstinence on trace fear conditioning and found that adult treatment resulted in deficits (6.3 and 12.6 mg/kg/day), but adolescent chronic nicotine treatment had no effect. In contrast, adolescent treatment with chronic nicotine (12.6 mg/kg/day) elicited a long-term (30 days) learning deficit, but adult chronic nicotine treatment did not. Using the elevated plus maze (EPM) we found no long-term changes in anxiety-related behavior after chronic nicotine exposure at either time-point. We investigated if chronic fluoxetine (FLX) could ameliorate adolescent chronic nicotine-associated long-term deficits in trace conditioning. We found that chronic FLX (160 mg/L) in drinking water ameliorated the long-term deficit in trace fear conditioning associated with nicotine exposure during adolescence. Additionally, in the same animals, we examined changes in total BDNF protein in the dorsal hippocampus (DH), ventral hippocampus (VH), and prefrontal cortex (PFC). Chronic FLX increased DH BDNF. Our data indicate nicotine administration during adolescence leads to late onset, long-lasting deficits in hippocampus-dependent learning that chronic FLX treatment ameliorate.

1. Introduction

Adolescence is associated with increased risk taking and impulsivity (Spear, 2000), along with rapid neurocognitive developmental changes (Gogtay et al., 2004). Furthermore, the adolescent brain responds differently to drugs of abuse compared to that of the adult (Faraday et al., 2001; Portugal et al., 2012; Reynolds et al., 2015; Slotkin et al., 2014; Xu et al., 2002). Increased impulsivity and risk taking during adolescence may be adaptive under normal conditions; however, it is also thought to drive experimentation with drugs, which can lead to dependence (Chassin et al., 1990; Counotte et al., 2011). For example, initiation of tobacco use most often occurs during adolescence and individuals that initiate smoking during adolescence show increased tobacco consumption later as adults (Chen and Millar, 1998). A critical feature of adolescent neurodevelopment is the maturation of dopaminergic, serotonergic, and cholinergic systems (An et al., 2008; Gould et al., 1991; Xu et al., 2002), and nicotine, the primary psychoactive compound in tobacco, is known to readily act on these neurotransmitter systems (Davis and Gould, 2007a; Dong et al., 2009; Xu et al., 2002). Therefore, during adolescence, individuals are both at risk for initiation of nicotine use and differentially susceptible to the effects of nicotine.

Nicotine can potently alter cognition. For example, acute nicotine enhances associative learning (Gould and Wehner, 1999; Wehner et al., 2004), via high-affinity nicotinic receptors (nAChRs) within the hippocampus and PFC (Kenney et al., 2012; Raybuck and Gould, 2010). In contrast, immediate abstinence (withdrawal) from chronic nicotine elicits deficits in hippocampus-dependent learning in adult mice (Davis et al., 2005; Davis and Gould, 2009; Gould et al., 2012; Raybuck and Gould, 2009). Adolescence appears be a time of unique vulnerability to nicotine-dependent changes in cognition. Importantly, adolescent animals

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treated with chronic nicotine present with hippocampal learning deficits after 30 days abstinence in adulthood, but adult nicotine-treated animals did not show long-term deficits (Holliday et al., 2016; Portugal et al., 2012). Thus, exposure to nicotine during adolescence can lead to age-dependent long-term alterations in cognition persisting into adulthood.

Chronic nicotine exposure during adolescence elicits long-term deficits in hippocampus-dependent contextual fear conditioning, but not delay cued fear conditioning, a non-hippocampal learning paradigm (Holliday et al., 2016; Portugal et al., 2012). However, it is not known if long-term deficits due to adolescent chronic nicotine are confined to contextual fear learning, or if these deficits present in other hippocampal fear learning tasks. Thus, we decided to examine the effects of prior chronic nicotine exposure on trace fear conditioning, which like delay conditioning also depends on forming a discrete CS-US association. However, unlike delay conditioning in which the CS and US temporally overlap, the CS and US in trace conditioning are temporally separated making the task hippocampus and PFC-dependent and may model aspects of working and declarative memory (Connor and Gould, 2016). Moreover, the PFC and hippocampus undergo maturation during adolescence (Gogtay et al., 2004) and have previously been shown to be altered by adolescent nicotine exposure (Goriounova and Mansvelder, 2012; Holliday et al., 2016; Portugal et al., 2012). Therefore, we hypothesized that chronic treatment during adolescence, but not adulthood would lead to long-term deficits in trace fear conditioning. In addition, because adolescent nicotine exposure can also elicit long-term affective changes (Holliday et al., 2016; Slawecki et al., 2003), we also probed for long-term changes in innate anxiety in the EPM after adolescent or adult chronic nicotine exposure. Finally, immediate abstinence has been shown to result in short-term withdrawal deficits in trace fear conditioning in adult mice (Raybuck and Gould, 2009). However, the effects of short-term abstinence on trace fear conditioning in adolescent animals has not been studied. Therefore, we also investigated whether these deficits emerge immediately after chronic nicotine treatment during adolescence or have a later onset.

Long-term negative effects of adolescent chronic nicotine exposure on cognition support the need to identify potential treatments. For example, treatment with cholinergic agents can reduce the detrimental effects of immediate chronic nicotine withdrawal on cognition in adult animals (Poole et al., 2014; Yildirim et al., 2015). However, pharmacological treatments for the long-term effects of adolescent chronic nicotine exposure on hippocampal learning have not been similarly investigated. Neurobiological evidence suggests that adolescent nicotine exposure can elicit long-lasting dysregulation of the serotonergic system (Slotkin et al., 2016; Xu et al., 2002). Fluoxetine, a selective-serotonin reuptake inhibitor, has been shown to act on neural substrates of learning and ameliorate cognitive deficits associated with chemotherapeutic agents and in a mouse model of Alzheimer's disease (Jin et al., 2016; Lyons et al., 2011). Adult fluoxetine (FLX) treatment also decreased negative affective outcomes in adulthood due to adolescent nicotine exposure (Iñiguez et al., 2008). Therefore, we hypothesized that chronic FLX treatment would ameliorate long-term trace fear conditioning deficits resulting from adolescent chronic nicotine exposure. In addition, FLX is known to increase brain derived neurotrophic factor (BDNF) gene expression in the PFC and hippocampus (Alme et al., 2007). BDNF is important for synaptic plasticity and learning and memory (Cunha et al., 2010; Korte et al., 1998; Mizuno et al., 2012) and the hippocampus sends BDNF containing projections to the PFC and BDNF within these regions plays an important role in associative learning (Heldt et al., 2007; Hoover and Vertes, 2007; Peters et al., 2010). Because nicotine has been shown to alter BDNF levels, we considered that cognitive deficits due to prior chronic nicotine exposure may be mediated in part by altered levels if BDNF, which could be rescued by treatment with FLX. Thus, we selected the dorsal, ventral and PFC as regions of interest to examine changes in BDNF protein after chronic FLX treatment.

2. Material and methods

2.1. Subjects

Male C57BL/6J mice (Jackson Laboratories, Bar Harbor, ME) were used in all experiments. Adolescent mice were PND 31 on day of delivery and adults were PND 49 ± 3. Mice were allowed to acclimate to the vivarium for at least 1 week prior to the start of experiments. Mice were housed 2–4 per cage and provided food *ad libitum*. A 12 h light-dark cycle was maintained throughout all studies. All behavioral procedures were approved by the Temple University Institutional Animal Care and Use Committee.

2.2. Drug administration and experimental design

For all experiments, doses of nicotine hydrogen tartrate salt (Sigma-Aldrich, St. Louis, MO) were dissolved in 0.9% saline and reported as freebase weight. Chronic nicotine (12.6 mg/kg/day and 6.3 mg/kg/day) and saline was administered via subcutaneous osmotic minipumps (Alzet, Cupertino, CA). These doses of chronic nicotine and the rout of administration are based on previous findings showing contextual learning deficits after adult and adolescent chronic nicotine administration (Holliday et al., 2016; Portugal et al., 2012). Additionally, these doses of nicotine are within a range of doses that result in blood plasma nicotine and cotinine levels similar to regular smokers (Cole et al., 2014; Davis et al., 2005; Portugal et al., 2012).

Fluoxetine hydrochloride (Sigma-Aldrich) was administered *ad libitum* in drinking water (filtered tap water) in opaque light-protected bottles. FLX solutions were changed and refreshed 3 times per week. We administered FLX at 160 mg/L, this concentration was previously shown to increase hippocampal BDNF protein in mice (Bath et al., 2012; Dulawa et al., 2004). Because animals were housed in groups, exact dosage is not known. However, a pilot analysis of consumption patterns indicated that mice consumed ~2–3 ml per day of 160 mg/L solution, resulting in estimated 15.25 mg/kg dose over the treatment period. This dose value is similar to that used by Bath et al. (2012), who estimated ~16 mg/kg using the same 160 mg/L concentration.

2.2.1. Age-dependent effects of short-term (24 h) and long-term (30 days) abstinence from chronic nicotine

For each dose of chronic nicotine (12.6 and 6.3 mg/kg/day), we used a separate a cohort of mice with aged match saline controls. Mice were treated with chronic nicotine at 12.6 mg/kg/day or 6.3 mg/kg/day for 12 days beginning at adolescence (PND 38) or adulthood (PND 56–63 ± 3). On day 12 of chronic administration, osmotic mini-pumps were surgically removed and animals were returned to the colony room in home cages. To examine age-dependent effects of chronic nicotine exposure on short-term abstinence, mice were trained in trace fear conditioning after 24 h abstinence and tested 24 h after training. In a separate set of animals, age-dependent long-term effects of chronic nicotine were tested first in the EPM on day 28 post pump removal, then 2 days later (30 days after pump removal) these same mice were trained in trace fear conditioning and tested 24 h later (Fig. 1).