Published in final edited form as:

Brain Res Bull. 2018 April ; 138: 50–55. doi:10.1016/j.brainresbull.2017.06.010.

Pre-adolescent and adolescent mice are less sensitive to the effects of acute nicotine on extinction and spontaneous recovery

Munir Gunes Kutlu^{1,*}, Dana Zeid¹, Jessica M. Tumolo², and Thomas J. Gould¹

¹Department of Biobehavioral Health, Penn State University, University Park, PA, USA

²Department of Psychology, Neuroscience Program, Weiss Hall, Temple University, Philadelphia, PA, USA

Abstract

Adolescence is a period of high risk for the initiation of nicotine product usage and exposure to traumatic events. In parallel, nicotine exposure has been found to age-dependently modulate acquisition of contextual fear memories; however, it is unknown if adolescent nicotine exposure alters extinction of fear related memories. Age-related differences in sensitivity to the effects of nicotine on fear extinction could increase or decrease susceptibility to anxiety disorders. In this study, we examined the effects of acute nicotine administration on extinction and spontaneous recovery of contextual fear memories in pre-adolescent (PND 23), late adolescent (PND 38), and adult (PND 53) C57B6/J mice. Mice were first trained in a background contextual fear conditioning paradigm and given an intraperitoneal injection of one of four doses of nicotine (0.045, 0.09, 0.18, or 0.36 mg/kg, freebase) prior to subsequent extinction or spontaneous recovery sessions. Results indicated that all acute nicotine doses impaired extinction of contextual fear in adult mice. Late adolescent mice exhibited impaired extinction of contextual fear only following higher doses of acute nicotine, and extinction of contextual fear was unaffected by acute nicotine exposure in pre-adolescent mice. Finally, acute nicotine exposure enhanced spontaneous recovery of fear memory, but only in adult mice. Overall, our results suggest that younger mice were less sensitive to nicotine's impairing effects on extinction of contextual fear and to nicotine's enhancing effects on spontaneous recovery of contextual fear memory.

Keywords

nicotine; adolescence; extinction; PTSD; learning; anxiety

*Corresponding Author Munir Gunes Kutlu, Ph.D. 219 Biobehavioral Health Building University Park, PA 16801: mgk14@psu.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1. Introduction

Adolescence is a period of significant behavioral and neurological maturation during which the highest incidence of initiation of regular tobacco product use occurs (Everett et al., 1999). It is estimated that more than 2,500 adolescents under the age of 18 try their first cigarette daily, and a large portion of these youth go on to develop lifetime nicotine dependence (Breslau and Peterson, 1996; SAMHSA, 2011). Further, 90% of adult smokers had their first cigarette while under the age of 20, and adults who report initiating tobacco use during adolescence are at greater risk of relapse following cessation attempts (Kandel & Logan, 1984; U.S. Department of Health and Human Services, 2014). Furthermore, reported use of tobacco-free nicotine products (e.g. “e-cigarettes”) among adolescents is steadily rising (Arrazola et al., 2015; Leventhal et al., 2015).

The increasing prevalence of nicotine consumption in adolescents is especially pertinent given that nicotine affects cognitive function through modulation of cholinergic signaling in the brain. Nicotine activates neuronal nicotinic acetylcholine receptors (nAChRs) in multiple brain regions involved in memory formation and emotional processing, including the hippocampus and the prefrontal cortex (Wada et al., 1989). Evidence consistently suggests that activation of these receptors by nicotine can alter fear learning (Davis & Gould, 2009; Gould & Higgins, 2003; Gould & Wehner, 1999; Kenney, Raybuck, & Gould, 2012; Kutlu & Gould, 2014; and see Kutlu & Gould, 2015 for review). Furthermore, animal models of nicotine exposure indicate that the neurological development occurring during puberty uniquely alters adolescent sensitivity to nicotine's effects on learning and memory (Abreu-Villaça et al., 2003; Holliday & Gould, 2016).

Adolescents are additionally at high risk for exposure to traumatic events. 47% of adolescents aged 12-17 report experiencing physical or sexual assault or witnessing violence (U.S. Department of Health and Human Services, 2012). A national survey of children ages 13-18 found that 5% of adolescents surveyed met criteria for post-traumatic stress disorder (PTSD) at some point in their lives (Merikangas et al, 2010). PTSD is a devastating mental disorder that emerges following exposure to a traumatic event. It is characterized by a range of debilitating psychological symptoms including flashbacks, hyperarousal, and avoidance of contexts similar to that of the traumatic event (Yehuda, 2002). Animal and human studies suggest that PTSD involves altered contextual fear processing such that these individuals suffer from deficits in their ability to extinguish trauma memories (Milad, Rauch, Pitman, & Quirk, 2006; Rougemont-Bücking et al., 2011). This is the basis of exposure therapy, a widely used PTSD treatment in which the affected individual is exposed to traumatic-event related stimuli (the conditioned stimulus) in a safe environment in order to extinguish the association between the event and their fear symptoms (Foa, Chrestman, & Gilboa-Schechtman, 2008). Despite initial extinction of fear in exposure therapy, individuals suffering from PTSD often experience a relapse of their fear response, a phenomenon referred to as spontaneous recovery in Pavlovian fear conditioning paradigms (Kutlu, Tumolo, Holliday, Garrett, & Gould, 2016).

In adult mice, chronic nicotine administration as well as withdrawal from this exposure has been found to impair contextual fear extinction (Kutlu et al., 2016). Acute nicotine

administration in adult mice enhances spontaneous recovery of contextual fear (Kutlu et al., 2016; Kutlu & Gould, 2014). On the other hand, pre-adolescent mice are less sensitive to impairment of contextual fear conditioning following withdrawal from chronic nicotine exposure (Portugal, Wilkinson, Turner, Blendy, & Gould, 2012). In sum, these results indicate that nicotine differentially impacts multiple components of contextual fear acquisition and retention in adolescents relative to adults.

PTSD and nicotine use are strongly correlated in adults: individuals who smoke prior to experiencing traumatic events have higher rates of PTSD, and daily smoking rates increase following trauma (Koenen et al., 2005; Thorndike, Wernicke, Pearlman, & Haaga, 2006). These statistics, combined with results from animal and human studies of the effects of nicotine on contextual fear memory processing, indicate that nicotine use could play a role in prolonged PTSD symptoms. Exposure to traumatic events additionally elevates risk of nicotine product use in adolescents and young adults (Feldner, Babson, & Zvolensky, 2007). Identification of nicotine's unique impacts on fear learning and memory in adolescents is critical for prevention efforts as well as for the development of therapies targeted at adolescents suffering from PTSD and/or nicotine dependence. In the present study, we investigated the developmental effects of acute nicotine administration during extinction training and spontaneous recovery in pre-adolescent, late adolescent, and adult mice. We hypothesized that the effects of acute nicotine exposure on extinction and spontaneous recovery would be altered in adolescent mice.

2. Method

Subjects

Subjects were pre-adolescent (postnatal day [PND] 23), late adolescent (PND 38), and adult (PND 53) male C57BL6/J mice (Jackson Laboratory, Bar Harbor, ME) that were group-housed and maintained in a 12 h light/dark cycle. They had access to food and water *adlibitum*. In mice, PND 28-40 has consistently been identified as an age range during which behaviors and biological changes associated with adolescence, such as initial signs of puberty, increased impulsivity, and social maturation, appear (Keene et al., 2002; Terranova et al., 1998). Preadolescence in mice (PND 23) is defined as the period just prior to adolescence. Past work has shown that behavioral and biological responses to nicotine during preadolescence are distinct from those observed in later adolescence and adulthood (Portugal et al., 2012). Training and testing occurred between 9:00 am and 7:00 pm. Behavioral procedures used in this study were approved by the Temple University and Penn State University Institutional Animal Care and Use Committees.

Apparatus

Behavioral experiments took place in four identical chambers (18.8 × 20 × 18.3 cm) placed in sound attenuating boxes (MED Associates). Ventilation fans mounted in the backs of the chambers produced a background noise (65 dB) and the 30-sec white noise (85 dB) that served as a conditioned stimulus (CS). The chambers were composed of Plexiglas and the chamber floors were metal grids (0.20 cm in diameter and 1.0 cm apart) connected to a shock generator, which delivered a 2-sec, 0.57-mA foot shock unconditioned stimulus (US).

The stimuli were controlled by an IBM-PC compatible computer running MED-PC software.

Drug

Nicotine hydrogen tartrate salt (0.045, 0.09, 0.18, and 0.36 mg/kg freebase, Sigma) dissolved in 0.9% physiological saline (saline) or saline alone were administered intraperitoneally (i.p.) 2–4 min prior to each extinction or spontaneous recovery session. Injection volumes were 10 mL/kg, as described in previous studies (e.g., Kutlu & Gould, 2014).

Behavioral procedures

The dependent variable was freezing to context, defined as the absence of any voluntary movement but respiration (Davis, James, Siegel, & Gould, 2005). A time sampling method was employed in which subjects were observed for 1 second every 10 seconds and scored as active or freezing. Experimenters were blinded to the drug conditions of the mice. Subjects were trained in contextual fear conditioning, in which they were placed in conditioning chambers, and baseline freezing levels were measured for 120 seconds before a white noise-footshock pairing, wherein a white noise and footshock co-terminated. Subjects were assessed for another 120 seconds before an additional white noise-footshock pairing. Mice remained in the chambers for an additional 30 seconds following the second footshock. The following day, mice were returned to chambers for 5 minutes to assess freezing to context in the absence of footshocks and white noise. For the next 5 days, subjects underwent daily extinction trials in which they were placed in the chambers for 5 minutes, and their freezing behavior was scored. Each extinction session was preceded by an i.p. acute nicotine or saline injection ($n = 6-9$ per group), and extinction to the context as a function of freezing behaviors was recorded.

In a separate group of animals, training was administered as described above, followed by extinction sessions that were not preceded by nicotine administration. Seven days after the last extinction session, mice were returned to the conditioning context to test for spontaneous recovery of freezing to the context. Animals were administered acute nicotine or saline i.p. 2–4 minutes prior to the spontaneous recovery session ($n=9-12$ per group).

Statistical Analysis

Following previous studies from our laboratory and others reporting the effects of nicotine on contextual fear extinction (Tian et al., 2008; Kutlu et al., 2016), freezing response during extinction sessions was normalized to the initial freezing response measured during the retention test ($\text{freezing} \times 100/\text{initial freezing}$). This ensured that potential between-group baseline differences in contextual freezing did not affect subsequent fear extinction curves. A 3-way repeated measures ANOVA (Age $\times$ Drug $\times$ Trial) was used to analyze normalized freezing scores followed by a separate Drug $\times$ Trial repeated measures ANOVA at $\alpha=0.05$. For the spontaneous recovery experiment, a two-way ANOVA (Age $\times$ Drug) was employed to analyze percent rebound levels (%Rebound; $\text{Re-test freezing} \times 100/\text{initial freezing}$). LSD post-hoc tests were used for all between-group comparisons in which the independent variable had more than 2 levels (nicotine groups vs. saline controls). Subjects showing no

extinction (no change in or increased freezing from first session to last extinction session) were excluded from the spontaneous recovery experiment. A total of 1 subject from the pre-adolescence group, 1 subject from the late adolescence group, and 6 subjects from the adult group were excluded. Group sizes are indicated in figure captions. All statistical analyses were run using SPSS 21.

3. Results

Adolescent mice are less sensitive to the impairing effects of acute nicotine on extinction of contextual fear

An initial one-way ANOVA showed that retention test freezing levels did not differ between age groups, suggesting that all three age groups acquired contextual fear conditioning at equal levels ($F(2,105)=0.247$, $p>0.05$). Additionally, a 2-way repeated measures ANOVA showed that freezing levels during extinction did not significantly differ between saline groups from the 3 age conditions ($F(10,85)=0.929$, $p>0.05$), suggesting that all three age groups showed similar levels of baseline extinction learning. A 3-way ANOVA comparing extinction of fear response (freezing) between all drug and age groups showed that the Age $\times$ Drug $\times$ Trial interaction was significant ($F(40,465)=1.973$, $p=0.01$). In order to isolate the source of the 3-way interaction, we tested individual Drug $\times$ Trial interactions for each age group. Separate 2-way ANOVAs indicated that the Drug $\times$ Trial interaction was significant for the adult ($F(5,160)=3.110$, $p<0.001$) and late adolescent groups ($F(20,175)=4.289$, $p<0.001$), but not for the pre-adolescent group ($F(20,130)=0.913$, $p>0.05$). LSD post-hoc tests showed that, in the adult group, 0.36 mg/kg group significantly differed from the saline controls during all 5 extinction session whereas the 0.18 mg/kg dose group significantly differed during the 2nd, 3rd, 4th, and 5th, the 0.09 mg/kg dose group differed only at the 5th, and the 0.045 mg/kg dose group differed on the 1st, 3rd, 4th, and 5th extinction sessions ($p<0.05$). In the late adolescent group, the 0.36 mg/kg dose group significantly differed from the saline controls during 3rd, 4th, and 5th extinction session, the 0.18 mg/kg dose group differed at the 2nd, 3rd, and 4th, and the 0.09 mg/kg group differed at the 4th and 5th extinction sessions ($p<0.05$). The 0.045 mg/kg nicotine group did not differ from the saline controls at any extinction session ($p>0.05$). Thus, acute nicotine had impairing effects on extinction of contextual fear for the adult and late adolescent groups, but not for the pre-adolescent group. Moreover, the effect of acute nicotine on extinction of contextual fear in the late adolescent group seemed to be weaker than the effect observed in adults, suggesting a linear effect of age.

Adolescent mice do not show the acute nicotine-induced enhancement of spontaneous recovery

An initial one-way ANOVA showed that the baseline rebound levels in the saline groups did not show an effect of age ($F(2,30)=0.032$, $p>0.05$). A two-way ANOVA comparing spontaneous recovery of fear response (freezing) between groups revealed no significant interaction between Age and Drug ($F(8,146)=1.148$, $p>0.05$), but both Drug ($F(4,146)=5.979$, $p<0.001$) and Age ($F(2,146)=13.525$, $p<0.001$) main effects were significant. Additionally, when only the adult and pre-adolescent groups or only the adult and late adolescent groups were compared, the main effect of Drug was significant

($F(4,101)=3.472$, $p<0.05$ and $F(4,104)=4.372$, $p<0.05$), respectively). Moreover, while the main effect of drug was significant for the adult group ($F(4,47)=3.429$, $p<0.05$), it was not significant for the pre- ($F(4,49)=2.325$, $p>0.05$), or late ($F(4,50)=2.437$, $p>0.05$) adolescent groups. LSD post-hoc tests showed that, in adult mice, there was a significant difference in spontaneous recovery between the saline and Nic 0.36 mg/kg groups ($p<0.05$), while saline-Nic 0.09 mg/kg ($p=0.065$) and saline-Nic 0.18 mg/kg ($p=0.087$) comparisons approached significance. These results indicate that nicotine produced greater enhancement of spontaneous recovery of contextual fear memory in adults relative to pre- and late adolescents.

4. Discussion

The results of the present study indicated that acute nicotine administration prior to contextual fear extinction sessions differentially affected extinction in the tested age groups. All acute nicotine doses (0.045, 0.09, 0.18, and 0.36 mg/kg) impaired extinction of contextual fear in adult mice, but late adolescent mice only exhibited impaired extinction of contextual fear following higher doses of acute nicotine (0.36, 0.18, and 0.09 mg/kg). Further, the impairing effect of nicotine on extinction of contextual fear memory seemed to be weaker in late adolescents relative to adults, as it emerged in fewer trials at the lower significant doses (0.09, 0.18 mg/kg) and in fewer trials at the highest significant dose in late adolescents compared to adults (0.36 mg/kg). Finally, extinction of contextual fear was largely unaffected by acute nicotine exposure in pre-adolescent mice. Spontaneous recovery results indicated that nicotine produced enhancement of spontaneous recovery of contextual fear memory in adults, but pre- and late adolescent mice showed no nicotine-induced enhancement of spontaneous recovery.

These findings suggest that adult mice are more sensitive to the impairing effects of nicotine exposure on extinction of contextual fear and on the enhancing effects of acute nicotine spontaneous recovery of contextual fear memory than late and pre-adolescent mice. Thus, adolescents may be protected from the immediate effects of acute nicotine exposure on PTSD-like impaired extinction of fear memories and enhanced spontaneous recovery of fear memories. These results are consistent with previous findings from our laboratory and others indicating that adolescents are differentially sensitive to nicotine's effects on cognition and their biological correlates. Together, these findings indicate that, relative to adult mice, adolescents can show both increased and decreased sensitivity to the effects of nicotine exposure depending on the behavioral paradigm in question as well as the length of nicotine exposure. For example, adolescent mice are differentially vulnerable to the effects of acute nicotine administration and withdrawal following chronic nicotine exposure on learning and memory. Specifically, pre-adolescent mice are less sensitive to the impairing effects of withdrawal from chronic nicotine exposure on contextual fear learning compared to adult mice whereas acute nicotine administration produces enhancement of hippocampus-dependent fear learning at a broader range of doses in adolescent mice (Portugal et al., 2012). Adolescent mice are additionally more sensitive to nicotine's anxiolytic and depressive effects than adults (Holliday et al., 2016; Iniguez et al., 2009; Kupferschmidt, Funk, Erb, & Lê, 2010), and adolescents are theorized to be especially vulnerable to the development of nicotine dependence (Schramm-Sapota, Walker, Caster, Levin, & Kuhn,

2009). In addition, findings from rodent models of nicotine addiction suggest that adolescents are more sensitive to the rewarding effects of nicotine, showing rapid acquisition of nicotine self-administration and enhanced conditioned place preference to contexts paired with nicotine exposure compared to adults (Chen, Matta, & Sharp, 2007; Kota, Martin, Robinson, & Damaj, 2007; Levin et al., 2007; O'Dell et al., 2006). While adolescent mice may be less sensitive to the aversive effects of withdrawal, adolescent nicotine exposure produces adult deficits in learning, increased adult depression symptoms, and altered hippocampal morphology (Portugal et al., 2012; Holliday et al., 2016). This suggests that, while there may be less immediate negative impact of adolescent nicotine use, adolescent nicotine exposure may increase the likelihood of adult mental health problems.

Several physiological differences between adults and adolescents may contribute to age-related differences seen in the effects of nicotine. Although metabolism of nicotine differs between developmental periods, these differences are not associated with differential alteration of learning by nicotine administration in adults versus adolescents (Portugal et al., 2012). Furthermore, in studies controlling for developmental variation in nicotine plasma levels following administration, the effects of increased sensitivity to nicotine reward and decreased sensitivity to withdrawal in adolescents persisted (O'Dell et al., 2006). Substantial development of neural circuitry during adolescence may result in differences in underlying cholinergic functioning between stages of adolescence and adulthood. Adolescent hippocampal nAChRs exhibit unique binding and expression patterns (Doura, Gold, Keller, & Perry, 2008; Trauth, Seidler, McCook, & Slotkin, 1999), and acquisition of nicotine self-administration in adolescents compared to adults was associated with greater increases in high affinity nAChR binding in the midbrain and striatum (Levin et al., 2007). In adult mice, learning impairments during chronic nicotine withdrawal were associated with upregulated hippocampal nAChR binding, while no such change was observed in pre-adolescents (Portugal et al., 2012). Cholinergic activity may play a prominent role in modulating the plasticity associated with adolescent neural maturation; thus, extended exposure to nicotine during early cholinergic system development may produce the long-term behavioral changes associated with early chronic nicotine exposure (Goriounova & Mansvelder, 2012). In the context of this study, cholinergic system differences across developmental stages may explain acute nicotine's differential effect on fear extinction and spontaneous recovery in adults and adolescents, although this should be explicitly tested in future work.

The outcomes of adolescent nicotine exposure may depend upon the affected underlying neural circuitry. For instance, acquisition, extinction, and spontaneous recovery of fear memory are thought to each involve distinct neural circuitry (Herry et al., 2010; Quirk, 2002). Specifically, while contextual fear memory acquisition involves amygdalar and hippocampal associative circuitry, extinction appears to involve the formation of a modulatory inhibitory memory including projections to and from the prefrontal cortex. Spontaneous recovery may involve a weakening of the inhibition that governs the original fear memory circuitry, resulting in a re-emergence of fear memories (Barad, Gean, & Lutz, 2006; Burgos-Robles, Vidal-Gonzalez, & Quirk, 2009). nAChRs are variably distributed throughout these regions, and their expression and functioning changes during adolescent development; thus, adolescent nicotine exposure can result in different alterations in cognition unique to this developmental period compared to adulthood (Azam, Chen, &

Leslie, 2007; Kota et al., 2007). In sum, neural development associated with adolescent maturation may contribute to the differences in the effects of nicotine on extinction and spontaneous recovery in adults versus pre-adolescents and adolescents. Our findings suggest that the plasticity associated with development may sometimes confer a degree of protection against the immediate detrimental effects of nicotine exposure on fear memory regulation. In contrast, no such protection was seen in adulthood and thus adult sufferers of PTSD may be especially vulnerable to nicotine's effects on extinction and spontaneous recovery of fear memories.

Acknowledgments

This work was funded with grant support from the National Institute on Drug Abuse (T.J.G., DA017949; 1U01DA041632), Jean Phillips Shibley Endowment, and Penn State Biobehavioral Health Department. We declare no potential conflict of interest.

References

- Abreu-Villaça Y, Seidler FJ, Qiao D, Tate CA, Cousins MM, Thillai I, Slotkin TA. Short-term adolescent nicotine exposure has immediate and persistent effects on cholinergic systems: critical periods, patterns of exposure, dose thresholds. *Neuropsychopharmacology*. 2003; 28(11):1935. [PubMed: 12784097]
- Arrazola RA, Singh T, Corey CG, Husten CG, Neff LJ, Apelberg BJ, et al. McAfee T. Tobacco use among middle and high school students-United States, 2011-2014. *MMWR Morbidity and mortality weekly report*. 2015; 64(14):381-385. [PubMed: 25879896]
- Azam L, Chen Y, Leslie FM. Developmental regulation of nicotinic acetylcholine receptors within midbrain dopamine neurons. *Neuroscience*. 2007; 144(4):1347-1360. [PubMed: 17197101]
- Bandiera FC, Richardson AK, Lee DJ, He JP, Merikangas KR. Secondhand smoke exposure and mental health among children and adolescents. *Archives of pediatrics & adolescent medicine*. 2011; 165(4):332-338. [PubMed: 21464381]
- Barad M, Gean PW, Lutz B. The role of the amygdala in the extinction of conditioned fear. *Biological psychiatry*. 2006; 60(4):322-328. [PubMed: 16919522]
- Breslau N, Peterson EL. Smoking cessation in young adults: age at initiation of cigarette smoking and other suspected influences. *American journal of public health*. 1996; 86(2):214-220. [PubMed: 8633738]
- Burgos-Robles A, Vidal-Gonzalez I, Quirk GJ. Sustained conditioned responses in prelimbic prefrontal neurons are correlated with fear expression and extinction failure. *Journal of Neuroscience*. 2009; 29(26):8474-8482. [PubMed: 19571138]
- Chen H, Matta SG, Sharp BM. Acquisition of nicotine self-administration in adolescent rats given prolonged access to the drug. *Neuropsychopharmacology*. 2007; 32(3):700-709. [PubMed: 16794562]
- Choi WS, Patten CA, Christian Gillin J, Kaplan RM, Pierce JP. Cigarette smoking predicts development of depressive symptoms among US adolescents. *Annals of behavioral medicine*. 1997; 19(1):42-50. [PubMed: 9603677]
- Davis JA, Gould TJ. Hippocampal nAChRs mediate nicotine withdrawal-related learning deficits. *European neuropsychopharmacology*. 2009; 19(8):551-561. [PubMed: 19278836]
- Davis JA, James JR, Siegel SJ, Gould TJ. Withdrawal from chronic nicotine administration impairs contextual fear conditioning in C57BL/6 mice. *Journal of Neuroscience*. 2005; 25(38):8708-8713. [PubMed: 16177040]
- Doura MB, Gold AB, Keller AB, Perry DC. Adult and periadolescent rats differ in expression of nicotinic cholinergic receptor subtypes and in the response of these subtypes to chronic nicotine exposure. *Brain research*. 2008; 1215:40-52. [PubMed: 18474362]

- Everett SA, Warren CW, Sharp D, Kann L, Husten CG, Crossett LS. Initiation of cigarette smoking and subsequent smoking behavior among US high school students. *Preventive medicine*. 1999; 29(5):327–333. [PubMed: 10564623]
- Feldner MT, Babson KA, Zvolensky MJ. Smoking, traumatic event exposure, and post-traumatic stress: A critical review of the empirical literature. *Clinical psychology review*. 2007; 27(1):14–45. [PubMed: 17034916]
- Foa, EB., Chrestman, KR., Gilboa-Schechtman, E. *Prolonged Exposure Therapy for Adolescents with PTSD Emotional Processing of Traumatic Experiences, Therapist Guide*. Oxford University Press; 2008.
- Fried PA, Watkinson B, Gray R. Neurocognitive consequences of cigarette smoking in young adults—a comparison with pre-drug performance. *Neurotoxicology and teratology*. 2006; 28(4):517–525. [PubMed: 16904287]
- Goriounova N, Mansvelder H. Nicotine exposure during adolescence alters the rules for prefrontal cortical synaptic plasticity during adulthood. *Frontiers in synaptic neuroscience*. 2012; 4:3. [PubMed: 22876231]
- Gould TJ, Higgins JS. Nicotine enhances contextual fear conditioning in C57BL/6J mice at 1 and 7 days post-training. *Neurobiology of learning and memory*. 2003; 80(2):147–157. [PubMed: 12932430]
- Gould TJ, Wehner JM. Nicotine enhancement of contextual fear conditioning. *Behavioural brain research*. 1999; 102(1):31–39. [PubMed: 10403013]
- Herry C, Ferraguti F, Singewald N, Letzkus JJ, Ehrlich I, Lüthi A. Neuronal circuits of fear extinction. *European Journal of Neuroscience*. 2010; 31(4):599–612. [PubMed: 20384807]
- Holliday ED, Gould TJ. Chronic nicotine treatment during adolescence attenuates the effects of acute nicotine in adult contextual fear learning. *Nicotine & Tobacco Research*. 2016; 19(1):87–93. [PubMed: 27613891]
- Holliday ED, Nucero P, Kutlu MG, Oliver C, Connelly KL, Gould TJ, Unterwald EM. Long-term effects of chronic nicotine on emotional and cognitive behaviors and hippocampus cell morphology in mice: comparisons of adult and adolescent nicotine exposure. *European Journal of Neuroscience*. 2016; 44(10):2818–2828. [PubMed: 27623427]
- Iniguez SD, Warren BL, Parise EM, Alcantara LF, Schuh B, Maffeo ML, et al. Bolanos-Guzmán CA. Nicotine exposure during adolescence induces a depression-like state in adulthood. *Neuropsychopharmacology*. 2009; 34(6):1609–1624. [PubMed: 19092782]
- Kandel DB, Logan JA. Patterns of drug use from adolescence to young adulthood: Periods of risk for initiation, continued use, and discontinuation. *American journal of public health*. 1984; 74(7):660–666. [PubMed: 6611092]
- Keene DE, Suescun MO, Bostwick MG, Chandrashekar V, Bartke A, Kopchick JJ. Puberty is delayed in male growth hormone receptor gene-disrupted mice. *Journal of andrology*. 2002; 23(5):661–668. [PubMed: 12185100]
- Kenney JW, Raybuck JD, Gould TJ. Nicotinic receptors in the dorsal and ventral hippocampus differentially modulate contextual fear conditioning. *Hippocampus*. 2012; 22(8):1681–1690. [PubMed: 22271264]
- Koenen KC, Hitsman B, Lyons MJ, Niaura R, McCaffery J, Goldberg J, et al. Tsuang M. A twin registry study of the relationship between posttraumatic stress disorder and nicotine dependence in men. *Archives of general psychiatry*. 2005; 62(11):1258–1265. [PubMed: 16275813]
- Kota D, Martin BR, Robinson SE, Damaj MI. Nicotine dependence and reward differ between adolescent and adult male mice. *Journal of Pharmacology and Experimental Therapeutics*. 2007; 322(1):399–407. [PubMed: 17446302]
- Kupferschmidt DA, Funk D, Erb S, Lê AD. Age-related effects of acute nicotine on behavioural and neuronal measures of anxiety. *Behavioural brain research*. 2010; 213(2):288–292. [PubMed: 20546793]
- Kutlu MG, Gould TJ. Acute nicotine delays extinction of contextual fear in mice. *Behavioural brain research*. 2014; 263:133–137. [PubMed: 24487010]
- Kutlu MG, Gould TJ. Nicotine modulation of fear memories and anxiety: Implications for learning and anxiety disorders. *Biochemical pharmacology*. 2015; 97(4):498–511. [PubMed: 26231942]

- Kutlu MG, Tumolo JM, Holliday E, Garrett B, Gould TJ. Acute nicotine enhances spontaneous recovery of contextual fear and changes c-fos early gene expression in infralimbic cortex, hippocampus, and amygdala. *Learning & Memory*. 2016; 23(8):405–414. [PubMed: 27421892]
- Leventhal AM, Strong DR, Kirkpatrick MG, Unger JB, Sussman S, Riggs NR, et al. Audrain-McGovern J. Association of electronic cigarette use with initiation of combustible tobacco product smoking in early adolescence. *Jama*. 2015; 314(7):700–707. [PubMed: 26284721]
- Levin ED, Lawrence SS, Petro A, Horton K, Rezvani AH, Seidler FJ, Slotkin TA. Adolescent vs. adult-onset nicotine self-administration in male rats: duration of effect and differential nicotinic receptor correlates. *Neurotoxicology and teratology*. 2007; 29(4):458–465. [PubMed: 17433619]
- Merikangas KR, He JP, Burstein M, Swanson SA, Avenevoli S, Cui L, et al. Swendsen J. Lifetime prevalence of mental disorders in US adolescents: results from the National Comorbidity Survey Replication–Adolescent Supplement (NCS-A). *Journal of the American Academy of Child & Adolescent Psychiatry*. 2010; 49(10):980–989. [PubMed: 20855043]
- Milad MR, Rauch SL, Pitman RK, Quirk GJ. Fear extinction in rats: implications for human brain imaging and anxiety disorders. *Biological psychology*. 2006; 73(1):61–71. [PubMed: 16476517]
- O'Dell LE, Bruijnzeel AW, Smith RT, Parsons LH, Merves ML, Goldberger BA, et al. Markou A. Diminished nicotine withdrawal in adolescent rats: implications for vulnerability to addiction. *Psychopharmacology*. 2006; 186(4):612–619. [PubMed: 16598454]
- Portugal GS, Wilkinson DS, Turner JR, Blendy JA, Gould TJ. Developmental effects of acute, chronic, and withdrawal from chronic nicotine on fear conditioning. *Neurobiology of learning and memory*. 2012; 97(4):482–494. [PubMed: 22521799]
- Quirk GJ. Memory for extinction of conditioned fear is long-lasting and persists following spontaneous recovery. *Learning & memory*. 2002; 9(6):402–407. [PubMed: 12464700]
- Rougemon-Bücking A, Linnman C, Zeffiro TA, Zeidan MA, Lebron-Milad K, Rodriguez-Romaguera J, et al. Milad MR. Altered processing of contextual information during fear extinction in PTSD: an fMRI study. *CNS neuroscience & therapeutics*. 2011; 17(4):227–236. [PubMed: 20406268]
- Schramm-Sapota NL, Walker QD, Caster JM, Levin ED, Kuhn CM. Are adolescents more vulnerable to drug addiction than adults? Evidence from animal models. *Psychopharmacology*. 2009; 206(1):1–21. [PubMed: 19547960]
- Substance Abuse and Mental Health Services Administration (SAMHSA). Results from the 2010 National Survey on Drug Use and Health: Volume I Summary of National Findings. Rockville, MD: Office of Applied Studies, SAMHSA; 2011.
- Terranova ML, Laviola G, de Acetis L, Alleve E. A description of the ontogeny of mouse agonistic behavior. *Journal of Comparative Psychology*. 1998; 112(1):3. [PubMed: 9528111]
- Thorndike FP, Wernicke R, Pearlman MY, Haaga DA. Nicotine dependence, PTSD symptoms, and depression proneness among male and female smokers. *Addictive behaviors*. 2006; 31(2):223–231. [PubMed: 15919158]
- Trauth JA, Seidler FJ, McCook EC, Slotkin TA. Adolescent nicotine exposure causes persistent upregulation of nicotinic cholinergic receptors in rat brain regions. *Brain research*. 1999; 851(1):9–19. [PubMed: 10642823]
- Tian S, Gao J, Han L, Fu J, Li C, Li Z. Prior chronic nicotine impairs cued fear extinction but enhances contextual fear conditioning in rats. *Neuroscience*. 2008; 153(4):935–943. [PubMed: 18440720]
- U.S. Department of Health and Human Services, Administration for Children and Families, Administration on Children, Youth and Families, Children's Bureau. *Child Maltreatment* 2011. 2012. Available from <http://www.acf.hhs.gov/programs/cb/research-data-technology/statistics-research/child-maltreatment>
- U.S. Department of Health and Human Services. The health consequences of smoking—50 years of progress: a report of the Surgeon General. Atlanta, GA: US Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health; 2014. p. 17
- Wada E, Wada K, Boulter JIM, Deneris E, Heinemann S, Patrick JIM, Swanson LW. Distribution of alpha2, alpha3, alpha4, and beta2 neuronal nicotinic receptor subunit mRNAs in the central nervous system: a hybridization histochemical study in the rat. *Journal of Comparative Neurology*. 1989; 284(2):314–335. [PubMed: 2754038]

Yehuda R. Post-traumatic stress disorder. New England journal of medicine. 2002; 346(2):108–114.
[PubMed: 11784878]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Highlights

- Acute nicotine impaired extinction and increased recovery of fear in adult mice.
- Acute nicotine impaired fear extinction but not recovery during late adolescence.
- Pre-adolescent fear extinction and recovery were not affected by acute nicotine.

Figure 1.

Preadolescent and Adolescent mice are less affected by the impairing effects of acute nicotine on contextual fear extinction. Upper panel: All doses of acute nicotine (0.045, 0.09, 0.18, and 0.36 mg/kg) disrupted contextual fear extinction in adult mice (n=8 per group). Middle Panel: Acute nicotine doses 0.09, 0.18, and 0.36 mg/kg but not the lowest dose of 0.045 mg/kg disrupted contextual fear extinction in late adolescent mice (n=8-9 per group). Lower Panel: Acute nicotine had no effect on contextual fear extinction in pre-adolescent mice (n=6-7 per group). Error bars indicate Standard Error of the Mean (SEM). (‡) denotes significant differences between Nic 0.045 mg/kg group and saline controls, (†) denotes significant differences between Nic 0.09 mg/kg and saline controls, and (+) denotes significant differences between 0.18 mg/kg and saline controls, and (*) denotes significant differences between Nic 0.36 mg/kg and saline controls at the $p < 0.05$ level.

Figure 2.

Preadolescent and Adolescent mice do not show the enhancing effects of acute nicotine on spontaneous recovery. Left panels: Extinction of contextual fear prior to nicotine administration during re-testing in adult (upper panel), late adolescent (middle panel), and pre-adolescent (lower panel) groups. Right panels: Spontaneous recovery of contextual fear represented as %Rebound in adult (upper panel), late adolescent (middle panel), and pre-adolescent (lower panel) groups. Acute-nicotine (0.36 mg/kg) administration prior to retesting enhanced spontaneous recovery in adult mice ($n = 9-12$ per group). Spontaneous recovery of contextual fear was unaffected in pre-adolescent ($n = 11$ per group) and late adolescent ($n = 10-12$ per group) mice. (*) denotes significant differences between compared to saline controls at the $p < 0.05$ level.