The Effects of Adolescent Nicotine Exposure on Adult Learning

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The Effects of Adolescent Nicotine Exposure on Adult Learning

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A Thesis Presented to the Graduate Faculty of the College of William and Mary in Candidacy for the Degree of Master of Arts

Department of Psychology

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Master of Arts

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The current experiments examined the effects of adolescent nicotine exposure on adult learning, specifically context conditioning, extinction learning, and latent inhibition. Nicotine was administered via subcutaneous osmotic minipumps or repeated intraperitoneal injections which provided a moderate or high dose of nicotine. Control animals were exposed to saline only. Adolescent nicotine, when administered via subcutaneous osmotic minipumps negatively affected performance on the context conditioning task, suggesting that the neural circuitry responsible for this hippocampus-dependent learning is affected by the present nicotine administration. No significant differences were observed between drug and saline treated animals on the extinction learning or latent inhibition tasks. When administered via repeated injections, animals exposed to the high dose of nicotine exhibited stronger learning on the context conditioning task when compared to saline control animals, suggesting that the stress associated with the injections impacts the relationship between nicotine and adult learning.
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The Effects of Adolescent Nicotine Exposure on Learning in Adult Rats

In 2000, polls showed that the frequency of smoking in the United States and other developed countries is declining; however, this overall decline reflects an increasing number of adults who are able to quit using tobacco products (Pierce et al., 2000). The rate of adolescent tobacco use has remained stable in the United States and has increased world-wide (Breslau et al., 2001). Nearly 3,000 children under the age of 18 begin smoking everyday in the United States and approximately three million teenagers smoke regularly (Centers for Disease Control and Prevention, 2004; Nelson, Giovino, Shopland, & Mowery, 1995). The majority of adult smokers report that they began smoking during their pre-teen or teen years; nine out of ten smokers become addicted before the age of twenty-one, and longitudinal studies have shown that between 50-80% of adolescent smokers continue to smoke daily as adults (Chen & Millar, 1998; Patton, Coffey, Sawyer, & Wakefield, 2006; Pierce & Gilpin, 1996). Typically, research examining the negative consequences of tobacco use focuses on diseases affecting the heart and lungs. Smoking has been identified as a major risk factor for heart attacks, strokes, chronic obstructive pulmonary disease (COPD), emphysema, and cancer (Centers for Disease Control and Prevention, 2004). In addition to these negative health outcomes, research has also begun examining the effects of nicotine on cognitive functioning.

The high rate of tobacco use has made nicotine one of the three most widely used psychoactive drugs (in addition to caffeine and alcohol). Although
nicotine is only one of about 4000 compounds released by the burning of cigarette tobacco, it is the primary active ingredient. Nicotine is responsible for the pharmacological effects of smoking (i.e. reduced anxiety and heightened concentration) and for the physiological dependency induced by cigarettes; however, the adverse cardiovascular, pulmonary and carcinogenic effects of smoking are related to other compounds in tobacco products (Centers for Disease Control and Prevention, 1990).

Nicotine is rapidly and widely dispersed throughout the brain and body. There are no barriers in the body to limit the distribution of nicotine and the drug easily penetrates the blood brain barrier and passes through all body fluids. This widespread delivery of nicotine exerts powerful effects on the brain, spinal cord, peripheral nervous system, and heart. Research has shown that nicotine’s actions on the central nervous system cause increases in psychomotor activity and alterations in cognitive functioning, sensorimotor performance, attention, and memory consolidation (Slotkin, 2002). Nicotine directly affects cognitive performance by acting as an agonist on the cholinergic system (Kumari et al., 2003; Rezvani & Levin, 2001).

After being ingested, nicotine binds to nicotinic acetylcholine receptors (nAChRs). nAChRs are a family of ligand gated ion channels which are expressed throughout the central and peripheral nervous systems. Neuronal nAChRs are composed of assemblies of α and β subunits which combine to form functional channels (Hogg & Bertrand, 2007). Nine α (α2-α10) and three β (β2-
β4) have been found in vertebrates. Nicotine has a high affinity for the nAChRs containing the α4β2 and α7 subunits (Kumari et al., 2003; Rezvani & Levin, 2001; Slotkin, Cousins, & Seidler, 2004). Electrophysiological evidence indicates that nAChRs are expressed on dendrites, cell bodies, and axons as well as in perisynaptic and presynaptic sites (Hogg & Bertrand, 2007). In addition, nAChRs are concentrated in brain regions that are vital for learning and memory, including the hippocampus and frontal cortex (Rezvani & Levin, 2001). Although prevalent in the cholinergic system, these receptors also modulate catecholaminergic transmission (Rezvani & Levin, 2001). Nicotine is the prototypic nAChR agonist and activates receptors that are expressed on or near nerve terminals which modulate the calcium dependent release of neurotransmitters including dopamine, norepinephrine, glutamate, GABA, and acetylcholine into the brain and bloodstream (Rezvani & Levin, 2001). By facilitating the release of these neurotransmitters, nicotine causes changes in learning and behavior.

Historically, researchers believed that the brain was fully mature by the time humans reached the adolescent stage in development (Strauch, 2003). Therefore, many researchers assumed nicotine would affect the adolescent brain in much the same way as it affected the brain in adulthood. However, recent findings in neurological research have shown that during adolescence, the brain goes through a massive renovation, exhibiting a magnitude of change similar to the degree observed during infant development (Strauch, 2003). Using MRI technology, neuroscientists have been able to view hundreds of adolescent brain
scans. During adolescence the structure of the brain is altered via changes in both gray matter (nerve cell bodies, glial cells, and dendrites) and white matter (myelinated nerve cell axons) (Strauch, 2003). Thus, brain development, in the form of cell acquisition, apoptosis, synaptogenesis and the programming of synaptic activity, appears to continue into adolescence (Slotkin, 2002). In addition to this discovery, human studies and epidemiological research have shown that teenage exposure to tobacco products leads to a greater susceptibility to the effects of nicotine, more persistent nicotine dependence and stronger addiction liability than adult exposure to tobacco (DiFranza, 2007; O’Loughlin, Kishchuk, DiFranza, Termblay, & Paradis, 2002). These findings led researchers to explore basic biological differences between the adult and adolescent brain related to nicotine exposure.

Nicotine is the primary agonist of nicotinic acetylcholine receptors (nAChRs) and the cholinergic system, which is involved in both cognitive function and reward, becomes fully functional and biochemically mature during the adolescent stage of development (Slotkin, 2002). Trauth and colleagues (1999) found that in a group of naive rats, nAChR binding and membrane protein concentration showed a continued developmental decline in the midbrain, cerebral cortex and hippocampus throughout the adolescent period. Nicotine, when ingested, mimics the actions of acetylcholine, and this exogenous stimulation of the cholinergic system can disrupt the timing of cellular events that occur in the developing brain. Slotkin and colleagues exposed adolescent rats (PD ~30-45) to
nicotine using osmotic minipumps and found that this administration significantly increased the upregulation of nAChRs (Trauth, Seidler, McCook, & Slotkin, 1999; Slotkin et al., 2004), altered synaptic activity (Slotkin, 2002), decreased cell packing density, cell number, and neuritic projections (Abreu-Villaca, Seidler, Tate, & Slotkin, 2003; Trauth, Seidler, & Slotkin, 2000a), and altered the developmental profile of p53 mRNA (Trauth et al., 2000a).

Adolescent nicotine exposure produces a wide and persistent upregulation of both α4β2 and α7 nAChRs (Trauth et al., 1999; Slotkin et al., 2004). Adolescent animals exposed to nicotine showed increases in H3 binding to nAChRs during and after the nicotine treatment period. Elevations in nAChR binding were prevalent in the midbrain, hippocampus, cerebral cortex and remained significantly elevated weeks after the cessation of treatment (PD 60). Greater sensitivity and persistence in the upregulation of α4β2 nAChRs are a hallmark of the development of nicotine dependence and intensified withdrawal symptoms (Trauth et al., 1999; Slotkin, 2002). Also, α7 nAChRs are specifically involved in neuritic outgrowth, neurotoxicity, neuroprotection and response to toxicant injury; the upregulation of the α7 nAChRs evokes neural cell injury (Slotkin et al., 2004).

Researchers examined choline acetyltransferase (ChAT) activity and Hemicholinium 3 (HC-3) binding, two biochemical measures of synaptic function, in adolescent animals exposed to nicotine (Slotkin, 2002). ChAT is an enzyme that synthesizes acetylcholine and serves as a marker for the density of
cholinergic innervations. HC-3 binding labels the high-affinity presynaptic choline transporter and is sensitive to neural impulse activity. During adolescence, nicotine exposure evoked significant reductions in ChAT activity within the midbrain, an area in the brain involved in reward and addiction. Decrements in ChAT are characteristic of the loss of cholinergic neuronal inputs in aging and neurodegenerative disorders. Therefore, adolescent nicotine exposure may evoke specific cholinergic neuronal damage (Slotkin, 2002). Adolescent nicotine exposure also led to substantial reductions in HC-3 binding in the hippocampus, an area involved in learning and memory, during the nicotine administration and for several weeks posttreatment. Therefore, nicotine negatively affects cholinergic synaptic function when exposure occurs during adolescence.

Adolescent nicotine treatment evokes decreases in cell packing density and total cell number, as assessed by DNA concentration and content measurements, and compensatory elevations in the total protein/DNA ratio (Abreau-Villaca et al., 2003; Trauth et al., 2000a). In addition, adolescent nicotine treatment also leads to reductions in neuritic projections as measured by the membrane/total protein ratio. These deficits were observed in the cerebral cortex, midbrain and hippocampus and persisted until one month posttreatment (Abreau-Villaca et al., 2003). These reductions in DNA reflect interference with cell proliferation and/or necrotic/apoptotic cell loss which contributes to profound deficits in cell number, alterations of synaptic function and eventual disruption in behavioral performance. Finally, adolescent nicotine treatment also altered the
developmental profile of p53 mRNA expression (Trauth et al., 2000a). P53 is a transcription factor involved with neuronal differentiation and the regulation of the cell cycle. P53 regulates the cell cycle by inducing growth arrest, activating DNA repair proteins, and initiating apoptosis; therefore, altering p53 can elicit neural damage.

The effects of nicotine appear to vary dramatically depending on the age of drug administration. Previous research examining the effects of fetal nicotine exposure has consistently shown that nicotine acts as a neuroteratogen that alters patterns of neural cell replication, differentiation, synaptogenesis and synaptic function maturation; in addition, nicotine damages developing brain cells and evokes permanent changes in synaptic activity and cell signaling (Levin and Slotkin, 1998). Brain development continues into adolescence and the adolescent brain remains vulnerable to the neurotoxic effects nicotine (Slotkin, 2002). The results presented above show that adolescent nicotine exposure produces long-term changes in the developmental trajectory of cholinergic systems that compromise function in a number of key brain regions involved in learning, memory and reward. Contrary to the findings regarding fetal and adolescent exposure to nicotine, adult exposure to nicotine results in neuro-protective effects, causing decreased cell death and stimulating nicotinic cholinergic receptors (Slotkin, 2002). Therefore, the developmental stage at which nicotine exposure occurs is critical in determining the outcome.
Although behavioral changes elicited by nicotine have been well characterized in adults and after fetal nicotine exposure, there is a paucity of information about how adolescent nicotine exposure affects learning and behavior. As recent research shows, adolescent nicotine exposure causes significant changes in cell development and synaptic function and these abnormalities alter behavioral and physiological performance. The present experiments are designed to further elucidate the long term effects of adolescent nicotine exposure on adult learning using an animal model. First, different types of learning (i.e. context conditioning, extinction learning, and latent inhibition) will be assessed which tax different brain regions and processes. Second, adolescent nicotine exposure will be compared to adult nicotine exposure to see how the timing of drug administration differentially affects later learning. Finally, both chronic exposure (via osmotic minipumps) and repeated intermittent exposure (IP injections) will be used to examine how different routes of nicotine administration affect later learning.

**Context Conditioning**

Contextual fear conditioning occurs when a previously neutral environment is paired with an aversive unconditioned stimulus. Following this treatment, the environment (or “context”) alone elicits a fear state. This type of conditioning involves multiple cognitive processes. First, the subject must form a representation of the novel environment. Next, the representation of the context must be associated with the aversive unconditioned stimulus (US). Finally, the
subject is re-exposed to the context and must retrieve the memory of the paired association. This memory elicits a variety of fear responses (Landeira-Fernadez, 1996).

Researchers have examined the neurological basis for context conditioning and have determined that this type of learning is dependent on a functioning hippocampus. Young rats that have a relatively immature hippocampus are incapable of forming a long-term memory for contextual cues, and hippocampus lesions have been shown to impair context conditioning (Rudy, 1996; Rudy & Morledge, 1994). In addition, studies have shown that modulation of the hippocampal cholinergic system (using scopolamine, a muscarinic antagonist) impairs contextual conditioning (Anagnostaras, Maren, & Fanselow, 1995; Rudy, 1996).

The hippocampus, located in the medial temporal lobe, consistently has been shown to be critically involved in memory processes. More recent theories have proposed that the hippocampus is involved in forming configural associations between stimuli such as those involved in integrating and processing spatial and contextual information (Kenney and Gould, 2008b). Therefore, the hippocampus is not only involved in memory, but also in connecting memories with other related information. This is critical to learning and remembering relationships that characterize spatial layouts, items in the particular context in which they have been experienced, and other associative, sequential or logical relationships among experiences (Eichenbaum, 1991).
Recent research has further examined the biological substrates of the processes involved in context conditioning. The representation of the context as a configuration of cues involves the dentate gyrus (DG) region within hippocampus (Rudy & O’Reilly, 1999; Fanselow, 2000), although it is believed that the representation is stored elsewhere in the brain. The amygdala and the hippocampal CA3 area are critically involved in the association between the the context and the US (Matus-Amat et al., 2007). Therefore, the dorsal hippocampus appears to be particularly important for forming contextual associations and the hippocampus and amygdala are vital for learning to associate the context with the US.

Numerous studies have shown that nicotine exposure has direct effects on the hippocampus and subsequent hippocampal functioning. nAChRs (including the α4β2 and α7 subtypes) are widely distributed in the hippocampus, and are expressed presynaptically and postsynaptically which suggests these receptors modulate processes involved in synaptic plasticity and facilitate neurotransmitter release (Kenney & Gould, 2008a). nAChRs containing the α7 subunit are present in all hippocampal subregions with the highest concentration in the DG; α4β2 nAChRs are located in the DG and CA1 regions. Gould and colleagues (2008) have found that nicotine alters contextual (hippocampus-dependent) but not cued fear conditioning (non-hippocampus-dependent) in adult mice. In addition researchers have found that nicotine affects other hippocampus-dependent tasks including spatial learning and spatial working memory using the Morris water and
radial arm mazes respectively (Levin & Rose, 1990; Socci, Sanberg, & Arendash, 1995). These findings support the theory that nicotine exposure causes changes in cell signaling within the hippocampus that modulate certain types of learning.

Nicotine's targeting of hippocampal cholinergic pathways is critical when examining the effects of adolescent nicotine exposure because cellular and synaptic development in this region continues prominently into adolescence. The proliferation and differentiation of hippocampus dentate gyrus cells occurs throughout adolescence (Trauth, Seidler, & Slotkin, 2000b). In rats, cholinergic systems in the hippocampus undergo specific maturational changes in nerve activity approximately five to six weeks after birth and show a consistent decrease in nAChR concentrations. In hippocampus cell cultures, nicotine increases neuritic branching while decreasing overall cell number and promoting apoptosis (Slotkin, 2002). As stated previously, adolescent nicotine administration in rats produces a distinct pattern of nAChR upregulation as well as cell loss and damage within the hippocampus. In addition, research has found long term substantial decreases in cholinergic activity (illustrated by decreased hemicholinium-3 (HC-3) binding) within the hippocampus after adolescent nicotine exposure (Slotkin, 2002).

Very few studies have examined the effect of adolescent nicotine exposure on hippocampus-dependent learning and even fewer have focused on context conditioning. Trauth and colleagues (2000b) examined the effects of adolescent nicotine on open field behaviors (locomotion, rearing and grooming) and passive
avoidance in rats. Nicotine exposure led to decreased grooming, locomotor activity and rearing both during and after treatment (Trauth et al., 2000b). Hippocampal nicotinic cholinergic pathways are essential to the acquisition of passive avoidance behaviors. Interestingly, nicotine exposure actually enhanced passive avoidance behaviors both during and after treatment (Trauth et al., 2000b). Smith and colleagues (2006) administered nicotine to rats during adolescence and tested hippocampal learning in adulthood and found that low doses of nicotine administered during adolescence led to enhanced context conditioning. However, it should be noted that Smith et al. (2006), presented a conditioned stimulus (CS; tone) concurrently with the contextual cues when animals were exposed to shock. Therefore, it may be that the tone overshadowed the association between the context and the footshock. In the current study, the context will be presented without the presentation of a CS, providing a more precise measure of context conditioning. Experiment 1 aims to further explore the nature of adolescent nicotine’s effect on adult context conditioning. Based on the damaging effects of nicotine on the hippocampus, it is hypothesized that adolescent nicotine exposure will cause deficits in later adult context conditioning.

*Extinction Learning*

Extinction refers to the weakening of a response to a stimulus that has previously acquired aversive or appetitive properties through learning (Quirk, Garcia, and Gonzalez-Lima, 2006). If the CS (e.g. a context or tone) is repeatedly
presented without the US (e.g. footshock), the animal will no longer exhibit the conditioned response (CR; e.g. freezing; the rodent's physiological fear response) to the CS. Rather than simply the reduction of previous associative learning, extinction exhibits new learning.

Reduction in the expectation that the US is associated with the CS is correlated to the reduction and eventual elimination of the CR. Therefore, extinction results in new learning about the CS-US expectancy (the CS no longer signals a US), which competes with the previously learned knowledge (the CS is paired with the US; Hofmann, 2008). Causal reasoning allows humans and animals to predict outcomes on the basis of observation and this cognitive process can modulate the learned association between the CS and US (Hofmann, 2008). Extinction of the CS-US association is caused by changes in expectancies and acquiring new contingency expectations.

Recent research has explored the biological basis of extinction behavior and found that this type of learning requires functional interactions between medial prefrontal cortex (mPFC), the amygdala, and to a lesser extent, the hippocampus (Quirk et al., 2006). The neural circuitry in these areas underlies extinction learning.

The prefrontal cortex (PFC) is located in the anterior part of the frontal lobes and is comprised of three distinguishable areas: the ventrolateral cortex (vl-PFC), the medial prefrontal cortex (mPFC) and the anterior prefrontal cortex (aPFC). Experts consider the PFC a 'higher' brain region that exerts inhibitory
control over 'lower' or more basic brain regions and serves as the orchestrator of thoughts and actions in order to formulate internal goals (Sotres-Bayon, Cain, and LeDoux, 2006). Research has shown that this brain region is critically involved in executive functioning which describes the ability to differentiate among conflicting thoughts, determine future consequences of current activities, and formulate predictions and expectations (Rozenweig, Breedlove, & Watson, 2004). The PFC is also strongly linked with the more basic emotional systems of the brain and damage to the PFC dramatically weakens emotional activity including natural responses to reward. In particular, the mPFC areas of the PFC are involved in adjusting behavior based on emotional/motivational cues (i.e. reward and punishment). Unit recording studies show that mPFC neuronal activity changes as reward changes and that damage to the mPFC alters perseveration (Sotres-Bayon et al., 2006). Perseveration describes the inability to switch behavioral choices when situations change. Therefore, patients with damage to the mPFC are impaired in using emotional information to guide decision making; they are unable to switch behavioral choices when reward information changes (Sotres-Bayon et al., 2006).

Researchers have observed that when the CS is repeatedly presented without the US, the mPFC receives excitatory inputs from the hippocampus, thalamus, and amygdala which results in long term potentiation in mPFC potentials (Quirk et al., 2006). In addition, Morgan, Romanski, and LeDoux (1993) found that rats with mPFC lesions are able to acquire fear learning
normally but showed impairment in subsequent extinction. Finally, the nicotinic receptor antagonist, mecamylamine, has been shown to disrupt extinction when infused directly into the PFC (Quirk et al., 2006).

Previous research has shown that tobacco smoking and cigarette craving modulate activity in the PFC and the amygdala in adults (Sotres-Bayon et al., 2006). Due to these findings, researchers have begun to explore the effects of nicotine on areas of the brain related to extinction learning. As stated previously, nAChRs are widely distributed in the central nervous system and this includes the PFC and the amygdala. Using animal models, nicotine exposure has been shown to stimulate the release of norepinephrine, producing structural plasticity and altering gene and protein expression in these areas (Tian, Gao, Fu, Li, & Li., 2008). Tian and colleagues (2008) found that adult animals exposed to nicotine showed long term deficits in cued fear extinction. Therefore, nicotine may cause structural and molecular adaptations in the PFC and amygdala that subsequently impair extinction learning performance.

Specifically, adolescent nicotine exposure has been shown to alter the dendritic structure in some mPFC neurons and also produces changes in gene expression in the hippocampus and PFC (Bergstrom, McDonald, French, & Smith, 2008; Polesskaya et al., 2007). In addition, Smith and colleagues (2006) found that adolescent rats exposed to low doses of nicotine showed significant decreases in frontal cortex α4 subunit mRNA when examined in adulthood compared to sham control and high nicotine dose animals. These animals also
showed impairments in cued fear extinction when tested as adults. Experiment 1 aims to further explore the nature of adolescent nicotine's effect on adult extinction learning. Based on the damaging effects of nicotine on the prefrontal cortex and the findings of Smith (2006) and Tian (2004), adolescent nicotine exposure is hypothesized to cause deficits in later extinction learning.

*Latent Inhibition*

If a CS is repeatedly presented to an organism prior to Pavlovian training (pairing of the CS with a US), the CR (fear) towards the CS is weaker than if the CS had not been presented prior to conditioning training (Gray et al., 1997). This retardation of Pavlovian conditioning is known as latent inhibition (also known as the CS Pre-Exposure effect) and can be used as a paradigm for assessing attentional deficits. Latent inhibition requires attention to relevant stimuli and the ability to filter irrelevant sensory information. Attentiveness to the pre-exposure stimulus (CS) decreases the strength and/or rate of acquiring the CS-US association. The ability to attend to potentially important stimuli contributes to more efficient learning (Gould, Collins, & Wehner, 2001).

The cholinergic and dopaminergic systems have been consistently linked to attention and the ability to filter irrelevant sensory information. The basal forebrain cholinergic system's (BFCS) primary projections innervate the hippocampus, cingulate cortex, and the neocortical mantle, as well as other areas (Rozenweig et al., 2004). These pathways are critical for attentional functioning including sustained attention, selective attention, and the ability to increase and
decrease attention to stimuli (Rozenweig et al., 2004). The dopaminergic system originates in the substantia nigra or ventral tegmental area and has two primary projections. The mesostriatal dopaminergic system originates in the substantia nigra and projects to the neostriatum. The mesolimbic system originates in the ventral tegmental area and provides DA innervations into the limbic system (including the nucleus accumbens) and frontal cortical areas. These pathways play a major role in motor control and attention (Newhouse, Potter, & Singh, 2004). In addition, these two neurotransmitter systems interact; several cholinergic projection systems provide input to dopaminergic cells in the substantia nigra and ventral tegmental area, and cholinergic stimulation leads to enhanced DA activity (Krause, Dresel, Krause, Fougere, & Achenheil, 2003). Dopaminergic transmission and particularly, nucleus accumbens dopamine has been found to directly impact latent inhibition (Gray et al., 1997).

A major function of nAChRs is to modulate the release of neurotransmitters including dopamine, glutamate, GABA, norepinephrine, and serotonin and this process is critical for cognitive and attentional processes. Nicotine exposure stimulates nAChRs located on DA neurons which results in increased activation of the central DA systems and leads to the enhancement of DA mediated functions (Newhouse et al., 2004). The nucleus accumbens, known as the pleasure center of the brain, plays an important role in reward, laughter, addiction and fear (Rozenweig et al., 2004). Nicotine, in addition to other drugs of abuse, increases dopamine release within the nucleus accumbens and has been
consistently shown to augment the cholinergic and dopaminergic systems (Grady et al., 2007). After directly infusing nicotine into dopaminergic nerve terminals, researchers have detected increases in DA release in the nucleus accumbens, striatum, and frontal cortex (Grady et al., 2007). In addition, chronic nicotine exposure leads to the upregulation of high-affinity nAChRs in the nucleus accumbens, cortical areas, and the hippocampus in mice (Even et al., 2008).

As stated previously, nicotine treatment during adolescence persistently alters activity of brain regions involved in reward and memory. Adolescent nicotine exposure causes nAChR upregulation and cell loss and damage in the DA-rich midbrain region of the brain. Chronic exposure to nicotine via osmotic minipumps led to long term (up to 21 days posttreatment) upregulation of high affinity nAChRs in cortical areas, caudate putamen, nucleus accumbens, hippocampus, ventral tegmental area, and superior colliculi (Doura, Gold, Keller, & Perry, 2008). In addition, Wang and colleagues (2008) compared the effects of adolescent nicotine exposure to adult nicotine exposure on dopamine release and, most relevant to the present experiment, found several distinct differences within the nucleus accumbens. Interestingly, adolescent nicotine exposure led to significantly greater DA activity within the nucleus accumbens shell than adult nicotine exposure. Similarly, within the nucleus accumbens, adolescent nicotine exposure increased total dendritic length and number of branches. In contrast adult nicotine exposure did not significantly alter total dendritic length or branch number. Finally, adolescent nicotine exposure has been linked to an increase in
FosB (a transcription factor) within the nucleus accumbens and this increase lasts until post-natal day 80 for some animals (Soderstrom, Qin, Williams, Taylor, & McMillen, 2007). Combined, these studies indicate that adolescent nicotine exposure has long term effects on DA activity within the nucleus accumbens.

Previous research has shown that nicotine’s effect on latent inhibition depends on when nicotine is administered during the conditioning process. When nicotine is administered prior to the CS pre-exposure phase only, the drug has been found to have no impact or enhance latent inhibition (Joseph, Peters, & Gray, 1993; Gould et al., 2001; Rochford, Sen, & Remi, 1996). When administered prior to CS pre-exposure and prior to Pavlovian conditioning, nicotine has been shown to disrupt latent inhibition (Joseph et al., 1993). When administered prior to Pavlovian conditioning only, nicotine also disrupts latent inhibition (Joseph et al., 1993). Combined, these findings are consistent with the argument that nicotine can potentially enhance latent inhibition by increasing attentional processes and the ability to detect relevant stimuli (i.e. the CS).

However, all of these studies have examined the acute effects of nicotine on latent inhibition. Very few studies have examined the long term effects of chronic nicotine on latent inhibition and there are no published studies to date on the long term effects of adolescent nicotine exposure on adult latent inhibition.

Experiment 2 aims to further explore the nature of adolescent nicotine’s effect on latent inhibition. Based on the damaging effects of nicotine on the midbrain and the effects of nicotine on the dopaminergic system, we hypothesize that
adolescent nicotine exposure will cause deficits in latent inhibition assessed
during adulthood.

Two groups of animals did not receive pre-exposure to the conditioned
stimulus and were considered control groups. Animals in the Delay group did not
receive pre-exposure to the CS but were exposed to delay conditioning during
shock training. Delay conditioning is a basic form of Pavlovian conditioning
and occurs when the conditioned CS (a tone) is presented while the organism is
exposed to the US (shock). This type of conditioning requires a functioning
amygdala and research has found that nicotine does not affect delay conditioning
(Rochford et al., 1996). Therefore, it is hypothesized that adolescent nicotine
exposure will not cause differences in delay conditioning in adulthood. In order
to observe that delay conditioning occurred, a third experimental group, the
Unpaired group did not receive pre-exposure to the CS and was not exposed to
delay conditioning during shock training. Instead, these animals were exposed to
the same number of shocks and tones but the two stimuli were not paired. It is
hypothesized that animals in the Delay group will show stronger fear to the CS
than animals in the Unpaired group.

*Adult versus Adolescent Exposure*

In both human and animal studies, adolescence appears to be a period of
development that is particularly vulnerable to the negative effects of nicotine.
Despite smoking significantly fewer cigarettes than adults, adolescents who use
tobacco products are more likely to meet the criteria for nicotine dependence than
are adults and often have worse outcomes in terms of ability to quit (Chen & Millar, 1998). As stated previously, researchers have been able to examine the adolescent brain using imaging technology and have found there is a significant amount of brain growth during the beginning of this period followed by a decrease in grey matter during the transition from adolescence to adulthood (Strauch, 2003). These changes also coincide with a gradual loss of synapses and subsequent strengthening of remaining synapses. In addition, adolescence marks a period in which the maturation of the central nervous system neurotransmitter pathways and functions are still taking place including the cholinergic and dopaminergic systems (Slotkin, 2002). Recent research examining brain development using animals models has shown that the neurochemical, neuroanatomical and behavioral changes that occur during adolescence in rats are similar to those seen in human adolescents (Doura et al., 2008). As the adolescent period of development comes to a close, the brain is more fully developed, less plastic and more resistant to the effects of various toxins and drugs. In fact, although animal studies indicate that nicotine acts as a neuroteratogen when exposure occurs during fetal and adolescent development, nicotine actually has neuro-protective qualities in the adult rat brain, causing decreased cell death and stimulating nicotinic cholinergic receptors (Slotkin, 2002). Because teenagers are more likely to begin using tobacco products than adults, and because nicotine damages the developing brain, adolescence represents a particularly vulnerable period of development to the effects of nicotine.
Recent research that has begun to directly compare the effects of adolescent and adult nicotine exposure on behavior focuses on the drug’s rewarding and emotional effects (i.e. on anxiety and depression). However, very few studies have focused on nicotine’s long term impact on learning. Levin and colleagues (2003, 2007) compared the amount of nicotine that rats self-administered during either adolescence or adulthood. Adolescent animals self-administered significantly more nicotine per kilogram of body weight than animals self-administering nicotine during adulthood. Similarly, Brielmaier and colleagues (2007) found that conditioned place preference (CPP) was established following a single pairing of nicotine injection with the initially non-preferred side of a place conditioning apparatus in early adolescent but not adult animals. Therefore, adolescent animals appear to be more sensitive to the rewarding properties of nicotine compared to adults. Researchers have also found that animals exposed to nicotine during adolescence show increased anxiety-like behaviors, evidenced by decreased exploration and activity in an open field, when compared to controls while animals exposed to nicotine during adulthood show no differences in anxiety-behaviors (Adrianai et al., 2004; Slawecki, Gilder, Roth, & Ehlers, 2003). Similarly, Smith and colleagues (2006) examined the long term effects of chronic adolescent versus adult nicotine exposure on fear related learning in the rat. Although adolescent animals exposed to nicotine showed long term changes in fear conditioning and extinction learning in adulthood; adult animals exposed to nicotine did not differ from age-matched saline-treated
animals on either task. Therefore, studies directly comparing nicotine exposure during adolescence and adulthood further illustrate that the adolescent brain is particularly vulnerable to the effects of nicotine.

Experiment 3 aims to further explore how nicotine differentially affects behavior depending on when drug exposure occurs. Animals were exposed to nicotine (via intermittent IP injection) during adolescence (PD 28-42) or adulthood (PD 90-104) and then tested on context conditioning and extinction learning eighteen days later. Based on previous research showing the damaging effects of nicotine on the developing adolescent brain and the lack of behavioral effects after adult exposure, it is hypothesized that adolescent nicotine exposure will cause long term deficits in context conditioning and extinction learning and that adult nicotine exposure will not cause long term changes on these assessments of learning.

Routes of Nicotine Administration

Smoking is a highly regulated behavior and smokers aim to maintain a steady state of nicotine levels within the brain to achieve optimal psychoactive effects. Smokers are able to self-regulate the level of nicotine in their system to produce desired effects (i.e. relaxation, increased concentration) and to avoid the unpleasant adverse effects associated with too high or too low concentrations (Sellers, 1998). The typical smoker consumes about 17 cigarettes per day, and the elimination half-life of nicotine in a chronic smoker is about two hours, so smokers require frequent administration of the drug to avoid withdrawal.
symptoms and craving (Benowitz & Jacob, 1984). Because smokers are in a state of nicotine deficiency when they wake up in the morning, they will smoke one or more cigarettes fairly rapidly and then continue smoking throughout the day to maintain this level.

Most cigarettes contain 0.5 - 2.0 milligrams of nicotine; however, only about 20% (between 0.1 and 0.4 milligram) of nicotine in a cigarette is actually inhaled and absorbed into the smoker's bloodstream (Matta et al., 2007). Therefore the average cigarette delivers roughly 10-30 µg kg⁻¹, typically resulting in 10-50 ng ml⁻¹ peak plasma levels. Nicotine is metabolized by the liver to six primary metabolites and in humans approximately 70-80% of nicotine is converted to the metabolite cotinine (Matta et al., 2007). Although animal studies are unable to directly imitate the route of administration primarily used by humans (smoking cigarettes), several paradigms are frequently used which closely mimic blood-nicotine levels and the somatic symptoms found in humans.

Rats provide an excellent experimental model to study the neurobiological mechanisms underlying the effects of nicotine exposure and are ideal for examining the drug's impact on behavior (Matta et al., 2007). Two of the most common methods of administering nicotine to rats are repeated injection and subcutaneous osmotic minipumps. Because the half-life of nicotine in the plasma is shorter in rats (45 minutes) than in humans (2 hours), the doses of nicotine administered via repeated injections and the osmotic minipumps need to be higher than those found in cigarettes in order to achieve similar blood-nicotine
concentrations. Additionally, systemic injections and subcutaneous osmotic minipumps do not deliver nicotine as rapidly to the brain as cigarettes (which are the most efficient mode of nicotine delivery to the brain) but still lead to similar neurochemical changes (Matta et al., 2007). There are strengths and weaknesses to both of these routes of administration.

Multiple injections are useful because the time of drug administration is well controlled and the dosage is dependent on the exact weight of each animal. In addition, when injections are given once or twice daily, nicotine is cleared entirely out of the system before the next injection is administered. Therefore, nAChRs are activated each time the drug is administered and this regimen mimics the pulsatile mode of delivery via smoking. When nicotine is administered by daily injections rats exhibit an inverted U-shaped dose-response curve and experience a peak response between 0.20 and 0.50 mg/kg (Matta et al., 2007). Previous studies have commonly employed doses between 0.10 and 0.60 mg/kg and have found that plasma nicotine levels associated with the 0.10 mg/kg are close to the typical plasma concentrations in inhaling cigarette smokers. However, the injection process is stressful and stress can affect a host of biological processes which impact the effects of nicotine. Acute nicotine injections to drug naïve rats have been shown to increase plasma levels of stress responsive hormones including corticosterone and norepinephrine (Benwell & Balfour, 1979).
Subcutaneous osmotic minipumps have the advantage of slowly and chronically releasing nicotine over an extended time period (up to 28 days) without the stress of repeated injections. In addition, this chronic nicotine exposure more closely models the chronic exposure experienced by habitual smokers. The dose of nicotine commonly administered via the osmotic minipumps is between 2.0 – 6.0 mg/kg/day which mimics the range of light (1/2 – 1 pack day) and heavy (two packs a day) smokers respectively (Matta et al., 2007). Although the osmotic minipump is the most commonly used method of administration for animal studies examining the effects of nicotine, there are several limitations to this procedure. First, two minor surgeries are required to implant and remove the pump, therefore the animals must go under anesthesia. Second, the animal’s weight increases during the course of nicotine administration (especially when this occurs during adolescent development) so the dose administered is actually the mean dose delivered over the course of drug exposure. Therefore, the amount of nicotine released is generally lower at the end of the exposure than at the beginning. Finally, chronic continuous nicotine exposure results in receptor desensitization whereas receptor function fluctuates during episodic smoking with periods of activation followed by desensitization then re-sensitization.

In Experiment 1, the animals were administered nicotine via the subcutaneous osmotic minipump during adolescence and in Experiment 3 the animals were administered nicotine via repeated intraperitoneal injections. In
both experiments the animals were later tested on context conditioning and extinction learning. The inherent differences between the subcutaneous osmotic minipumps and the repeated injections may differentially impact long term learning. However, due to the previous research illustrating the damaging effects of nicotine during adolescence, it is hypothesized that nicotine exposure, via both routes of administration will cause long term deficits in learning.

Method

Subjects

Litters of Sprague-Dawley rats born at the vivarium of the Psychology Department at the College of William and Mary, Williamsburg, VA were used in these experiments. Male and female breeder pairs were housed together in polycarbonate cages with wire lids. Pine chip bedding was provided and food (Formulab Diet 5008; W.F. Fisher & Son, Somerville, NJ) and water were available ad libitum. Cages were checked daily for new births and the day of birth was designated as Postnatal Day (PD) 0. Litters were culled to 8-10 pups on PD 2. On PD 21 rats were weaned and maintained in 50.8 x 40.6 x 21.6 cm (l x w x h) clear polycarbonate cages with wire lids. Rats were housed as a litter until PD 42 when males and females were kept in separate polycarbonate cages. The vivarium was temperature controlled and maintained on a 14:10 light/dark cycle, with light onset at 6 am.

In Experiment 1, the subjects were 89 Sprague-Dawley rats (45 males and 44 females) and experimental group size ranged from 12-13 animals. In
Experiment 2, the subjects were 86 Sprague Dawley rats (46 males and 40 females) and experimental group size ranged from 9-11 animals. In Experiment 3a, the subjects were 68 Sprague-Dawley rats (34 males and 34 females) and experimental group size ranged from 10-15 animals. In Experiment 3b, the subjects were 47 Sprague-Dawley rats (23 males and 24 females) and experiment group size ranged from 8-11 animals. All experimental procedures were approved by the Institutional Animal Care and Use Committee at the College of William and Mary.

Animal Treatments

Osmotic Minipumps. In Experiments 1 and 2, drug treatments were administered by subcutaneous osmotic mini-pump infusions beginning on postnatal day (PD) 28. Each animal was anesthetized with an injection containing ketamine (90.0 mg/kg) and xylazine (9.0 mg/kg). A small area on the back was shaved and an incision was made to permit the subcutaneous insertion of osmotic minipumps (Alzet micro-osmotic pump model 1002, DURECT Corporation, Cupertino, CA). Pumps were prepared with nicotine bitartrate (Sigma Chemical Co., St. Louis, MO) dissolved in saline to deliver an initial dose rate of 0 (saline), 3.0 or 6.0 mg/kg of nicotine per day, based on an estimate of the animals weight on PD 35. Weight estimates were obtained by weighing PD 35 animals in the vivarium and taking the average (male = 169.5 g, female = 134.9 g). The nicotine doses were chosen to match plasma levels seen in moderate and heavy smokers respectively (Matta, et al., 2007). The incision was closed with wound clips and
the animals were permitted to recover in their home cages. Osmotic minipumps delivered nicotine at a constant rate (average pumping rate \( Q = 0.25 \mu l/hr \)) for 14 days and were removed on PD 42.

**Intraperitoneal injections.** In Experiment 3, drug treatments were administered by repeated intraperitoneal injections beginning on either PD 28 (Experiment 3a) or PD 90 (Experiment 3b) and ending on PD 42 (Experiment 3a) or PD 104 (Experiment 3b), respectively. Injections were administered every other day between 10 AM and Noon during this period (8 total injections).

Nicotine was prepared with nicotine bitartrate (Sigma Chemical Co., St. Louis, MO; doses based on free base) dissolved in saline to deliver a nicotine dose of 0.15 mg/kg (moderate dose) or 0.40 mg/kg (high dose). All nicotine solutions were pH balanced to approximately 7.2. On injection days, animals were weighed and then given an IP injection containing either saline only, or nicotine (1.0 ml/kg).

**Behavioral Testing Apparatus**

In Experiments 1 and 3, training and testing for context conditioning and extinction occurred in identical Med Associates™ (St. Albans, VT) modular conditioning chambers measuring 30.5 x 24.1 x 21.0 cm (l x w x h). The front wall (which also served as the door to the chamber) and the back wall were constructed of clear plexiglass. The two side walls were constructed of aluminum. The floor consisted of parallel stainless steel rods that were connected by an electrical grid. The rods were 0.7 cm in diameter and were spaced 1.5 cm
apart, center to center. A 100-mA, 28-V DC houselight centered on the left aluminum wall and positioned 2.5 cm below the ceiling illuminated each chamber. The houselight bulb was contained within a cylindrical diffuser that projected light toward the top of the chamber ceiling. Background noise from a ventilation fan, was 74 dB (C). Each of the twelve chambers was contained within a separate sound attenuating chamber.

Each chamber could be equipped with a water-filled lick tube. When inserted, the lick tube protruded 2.0 cm into a square drinking recess on the right aluminum wall. Each recess was 5.1 cm x 5.1 cm (length x height) and 3.0 cm deep. The recess was centered on the aluminum wall with its center 3.5 cm above the chamber floor. An infrared photobeam was projected across the tip of the lick tube. Subjects had to insert their heads approximately 1 cm into the recess in order to drink from the lick tube, thereby breaking the beam. The duration that subjects were accessing the lick tube could be recorded with a computer program using MED-PC software (Med Associates, Inc., St. Albans, VT).

In Experiment 2, training and testing occurred in two different contexts within the same chamber: Context Train and Context Test. Context Train served as the context for conditioning and was identical to chambers used in experiments 1 and 3. Context Test was created by installing a small rectangular Plexiglas insert into the chamber for the purpose of CS testing. The insert measured 24.5 cm x 8.5 cm x 15.5 cm (l x w x h). The floor, one side wall, and the rear wall were constructed of clear Plexiglas. The ceiling and other side wall of the insert
were constructed of steel wire mesh. The insert was positioned such that the front end of the insert would seat tightly against the front wall of the chamber to permit access to the lick tube that was present in the chamber. Context Test was always illuminated by a dim stimulus light (100-mA, 28-V DC, 2.5 cm diameter) centered on the left panel of the right wall of the chamber. The panel light bulb projected light through a white opaque light diffuser creating dim diffuse illumination of Context test. In both Context Train and Context Test, a 2900-Hz, pure tone with amplitude of 82 dB could be delivered by means of a Med Associates TM sonalert tone module (ENV-223AM). The tone module was positioned on the left aluminum wall of Context Train.

*General Behavioral Testing Procedure*

In the current study a lick suppression paradigm was used to assess context conditioning, extinction learning, and latent inhibition. Lick suppression involved three phases. The first was to establish a baseline level of a measurable activity, the rate of drinking. A baseline measure must be obtained in order to ensure that all rats are performing at an equal level prior to training and testing. This is accomplished by placing thirsty rats into an operant chamber with access to a lick tube and measuring the first five cumulative seconds of drinking. The second phase was to institute Pavlovian conditioning; either a neutral context (operant chamber) or a salient cue (tone) is paired with an aversive stimulus (footshock). During this phase the rat learns to associate the context or cue with the negative stimulus. Finally, thirsty rats were returned to the operant chamber
with access to the lick tube and the first five cumulative seconds of drinking were again measured. Conditioned lick suppression measures the extent to which the learned fear interferes with the ongoing motivation to drink by thirsty rats. Therefore longer latencies in drinking represent stronger fear learning on test days.

Before training and testing, all animals were removed from gang tubs and placed in individual wire cages. While in the individual wire cages, animals were handled daily and progressively deprived of water so that at the beginning of behavioral training, animals were limited to 20 minutes of water access per day.

**Experiment 1 Procedure**

Behavioral training began 23 days after the osmotic minipumps were removed (see Figure 1). The behavioral procedure for Experiment 3 was identical to Experiment 1. Animals were exposed to two acclimation days. During the first two days of behavioral training, all subjects became acclimated to the Context Train chambers. On each acclimation day, subjects were placed in the chamber for 60 minutes and were allowed to drink from the water-filled lick tubes. The time (s) it took for each subject to drink for 5 cumulative seconds was recorded and provided a baseline measure of drinking behavior. As later indicated, nicotine treated animals did not significantly differ from saline treated animals on either pre-conditioning acclimation day. All animals were performing at the same level prior to shock training.
On the third day of behavioral training, context conditioning was conducted. All subjects were placed in the Context Train chamber for 23 minutes with water-filled lick tubes removed. There were five groups which differed on conditioning treatment and prior adolescent drug exposure. Two “No Shock” groups were exposed to the context only with no shock presented during the conditioning session. Group saline-No shock had been exposed previously to saline and Group 6.0 mg/kg/day-No Shock had been exposed previously to nicotine. Three “Shock” groups were exposed to 10 unsignaled shocks (1.0 mA, 1 s) during the session with a mean ITI of 100 s (range: 65 – 135 s). Groups Saline-Shock had been previously exposed to saline and groups 3.0 mg/kg/day-Shock and 6.0 mg/kg/day-Shock had been previously exposed to nicotine.

On the fourth-sixth days of behavioral training, the tests for context conditioning and extinction occurred. All subjects were placed in the chamber for 60 minutes with access to the water filled lick tubes and suppression of drinking in the presence of context cues was assessed. On each of the three test days, latency to complete the first five cumulative seconds of drinking from placement in the chamber was recorded. Higher latencies reflect proportionally more suppression of drinking (i.e., higher context-elicited fear). Test day 1 served as the primary test for context conditioning. Tests 2 and 3 were identical to Test 1 and were intended to assess extinction of learned context fear across continued non-reinforced exposure to the contextual cues.
Experiment 2 Procedure

Behavioral training began 18 days after the osmotic minipumps were removed (see Figure 2). On the first day of behavioral training, all animals were acclimated to the Context Test chamber for 60 minutes and had access to the lick tube. The time (s) it took for each subject to drink for 5 cumulative seconds was recorded and provided a baseline measure of drinking behavior in the lick suppression paradigm. As later indicated, nicotine treated animals did not significantly differ from saline treated animals on either pre-conditioning acclimation day. All animals were performing at the same level prior to CS pre-exposure and shock training.

On the next four days of behavioral training, animals were exposed to the Context Test chamber for 60 minute sessions per day with the water-filled lick tube removed from the chamber. Three “CS Pre-Exposure” groups (CS Pre-Exposure-Saline, CS Pre-Exposure-3.0 mg/kg/day, and CS Pre-Exposure-6.0 mg/kg/day) were exposed to 30 tones with a mean ITI of 88s (range: 68-108s) during the four 60 minute sessions in Context Test. Three “Delay” groups (Delay-Saline, Delay-3.0 mg/kg/day, and Delay-6.0 mg/kg/day) and three “Unpaired” groups (Unpaired-Saline, Unpaired-3.0 mg/kg/day, and Unpaired-6.0 mg/kg/day) were exposed to the Context Test chamber for four 60 minutes for acclimation purposes only (without exposure to the tone).

On day six of behavioral training, animals were exposed to tone-shock conditioning in the Context Train chamber during a 60 minute session with the
water-filled lick tube removed from the chamber. The three “CS Pre-Exposure”
groups and the three “Delay” groups were exposed to delay conditioning which
consisted of four tone-shock conditioning trials with a mean ITI of 794 sec
(range: 594-1014). The tone was a 15-sec, 3000-Hz, pure tone with an amplitude
of 82 dB (C) and shock was 1.0-mA, 1.0-sec in duration. Shock occurred during
the last second of each tone presentation. The three “Unpaired” groups were
exposed to the same four tones and the same four shocks but the two stimuli were
not paired and served as a control group.

On day seven and eight of behavioral training, animals were exposed to
two 60-min recovery sessions in the Context Test chamber. During each recovery
session, rats were allowed to drink from water-filled lick tubes. No discrete CS or
US was presented. The purpose of recovery sessions was to restabilize drinking
behavior following shock sessions prior to target CS testing. As later indicated,
there were no significant differences between drug groups on Pre-CS latencies; all
animals were performing at the same level in the Context Test chamber prior to
CS testing.

On the final day of behavioral training, conditioning to the tone was
assessed. Tone testing occurred in the Context Test chamber. Animals were
placed in the chamber with access to the lick tube. After drinking for five
cumulative seconds (pre-CS period), the tone CS was presented (CS period) and
remained on until the animal completed an additional five cumulative seconds of
drinking in the presence of the tone CS. Suppression of drinking in the presence
of the tone was taken as a measure of learned fear. Again, higher latencies reflect proportionally more suppression of drinking (i.e., higher context-elicited fear).

**Experiment 3 Procedure**

Behavioral training began 18 days after the IP injection period ended (see Figure 3). The behavioral training procedure for Experiment 3 was identical to Experiment 1. During the first two days of behavioral training, all subjects became acclimated to the Context Train chambers. As in Experiment 1, nicotine treated animals did not significantly differ from saline treated animals on either pre-conditioning acclimation day. All animals were performing at the same level prior to shock training.

On the third day of behavioral training, context conditioning was conducted. Two “No Shock” groups were exposed to the context only, no shock were presented during the conditioning session. Group saline-No shock had been exposed previously to saline and Group 0.40 mg/kg/day-No Shock had been exposed previously to nicotine. Three “Shock” groups were exposed to 10 unsignaled shocks (1.0mA, 1 s) during the session with a mean ITI of 100 s (range: 65 – 135 s). Groups Saline-Shock had been previously exposed to saline and groups 0.15 mg/kg/day-Shock and 0.40 mg/kg/day-Shock had been previously exposed to nicotine.

On the fourth-sixth days of behavioral training, the tests for context conditioning and extinction occurred. All subjects were placed in the chamber for 60 minutes with access to the water filled lick tubes and suppression of drinking
in the presence of context cues was assessed. Again, higher latencies reflect proportionally more suppression of drinking (i.e., higher context-elicited fear).

**General Statistical Procedures**

To control for litter effects, no more than two animals from each litter (one female, one male) was represented in each treatment group. When more than one male or female from a litter was assigned to a particular group, a mean from those animals was computed and served as the unit for data analysis. Latency data were normalized using a log (base 10) transform. A mixed ANOVA was conducted on test data followed by planned comparisons using the overall error term. A level of $\alpha = 0.05$ was used to determine statistical significance.

**Results**

**Experiment 1**

Acclimation latencies (mean + S.E.) in groups Saline-Shock, 3.0 mg/kg/day-Shock, 6.0 mg/kg/day-Shock, Saline-No Shock, 6.0 mg/kg/day-No Shock were 2.0 (+.08), 1.79 (+.06), 1.96 (+.07), 1.88 (+.06), and 1.85 (+.10), respectively, on Acclimation Day 1; and were 1.42 (+.10), 1.33 (+.05), 1.32 (+.09), 1.32 (+.08), and 1.33 (+.10), respectively, on Acclimation Day 2. None of the groups differed in lick latency on either preconditioning acclimation day ($F$s < 1.34). A 5 (Group) X 3 (Test Day) mixed ANOVA was subsequently conducted on test data. The between-subjects factor was Group (Saline-Shock, 3.0 mg/kg/day-Shock, 6.0 mg/kg/day-Shock, Saline-No Shock, 6.0 mg/kg/day-No Shock) and the within-subjects factor was Test Day (Test 1, Test 2, Test 3). The
analysis revealed significant main effects of Group \( (F(4, 56) = 9.49, p < .001) \) and Test Day \( (F(2, 112) = 58.38, p < .001) \) as well as a significant Group X Test Day interaction, \( (F(8, 112) = 9.92, p < .001) \). As can be seen in Figure 4, shock exposed groups supported greater suppression to the context compared to no-shock groups.

Planned comparisons revealed that Test 1 latencies in Groups Saline-Shock and 6.0 mg/kg/day-Shock were significantly higher than in corresponding no-shock groups, \( (Fs(1, 112) = 48.87 \) and 81.64, respectively, \( ps < .001) \) indicating shock treatment was effective at establishing context conditioning. Test 1 latency in Group 3.0 mg/kg/day was compared to the mean of the two no-shock groups and this difference was also significant, \( (F(1, 122) = 23.75, p < .001) \). The two no-shock groups did not differ \( (F(1, 112) < 1) \). Of critical interest, Figure 4 also suggests that levels of context learning assessed on Test 1 were not the same in groups exposed to different drug treatments during adolescence. Both shock groups receiving nicotine during adolescence (Group 3.0 mg/kg/day and Group 6.0 mg/kg/day) had significantly lower latencies compared with the Saline-Shock group, \( (Fs(1, 112) \geq 4.65, ps < .05) \). Therefore, adolescent nicotine treatment impaired later adult context fear learning.

Patterns of context suppression across subsequent extinction testing on Test 2 and Test 3 suggest that extinction of context fear in all groups was relatively rapid and nearly complete by Test 2 (see Figure 4). Each of the three shock groups had significantly lower suppression on Test 2 compared to Test 1
(Fs (1, 112) > 22.54, ps < .01) and there was no further reduction in latencies on Test 3 compared to Test 2, (Fs (1, 112) < 2.22).

**Experiment 2**

In this experiment both between-subjects factors (Drug and Condition) had three levels creating nine total groups: Saline-CS Pre-Exposure, Saline-Delay, Saline-Unpaired, 3.0 mg/kg/day- CS Pre-Exposure, 3.0 mg/kg/day-Delay, and 3.0 mg/kg/day-Unpaired, 6.0 mg/kg/day-CS Pre-Exposure, 6.0 mg/kg/day-Delay, and 6.0 mg/kg/day-Unpaired. A 3 (Drug) X 3 (Condition) ANOVA was conducted on the latency to drink for five cumulative seconds before (Pre-CS) and after (Post-CS) the tone was presented on the critical test day. There were no significant differences between drug or condition groups on the Pre-CS latency data (all Fs <1), indicating that all animals were performing at the same level prior to receiving the tone. Data analysis for the Post-CS data revealed a significant main effect for Condition (F (2, 64) = 7.72, p < 0.01), however the main effect of Drug and the Drug X Condition interaction were not significant (Fs <1) (see Figure 5).

Planned comparisons using the overall error term revealed that animals in the Delay condition group exhibited significantly longer latencies than animals in the Unpaired condition group (F (1, 47) = 11.07, p < 0.01). In addition, animals in the CS Pre-Exposure condition group exhibited significantly shorter latencies than animals in the Delay condition group (F (1, 47) = 11.38, p < 0.01) and did not differ from animals in the Unpaired condition group (F < 1). These findings demonstrates that paired associative learning occurred in the Delay condition
group and that the CS pre-exposure effect was established in the CS Pre-Exposure condition group. In order to focus on latent inhibition, a planned one-way ANOVA was run within the CS Pre-Exposure group. The 3.0 mg/kg/day had slightly higher latencies than the saline and 6.0 mg/kg/day groups, indicating a possible deficit in latent inhibition; however, this difference was not statistically reliable.

*Experiment 3a*

Acclimation latencies (mean + S.E.) in groups Saline-Shock, 0.15 mg/kg/day-Shock, 0.40 mg/kg/day-Shock, Saline-No Shock, 0.40 mg/kg/day-No Shock were 1.91 (+.09), 1.98 (+.11), 1.86 (+.09), 1.90 (+.09), and 1.95 (+.09), respectively, on Acclimation Day 1; and were 1.38 (+.08), 1.38 (+.10), 1.38 (+.08), 1.29 (+.08), and 1.34 (+.08), respectively, on Acclimation Day 2. None of the groups differed in lick latency on either preconditioning acclimation day ($F$s < 1). A 5 (Group) X 3 (Test Day) mixed ANOVA was subsequently conducted on test data. The between-subjects factor was Group (Saline-Shock, 0.15 mg/kg/day-Shock, 0.40 mg/kg/day-Shock, Saline-No Shock, 0.40 mg/kg/day-No Shock) and the within-subjects factor was Test Day (Test 1, Test 2, Test 3). The analysis revealed significant main effects of Group ($F(4, 63) = 7.73, p < .01$) and Test Day ($F(2, 62) = 21.13, p < .001$) as well as a significant Group X Test Day interaction, ($F(8, 126) = 3.52, p < .01$). As can be seen in Figure 6, shock exposed groups supported greater suppression to the context compared to no-shock groups.
Planned comparisons revealed that Test 1 latencies in Groups Saline-Shock and 0.40 mg/kg/day-Shock were significantly higher than in corresponding no-shock groups, \( F(1, 28) = 17.00 \) and \( 38.20 \), respectively, \( p < .001 \) indicating shock treatment was effective at establishing context conditioning.

Test 1 latency in Group 0.15 mg/kg/day was compared to the mean of the two no-shock groups and this difference was also significant, \( F(1, 27) = 14.23, p < .001 \). The two no-shock groups did not differ \( F(1, 28) < 1 \). Of critical interest, Figure 6 also suggests that levels of context learning assessed on Test 1 were not the same in groups exposed to different drug treatments during adolescence.

Shock groups receiving the low dose of nicotine during adolescence did not significantly differ when compared with the Saline-Shock group, \( F(1, 23) < 1 \). However, the shock group receiving the high dose of nicotine during adolescence exhibited significantly higher latencies when compared with the Saline-Shock group \( F(1, 28) = 4.08, p < .05 \).

Patterns of context suppression across subsequent extinction testing on Test 2 and Test 3 suggest that extinction of context fear in all groups was relatively rapid and nearly complete by Test 2 (see Figure 6). Each of the three shock groups had significantly lower suppression on Test 2 compared to Test 1 \( (F s(1, 28) > 8.24, p < .01) \) and there was no further reduction in latencies on Test 3 compared to Test 2, \( F(1, 28) < 1.01 \).
Experiment 3b

Acclimation latencies (mean + S.E.) in groups Saline-Shock, 0.15 mg/kg - Shock, 0.40 mg/kg -Shock, Saline-No Shock, 0.40 mg/kg-No Shock were 1.89 (+.10), 1.85 (+ .09), 1.93 (+ .10), 1.90 (+.09), and 1.90 (+.10), respectively, on Acclimation Day 1; and were 1.50 (+.10), 1.54 (+.09), 1.67 (+.09), 1.61 (+.08), and 1.38 (+.09), respectively, on Acclimation Day 2. None of the groups differed in lick latency on either preconditioning acclimation day (Fs < 1.52). A 5 (Group) X 3 (Test Day) mixed ANOVA was subsequently conducted on test data. The between-subjects factor was Group (Saline-Shock, 0.15 mg/kg-Shock, 0.40 mg/kg-Shock, Saline-No Shock, 0.40 mg/kg-No Shock) and the within-subjects factor was Test Day (Test 1, Test 2, Test 3). The analysis revealed significant main effects of Group (F(4, 42) = 19.00, p < .001) and Test Day (F (2, 42) = 90.53, p < .001) as well as a significant Group X Test Day interaction, (F (8,42) = 6.01, p < .001). As can be seen in Figure 7, shock exposed groups supported greater suppression to the context compared to no-shock groups.

Planned comparisons revealed that Test 1 latencies in Groups Saline-Shock and 0.40 mg/kg -Shock were significantly higher than in corresponding no-shock groups, (Fs (1, 17) = 43.66 and 38.43, respectively, ps < .001) indicating shock treatment was effective at establishing context conditioning. Test 1 latency in Group 0.15 mg/kg was compared to the mean of the two no-shock groups and this difference was also significant, (F (1, 19) = 46.00, p < .001). The two no-shock groups did not differ (F (1, 19) < 1). Of critical interest, Figure 7 also
suggests that levels of context learning assessed on Test 1 were the same in groups exposed to different drug treatments during adulthood. Both shock groups receiving nicotine during adulthood (Group 0.15 mg/kg and Group 0.40 mg/kg) did not significantly differ from the Saline-Shock group, \( F(1, 16) < 1.00 \). Therefore, adult nicotine treatment did not affect later context fear learning.

Patterns of context suppression across subsequent extinction testing on Test 2 and Test 3 reveal that context fear was still present for all shock groups on Test 2 (see Figure 7). Planned comparisons revealed that Test 2 latencies in Groups Saline-Shock and 0.40 mg/kg-Shock were significantly higher than in corresponding no-shock groups, \( F(1, 19) = 6.22 \) and 6.20, respectively, \( ps < .05 \). After Test 2 all conditioned fear was extinguished; there were no significant differences between shock and no-shock groups and there were no significant differences between drug groups on Test 2 or Test 3.

Discussion

Experiment 1

When nicotine was administered using subcutaneous osmotic minipumps, adolescent nicotine exposure produced deficits in context conditioning, a form of learning dependent upon the hippocampus (Kim & Fanselow, 1992; Phillips & LeDoux, 1992). Rats exposed to subcutaneous osmotic pumps containing both moderate (3.0 mg/kg/day) and high (6.0 mg/kg/day) doses of nicotine for a two-week period during adolescence displayed evidence of impaired context learning when tested later as adults, compared to rats that were not exposed to nicotine.
None of the groups differed in lick latency during the preconditioning acclimation sessions. Furthermore, no-shock controls previously exposed to nicotine (6.0 mg/kg/day-No Shock) versus saline (Saline-No Shock) did not differ on any test day. The lack of group differences in lick latencies prior to shock exposure and between groups not exposed to shock suggest that the nicotine-related shorter lick latencies observed on the initial test day cannot be attributed to changes in locomotor activity or in motivation for water induced by adolescent nicotine administration. Because nicotine treatment ended 23 days prior to testing, the effects observed were related to either past nicotine exposure or past nicotine withdrawal, but not to direct nicotine withdrawal (Matta et al., 2007).

In addition, the Test 1 results cannot be explained by differences in pain sensitivity or anxiety. Tian and colleagues (2008) administered nicotine to adult Sprague-Dawley rats using repeated injections and then tested foot-shock sensitivity 14 days posttreatment. Animals were placed in chambers and received unsignaled footshocks of increasing amplitude. Monitors scored the animal’s response to each footshock and found that there were no significant differences between nicotine and saline treated animals on any responses to the footshocks (Tian et al., 2008). Smith et al. (2006) found that adolescent nicotine exposure decreases time spent in the center of an open field when tested during adulthood. This finding suggests that adolescent nicotine exposure may have increased anxiety. If increases in anxiety occurred in the present experiment, it would be expected that nicotine-exposed animals would demonstrate higher levels of
context conditioning. The opposite was observed in the present experiment; therefore, it seems unlikely that the nicotine-induced decrease in context conditioning is due to concomitant effects on anxiety.

Interestingly, results show that the animals exposed to the moderate dose of nicotine exhibited the poorest performance on context conditioning followed by animals exposed to the high dose of nicotine and control animals respectively. This finding may be due to a malfunction with the high dose osmotic minipumps. The amount of nicotine needed to be dissolved into saline in order to deliver 6.0 mg/kg/day is nearing the maximum amount of nicotine than can be dissolved in solution. Therefore, the solution was quite thick and may have crystallized during the nicotine administration, preventing all of the nicotine to be properly distributed. If in fact the high dose minipumps were functioning, another possible explanation for the inverted dose response involves the activation of various nAChR subunits. In adult animals, nicotine can enhance context conditioning, an effect that appears to be mediated through the α4β2 nicotinic receptor subtype in the hippocampus (Davis, Porter, & Gould, 2006). Thus, it may be that the α4β2 nicotinic receptor subtype is relatively more critical for context conditioning than other nAChR subtypes. It may be that the moderate dose of nicotine had the most direct effects on the nicotinic receptor subtype involved in context conditioning whereas the 6.0 mg/kg/day dose, nicotine may have had more potent effects at other receptor subtypes that attenuated the actions of nicotine at the α4β2 subtype.
Of the few studies that have examined the effects of adolescent nicotine exposure on hippocampus dependent memory tested in adulthood, Smith et al. (2006) did not find evidence of impaired context conditioning. There are many possibilities for the different results obtained in the present experiment and that of Smith et al. (2006). First, higher doses of nicotine were used in the current experiment (2.0 mg/kg/day was the highest dose in Smith et al., 2006). In addition, a lick suppression paradigm was employed in the current study compared to freezing behavior, which was used as the dependent measure in Smith et al. (2006) and there is evidence that different dependent measures vary in the sensitivity of fear to the effects of nicotine (see also Kenney & Gould, 2008a). Finally, as stated previously, Smith et al. (2006) presented a conditioned stimulus (tone) concurrently with the contextual cues when animals were exposed to shock. Animals had to attend to both the CS and the context which may have inhibited the amount of attention directed toward the context. The current experiment, which exposed the animal to the context only during shock training, may have been more sensitive to the effects of nicotine on context conditioning.

As stated previously, adolescent nicotine exposure significantly increases the upregulation of nAChRs, alters synaptic activity, and decreases cell packing density, cell number, and neuritic projections within the hippocampus and much of this damage is long lasting (Abreu-Villaca et al., 2003; Slotkin, 2002). Therefore, our findings indicate that adolescent nicotine exposure activates nicotinic receptors located on the hippocampus and generates cellular and
molecular processes that disrupt hippocampus-dependent learning in the long-term.

Nicotine administered using the osmotic minipumps during adolescence did not have a subsequent effect on adult extinction learning. There were no significant differences between nicotine and saline treated animals during Test 2 and Test 3, with all groups exhibiting extinction rapidly after the initial test day. The available literature suggests that previous nicotine exposure has a long-term adverse effect on the animal’s ability to extinguish a learned behavior (Smith et al., 2006; Tian et al., 2008). The null finding in the current study may be due to a methodological flaw. Each test session was 60 minutes, so the animal was exposed to a very long duration in which the CS (context of the chamber) was not paired with the US (footshock). Extinction learning occurred rapidly for all animals under these conditions and it is possible that the procedures were not sufficiently sensitive to detect nicotine-related effects on extinction. In order to further explore the long term effects that nicotine has on the mPFC, future studies should examine how nicotine affects extinction learning under conditions that produce slower extinction rates (e.g. shorter sessions), and other tasks which tax the mPFC more specifically (e.g. five-choice serial reaction time task).

**Experiment 2**

There were no significant differences between nicotine and saline treated animals on delay conditioning or latent inhibition. The lack of effects of nicotine exposure on delay conditioning was expected and provides further evidence that
nicotine-treated animals were not differentially sensitive to the shock (see also Carstens, Anderson, Simons, Carstens, & Jinks, 2001; Yang, Wu, & Zbuzek, 1992). Of the animals that were pre-exposed to the CS prior to testing, there were no significant differences between drug groups in latency to drink after the CS was presented. As stated previously, research regarding the effects of nicotine on latent inhibition has been mixed with some studies showing that nicotine enhances latent inhibition while others show that nicotine disrupts latent inhibition. Gould and colleagues (2001) note that the number of pre-exposures, nicotine dosage, and/or the species used as the experimental model may lead to these differences. In addition, these prior studies focused on the acute effects of the drug rather than the long term effects of chronic exposure. Although nicotine may impact latent inhibition in the short term, chronic exposure to nicotine may not have long term effects on tasks dependent upon dopaminergic transmission within the nucleus accumbens.

It should be noted that the animals previously exposed to the moderate dose of nicotine did show signs of impaired latent inhibition, although these differences were not significant. Future studies should adjust the number of pre-exposures to further explore the possible effect nicotine has on latent inhibition and also employ other learning paradigms that tax dopaminergic function within the nucleus accumbens (e.g. delayed reward reinforcement).
Experiment 3

When nicotine was administered using repeated IP injections we found that adolescent nicotine exposure actually enhanced context conditioning in adulthood and that adult nicotine exposure had no long term effects on context conditioning. Rats exposed to repeated injections containing the high (0.40 mg/kg) doses of nicotine for a two-week period during adolescence displayed evidence of enhanced context learning when tested later as adults. There were no significant differences between animals to the moderate dose of nicotine (0.15 mg/kg) and animals exposed to saline only. Similar to the findings using the osmotic minipumps, the lack of group differences in lick latencies prior to shock exposure and between groups not exposed to shock suggest that the nicotine-related longer lick latencies observed on the initial test day cannot be attributed to changes in locomotor activity or in motivation for water induced by adolescent nicotine administration. Identical to the results from Experiment 1, adolescent nicotine exposure via repeated IP injections did not differentially affect extinction learning.

As expected, adult exposure to nicotine had no long term effects on context conditioning, providing further evidence that the brain is particularly vulnerable to the effects of nicotine during adolescence. Adult exposure to nicotine also had no long term effect on this mPFC-dependent form of learning. Because the same behavioral testing paradigm was used for both experiments, our null finding may be due to the methodological flaws mentioned previously.
Stress, Nicotine and Context Conditioning. Adolescence is increasingly being viewed as a significant period of developmental vulnerabilities. In addition to being susceptible to the harmful effects of nicotine, the adolescent brain is sensitive to the negative effects of stress. The relationship between stress and nicotine may help to explain our contradictory findings. Although nicotine impaired context conditioning when administered via osmotic minipumps, nicotine enhanced context conditioning when administered via repeated injections. The injection process is stressful and nicotine injections to drug naïve rats have been shown to increase plasma levels of stress responsive hormones including corticosterone and norepinephrine (Benwell & Balfour, 1979). The stress induced by the injections may have modulated the effects of nicotine on the adolescent brain.

Romeo and McEwen (2006) examined how stress impacts the adolescent brain and found that this is a particularly vulnerable period because stress reactivity is heightened during pubertal development and because brain regions implicated in stress and emotionality are continuing to develop during this time (i.e. hippocampus, mPFC and amygdala). Studies comparing responsiveness to an acute stressor in adolescent and adult animals have demonstrated that basal and stress-induced adrenocorticotrophic hormone and corticosterone levels are similar; however, adolescent animals exhibit much more prolonged adrenocorticotrophic hormone and corticosterone levels in response to a stressor (Romeo & McEwen, 2006). Researchers also found substantial differences between adult and
adolescent animals when comparing responsiveness to chronic stress. In adult animals, repeated exposure to a stressor led to habituation of the stress response; peak stress hormone levels became blunted with each exposure. Adolescent animals showed less of a blunted response with repeated exposure to the stressor but exhibited a faster return to baseline (Romeo & McEwen, 2006). In addition, both acute and chronic stress led to significantly larger activation of corticotrophin-releasing hormone in adolescent animals when compared to adult animals (Romeo & McEwen, 2006). Finally, when administered equivalent doses of corticosterone, adolescent animals exhibited increased hippocampal NMDA receptors subunit expression to a greater degree than adult animals (Romeo & McEwen, 2006). The hippocampus, frontal cortex and amygdala are highly sensitive to corticosterone and are continuing to develop during adolescence.

As stated previously, the hippocampus is critically important in learning and memory, and necessary for context conditioning. This brain region continues to develop during adolescence and studies have shown that exposure to stress can disrupt this development. In adult male rats chronic restraint or social stress significantly reduced branching of the apical dendrites within the CA3 region of the hippocampus. Interestingly these effects of stress on hippocampal structure were reversible, and 10 days after the last stress session dendritic branching reverted to pre-stress levels. This stress induced dendritic atrophy adversely affects spatial cognition by impairing spatial memory when learning was assessed shortly after the animals experienced stress (Conrad et al., 1996). After stress
exposure, adolescent animals also exhibited volumetric deficits in CA1 and CA3 pyramidal cell layers as well as the dentate gyrus of the hippocampus. This reduction in hippocampal volume may have been due to stress blocking the normal maturational increase in hippocampus volume. Contrary to the adult response, the damaging effects of stress on the adolescent hippocampus were not observed until 3 weeks after the stress sessions were terminated (Romeo & McEwen, 2006). This finding indicates that the effects of chronic stress on the developing adolescent brain are delayed and long-lasting. Similar to the adult findings, the decrease in hippocampal volume was associated with deficits on the Morris water maze (Isgor et al., 2004). Therefore, the stress induced by the repeated injections given during adolescence may have caused long term damage to the hippocampus and deficits on hippocampus-dependent learning.

In the current study, control animals (exposed to saline only) that were exposed to the stress of repeated injections performed more poorly than control animals implanted with the osmotic minipumps, lending evidence to suggest that stress did lead to deficits in context conditioning. Although animals exposed to the high dose of nicotine (0.40 mg/kg) showed significantly stronger context conditioning compared to animals exposed to the moderate dose of nicotine and saline injections, the actual latency to drink exhibited by these animals was similar to that exhibited by the animals exposed to the high dose osmotic minipumps (see Figures 4 and 6). Therefore, animals exposed to the high dose nicotine injections still showed impaired learning when compared to the osmotic
minipump control animals. These results imply that the high dose of nicotine did not necessarily enhance context conditioning, but may have prevented stress-induced deficits on this hippocampus-dependent task. When ingested, nicotine leads to feelings of relaxation, calmness and alertness (Matta et al., 2004). The high dose of nicotine may have reduced the stress of receiving the injections, thus preventing stress-induced hippocampus damage and subsequent deficits on context conditioning. In addition, studies have shown that the stimulation of nicotinic cholinergic receptors in mature cells can actually decrease the cell death elicited by injurious treatments, potentially by the induction of neurotrophic factors (Slotkin, 2002). Therefore, nicotine may have had neuroprotective qualities in the stress-altered adolescent brain but neurotoxic effects on the intact adolescent brain.

**Summary**

Chronic exposure to nicotine does have a long term impact on context conditioning but not extinction learning, delay conditioning or latent inhibition. As expected, the adolescent brain was more vulnerable to the effects of nicotine exposure than the adult brain. Although adolescent nicotine exposure affected context conditioning when tested in adulthood, nicotine exposure during adulthood had no long term effects on context conditioning or extinction learning. Finally, the route of administration differentially impacts the effect nicotine has on later hippocampus-dependent learning. Although nicotine exposure via the osmotic minipump caused deficits in context conditioning, nicotine exposure via
repeated IP injection (high dose only) enhanced context conditioning when learning was assessed in adulthood.

**Future Directions and Implications**

Research shows that hippocampus-dependent tasks are particularly vulnerable to the effects of nicotine (Kenney & Gould, 2000a). When exposure occurs during adolescence nicotine impairs performance on hippocampus-dependent tasks when learning is assessed in adulthood. In the current experiments, context conditioning, a non-spatial task was used to assess adolescent nicotine’s effect on hippocampus-dependent learning. Future research should employ the same drug administration regimen and use other hippocampus-dependent tasks that do not involve fear learning in order to expand the generalizability of the current findings. Because the hippocampus is so integrally involved in spatial learning, it would be interesting to focus on tasks like the Morris water maze or the radial arm maze. In addition, future research should begin to examine how long adolescent nicotine’s effect on hippocampus-dependent learning persists. Animals could be exposed to nicotine during adolescence and then different groups could be tested on context conditioning at varying time points (e.g. PD 60, 75, 90 and 105). Finally, further experimentation is needed to understand the inverted dose response observed in Experiment 1. The same experiment could be replicated except a larger number of varying doses could be used (e.g. 1.0, 3.0, 5.0, and 6.0 mg/kg/day) in order to further understand how this may impact later context conditioning.
Experiment 3 was conducted in order to identify adolescence as a particularly vulnerable period of development to the effects of nicotine. As expected adult exposure to nicotine did not have any long term impact on context conditioning or extinction learning. However, in this experiment adolescent nicotine exposure did not cause deficits in context conditioning compared to the saline control group. Future studies should administer nicotine to adult animals using the subcutaneous osmotic minipumps to ensure that the deficits we observed in Experiment 1 were specific to adolescent exposure. The subcutaneous osmotic minipumps used in the current experiments were not large enough to provide a steady state of the 6.0 mg/kg/day dose of nicotine in the adult animals due to the increased body weight. Therefore, larger minipumps would need to be used in order to accomplish this or smaller doses would need to be administered to both adolescent and adult animals.

Finally, future research should further investigate the interaction between adolescent stress and nicotine exposure. The current experiment employed stress somewhat accidentally by using repeated IP injections and found that this method differentially affected context conditioning in adulthood. First, it should be firmly established that adolescent stress exposure leads to long term deficits on context conditioning and second, that nicotine can reduce these deficits. Researchers could then begin to understand at what point during stress exposure nicotine needs to be administered in order to reduce the effects of stress and what molecular processes take place for this to occur.
The results of our research are consistent with the view that there is a continuum of toxicity for nicotine, and that adolescence is a period of development that is still vulnerable to the neurotoxic effects of nicotine. Although adolescent nicotine exposure impacted adult context conditioning, adult nicotine exposure had no long term effect on context conditioning. Although it is difficult to directly apply the findings from studies with animal subjects to human behavior, recent research examining the effects of tobacco use on learning in humans has shown a similar pattern. When comparing adult never-smokers to adult smokers and ex-smokers (both with an average onset of smoking at approximately age 15), never-smokers performed significantly better on cognitive assessments (differences between these groups could not be attributed to age, gender, socioeconomic status, IQ level, or severity of psychopathology; Ernst, et al., 2001). Similarly, Jacobsen et al. (2005) found that the age of smoking onset was significantly positively related to working memory performance accuracy, those who began smoking later in life showed less impairment than those who began smoking earlier. Nearly 3,000 children under the age of 18 begin smoking everyday in the United States and if nicotine produces long term deficits in hippocampus functioning, these teenagers may exhibit cognitive deficits, such as impaired spatial memory and navigation, when they reach adulthood.
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Figure Captions

Figure 1. Timeline depicting procedure for Experiment 1. Animals were exposed to nicotine via subcutaneous osmotic minipumps from PD 28-42 and then were tested on context conditioning and extinction learning from PD 65-70.

Figure 2. Timeline depicting procedure for Experiment 2. Animals were exposed to nicotine via subcutaneous osmotic minipumps from PD 28-42 and then were tested on latent inhibition, delay conditioning or unpaired conditioning from PD 60-68.

Figure 3. The first timeline depicts the procedure for Experiment 3a and the second depicts the procedure for Experiment 3b. In Experiment 3a animals were exposed to nicotine via repeated IP injections administered every other day from PD 28-42 and then were tested on context conditioning and extinction learning from PD 60-65. In Experiment 3b animals were exposed to nicotine via repeated IP injections administered every other day from PD 90-104 and then were tested on context conditioning and extinction learning from PD 122-127.

Figure 4. Mean latency (log s) to complete five cumulative seconds of drinking in the presence of context cues on each of three consecutive test days when subjects were tested as adults (PD 68-70). Lower values reflect comparatively weaker context conditioning. Shock groups received 10 unsignaled shocks on the
conditioning day and No Shock groups received context exposure in the absence of shock. The groups further differed in whether they had received saline or nicotine (3.0 mg/kg/day, or 6.0 mg/kg/day) during adolescence (PD 28-42).

*Figure 5.* Mean latency (log s) to complete five cumulative seconds of drinking in the presence of the conditioned stimulus, a tone, when subjects were tested as adults (PD 69). Lower values reflect comparatively weaker context conditioning. Animals previously exposed to the CS prior to delay condition exhibited significantly shorter latency compared to animals that were not to the CS (Delay Conditioning group). There were no significant differences between drug groups on delay conditioning, latent inhibition or unpaired conditioning.

*Figure 6.* Mean latency (log s) to complete five cumulative seconds of drinking in the presence of context cues on each of three consecutive test days when subjects were tested as adults (PD 68-70). Lower values reflect comparatively weaker context conditioning. Shock groups received 10 unsignaled shocks on the conditioning day and No Shock groups received context exposure in the absence of shock. The groups further differed in whether they had received saline or nicotine (0.15 mg/kg, or 0.40 mg/kg) during adolescence (PD 28-42).

*Figure 7.* Mean latency (log s) to complete five cumulative seconds of drinking in the presence of context cues on each of three consecutive test days when subjects were tested as adults (PD 125-127). Lower values reflect comparatively
weaker context conditioning. Shock groups received 10 unsignaled shocks on the conditioning day and No Shock groups received context exposure in the absence of shock. The groups did not differ in whether they had received saline or nicotine (0.15 mg/kg, or 0.40 mg/kg) in earlier adulthood.
Figure 1

Minipumps Implanted
Minipumps Removed
Water Deprivation
Acclimation
Shock Training
Test Days

PD 28 42 60-64 65-66 67 68-70
Figure 2

- Minipumps Implanted
- Minipumps Removed
- Water Deprivation
- Acclimation
- CS Pre-Exposure
- Shock Training
- Recovery Sessions
- Test

PD 28 42 53-59 60 61-64 65 66-67 68
Figure 3

- IP Injections
- Water Deprivation
- Shock Training
- Acclimation
- Test

PD 28-42
PD 90-104

55-59 60-61 62 63-65
117-121 122-123 124 125-127
Figure 4

- SAL/NS
- 6.0/NS
- SAL/SH
- 3.0/SH
- 6.0/SH

Mean Latency (log s)

Test 1  Test 2  Test 3
Figure 5

- **SAL**
- 3.0 mg/kg/day
- 6.0 mg/kg/day

Mean Latency (log s)

- No Pre Exposure (Delay)
- CS Pre Exposure
- Unpaired Control
Figure 7

- SAL/NS
- 0.40/NS
- SAL/SH
- 0.15/SH
- 0.40/SH

Mean Latency (log s)

Test 1  Test 2  Test 3