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Research Report

Adolescent mice are less sensitive to the effects of acute nicotine on context pre-exposure than adults

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ABSTRACT

Adolescence is a critical developmental period associated with both increased vulnerability to substance abuse and maturation of certain brain regions important for learning and memory such as the hippocampus. In this study, we employed a hippocampus-dependent learning context pre-exposure facilitation effect (CPFE) paradigm in order to test the effects of acute nicotine on contextual processing during adolescence (post-natal day (PND) 38) and adulthood (PND 53). In Experiment 1, adolescent or adult C57BL6/J mice received either saline or one of three nicotine doses (0.09, 0.18, and 0.36 mg/kg) prior to contextual pre-exposure and testing. Our results demonstrated that both adolescent and adult mice showed CPFE in the saline groups. However, adolescent mice only showed acute nicotine enhancement of CPFE with the highest nicotine dose whereas adult mice showed the enhancing effects of acute nicotine with all three doses. In Experiment 2, to determine if the lack of nicotine's effects on CPFE shown by adolescent mice is specific to the age when they are tested, mice were either given contextual pre-exposure during adolescence or adulthood and received immediate shock and testing during adulthood after a 15 day delay. We found that both adolescent and adult mice showed CPFE in the saline groups when tested during adulthood. However, like Experiment 1, mice that received contextual pre-exposure during adolescence did not show acute nicotine enhancement except at the highest dose (0.36 mg/kg) whereas both low (0.09 mg/kg) and high (0.36 mg/kg) doses enhanced CPFE in adult mice. Finally, we showed that the enhanced freezing response found with 0.36 mg/kg nicotine in the 15-day experiment may be a result of decreased locomotor activity as mice that received this dose of nicotine traveled shorter distances in an open field paradigm. Overall, our results indicate that while adolescent mice showed normal contextual processing when tested both during adolescence and adulthood, they are less sensitive to the enhancing effects of nicotine on contextual processing.

1. Introduction

Adolescence is a critical developmental period associated with increased vulnerability to substance abuse including nicotine addiction (Giovino, 2002; Nelson et al., 2008; see Spear (2000) for a review). For example, the majority of smokers try their first cigarettes before the age of 18 (Everett et al., 1999; Johnston et al., 2009; Lantz, 2003). Furthermore, there is evidence demonstrating that earlier onset of smoking is predictive of more severe nicotine dependence later in life (Everett et al., 1999), suggesting that nicotine use starting in this period has a higher impact on lifelong nicotine addiction when compared to nicotine use started during adulthood.

Adolescence is also a key developmental stage for the maturation of the brain regions important for learning and memory (Benes, 1989; Wolfer and Lipp, 1995; Dumas and Foster, 1998; Eriksson et al., 1998). One such brain region is the hippocampus, a unique brain structure heavily involved in episodic memory, spatial learning, contextual learning, and spatial working memory (Aggleton et al., 1986; Jung and McNaughton, 1993; Burgess et al., 2002; Daumas et al., 2005). Using animal models, numerous studies have shown that acute nicotine enhances hippocampus-dependent forms of learning and memory such as contextual and trace fear conditioning (Gould and Higgins, 2003; Davis et al., 2005, 2006, 2007; Davis and Gould, 2006; Raybuck and Gould, 2010), spatial object recognition (Kenney et al., 2011), spatial learning in Morris Water Maze (Abdulla et al., 1996; Sharifzadeh et al., 2005), and spatial working memory in Radial Arm Maze (Levin et al., 1997,1998; Levin and Torry, 1996). Importantly, Portugal et al. (2012) investigated the effects of nicotine exposure during adolescence on hippocampus-dependent learning and showed that mice had altered sensitivity to the enhancing effects of nicotine on contextual fear conditioning across adolescence.

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Together, the studies mentioned above suggest that acute nicotine enhances hippocampus-dependent learning and memory but the cognitive-enhancing effects of acute nicotine is altered during adolescence.

Another hippocampus-dependent learning paradigm that can be used to examine the effects of nicotine on contextual processing separate from context-shock learning is context pre-exposure facilitation effect (CPFE; Rudy et al., 2002; Matus-Amat et al., 2007; Schiffino et al., 2011). CPFE is a learning task where pre-exposure to the context facilitates contextual fear conditioning induced by a single immediate foot-shock (Fanselow, 1990) up to 28 days after pre-exposure (Rudy and Wright-Hardesty, 2005). Importantly, Kenney and Gould (2008) showed that in adult mice, acute nicotine administrations prior to both context exposure and retrieval test enhanced CPFE whereas nicotine injections prior to context-shock learning and testing had no effect. This suggests that acute nicotine specifically enhances contextual learning but not the context-shock association. However, the effects of acute nicotine on CPFE in adolescent mice are unknown. Therefore, in this study, employing a CPFE paradigm, we investigated the effects of acute nicotine on contextual processing during adolescence and adulthood. Moreover, we tested nicotine's effects on CPFE when mice were given nicotine exposure during adolescence but trained and tested during adulthood. This study informs on the age-specific effects of acute nicotine on short-term (24 h) and long-term (15 days) contextual processing.

2. Results

2.1. Experiment 1: Acute nicotine enhances CPFE in adult but not in adolescent mice

In Experiment 1, adolescent (PND 38) and adult mice (PND 53) were given pre-exposure to the conditioning context (PRE groups) following intraperitoneal injections of either nicotine (0.09, 0.18, or 0.36 mg/kg) or saline. Another group of mice stayed in their homecages after the injections (No-PRE groups). Twenty-four hours later, mice were trained in an immediate shock paradigm and the following day the mice were tested for freezing behavior to the same context (Fig. 1, Upper Panel).

In order to assess the effects of nicotine injections on CPFE in adolescent and adult mice, we conducted a 4 (Drug; 0.09, 0.18, 0.36 mg/kg Nicotine vs. Saline) × 2 (Pre-exposure; No-PRE vs. PRE) ANOVAs for each age group. This way we aimed to identify the dose-response curve for each age group. Our results showed that the adolescent mice were less sensitive to the effects of acute nicotine on CPFE. A 4 × 2 ANOVA showed that the Drug × Pre-exposure interaction was significant for the adult group, $F(3,58)=7.58$, $p<0.001$. Another 4 × 2 ANOVA did not yield a significant Drug × Pre-exposure interaction ($F(3,65)=2.45$, $p>0.05$) for the adolescent group but both drug ($F(3,65)=3.08$, $p=0.033$) and Pre-exposure ($F(1,65)=63.86$, $p<0.001$) main effects were significant. Separate planned t-tests showed a significant difference between Sal PRE and Nic 0.09 mg/kg PRE ($t(16)=3.42$, $p<0.05$), Sal PRE and Nic 0.18 mg/kg PRE ($t(16)=3.02$, $p<0.05$), and Sal PRE and Nic 0.36 mg/kg PRE ($t(16)=5.41$, $p<0.01$) groups in the adults, which shows a significant enhancement of CPFE by all doses of nicotine in adult mice. In adolescent PRE groups, there was only a significant difference between groups that received saline and 0.36 mg/kg nicotine ($t(17)=2.12$, $p<0.05$) while 0.09 mg/kg ($t(20)=0.96$, $p>0.05$) and 0.18 mg/kg ($t(17)=0.27$, $p>0.05$) nicotine groups showed no difference from the saline group. No significant differences were found between the saline and nicotine No-PRE conditions ($ps>0.05$). Also, both adult and adolescent saline group mice showed significant differences between PRE and No-PRE groups (Adults, $t(17)=5.54$, $p<0.01$; Adolescents, $t(22)=3.42$, $p<0.01$), which indicates CPFE in both age groups. Finally, we found a significant difference of baseline CPFE levels between saline treated adolescent and adult mice ($t(18)=2.22$, $p<0.05$). However, no interaction between age and pre-exposure was found for saline treated animals ($F(3,39)=2.139$, $p>0.05$), which suggests that although baseline Sal PRE group responses differed between ages this effect was not strong enough to affect overall CPFE between age groups. These results replicate Kenney and Gould's (2008) study showing acute nicotine-induced enhancement of CPFE in adult mice. However, our results also show that adolescent mice did not display the acute nicotine-induced enhancement of CPFE except at the highest dose of nicotine (0.36 mg/kg), suggesting that the dose response for nicotine effects on CPFE in adolescent mice is shifted to the right (Fig. 2).

2.2. Experiment 2: Acute nicotine enhances the effects of adult but not adolescent contextual pre-exposure when tested during adulthood

The results of Experiment 1 showed that adolescent mice were less sensitive to the enhancing effects of acute nicotine on CPFE. However, it is unclear whether the lack of acute nicotine effects on CPFE shown by adolescent mice was specific to the age when they were tested. Therefore, in Experiment 2, mice were given pre-exposure to the context during adolescence but received immediate shock and were tested during adulthood (Fig. 1, Lower Panel) following acute nicotine (0.09 and 0.36 mg/kg) or saline injections.

In order to identify the effective doses of acute nicotine enhancing CPFE following a 15 day delay, we conducted a 3 (Drug; 0.09, 0.36 mg/kg Nicotine vs. Saline) × 2 (Pre-exposure; No-PRE vs. PRE) ANOVAs for each age group. Our results showed that the mice that received pre-exposure to the training context during adolescence required a higher acute nicotine dose (0.36 mg/kg) for the effects of acute nicotine on CPFE whereas both acute nicotine doses were effective in the group that received pre-exposure during adulthood. Two 3 × 2 ANOVAs showed that the Drug × Pre-exposure interaction was significant for the group that received pre-exposure as adults, $F(2,43)=6.65$, $p<0.05$, but not for the group that received pre-exposure as adolescents $F(2,45)=2.661$, $p>0.05$. For the group that received adolescent pre-exposure, the main effect of Pre-exposure was significant ($F(1,45)=52.72$, $p<0.05$) but the main effect of drug was not significant ($F(2,45)=6.54$, $p>0.05$). Separate planned t-tests showed significant difference between Sal PRE and Nic 0.09 mg/kg PRE ($t(16)=2.94$, $p<0.05$), and Sal PRE and Nic 0.36 mg/kg PRE groups that received adult pre-exposure ($t(16)=4.48$, $p<0.01$). In the groups that received pre-exposure as adolescents, the difference between Sal PRE and Nic 0.36 mg/kg PRE groups was significant ($t(17)=2.72$, $p<0.05$) but not between Sal PRE and Nic 0.09 mg/kg PRE ($t(16)=1.66$, $p>0.05$). Also, both groups that received pre-exposure and saline treatment during adolescence and adulthood showed a significant difference between PRE and No-PRE groups (Adults, $t(16)=7.05$, $p<0.01$; Adolescents, $t(17)=4.17$, $p<0.05$), indicating basic CPFE in both age groups whereas there was no significant difference between baseline CPFE levels between those pre-exposed as adults and adolescents ($t(18)=0.08$, $p>0.05$). Furthermore, no significant differences were found between the Sal No-PRE and Nic 0.09 mg/kg No-PRE conditions in the groups that received pre-exposure either during adolescence and adulthood ($t(15)=0.28$, $p>0.05$ and $t(14)=0.30$, $p>0.05$, respectively). However, Nic 0.36 mg/kg No-Pre groups' freezing was significantly higher than the Sal No-Pre groups that received pre-exposure both as adolescents and adults ($t(15)=2.48$, $p<0.05$ and $t(13)=3.46$, $p<0.05$, respectively) suggesting a locomotor effect at the 0.36 mg/kg dose. Therefore, following the same timeline for the