EFFECT OF STRESS ON NICOTINE SELF-ADMINISTRATION IN
ADOLESCENT AND ADULT RATS

by

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A thesis submitted in conformity with the requirements for the degree of Master of Science
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Abstract

Initiation of smoking mainly occurs during adolescence. Adolescents experience more stressful life events; therefore, stress may be a factor that contributes to this high risk of smoking initiation. The current study examines the effects of three different stressors (yohimbine, intermittent footshock and social defeat) on nicotine self-administration (NSA) in adolescent and adult rats. The effects of yohimbine and footshock were examined after the establishment of NSA behavior, while the effect of social defeat was tested on the initiation of NSA behavior. Yohimbine increased NSA, but the other two stressors did not. The increase in NSA induced by yohimbine tended to be higher in adults than in adolescents. No marked age differences in response to the other two stressors were observed. These results suggest that stress increases NSA in a stressor-specific manner, and that adolescents do not show enhanced vulnerability to the effect of stress on NSA.
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CHAPTER 1: INTRODUCTION

1.1 A High Smoking Initiation Rate during Adolescence

Smoking causes a substantial health and economic burden on society, leading to hundreds of thousands of premature deaths and billions of dollars loss each year (CDC 2008). Vast numbers of people smoke and suffer from its consequences. By 2003, there was an estimated 1.3 billion daily smokers worldwide (Guindon 2003). Smoking leads to the development of many diseases, such as cancer, cardiovascular disease, respiratory disease and negative reproductive outcomes (Wipfli and Samet 2009). In 2002, the number of premature deaths caused by smoking was estimated to be over 6 million annually (Mathers and Loncar 2006). Nicotine, a component of tobacco, is thought to be the major factor that is responsible for addiction to smoking (Stolerman 1991; Stolerman and Jarvis 1995).

Epidemiological data indicate that initiation of smoking primarily occurs during the adolescent period, whereas it rarely happens in adulthood (DHHS 1989). In Canada, 90% of the smokers aged 25 and over consumed their first cigarette before the end of their teens (Health Canada 2003). Similarly, the average age of first cigarette use was 16.9 years among recent smoking initiates between age 12 to 49 in the United States, according to a survey in 2007 (SAMHSA 2008). In addition to this higher rate of smoking initiation, adolescents are also particularly vulnerable to becoming dependent on nicotine. It has been shown that although they smoked significantly fewer cigarettes, adolescents experienced higher levels of nicotine dependence compared to adults (Kandel and Chen 2000). Early onset of smoking is also associated with other long-term consequences, such as greater daily cigarette consumption, lower
probability of quitting and a higher risk of relapse later in life (Breslau et al. 1993; Breslau and Peterson 1996; Chen and Millar 1998; Cui et al. 2006). Furthermore, cigarettes may serve as the “gateway drug” that facilitates the progression into use of other drugs of abuse (Kandel et al. 1992). Therefore, it appears that compared to other age groups, adolescents have an elevated risk of initiating smoking and the negative long term consequences bundled with it.

1.2 Behavioral and Neurobiological Changes during Adolescence

Adolescence is a transition period from childhood to adulthood. However, the development that takes place during adolescence is more than just increased physical growth. In the following section, I will describe the behavioral and neurobiological changes during adolescent period in human. Parallel discussions concerning the changes during this developmental period in rodents will also be presented, as rats were used to investigate the relationship between adolescence and nicotine intake in the present work.

1.2.1 Human

In humans, the adolescent period is typically considered to be from age 12 to 18 years (Spear 2000), but it is not uncommon to consider adolescence to extend from puberty to the early 20s, when adult independence occurs (Arnett 1992). It should also be noted that the boundaries of adolescence depend on gender, with females generally maturing more rapidly than males across difference mammalian species (Savin-Williams 1989).
Behavioral Characteristics

Human adolescents show increased risk-taking behaviors (Deakin et al. 2004; Spear 2000; Trimpop et al. 1998). Adolescents are commonly described as “reckless”. Indeed, human adolescents exhibit more problem behaviors compared to other age groups, including disobeying parents, school misconduct, substance use, theft and fighting (Maggs 1995). With these characteristics, adolescents as a group bear more negative consequences, which is reflected by an increased mortality from early to late adolescence (Irwin 1993; Irwin 1992). Other negative outcomes include drug use, unwanted pregnancy, or injury (see Arnett 1992). However, compared to other age periods, adolescence may represent an “optimal age-span” for an individual to gain rational decision making skills by experimenting with risk-taking behaviors and obtaining social experiences early in life (Gardner 1993). Therefore, risk-taking behaviors may be associated with some potential benefits in this age group.

A term closely linked to risk-taking and reckless behavior is sensation seeking. By definition, sensation seeking is characterized by “the need for varied, novel, and complex sensations and experiences, and the willingness to take physical and social risks for the sake of such experiences” (see Zuckerman 1979 p.10). Such desire for novel experiences is much higher in adolescents compared to adults (Arnett 1994; Zuckerman et al. 1978). It was speculated that sensation seeking is achieved by risk-taking behaviors; conversely, it could be a predisposition for the development of risk-taking behaviors (Arnett 1996). Regardless of the causal relationship between sensation seeking and risk-taking, the desire for novelty is extremely important in the initiation of drug use during adolescence, because the most commonly reported reason for initiating drug use is to satisfy curiosity or to experience something new or different (Zuckerman 1992). Nevertheless, while some individuals engage in risk taking activities to seek the positive
arousal effect, some adolescents use it as a means to reduce depressive feelings and/or cope with stress (Irwin 1992).

Adolescents also show elevated impulsivity compared to other age groups (Chambers and Potenza 2003; Chambers et al. 2003; Romer 2010). For example, problem gambling, which is mediated in part by impaired impulse control, has a high prevalence during adolescence and in early adulthood (Derevensky and Gupta 2000; Gupta and Derevensky 2000; Shaffer and Bethune 2000; Shaffer et al. 1999). In clinical psychology, impulsivity is often measured by the use of self-report questionnaires; however, no single definition of impulsivity exists. Generally, it can be described as a “goal-directed behavior characterized by poor judgment in the attainment of rewards” (Evenden 1999). More specifically, some common characteristics of impulsivity include decreased inhibitory control, intolerance of delay to reward, quick decision-making due to lack of consideration, and poor attentional ability (Winstanley et al. 2006). Impulsivity during adolescence is closely linked to risk taking behaviors (Arnett 1992), both of which could promote the initial experimentation with illicit substances. Moreover, some investigators have suggested that the neural substrates for decision making may also participate in the control of drug use (Crews and Boettiger 2009; Dalley et al. 2008), and the neurochemical changes during adolescence have been suggested to resemble a pathological state of poor decision making, rendering adolescents more vulnerable to drug abuse (Chambers and Potenza 2003; Chambers et al. 2003).

Other than elevated risk taking and impulsive behaviors, intimacy with family members is replaced by more social interactions with peers during adolescence, and interactions with friends are perceived as more rewarding in adolescence than in childhood (Larson and Richards 1991). Changes in the proportion of time spent with family and friends and conformity with peers and
parents occurs during adolescence. While conformity with parents decreases with age, conformity with peers peaks at middle adolescence, suggesting peer interaction helps adolescents acquire independence from the home environment. It may also facilitate the engagement of antisocial behaviors (Berndt 1979). Cognitive functions such as problem solving and decision making are immature and continue to develop throughout the adolescent period (Byrnes 2002; Happaney et al. 2004), serving as another explanation for the reckless behavior during adolescence (Arnett 1992).

**Neurochemical Changes**

Massive neuroanatomical development occurs during adolescence. It is speculated that these ontogenetic changes not only underlie the distinct personality traits that are seen during this phase of life, but also allows youngsters to adapt better to the changes in the environment during this transition period (see Spear 2000 for review). Due to ethical reasons, most of our understanding about the adolescent brain comes from animal experiments. On the other hand, studies on the human adolescent brain mainly rely on magnetic resonance imaging (MRI) techniques, which measure activity in different brain structures (Casey et al. 2008).

Globally, the volume of neuronal grey matter (GM) in each lobe follows an inverted U-shaped trajectory, showing a pre-pubertal increase followed by a post-pubertal loss (Giedd 2004; Giedd et al. 1999), which is widely assumed to be a result from synaptic pruning during late adolescence and early adulthood (Paus 2005). Furthermore, GM volume in each lobe peaks at different times, with females preceding males (Giedd et al. 1999). In the frontal cortex, GM volume peaks around age 11 in both genders (Giedd et al. 1999).
White matter (Millan et al. 2000), which consists of axons that connect neurons, enables the smooth-flow of electrical signals from neuron to neuron. Unlike GM, WM growth is roughly linear during adolescence (Giedd 2004), and continues into the 4th decade of life, with a peak around mid-forty (Bartzokis et al. 2008). By using diffusion tensor imaging (DTI), it is found that the microstructure of WM matures throughout adolescence as well (Paus 2009).

Among all the cortical regions, the prefrontal cortex has received the most attention, due to its significant development during adolescence and it association with cognitive function (Steinberg 2009). Using functional MRI (fMRI), Rubia and her group demonstrated that the maturation of the prefrontal region correlates linearly with age, as cognitive control functions develop from childhood into adulthood (Rubia et al. 2000; Rubia et al. 2006). However, this linear frontal cortex development may be inconsistent with behavioral changes (Casey et al. 2008), with adolescents displaying more reckless and risk-taking activities than both children and adults (Arnett 1992; Arnett 1996).

Subcortical regions also mature during adolescence (Giedd 2004; Giedd et al. 1999; Schmithorst and Yuan 2009). The nucleus accumbens (NAcc), part of basal ganglia involved in reward-seeking, shows greater activity in adolescents compared to adults (Ernst et al. 2005). It appears that during adolescence, the maturation of limbic subcortex exceeds that of the prefrontal cortex, a region that is still gradually remodeling (Casey et al. 2008). Therefore, some investigators propose that adolescents show greater reward-seeking and poor decision-making behavior because of this non-parallel maturational trajectory in different brain regions (Casey et al. 2008; Steinberg 2009).
1.2.2 Rodents

The adolescent period is not unique to humans. Indeed, many mammalian species experience similar growth spurts and transitions in their behavioral repertoires during this period (see Spear 2000). For this reason, experiments investigating various aspects of adolescence, which could not be conducted on humans due to ethical reasons, can be done on laboratory animals. Adolescent rodents are one of the most widely used animal models of human adolescents, because they share similar behavioral and neurological characteristics. A more detailed discussion about the rodent adolescent period is given below.

In rodents, the term “periadolescent” is defined from “7-10 days prior to the onset of puberty at about 40 days postnatal (PD), to the first few days thereafter” (Spear and Brake 1983). However, to catch the full scope of the ontogenetic changes occurring at this stage, adolescence in rodents is considered to be the period from PD21 to PD60. More specifically, rodent adolescence is segmented into three age-intervals: early-adolescence (PD 21- to-34); middle adolescence (PD34- to-46); and late adolescence (PD46-to-59) (Laviola et al. 2003). The classification of these intervals is made according to the distinct behavioral and psychopharmacological characteristics at each stage (Laviola et al. 2003; Spear and Brake 1983).

It is not uncommon to find that the term “juvenile” used to describe young animals after weaning. The juvenile period usually refers to the age span from weaning to puberty (Pereira 1993), which partially overlaps with the early adolescent period. Again, while the boundaries of the juvenile period are defined by sexual maturity and reproductive ability (Crockett and Pope 1993), adolescence describes a transitional period from weaning to adulthood, encompassing physiological, neurobehavioral and hormonal changes during this period. In addition, the term
juvenile is more often used to describe non-human primates (see Spear 2000); thus, in the following discussion which focuses on rodents, the term “adolescent” will be used exclusively.

**Behavioral Characteristics**

Adolescent rodents display similar social behavioral characteristics as humans, such as novelty seeking and risk-taking. For instance, after spending equal time in the familiar compartment, adolescent mice (P33-43) showed a more marked preference for a novel environment compared to their adult counterparts once they were allowed to explore a new compartment on the test day (Adriani et al. 1998). Further, the same group of investigators also found that adolescent male mice (P48) spent equal amounts of time in the open and closed arm of the plus-maze apparatus, while younger (P35) and older (P61) subjects preferred to stay in the closed and protected arm (Macrì et al. 2002). Because staying in the open and unprotected arm produces fear and anxiety, a natural aversion to the open arm is often reported in rodents (Cruz et al. 1994; Holmes and Rodgers 1999). Thus, the authors interpreted this elevated exploration activity in the open arm as a sign of risk-taking behavior in adolescent mice (Laviola et al. 2003). This universal risk-taking and novelty seeking behavior in mammalian adolescents may have evolutionary significance. Since adolescence is the age for young animals to leave home and acquire new food sources, an eagerness for the wide world and a fearless attitude may help them gain necessary skills in obtaining independence from parents and establish mature roles. In addition, leaving home is also an efficient way to avoid inbreeding, as adolescence is also marked by sexual maturity (see Spear 2000).

It was also found that adolescent mice are also more impulsive than adults (Laviola et al. 2003). Impulsivity was measured using the intolerance-to-delay paradigm (Evenden and Ryan 1996). Briefly, operant chambers with two nose-poking holes were provided: nose-poking in one
hole would result in the delivery of a small amount of food immediately; nose-poking in the other hole would lead to the delivery of a larger amount of food, but with some delay. After a few days of testing, adolescents displayed a preference for the small and immediate reward, which is seen as a manifestation of elevated impulsivity (Adriani and Laviola 2003). It is worthwhile to mention that during the test, adolescents also exhibited elevated nonreinforced responses, nose-poking during the delay where responses were not reinforced, which showed that they were less able to control their behavior compared to adults (Adriani and Laviola 2003).

Social play behavior in rats also peaks during adolescence (Panksepp 1981). The targets of this play behavior can be the same sex, opposite sex and mother rats, and it involves “running, jumping, climbing, fierce sham fights (no anger), with biting, clawing, and pummeling, running over the mother and biting her ears, digging in corners, gnawing at the cage, sex-motions, picking, licking and fondling each other” (Small 1899). Among the male adolescents, play “fighting” behavior between same sex individuals dominates during this period (Meaney and Stewart 1981); its development follows an U-shaped function: increasing from PD18-28, peaking between 32 to 40 days of age, and gradually declining thereafter (Panksepp 1981). It should be noted that this kind of play fighting behavior is assertive, but never truly aggressive (Panksepp 1981); this “friendly” form of social interaction can be considered as practice for the adult sexual and aggressive behavior without bearing its harmful consequences (Thor and Holloway 1984).

Finally, cognitive function is still maturing during adolescence. For instance, adolescent rats perform better in simple active-avoidance tasks than other age groups (Bauer 1980; Myslivecek and Hassmannova 1979), because of their high level of spontaneous activity; on the other hand, they have difficulty learning complex goal-orientated tasks (Niemi and Thompson 1980), which
could be explained by insufficient attention paid to salient stimuli or insensitivity to reward contingencies (Spear and Brake 1983).

**Neurochemical Changes**

Neuroanatomical changes similar to those in human adolescents occur in young rodents as well. In early puberty, there is a marked overproduction of axons and synapses, followed by rapid pruning in the prefrontal cortex and other cortical regions during later adolescence (Huttenlocher 1979; van Eden et al. 1990). In contrast to human studies, where only data from gross examination of regional development are available, there has been extensive investigation concerning the developmental changes of various neurotransmitter systems in rodents.

The mesolimbic dopamine (DA) projects from the midbrain ventral tegmental area (VTA) to limbic structures including the nucleus accumbens, amygdala, and hippocampus, as well as the medial prefrontal cortex (Dahlstrom and Fuxe 1964; Wahlstrom et al. 2009). It plays important roles in the control of reward-seeking and motivational behaviors (Robinson and Berridge 1993; Wise 2004; Wise and Rompre 1989). Development of the dopaminergic innervations in the prefrontal cortex continues into early adulthood (Kalsbeek et al. 1988), while the basal dopamine synthesis in this area peaks at PD30 in rats (Andersen et al. 1997). In contrast, during adolescence, basal level of DA synthesis increases markedly with age in the corpus striatum and nucleus accumbens, such that the basal level of DA at PD40 is higher than that of PD30 (Andersen et al. 1997). Dopamine receptor expression also changes throughout adolescence. D1 and D2 receptor density in the striatum peaks at approximately PD40 and decreases by 58-57% by PD120 (Gelbard et al. 1989; Teicher et al. 1995). Similar patterns of overproduction and elimination of D1 and D2 receptors are also observed in the prefrontal cortex, except that later elimination is more extensive and protracted in this area (Andersen et al. 2000). In the nucleus
accumbens, D1 and D2 receptor density peaks at PD40 as well; however, it fails to show pruning afterwards (Teicher et al. 1995). D3 receptor expression is elevated through adolescence, but does not reach maximal levels until adulthood (PD60) in the striatum, nucleus accumbens and olfactory tubercle (Stanwood et al. 1997). Dopamine transporters (DAT), which mediate reuptake of dopamine into presynaptic terminals, reach adult levels at P35 and continue to increase steadily until PD60 in the caudate-putamen and nucleus accumbens (Tarazi et al. 1998). Finally, the sensitivity of dopamine autoreceptors, presynaptic receptors that regulate dopamine synthesis, drops significantly during adolescence, e.g. activation of D2 autoreceptors by 7-OH-DPAT prominently decreased dopamine synthesis during P10-30, but was ineffective at P40 in the prefrontal cortex (Andersen et al. 1997). Similarly, in striatum and nucleus accumbens, there is also a decline of the inhibitory effects of dopamine autoreceptors during adolescence (Andersen et al. 1997). It is therefore evident that the mesolimbic dopamine system undergoes extensive reorganization during adolescence, and its function seems to peak during this stage. However, to estimate the impact of these neurobiological changes on reward-related behaviors, one has to look at changes that occur in other neurotransmitter systems as well.

The acetylcholinergic (Ach) system also demonstrates distinct age differences. The Ach system in the brain is extensively involved in and affected by nicotine addiction (see Slotkin 2002 for review). Overall, acetylcholine (Ach) levels in the whole brain increase steadily after gestation and reaches adult levels by the 4th week postpartum (Coyle and Yamamura 1976). In the striatum specifically, Ach levels begin to increase dramatically at PD7 and peaks at PD28. Cholinergic neurons in all forebrain regions experience progressive soma and proximal-dendrite hypertrophy, followed by shrinkage during adolescence, and reaches adult configurations by the 5th postnatal week (Gould et al. 1991). Nicotinic acetylcholine receptors (nAchRs) show ontogenetic difference as well. Using the autoradiographic methods, it was shown that adolescent
rats (PD42) had higher levels of α4β2 and β7 nAchRs compared to adults (PD83-103); however, the levels of α6 nAchRs in both ages were quite similar throughout the brain (Doura et al. 2008). Consistently, nicotine-stimulated striatal dopamine release was found to peak at PD30 in rats (Azam et al. 2007). This is probably associated with elevated levels of α4β2 nAchRs in both dopamine cell body and terminal regions in adolescent rats (Doura et al. 2008), because nicotine-induced dopamine release is mainly triggered by activation of presynaptic α4β2 nAchRs in vivo (Champtiaux et al. 2003; Marks et al. 1999). Using different radioactive reagents, other groups of investigators showed that nAchR levels in cerebral cortex and hippocampus was higher at PD45 compared to adult rats (Trauth et al. 1999). More recently, Yu and colleagues (2007) found that α3 and α4 nicotinic receptor levels in the cortex and hippocampus increased from PD7, reached a peak at PD21 and then declined to adult level at PD28 in mice. Overall, it appears that the level of certain nAchRs subtypes peaks during early adolescence, which maybe associates with elevated sensitivity to the rewarding effects of nicotine in adolescent rodents (Belluzzi et al. 2004; Vastola et al. 2002).

The activity of the serotonergic (5-HT) system is believed to decline during adolescence. A human postmortem study revealed that the most dramatic reduction of 5-HT1A receptors occurs during the adolescent period (Dillon et al. 1991). Another study indicated that CSF concentrations of DA and 5-HT metabolites declined to adult level around age 16, but the 5-HT metabolite, 5-hydroxyindoleacetic acid, decreased more dramatically compared to DA metabolites, indicating higher rate of DA to 5-HT turnover (Takeuchi et al. 2000). In rats, the 5-HT turnover in the anterior cingulate cortex (ACC) in adolescents has been estimated to be significantly lower than in either younger and older rats (Teicher and Andersen 1999). It was found that decreased level of brain 5-HT is associated with increased impulsive behavior in humans (Brown and Linnoila 1990; Nordin and Eklundh 1999; Virkkunen et al. 1994). In this
case, developmental changes in the 5-HT system may contribute to behaviors, such as impulsivity and sensation seeking, exhibited during adolescence (Chambers et al. 2003).

The primary excitatory neurotransmitter glutamate and inhibitory neurotransmitter γ-aminobutyric acid (GABA) systems also change during adolescence. For example, glutamate binding to NMDA receptors in the frontal cortex and subcortical regions peaks during early adolescence and declines thereafter, losing 1/3 of NMDA receptors by P60 (Insel et al. 1990). Changes in the glutamate system are believed to play a significant role in many adolescent behaviors. The glutamate projection from the prefrontal cortex to the nucleus accumbens is involved in modulating motivational drive and decision making. Therefore, a decline of glutamate activity may be associated with the increases in impulsivity and substance use during adolescence (see Chambers et al. 2003). GABA\(\alpha\) receptor subunits also show a similar pattern of development, such that the \(\alpha_1, \alpha_2, \alpha_3\) and \(\gamma_2\) subunits of GABA\(\alpha\) receptor show increases up until early adolescence (PD30) and declines thereafter and finally stabilizes in adulthood in the neocortex (Yu et al. 2006). Alpha 5 GABA receptor subunit has slightly different developmental trajectory. Its density in the neocortex reaches peak at PD10 and shows continuous reduction until 3 months of age (Yu et al. 2006).

1.2.3 Rodent models are appropriate tools for the study of human adolescence

The validity of an animal model is often assessed by using 3 criteria: face validity, construct validity, and predictive validity. In simple terms, face validity surveys how much a model resembles what it intended to measure. Construct validity asks whether the model reflects the underlying mechanism that leads to the clinical syndrome being modeled. Predictive validity, as
the name suggests, measures the accuracy that the model will forecast the future outcome (Spear
2000). However, due to the nature of the existing studies on adolescent addiction, the predictive
validity of the rodent model has been scarcely assessed and can only be speculated upon.
Therefore, this last criterion will be excluded from the following discussion.

To demonstrate the face validity of the rodent model, we should first reveal this model’s
“behavioral features that resemble those found in human adolescents” (Laviola et al. 2003). As
discussed above, rodent adolescents share with their human counterparts similar behavioral
characteristics that are remarkably pronounced during this critical period of time, including
increased risk-taking, novelty-seeking and impulsivity (Adriani et al. 1998; Chambers and
Potenza 2003; Deakin et al. 2004; Laviola et al. 2003; Macri et al. 2002). Humans with these
characteristics display a higher tendency to experiment with and initiate substance use (see
Chambers et al. 2003). Moreover, adolescent rodents show similar social interaction profiles
compared to human teenagers, with peer interaction replacing intimacy with family members
during this stage (Berndt 1979) (Thor and Holloway 1984). All these behavioral changes reflect
a universal function of adolescent period across different mammalian species; that is to allow
young individuals obtain independence from their parents and prepare them for their future
social roles in the community.

To address the construct validity, one needs to be specific about the clinical syndrome that
the model intended to simulate. In my study, rodents were used as a model for the development
of nicotine-taking behavior in adolescents. However, as the mechanism that leads to elevated risk
to nicotine use during adolescence is still unknown in humans, which is the reason for the current
experiments, it seems impossible to examine the construct validity of our rodent model.
Nevertheless, all effects of nicotine that are present in human subjects, such as anxiolytic,
anxiogenic, analgesic and most importantly its incentive effect, have been demonstrated in rodents (Caggiula et al. 1995; File et al. 1998; Koob and Le Moal 2006). The acetylcholine and mesolimbic dopamine system, which underlie the motivational effects of nicotine in human, are also activated by administration of nicotine and mediate its reinforcing effect in rodents (see review Benowitz 2008). Moreover, as discussed above, as in human adolescents, many brain systems, especially those involved in the effect of nicotine, are extensively changed during rodent adolescence. Therefore, if these neurochemical changes render human adolescents at high risk of smoking, there should be a high possibility that one would observe a similar trend in rodents. Even though it is hard to assess the construct validity at the present stage, similar mechanisms of action of nicotine, as well as similar neurochemical changes occurring in human and rodent adolescence, support rodents as an appropriate tool for studying nicotine-taking in human adolescents.

We understand that human adolescents face a dynamic environment that is far more complicated than that encountered by any other species, which makes the use of animal models limited. However, the face and construct validity of this rodent model of human adolescents are continuously being reassessed as more data are being generated. Indeed, numerous studies have already made progress on understanding the mechanism of drug-taking and seeking during adolescence by using this rodent model.
1.3 Nicotine

1.3.1 Pharmacokinetics

Absorption and Distribution

Nicotine, whose chemical name is S-3- (1-methyl-2-pyrrolidinyl) pyridine, has a molecular weight of 162.23 Da. It is naturally found in tobacco leaves (Soloway 1976), and 99.9% of nicotine exists as the levorotary (S)-isomer (Armstrong et al. 1998). Nicotine is weakly basic with a pKa of 8.0 (Fowler 1964), and absorption of nicotine across membranes depends on the environmental pH. Smoke from burning pipes, cigar and cigarettes is alkaline; therefore, most of the nicotine is in its unionized form (Sensabaugh and Cundiff 1967). Each cigarette contains approximately 10-20 mg of nicotine, 25% of which will be available in mainstream smoke (Benowitz 2008). Up to 90% of the nicotine in tobacco smoke will be readily absorbed through inhalation (Armitage et al. 1975), reaching the brain in less than 10s, almost as fast as via intravenous injection (Benowitz et al. 1988). This rapid absorption rate partially accounts for the high addictive property of nicotine, and it also allows the smokers to adjust their smoking behavior on a puff-by-puff basis (Benowitz 1990).

Nicotine is distributed extensively to body tissues with a volume of distribution of around 3l/kg (Hukkanen et al. 2005). Nicotine binds to liver and brain tissues with high affinity, the former moderately exceeding the latter (Grusz-Harday 1967). The plasma nicotine concentration ranges from 20-25ng/ml in cigarette smokers (Koob and Le Moal 2006).

Metabolism and Elimination

In humans, the half-life of nicotine after cigarette smoking or via intravenous injection is about 2 hours (Hukkanen et al. 2005) and it is extensively metabolized by the liver. A number of
enzymes contribute to the biotransformation of nicotine. About 70 to 80% of the nicotine is oxidized into cotinine by cytochrome P450s (CYP) and aldehyde oxidase (AOX1) (Brandange and Lindblom 1979; Messina et al. 1997). Cotinine is further metabolized into trans-3’-hydrozycotinine through oxidation by CYPs (Nakajima et al. 1996). Together, some of the nicotine and its metabolites are subject to glucuronidation by UDP-glucuronosyltransferases (UGTs) (Yamanaka et al. 2005b). Another major metabolite of nicotine is nicotine N’-oxide, which accounts for about 4–7% of the total nicotine absorbed. This process involves an enzyme called monooxygenase 3 (FMO3) (Cashman et al. 1992). Trivial amounts of nornicotine and norcotinine are also produced by CYPs through demethylation (Yamanaka et al. 2005a).

CYP2A6 is the human hepatic microsomal enzyme primarily responsible for C-oxidation of nicotine and cotinine (Raunio et al. 2001), and the CYP2A6 gene is highly polymorphic. The wild-type CYP2A6 gene is denoted CYP 2A6*1A, and at least 30 alternative CYP2A6 alleles (CYP2A6*2 to 31) and 2 duplications (CYP2A6*1X2A and 1X2B) have been described (see Mwenifumbo and Tyndale 2009 for review). Expression of CYP2A6 is quite liver-specific (Raunio et al. 2001), however, nicotine metabolism does happen in other organs as well. In the brain, to where nicotine binds with high affinity and exerts its psychopharmacological effects, CYP2B6, CYP2D6 and CYP2E1 are expressed (Gervot et al. 1999; Howard et al. 2003; Siegle et al. 2001). CYP2B6 and CYP2E1 are also found in human lung and other organs (Gervot et al. 1999; Godoy et al. 2002; Hukkanen et al. 2002). Even though these extra-hepatic nicotine metabolism pathways do not significantly affect total body clearance, their expression is of importance at the site where nicotine exerts its pharmacological effect and modifies behaviors.

Age affects the rate of nicotine metabolism, such that in the elderly, the total clearance rate is reduced by 23% compared to young adults (Molander et al. 2001). This reduction in metabolism
may be caused by decreased liver blood flow (Hukkanen et al. 2005). However, CYP2A6-mediated metabolism does not differ significantly between adults and older children (Krul and Hageman 1998). Furthermore, decreased nicotine metabolism rate is associated with reduced risk of becoming nicotine-dependent in both adults (O'Loughlin et al. 2004) and adolescents (Rubinstein et al. 2008).

The nicotine metabolism profile in rodents is distinctly different from that in humans. In mice, nicotine clearance is extremely rapid, with half-life averages at 6 to 9 min (Siu and Tyndale 2007), and oxidation of nicotine and it metabolites is mediated by CYP2A5. In rats, hepatic CYP2A enzymes do not metabolize nicotine (Hammond et al. 1991). Instead, rat CYP2B1 and CYP2B2 take over the job and transform nicotine to cotinine (Nakayama et al. 1993). Moreover, the primary metabolite of nicotine is not cotinine, but nicotine-$N'$-oxide, which means that N-oxidization pathway takes a major role in nicotine metabolism (Kyerematen et al. 1988b). The nicotine half-life in Sprague-Dawley rats is around 1.3 hours in male and 1.8 hours in female (Kyerematen et al. 1988a).

Finally, nicotine and cotinine are excreted by glomerular filtration and subject to tubular reabsorption; therefore, the renal clearance rate of nicotine and it metabolite depends on the urine pH.

1.3.2 Pharmacodynamics

In the brain, nicotine binds to nicotinic acetylcholine receptors (nAchRs), which are located throughout the central nervous system (Koob and Le Moal 2006). The nAchR has a pentameric structure (Numa et al. 1983). In mammals, 9 $\alpha$-subunits ($\alpha2$ to $\alpha10$) and 3 $\beta$-subunits ($\beta2$ to $\beta4$)
of nAchR have been found. The most abundant receptor subtypes in the brains of human are α4β2, α3β4, α7 (Benowitz 2008).

Like other drugs with addictive properties, nicotine likely exerts its psychopharmacological effect by activating the mesolimbic dopaminergic pathway (Corrigall et al. 1994; Corrigall et al. 1992). Nicotine increases dopamine levels in the ventral tegmental area (VTA), nucleus accumbens (Rice and Cragg 2004) and prefrontal cortex (Rao et al. 2003). Among these regions, the VTA may be the main site where nicotine exerts its positive reinforcing effects (Benowitz 2008; Koob and Le Moal 2006). In rats, nicotine self-administration is inhibited by introducing nicotinic acetylcholine receptor (nAchR) antagonists in the VTA, but it is not affected by antagonists in the nucleus accumbens (Corrigall et al. 1994). The predominant nAchR subtype expressed on VTA dopamine neurons are presynaptic heteromeric α4β2 receptors, which have high affinity for nicotine (Champtiaux et al. 2003; Marks et al. 1999). Mutant mice without β2 subunit do not show nicotine self-administration (Picciotto et al. 1998), while mice with artificially engineered α4 subunit demonstrate hypersensitivity to the rewarding effects of nicotine in place preference tests (Tapper et al. 2004).

Besides directly binding to nAchRs on dopamine cells and triggering DA release, nicotine also stimulates other neurotransmitter systems in the VTA, which indirectly modulate dopamine function. Nicotine activates glutamatergic transmission projecting from the prefrontal cortex to VTA (McGehee et al. 1995; Sesack and Pickel 1992), as well as the gamma-aminobutyric acid (GABA) afferents from nucleus accumbens to the VTA and the GABA interneurons within VTA (Kalivas et al. 1993; Walaas and Fonnum 1980). These two neurotransmitter systems have opposite effects on the activity of mesolimbic dopamine: glutamate increases the mesolimbic dopamine level (McGehee et al. 1995), while GABA inhibits dopamine activity (Dewey et al.
It was found that the nAchR expressed on GABA interneurons in VTA are of the $\alpha_4\beta_2$ subtype (Klink et al. 2001), whereas on the glutamatergic terminals, the $\alpha_7$ subunit predominates (Jones and Wonnacott 2004).

It was speculated that when nicotine is chronically administered, nAchRs are first activated, and then a portion of the receptors are deactivated through a process called desensitization, followed by an upregulation during withdrawal, which is represented by an increase in the number of receptors as a compensatory pathway for desensitization (Wonnacott 1990). This decline in the number of functioning $\alpha_4\beta_2$ receptors on VTA dopamine neurons is thought to be the mechanism for acute tolerance (Benowitz 2008), such that the smokers describe that the first cigarette of the day as the most pleasurable (Russell 1989). However, a staggered rate of nAchR desensitization on difference types of VTA neuron cells could help to explain the mechanism of nicotine dependence. The $\alpha_4\beta_2$ receptors on GABA-ergic neurons quickly desensitizes, while $\alpha_7$ subunits on glutamatergic terminals are slower to desensitize and induce a long-term potentiation of excitatory input into the dopamine neurons. Overall, the excitatory signal to the mesolimbic dopamine systems exceeds the inhibitory one, and therefore produces a powerful and prolonged dopamine activation (Mansvelder et al. 2002). However, as Ortells and Barrantes (2009) recently point out, desensitization might be the step that initiates the mechanism for nicotine dependence, but nAchR upregulation is the process that completes it. They propose that $\alpha_4\beta_2$ receptors are more sensitive to the effect of nicotine than $\alpha_7$, therefore upregulation on the inhibitory pathway following desensitization outdoes that on the excitatory input to mesolimbic dopamine system, which promotes more nicotine consumption to normalize dopamine levels in the mesolimbic region (Ortells and Barrantes 2009).
1.4 Animal Model of Drug Taking and Relapse

The term “dependence” has been defined by many health organizations and authorities. The World Health Organization (WHO) defines drug dependence as “a behavioral pattern in which the use of a given psychoactive drug is given a sharply higher propriety over other behaviors which once had a significantly higher value” (Benowitz 2008). Simply speaking, individuals who experience drug dependence lose control over their behaviors, which are instead dictated by their states of drug taking. More specifically, the Diagnostic and Statistical Manual of Mental Disorders version IV [DSM-IV] has given a definition of drug dependence as a diagnostic tool. According to DSM-IV, for a diagnosis of drug dependence, a patient must present three of the following seven characteristics:

1. Tolerance; 2. Withdrawal; 3. The substance is taken in larger amounts or over a longer period than was intended; 4. There is a persistent desire or unsuccessful efforts to cut down or control substance use; 5. A great deal of time is spent in activities necessary to obtain substance, use the substance, or recover from its effects; 6. Important social, occupational, or recreational activities are given up or reduced because of substance use; 7. The substance use is continued despite knowledge of having a persistent or recurrent physical or psychological problem that is likely to have been caused or exacerbated by the substance.

The time course for developing drug dependence varies with the types of substance used, interpersonal difference and environment. However, all dependence begins necessarily with experimentation with drug. Consequently, the positive rewarding/reinforcing effect of the substances leads to continuing drug use and maintenance of the drug-taking behavior. However, there is a distinct difference between limited and controlled recreational drug use
and uncontrolled drug abuse (Koob and Volkow 2010). Many people take drugs with addictive properties but never become dependent on them. For instance, 15.6% of the adult population in the United States used non-medical or illicit drugs at some point during their lifetime, however, only 2.9% of this population developed substance dependence (Grant and Dawson 1998; Grant et al. 2004). Nevertheless, the positive reinforcing effect is especially important in the early stage of drug dependence. Therefore, studying the reward/reinforcing property of drugs can yield insights in the development of drug dependence. The following sections will describe and evaluate a number of procedures used to measure the rewarding properties of drugs in laboratory animals.

1.4.1 Conditioned Place Preference (CPP)

In the previous discussion, reward and reinforcement have been used interchangeably. However, it is now important to define each term and point out their differences. A reinforcer increases the likelihood of the behavior that produces it, whereas reward usually refers to a stimulus of an appetitive nature (Mackintosh 1974). The conditioned place preference (CPP) procedure measures the rewarding properties rather than reinforcement of drug, with the assumption that animal will approach the environment associated with a rewarding effect (Bardo and Bevins 2000; Tzschentke 1998).

CPP utilizes principles of classical conditioning, a process in which the response normally elicited by one stimulus (termed the unconditioned stimulus US) comes to be controlled by another stimulus (termed the conditioned stimulus CS) that has been paired with US. During the CPP procedure, two compartments with distinct contextual cues (e.g. color, floor patter, odor etc)
are available. Pairing involves the animal receiving the stimulus of interest (US), such as a drug injection, in one compartment (CS); whereas in the other compartment, the animal receives a neutral stimulus (e.g. saline injection). Following several pairing sessions, the animal is given a free choice between the two environments in the absence of the US. An increase in time spent in the compartment paired with US is an indication that the stimulus of interest has a rewarding effect (Bardo and Bevins 2000). A variety of substances with incentive properties have been shown to produce CPP in animals. A list of them includes but is not restricted to food (Agmo et al. 1993; Spyraki et al. 1982a), water (Agmo et al. 1993), novel stimuli (Bevins and Bardo 1999), and drugs of abuse such as cocaine (Spyraki et al. 1982b), amphetamine (Spyraki et al. 1982c), morphine (Bardo et al. 1984), and nicotine (Shoaib et al. 1994).

Compared to other procedures that measure drug reward, CPP is a rapid and inexpensive method that requires little training and no surgery (Katz and Gormezano 1979). For example, a CPP effect can be observed following a single pairing trail with morphine or amphetamine (Bardo and Neisewander 1986; Bardo et al. 1999). Furthermore, CPP does not only measure the rewarding property, but it also has the ability to detect the aversive effect associated with drug withdrawal (Suzuki et al. 1996).

CPP is not without limitations. First of all, CPP is not sensitive to changes in drug dose, and only one data point can be generated using one subject, which means that a within-subject dose-response curve is difficult or impossible to obtain (Bardo and Bevins 2000). Secondly, animals often show a preference for one of the two environments prior to the start of the conditioning, which makes the choice of pairing of drug with either of the two environments problematic: pairing the drug with the preferred environment would reduce the power to detect potential rewarding property due to ceiling effect (Cunningham et al. 2003); on the other hand, if a CPP
occurs after pairing drug with the non-preferred environment, it would be hard to tell the result is due to a reduction in the aversion or a true reward effect of the drug (Tzschentke 1998). The fundamental drawback of CPP is that it involves passive administration of drug, whereas in the self-administration procedure (discussed in detail later), drug is actively obtained by the animals. In the natural setting, humans take drugs by voluntarily seeking and administering them. In addition, evidence points out that the neurochemical profile in the mesolimbic system after passive administration of a drug is different from that after self-administration, suggesting a difference in motivational states with different experimental protocols (Stefanski et al. 1999). Therefore, the CPP procedure has low face and construct validity; yet, it is a highly useful and effective tool to screen drugs for reward value.

1.4.2 Intravenous self-administration (SA) Procedure

The self-administration (SA) procedure is the gold standard for measuring the reinforcing effect of drugs. This procedure is based on the theory of operant conditioning, a form of learning where the frequency of the behavior is affected by its consequences (Bozarth 1990). The SA procedure takes place in operant boxes (or Skinner boxes), where animals can perform an action (nose poking or bar pressing) to obtain reinforcers. There are usually two bars (or nose-poking holes) in each operant box: the one that leads to the delivery of reinforcer is termed the active lever; the other one is termed the inactive lever, because it does not produce any consequences. The function of this inactive lever is to control for nonspecific responses, so a dramatic increase in the response on the active lever indicates a true rewarding effect of the drug (Gardner 2000). In addition, the delivery of each reinforcer is usually paired with cues (e.g. visual, auditory). This cue may become associated with availability of the reinforcer and develop conditioned
reinforcing property, which later on enables the study of the motivational effect of environmental context on drug-seeking (Stewart et al. 1984).

Unlike CPP, in intravenous SA procedures, the substance is actively administered and the intravenous route of administration has a fast onset of action (Benowitz et al. 1988); therefore, it has close correspondence with addiction behaviors in a natural environment, and therefore a high face validity (Griffiths and Balster 1979; Panlilio and Goldberg 2007). In addition, even though most of the drugs that produce CPP are also self-administered by rats, and vice versa, CPP and SA are not isomorphic (Bardo and Bevins 2000). Indeed, evidence indicates that CPP and SA measure the motivational effects of drug that are governed by distinct neuropharmacological mechanisms. For instance, in rats, the reinforcing effect of cocaine is diminished in self-administration with the presence of D2 antagonist (Corrigall and Coen 1991; Ettenberg et al. 1982; Phillips et al. 1994; Roberts and Vickers 1984), but CPP produced by cocaine is unaffected (Baker et al. 1996; Mackey and van der Kooy 1985; Spyraki et al. 1982b). Compared to CPP, self-administration is a more appropriate method for examining the initiation and maintenance of drug-taking behavior. However, it is relatively more expensive, and its utility is limited by the short duration of the patency of the intravenous catheter (Panlilio and Goldberg 2007).

In SA, there is a relationship between the quantity of responses required and its consequent reinforcer, which is termed the schedule of reinforcement. When the number of responses required to obtain a single reinforcement is constant through the period of one session, it is called a fixed-ratio (FR) schedule. Many stimulants and opioids have been shown to sustain a stable FR self-administration pattern (Dougherty and Pickens 1973; Pettit et al. 1984; Piazza et al. 1989). It was suggested that FR serves as a good method to detect the reinforcing property of drug;
however, it is far from a tool to measure the magnitude of the reinforcing effect and can be very ambiguous when the direction of change in the reinforcing effect are to be analyzed from the pattern of FR self-administration (Arnold and Roberts 1997). The classical view supports that when reinforcing efficacy decreases, animals increase the frequency of drug intake to compensate for the reduced appetitive effect (Yokel and Wise 1975; 1976). However, this interpretation is challenged by experiments showing that disruption of DA neurons by the neurotoxin 6-OHDA failed to increase cocaine self-administration frequency in rats (Roberts and Koob 1982; Roberts et al. 1980), since decreases in DA level decrease the reinforcing efficacy of cocaine.

In comparison, the progressive-ratio (PR) schedule is considered to be a better method of quantifying reinforcing efficacy (Arnold and Roberts 1997; Cohen et al. 1994). During a PR session, the number of responses required for the next reinforcer increases upon each successive delivery (Hodos 1961). The escalation of required responses usually follows an exponential formula, which means that the cost required to obtain the next reinforcement is much greater than the previous one. Therefore, in PR, animals are asked to demonstrate the magnitude of their willingness to obtain reinforcement. This motivation is reflected by a parameter called breakpoint, which is defined as the final ratio of responses successfully completed (Cohen et al. 1994; Depoortere et al. 1993; Hodos 1961). PR has been used to study the reinforcing properties of various drugs of abuse, such as cocaine, heroin and nicotine (Depoortere et al. 1993; Roberts and Bennett 1993; Shram et al. 2008a). Unlike in FR, in the PR schedule, increases and decreases in DA level lead to higher and lower breakpoint on cocaine taking, respectively (Richardson et al. 1994; Roberts et al. 1987). Therefore, the PR schedule is more sensitive to changes in reinforcing efficacy, and therefore has proved to be a reliable tool in assessing the magnitude of the motivational effect of drugs.
1.4.3 Reinstatement Procedure

Drug dependence is marked by repeated episodes of relapse, which is the re-initiation of drug taking after protracted periods of abstinence; indeed, relapse is thought to be the most difficult clinical problem in the treatment of drug addiction (Hunt et al. 1971; O'Brien 1997). Many theories have been proposed to explain the psychological process of relapse. One of these is the negative reinforcement theory, which suggests that the resumption of drug use is to alleviate the negative physical and emotional state elicited by drug withdrawal (Wise and Bozarth 1987). In comparison, another school of thought suggests that relapse is ultimately caused by sensitization to the incentive salience of drug, or simply termed as “wanting” drug (Robinson and Berridge 1993). Even though the mechanism of relapse remains a mystery, it is still important to study drug reinstatement using animal models because it may eventually help to develop a more effective treatment for drug dependence.

Several procedures have been used to study the reinstatement of drug-seeking. The following discussion will be focused on the reinstatement procedure that is based on the self-administration model (de Wit and Stewart 1981; 1983). Animals are first trained to self-administer drug for a period of the time. After the drug-taking behavior becomes stable, animals undergo extinction, where responses do not lead to reinforcement. This process leads the animals to extinguish their drug-taking behavior. Upon successive extinction, the resumption of drug taking behavior is tested by introducing animals to factors that are known to induce reinstatement.

This reinstatement procedure in rodents corresponds closely to relapse behavior seen in clinical trials, in which factors known to induce relapse in human, including re-exposure to drug (priming), cues previously associated with drug taking, and the presence of stress (Jaffe et al. 1989; Ludwig et al. 1974; Sinha 2001), cause a return to drug-seeking in animals whose drug-
seeking behavior had been extinguished. However, differences exist between reinstatement phenomena in rodents and human relapse. First of all, the motivational causes for human and animal abstinence/extinction are different (Shaham et al. 2003). Usually, humans abstain from drug to avoid negative consequences (e.g. health condition, or loss of job). However, experimental animals stop responding because of the unavailability of drugs. It is not clear how much this difference in motivational state affects the validity of the reinstatement model.

Secondly, throughout the extinction phase, animals perform the drug-seeking behavior without obtaining drug, which is rarely the case in abstinent humans. Finally, animals do not receive drug (drug free) during reinstatement (except in the case of priming), whereas in humans, relapse implies that the drug has been re-taken (Shaham et al. 2003). Therefore, it is arguable whether reinstatement procedures measure drug “craving” or relapse. However, the drug free state in the animals is sometime crucial to the interpretation of data because it avoids the potential confounding effects of drug. Overall, although suffering from some limitations in face and construct validity, the reinstatement procedure is an effective tool in studying the relapse process in humans.

Drug dependence is a complicated behavioral and neurological disease, and its underlying mechanisms remain unclear. So far, no experimental methods can model every aspect of dependence; the animal models introduced above each targets individual facets of drug abuse. When used in combination, however, they can provide more detailed insights into drug dependence.
1.5 Stress

We often describe stress as events that threaten our well-being. Stress refers to the process involving perception, appraisal, and response to aversive and threatening stimuli (Cohen et al. 1995). For the purpose of clarity, the aversive stimulus is termed stressor; the reaction to the stressor, e.g., behavioral and physiological changes, is termed response. Stressors can be categorized according to different criteria. Some commonly used types of stressors are physical versus psychological stressors. Examples of physical stressors include hunger and body injury, while social challenge and traumatic events are viewed as psychological stressors. The stress response prepares the organism to fight or escape from dangerous situations and thus is of great importance for the survival of the organism (Linsky et al. 1995). Sometimes, stress can generate positive and exciting feelings if perceived and managed properly (Levine 2005; McEwen 2007); but most often, when the nature of the stressor is chronic or exceeds one’s controllability, the stress response system would eventually fail after prolonged resistance, leaving organisms in devastating physiological and psychological conditions (Selye 1936; 1946). In the following section, the major neural and hormonal systems that are involved in stress perception and responses will be described.

1.5.1 Hypothalamic-pituitary-adrenal axis

A key element of the stress response lies in the hypothalamic-pituitary-adrenal axis. It elicits cascades of physiological actions, helping the organism to deal with real or potential threat and therefore increasing survival rate. Briefly, upon perceiving stress, the paraventricular nucleus (PVN) of the hypothalamus is activated, which then releases corticotrophin releasing hormone (CRH) into the portal blood vessels that connect to the anterior pituitary gland (Rivier and Vale
CRF binds to the CRF1 receptor on the pituitary and leads to the secretion of adrenocorticotropic hormone (ACTH) into the bloodstream. In the systemic circulation, ACTH meets the adrenal gland and stimulates secretion of glucocorticoids from the adrenal cortex (see Smith and Vale 2006). Glucocorticoids, steroid hormones, bind to the intracellular receptors located throughout the body, and consequently regulate metabolic, cardiovascular, immune, and behavioral processes (Charmandari et al. 2005; Munck et al. 1984; Sapolsky et al. 2000). This is not only important to prepare the body to cope with threatening situations, but also crucial for normal physical growth (Gaillard and Wehrenberg 1996).

Glucocorticoids also inhibit HPA activity through negative feedback, which is mediated by glucocorticoid receptors (GRs) expressed mainly in neurons of the hippocampus. The HPA axis is also subject to neuronal regulation. Brain stem catecholaminergic centers, cell groups of the lamina terminalis, and forebrain limbic structures all send inputs to the PVN (see Smith and Vale 2006). Among them, the limbic system has very few direct connections to the neurons in PVN. Instead, it regulates the HPA axis through intermediary neurons in the bed nucleus of stria terminalis (BNST), hypothalamus, and brainstem (Herman et al. 2004; Herman et al. 2005; Sawchenko et al. 1993). Different brain regions are activated and responsible for responses to different types of stressors. For example, the amygdala promotes the activation of the HPA stress system when the organism is exposed to either physical or psychological stressors (Herman et al. 2005). In contrast, hippocampus and prefrontal cortex have an inhibitory role on the HPA axis when challenged with psychological stressors (Pruessner et al. 2008; Wang et al. 2005). The brainstem, especially the noradrenergic neurons originating from there, elicits the stress response when the organism is exposed to physical stressors (Cunningham and Sawchenko 1988; Palkovits et al. 1999)
1.5.2 Extrahypothalamic CRF system

CRF neurons and receptors also exist outside of the HPA axis, namely the extrahypothalamic CRF system. CRF-like immunoreactivity has been shown to be present in the following areas, the neocortex, extended amygdala, medial septum, hypothalamus, thalamus, cerebellum, and ventral tegmental area (Charlton et al. 1987; Fischman and Moldow 1982; Swanson et al. 1983). Two components of the extended amygdala, the bed nucleus of the stria terminalis (BNST) and central nucleus of amygdala (Stewart et al. 1990) have received considerable attention due to their important role in general approach behaviors and fear and anxiety (Everitt et al. 1989; Kelley 1999; Lee and Davis 1997; Numan 1996). The reason that these two regions are grouped together is because they share similar morphological features and connectivity (Alheid and Heimer 1988). BNST and CeA receive input from lateral tegmental nuclei (LTN) through the ventral noradrenergic pathway (Moore and Bloom 1979), such that when rats experience footshock, the ventral pathway releases noradrenaline in the BNST, which subsequently triggers secretion of CRF (Erb et al. 2001a). Some evidence of this connection comes from Phelix and groups’ work (1992; 1994) showing direct synaptic interactions between noradrenaline axons and dendritic terminals of CRF-containing cells in the ventrolateral BNST, using combined light and electron microscopy.

Some neurons in BNST and CeA connect to each other intrinsically (Sakanaka et al. 1986), while other neurons projects to various hypothalamic and brain stem regions that are implicated in general approach behaviors (see Erb et al. 2001b for review). For instance, the lateral hypothalamus, which controls feeding behavior (Stanley et al. 1996), receives projections from dorsolateral and ventrolateral BNST (Glickman and Schiff 1967). The lateral division of BNST also projects to the midbrain central grey which may involved in aggression induced by stress.
The ventral BNST, however, has projections directly to the nucleus accumbens (NA) (Brog et al. 1993), which is the reward and motivation center as previously mentioned. The BNST also controls nucleus accumbens function by directly innervating the ventral tegmental area (VTA), which sends dopaminergic fibers to the accumbens. It is worthwhile mentioning that some of the intrinsic interconnections and extrinsic projections from the BNST and CeA are inhibitory in nature (Jia et al. 1997; Pickel et al. 1995; Sun and Cassell 1993; Sun et al. 1994), such that regions receiving input from BNST and CeA are under a constant inhibitory tone by GABA. Therefore, it is speculated that the function of CRF could be to remove the inhibitory effect of BNST on other brain areas after exposure to stress, and thus indirectly stimulate the appropriate behavioral response (Erb et al. 2001b).

1.6 Stress and Addiction

Stress increases vulnerability to addiction. Extensive clinical and epidemiological studies support the positive association between adverse life experiences with drug abuse problems (for review see Sinha 2001). For instance, Lloyd and colleagues report that in adolescents and young adults, the cumulative number of stressful events is a significant predictor of alcohol and drug-dependence in a dose-dependent manner (Lloyd and Turner 2003; 2008). Smoking behavior is also increased by stress (Colamussi et al. 2007; John et al. 2006; Pomerleau and Pomerleau 1991). Furthermore, many studies also associate mood and anxiety disorder, such as depression and post-traumatic stress disorder, with a high risk of drug addiction (King et al. 1996; Riggs et al. 1999). Many theories have been proposed to link stress with the high incidence of addiction seen in human. The popular self-medication hypotheses suggest that people use addictive drugs to alleviate the negative emotional state that is associated with chronic stress disorder (Khantzian
1985). Others have proposed that people take drugs to reduce distress as well as experience the increased positive affect (Shiffman 1982; Tomkins 1966), both of which increase the motivation to obtain drug. More recently, it has been hypothesized that stress changes the brain reward circuit based on the observation that stress can activate the mesolimbic DA system (see Piazza and Le Moal 1998), and therefore it enhances the sensitivity to the reinforcing effect of drugs of abuse (Koob and Le Moal 1997). So far, the exact mechanism of how stress increases the risk of drug dependence is still unknown; however, numerous animal studies have demonstrated that stress could facilitate the initiation of self-administration behavior of drugs, elevate the level of intake, as well as reinstate drug-seeking behavior after extinction.

1.6.1 Stress and initiation of drug self-administration

*Repeated physical stress and initiation of drug self-administration*

A stimulating effect of stress on drug self-administration was reported (Piazza and Le Moal 1998), however, studies on the effect of physical stressors on the acquisition phase of psychostimulant use have been scarce. For instance, existing data show that repeated footshock and tail-pinching facilitate initiation of cocaine (Goeders and Guerin 1994) and amphetamine (Piazza et al. 1990) self-administration, respectively. There are more studies concerning the effect of physical stressor on alcohol taking; however, reports of increases, decreases or no change in alcohol consumption exist in studies using intermittent footshock (Anisman and Waller 1974a; Cicero et al. 1968; Myers and Holman 1967), and restraint stress (Derr and Lindblad 1980; Krishnan et al. 1991; Nash and Maickel 1985; Rockman et al. 1986). Moreover,
none of the studies were conducted with the self-administration model. Therefore, the effect of physical stressors on the initiation of drug-taking is inconclusive.

*Social stress and initiation of drug self-administration*

Compared to physical stressors, the effect of social stress, such as social isolation or social defeat, has been more commonly studied on the initiation phase of drug self-administration. Social stress, compared to physical stress, may have more ethological relevance, as it is a stressor that animals would experience from their natural environment. Moreover, because they models the day-to-day stress encountered in life, studies with social stress may be more relevant to the studies of human addiction. For instance, most of the types of stress experienced by human adolescents are psychological, rather than physical in nature (Simmons et al. 1988; also see Spear 2000 for review).

In social animals such as rats, isolation is stressful (Brain and Benton 1979; Levine 1993). In most of the studies, social isolation is chronic in nature and was done by single-housing the animals from weaning and testing its effects during adulthood. Studies with social isolation and drug self-administration report variable results, and the direction of effect generally depends on the infusion dose of self-administered drug. It was first found that isolated rats reliably initiated cocaine taking when the dose is comparatively high (0.5 and 1.0 mg/kg/infusion) (Schenk et al. 1987). But later studies contradicted this finding. Howes and colleagues found that isolation housing facilitated acquisition of cocaine self-administration at the lowest dose (0.083mg/kg/infusion), but retarded acquisition at the highest (1.5 mg/kg/infusion) in comparison to group-housed rats (Howes et al. 2000). Consistent with this observation, social isolation also led to higher infusion rates during initiation of amphetamine self-administration when the dose was low (0.03mg/kg/infusion), but not when the dose was high
(0.1mg/kg/infusion). Taken together, social isolation appears to enhance the initiation of drug taking when the drug doses used are comparatively low.

Another social stressor that has been frequently examined on acquisition of drug self-administration is social defeat (SD). Compared to social isolation, social defeat, as a consequence of aggressive confrontations between two individuals, is a naturally occurring form of social stress across many species, including primates and rodents (Miczek et al. 2008). In addition, different from social isolation which extends from few weeks to few months, the exposure period for social defeat, as typically implemented is usually brief, ranging from 4 to 10 days. For example, 4 episodes of social defeat given over the course of a week increased infusion rates during initiation of cocaine self-administration in both male and female rats (Haney et al. 1995). Similarly, in rats that previously experienced 4 episodes of social defeat over 4 consecutive days, the time required to acquire cocaine self-administration was half of that used in non-defeated rats (Tidey and Miczek 1997). Therefore, the stimulating effect of social defeat on the initiation of cocaine self-administration is reliably demonstrated.

1.6.2 Stress on drug-taking after establishment of self-administration behavior

*Acute social stress on maintenance of drug self-administration*

Many experiments have looked at the acute effect of stress on the level of drug-taking after self-administration behavior was established. It is usually done by applying the stressor immediately prior to the start of the self-administration session. Because most studies have shown that multiple exposures to defeat are required for effects, social stress is not often used acutely. It was shown that acute exposure to defeat increased responding reinforced by cocaine
self-administration in rats (Miczek and Mutschler 1996), but consistently decreased alcohol self-administration (Funk et al. 2005; van Erp and Miczek 2001). Therefore, it appears that drug type is critical in determining the acute effect of social stress on drug-self-administration.

**Acute exposure to intermittent footshock on maintenance of drug self-administration**

The acute effects of footshock on alcohol self-administration have been little studied. More studies have used the two-choice paradigm, and shown the effect of footshock to be inconsistent (Ng Cheong Ton et al. 1983; Volpicelli et al. 1990). However, it is consistently reported that footshock increases alcohol consumption after an alcohol deprivation period (Funk et al. 2004; Matthews et al. 2008; Vengeliene et al. 2003). One experiment examining alcohol self-administration after acute exposure to footshock in mice showed that the effect of stress is dependent on the strain of the mice, and when footshock did increase alcohol intake, the magnitude is rather small (Matthews et al. 2008). The effect of footshock on opioid self-administration is more consistent. Shaham and colleagues have shown that acute footshock (0.8mA, 10min) increased oral fentanyl self-administration (Shaham et al. 1993). The same group also demonstrated that intermittent footshock stress (0.5mA, 10min) significantly increased heroin self-administration in rats; this effect was only observed under PR schedules, not low-ratio FR schedules (Shaham and Stewart 1994). With both drugs, however, the increases in the level of intake produced by acute exposure to footshock appeared to be moderate.

**Effect of yohimbine on maintenance of drug self-administration**

To our knowledge, yohimbine has been the only stressor that profoundly increases levels of drug taking during the maintenance phase of self-administration. Yohimbine, a pharmacological stressor (Bremner et al. 1996a; b), is an alpha-2 adrenoceptor antagonist that can increase
noradrenaline (NA) cell firing and noradrenaline release (Abercrombie et al. 1988; Aghajanian and VanderMaelen 1982). It has been shown to reliably and profoundly elevate alcohol self-administration on a FR3 schedule in rats (Le et al. 2009; Le et al. 2005; Marinelli et al. 2007). The ability of yohimbine to enhance alcohol taking, however, does not seem to be produced by its action on the NA system, because destruction of NA bundle by 6-OHDA or administration of alpha-2 adrenoceptor agonist did not reverse the effect of yohimbine on operant alcohol taking (Le et al. 2009). On the other hand, administration of CRF1 receptor antagonist, antalarmin, and 5-HT1A receptor antagonist, WAY100,635 significantly attenuated the increase in alcohol self-administration produced by yohimbine (Le et al. 2009; Marinelli et al. 2007), suggesting roles for CRF and 5-HT in the effects of yohimbine on alcohol self-administration.

**Mechanism of acute or repeated stress enhance drug self-administration**

The studies discussed above suggest that the exposure to stress generally enhances drug self-administration behavior, especially when the stress is repeated in nature (yohimbine is an exception to this). Even though the underlying mechanism for this effect is yet to be elucidated, some hypotheses have been proposed and they deserve to be mentioned at this point. Piazza and Le Moal (1998) have proposed that the ability of stress to stimulate drug taking lies in the activation of HPA axis. Acute stress exposure elicits glucocorticoid release, which subsequently increase the secretion of dopamine in the mesolimbic pathway (Piazza et al. 1996a). This surge of dopamine release may sensitize animals to the reinforcing effect of drugs of abuse, but as glucocorticoid levels return to baseline though negative-feedback, the dopamine levels drops. Repeated stress, on the other hand, impairs glucocorticoid negative-feedback system, and elevates baseline glucocorticoid levels. Therefore, after repeated exposure to stress, the increase in sensitivity to drugs may be longer-lasting due to this. However, deregulation in HPA axis may
not be the only mechanism mediating the effects of repeated stress on drug taking. Because acute stress can stimulate the mesolimbic system and increase the level of dopamine in the brain (Kalivas and Duffy 1989), repeated stress may induce neurochemical alternations in mesolimbic circuits and produce sensitization to the reinforcing effect of drugs (Covington et al. 2008; Nikulina et al. 2008).

1.6.3 Stress on reinstatement of drug-seeking behavior

Intermittent footshock is probably the most commonly used stressor in studies of drug relapse. It is applied prior to the test session and reliably reinstates heroin, cocaine, alcohol and nicotine seeking behavior after extinction (e.g. Buczek et al. 1999; Erb et al. 1996; Le et al. 2000; Le et al. 1999; Shaham and Stewart 1995). In the case of pharmacological stressors, CRF infused into the lateral ventricles or BNST reinstated heroin or cocaine self-administration (Erb and Stewart 1999; Shaham et al. 1997). Yohimbine has also been shown to reinstate cocaine seeking in squirrel monkeys (Lee et al. 2004), and methamphetamine (Shepard et al. 2004), alcohol (Le et al. 2009; Le et al. 2005; Shepard et al. 2004), and food seeking in rats (Ghitza et al. 2006; Nair et al. 2006). The effects of other types of acute environmental stressors, such as social defeat and restraint, on reinstatement have been tested, but they failed to affect drug-seeking behavior (Funk et al. 2005; Wang 2002). Unlike the effect of stress on self-administration, it has been suggested that CRF pathways play a critical role in stress induced reinstatement of alcohol (Le et al. 2000), heroin (Shaham et al. 1997), cocaine (Erb et al. 1998), and nicotine seeking (Zislis et al. 2007b). NA and 5-HT systems also appear to be involved in relapse to drugs such as alcohol (Le et al. 2009) and nicotine (Zislis et al. 2007b).
1.7 Stress and Adolescence

Human adolescence is a period marked by increased level of stress. Reports of depressed mood and affective disturbances climax during adolescence (Rutter et al. 1976), and the total number of stressful life events is also significantly higher during adolescences in both boys and girls, compared to the number in childhood (see Spear 2000 p.429). In addition to elevated total numbers of stressful events, animal studies also indicate that the adolescent stress response system is different from that of adults. At the beginning of early adolescence (PD21), many parameters of the HPA axis reach adult levels (see McCormick et al. 2009 for review), but other parameters, such as the CRH responsive cells in the pituitary, continue to mature throughout adolescence (Senovilla et al. 2005). The basal level of corticosterone in adult (PD69) and adolescent (PD37) rats is very similar (Cruz et al. 2008), but after challenge with acute restrain stress, prepubertal (PD25-28) rats demonstrated a delayed and prolonged ACTH and corticosterone response compared to their adult counterparts (>PD65) (Romeo et al. 2006b). Moreover, with repeated exposure to stress, male rats that were 28-day-old showed a higher peak levels of plasma ACTH and corticosterone than adult animals, but this returned to baseline levels at a faster rate, leaving the ACTH and corticosterone level in adult rats higher than those in the prepubertal rats 45min after the last episode of restrain stress (Romeo et al. 2006a). In addition to the hormonal response, adolescent rodents also display a more pronounced behavioral response to the effect of stress. For example, acute restraint stress (30min) suppressed play behavior and enhanced huddling in prepubertal rats, but not in adults (Romeo et al. 2006b). Interestingly, a follow-up experiment showed that the suppression of play behavior by acute stress was short-lived (1hour), while repeated restraint stress during adolescence decreased social investigation, while leaving play behavior intact (Klein et al. 2010). Furthermore, using in situ hybridization, Romeo et al (2007) demonstrated that, after acute restraint stress, the increase in CRF mRNA
expression in the hypothalamus (PVN) was more pronounced in prepubertal rats (PD28) than
adults (PD77). Contradictory results were also observed. For example, after 15 minutes exposure
to the open arm of the elevated plus maze (EPM), which serves as mild stressor, adult male rats
(PD70) had a higher level of plasma corticosterone than rats in late adolescence (PD45)
(McCormick et al. 2008). The authors of this report speculated that a perceived narrower arm in
EPM for the adult rats because of the larger body size, could contribute to the differences in the
stress response. Nevertheless, this group also observed a higher corticosterone level 90 min after
the EPM stress in adolescent group, which is consistent with previous studies showing a
prolonged release of corticosterone after acute stressor in adolescent group (McCormick et al.
2008; Romeo et al. 2006a).

Overall, these results suggest that there are age differences in hormonal and behavioral
responses to stressors; however, this difference depends on the nature of the stressor, as well as
the time point when the measurements were taken. One phenomenon that needs to be pointed out
is the gender difference in the response to stress. Young girls perceive events to be more stressful
than same-age boys (see Spear 2000 for review). Evidence also suggests that female adolescent
rodents respond to stressors differently from males, in a fashion that is not controlled by sex
hormones (see McCormick and Mathews 2007 for review; McCormick et al. 2008).

1.8 Theory of the Biological Vulnerability to Nicotine Addiction during Adolescence

As discussed in the early part of this introduction, epidemiological data demonstrate that the
initiation of smoking mainly occurs during adolescence, and this early onset of smoking is
associated with more severe long-term consequences. Possible factors contributing to this
elevated vulnerability to smoking involve environmental factors, such as family and peer
influence, genetic variance, as well as behavioral characteristics that are typical during adolescence, such as impulsivity and risk-taking. However, one other theory to explain this phenomenon suggests that adolescence is a period of biological vulnerability to the psychopharmacological effect of nicotine. This may be because many brain regions, especially those that govern reward and motivation, are still under development throughout adolescence. The validity of this theory has been tested using various procedures using different animal models.

The results from CPP studies are consistent, with adolescent rats demonstrating more pronounced CPP than adults (Belluzzi et al. 2004; Shram et al. 2006; Vastola et al. 2002). Furthermore, the age difference in CPP does not depend on the route of administration, as recently, Shram (2009) has shown more robust CPP in adolescence than in adulthood when nicotine was administered intravenously. On the other hand, young rodents are less sensitive to the aversive effects of nicotine (Shram et al. 2006; Wilmouth and Spear 2004). These two pieces of evidence lead to the speculation that the rate of smoking initiation is high during adolescence because they experience an enhanced rewarding effect, but a reduced aversive effect of nicotine.

In comparison, data obtained using the intravenous self-administration procedure are less consistent. By using the relatively low-response FR schedule (FR1 and FR2), adolescent rodents self-administrate more nicotine than adults in some studies (Adriani et al. 2002; Chen et al. 2007; Levin et al. 2003), but not in the others (Shram et al. 2008a; Shram et al. 2008b). However, when the ratio is increased to FR5, adolescent rats are less willing to respond for nicotine (Shram et al. 2008a). When looking at the self-administration profile during PR, however, adults and adolescents differ distinctively. Adults achieve higher breakpoint for nicotine infusion compared
to adolescents, suggesting that the reinforcing effect of nicotine is higher in adult rats (Shram et al. 2008a).

The results from the self-administration paradigm are inconsistent with those obtained with CPP. Maybe it is because the two paradigms measure different aspects of the addiction property of nicotine. The results with PR disagree with the biological vulnerability theory, and do not coincide with the clinical observation that adolescent humans are at elevated risk of initiating smoking. The reasons for this are not known.

One possible phenomenon that may help to explain the increased initiation of smoking in adolescent is stress. As previously discussed, stress is associated with increased rate of drug abuse in human, and stress also promotes drug-taking behaviors in animals. The adolescent period is a transitional stage with the young individuals facing new challenges; therefore, adolescence is a stressful period (see Spear 2000). Moreover, during human adolescence, stressful life events are positively correlated with higher risk of smoking initiation. Therefore, it is plausible to speculate that stress, among other factors, promotes the development of smoking behavior. One way stress could do this is by interacting with nicotine and promoting its reinforcing effect. In addition, because the adolescent brain is at a unique transition period, the manner or the magnitude of such interaction could differ from that in adults, which renders adolescents with a higher risk to beginning smoking.
CHAPTER 2: PURPOSE OF INVESTIGATION

Epidemiological data reveal that initiation of smoking occurs mainly in adolescence, whereas it rarely happens in adulthood. Experimental studies with animals have suggested that adolescents are more sensitive to the reward/reinforcing effect of nicotine, as measured by CPP, but are less motivated to self-administer nicotine than adult rats during a demanding self-administration schedule (Shram et al. 2008a). Stress, among other factors, is strongly associated with smoking behavior. It was shown that negative life events during adolescence are positively correlated with initiation of smoking (Byrne et al. 1995). However, to our knowledge, no study has looked at the impact of stress on sensitivity to the reinforcing effect of nicotine during the adolescent period, despite the fact that adolescents encounter more stressful life events and their stress responses differ from adults.

Therefore, the current studies were proposed to investigate the age difference in nicotine self-administration in response to stress in adolescent and adult rats. We hypothesized that exposure to stress would increase the reinforcing effect of nicotine, and adolescents would be particularly vulnerable to this effect. To this end, we examined whether or not acute exposure to stress would increase nicotine self-administration and whether or not prior exposure to stress would facilitate initiation of nicotine self-administration in both adolescent and adult rats. Three experiments were conducted to address these questions:

1) Acute effects of a pharmacological stressor, yohimbine, on the rewarding efficacy of nicotine were studied on a PR schedule in adolescent and adult rats that have been trained to self-administer nicotine.
2) The second experiment was designed to examine the generality of the effect of acute stress on nicotine self-administration. The effect of inescapable intermittent footshock stress on nicotine self-administration was examined on PR performance in adolescent and adult rats that have been trained to self-administer nicotine.

3) In the third experiment the effects of prior exposure to repeated social defeat stress on the acquisition of nicotine self-administration behavior was examined in adolescent and adult rats.

Yohimbine was chosen because it is the only stressor that has been shown to vigorously increase the level of alcohol self-administration as well as reinstate alcohol seeking after acute exposure (Le et al. 2005; Marinelli et al. 2007). Similarly, acute intermittent footshock was chosen because it reliably induces reinstatement behavior across a wide range of drug classes (Buczek et al. 1999; Erb et al. 1996; Le et al. 1998), and increases opioid and alcohol self-administration in some studies (Matthews et al. 2008; Shaham and Stewart 1994). Finally, social defeat stress was chosen, because studies with cocaine in adult rats have shown that repeated social defeat prior to drug exposure facilitated the acquisition of cocaine self-administration behavior, as well as elevating breakpoints under a progressive-ratio schedule (Covington and Miczek 2005; Tidey and Miczek 1997).
CHAPTER 3: GENERAL MATERIAL AND METHODS

3.1 Subjects

3.1.1 Adult rats

Male Long Evans rats (200-250g, approximately 60-days old) were purchased from Charles River laboratories (QC, Canada). Upon arrival, animals were group-housed (2 per cage) in plastic “shoebox” cages (18 x 19 x 22 cm) in a humidity- and temperature-regulated vivarium. Rats were maintained on a reversed 12h light/dark cycle (lights on from 7pm to 7am) throughout the experiment. They had free access to water and Purina rat chow until catheterization surgeries were done on them. They were then single-housed and fed 20g of rat chow per day.

3.1.2 Adolescent rats

Pregnant Long Evans dams (Charles River Laboratories, QC, Canada) were single-housed in Plexiglas cages (51 x 41 x 20 cm), and they were expected to give birth to pups 2 weeks after arrival. The dams were used as sources for juvenile rats in order to avoid stress caused by transportation to the adolescents. Pups were weaned on P20-22. They were housed by litter, giving free access to food and drink. Only male pups were used in the experiments. Following catheterization surgery, adolescent rats were single-housed and maintained on 20g of rat chow per day. The housing conditions of the adolescent rats were identical to those of adult rats.
3.2 Apparatus

3.2.1 Nicotine self-administration

Twenty-four identical computer-interfaced operant chambers (Med Associates, St Albans, VT) were used for nicotine self-administration. Each chamber was equipped with one active lever and one inactive lever; both located 2.5 cm above the removable grid floor. A house light was located on the opposite side to the levers in the chamber. A white cue light was 7.5 cm above the active lever, and a tone generator (2900 Hz) was located directly above that. The house light was turned on at the start of every operant session and was kept on for the entire session. During a session, pressing on the active lever lead to the activation of a high-speed microliter syringe pump (PHM-104, Med Associates), which was located outside of the operant chamber. The pump delivered nicotine solution into the intravenous catheter in the rats through a 23-gauge cannula. The cannula was connected to the pump by Tygon tubing that was protected by a metal spring. When nicotine solution was delivered, the cue light (40 s) and tone (1 s) were turned on, and the house light was turned off. Each drug delivery was followed by a 40 s time-out period, during which presses on the active lever were recorded but had no programmed consequences. The responses on the inactive lever were recorded throughout the session, but did not lead to presentation of cue light and tone, or pump activation.

3.2.2 Food training

Sucrose pellet training was conducted in 16 similar operant chambers, with the exception that a food receptacle was located between the levers. The receptacle was connected to a food magazine outside of the chamber that could dispense 45 mg sucrose pellets (Bioserv, Frenchtown, NJ). During a food training session, responses on the active lever lead to delivery of sucrose
pellets (one at a time) to the food receptacle. Upon each successive delivery, the cue light (6s) and tone (1s) were turned on, and the house light turned off. Presses on the inactive lever were recorded but had no programmed consequences.

### 3.3 Catheterization surgery

#### 3.3.1 Intravenous catheter construction

Intravenous catheters were implanted to deliver solution intravenously from the drug pump; therefore, one end of the catheter was inserted into the right jugular vein of the rats, and the other end protruded from the back of the rats, and was connected to the Tygon drug line.

The intravenous end of the catheter was constructed with a short length of silicon rubber tubing (0.012”ID x 0.025” OD; Dow Corning, Midland, MI, USA) connected to a 65mm long piece of polyethylene tubing (PE10; 0.28mm ID; Beckton Dickinson, Sparks, MD, USA). About 7mm of the silastic tubing was wrapped outside of the PE10. A 6mm long piece of heat shrink tubing (3/64”; Electrosonic, Willowdale, ON) was heat-shrunk to permanently secure this joint. During the catheterization surgery, the silastic end was completely inserted into the jugular vein because of its softness and flexibility. The length of the silastic was determined according to the size of the rats. For adolescent rats, the silicon silastic was 27mm long, and for adult rats, it was 37mm long.

The other end of the catheter that was to be connected to the drug line was made with a 170mm long piece of polyethylene tubing (PE20; 0.28mm ID; Beckton Dickinson, Sparks, MD, USA), a 16mm long shrink tube (3/64”; electrosonic, Willowdale, ON), a drilled machine bolt, and a small oval-shaped piece of biocompatible (Marlex, C.R.Bard, Cranton, RI, USA). Half of
the shrink tube (about 8mm) was fed through the PE20. A hub made with a 23 gauge hypodermic needle was fed into the other half of the shrink tube. When heat was applied to the shrink tube, one end was permanently joined to the PE20, and the other end would be used as the input opening of the catheter after the hub was pulled out. A bolt and mesh were adhered onto the shrink tube on the PE20 by using dental acrylic. A catheter was completely constructed by fusing the end of PE10 and PE20 together with heating.

3.3.2 Surgery preparation

Cotton swabs, sponges, drapes, 4-0 silk sutures and 5-0 silk sutures (Surgical Specialties, Reading, PA, USA) were autoclaved prior to surgery. Surgical instruments required for catheterization were sterilized by soaking in Zephiran Chloride solution at least 20 min prior to the start of the surgery. Instruments were re-sterilized between operations in the same fashion. Intravenous catheter and injectors were soaked in and filled with Zephiran Chloride solution for a minimum of 20 min, and then washed with sterile saline solution prior to implantation. Surgical gloves were worn throughout the operation, and a new pair was used for each surgery.

3.3.3 Anesthesia

Rats were then anesthetized with a Ketamine/ Xylazine mixture (75mg/kg ketamine/10mg/kg xylazine, i.p). For the adult rats, volume ratio of each component in the mixture was Ketamine:dH2O:Xylazine=3:3:2 (2ml/kg). Because of adolescents’ considerably smaller body size, the anesthetic solution was modified by adding extra dH2O to the mixture (Ketamine:dH2O:Xylazine=3:11:2). And each adolescent was therefore injected at 4ml/kg of the
mixture. After the animal appeared to be anesthetized, the level of anesthesia was tested by toe-pin. The procedure proceeded only if the rat did not show any response to the test. Otherwise the rat was allowed to recover and had the operation done on another day.

3.3.4 Intravenous catheterization surgery

Hair on the back and neck (ventral) region of the rats was carefully shaved. Subsequently, Betadine scrub solution, 70% ethanol, Betadine with 10% porcidone-iodine solution were applied to these areas in the presented order. Incision sites were also treated with a local anesthetic, 0.125 % Marcaine (0.1ml per site, s.c.). Eye gel was used to protect rat eyes from dehydration during surgery and recovery from anesthesia. Saline (1ml) was subcutaneously injected to prevent excess loss of body water during surgery.

The rat was place on a sterile drape dorsal side up. A 1-centimeter long cut was made between the scapulae and a subcutaneous pocket on the back was created by using hemostat. Then, the rat was flipped around to expose the shaved ventral neck region. A 3-millimeter long incision was made through the skin on the neck. Subsequently, a hemostat was used to create a subcutaneous tunnel from the cut on the back to the incision at the neck. A catheter connected to a saline syringe was pulled through the tunnel. After successfully locating the jugular vein, the silastic end of the catheter was inserted in to the vein. A piece of 5-0 silk suture was used to tie the venous wall to the silastic tubing. After the vein was placed back to its original position beneath the tissue, the incision was sutured by using 4-0 silk suture.

The rat was placed facing down again. The Marlex mesh was inserted through the incision, with PE20 looped inside the subcutaneous pocket. Then, the incision is closed using 4-0 silk
suture. Antibiotic powder, Cicatrin (Glaxo Wellcome, Picking, ON) was applied to the wounds on the neck and back to reduce infection and help recovery. The analgesic Buprenorphine (0.01mg/kg) was injected subcutaneously to help control post-operation pain. Rats were allowed to recover for 6-7 days after the surgery.

3.3.5 Maintenance and verification of catheter patency

Catheters were flushed daily with anticoagulant (0.1 ml sterile heparin-saline solution (50U/ml, Sigma, St. Louis, MO, USA). Generally, successfully injecting the solution into the catheter easily was an indicator of patency. However, a patency test was given at the end of the experiment to verify that catheters delivered drug intravenously. Sodium methohexital (0.05mg/kg), a short-acting and fast-onset anesthetic, was injected into the intravenous catheter. Rats with catheters patent and properly positioned should show sign of anesthesia within 2-3 s, and would recover from paralysis with a few seconds.

3.4 Drug preparation

Nicotine solutions were prepared fresh daily by dissolving nicotine bi-tartrate (Sigma-Aldrich, Oakville, ON, Canada) in 0.9% saline solution. Then, nicotine solution was titrated to pH 7 (+/- 2) using 1N NaOH. Sterilization was achieved by filtering the solution through a 0.2μm PES syringe filter (Whatman, Florham Park, NJ, USA). For the yohimbine and footshock experiments, the unit doses for the intravenous nicotine self-administration were 15, 30, and 60 μg/kg per infusion. For the social defeat experiment, the 30 μg/kg per infusion dose was used.
3.5 General experimental procedure

3.5.1 Food training

Before nicotine self-administration sessions started, adult rats and adolescent rats were trained to obtain 45mg sucrose pellets on a FR1 reinforcement schedule in the operant chambers designated for sucrose pellet training. This procedure helps the rats to acquire the ability to differentiate the active lever from the inactive lever. Rats could earn up to 400 pellets in each daytime training session (7h) or 600 pellets in each night training session (16h). They had free access to water throughout the session. Each rat was given 1 daytime and 1 night training session on alternating days. Rats were considered to have successfully learned to bar-press if they obtained more than 50 sucrose pellets in a one-hour consolidation test session done after training. Furthermore, rats were deemed to have learned to differentiate the active from inactive levers if the responses on active lever exceed that on the inactive lever significantly.

3.5.2 Nicotine self-administration

Once finished the sucrose pellet training phase, rats were trained to self-administer nicotine (30μg/kg) on a FR1 schedule 1 hour daily for 4 consecutive days. During the self-administration sessions, timeout following each nicotine infusion was 40s and pressing on the inactive lever would not lead to any programmed consequences but was recorded. Then, the rats were placed on FR2 schedule for 4 days (1h daily). In the yohimbine and footshock experiment, on the last day of FR2, rats were randomly assigned into 3 groups, and each group would self-administer a different dose of nicotine (15, 30, or 60 μg/kg/infusion) from this point on.
Following the FR2 schedule, the adult and adolescent rats were allowed to respond to the dose of nicotine assigned to them on a PR schedule for number of days. The sequence of the number of required responses on the active lever to obtain one infusion was determined by the exponential formula \(5 \exp(0.2 \times \text{infusion number})-5\). Therefore, the number of responses required during a PR session increased in the following order: 3, 6, 10, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 179, 219, 268, 328, 402, 492, 603 and so on. Sessions were terminated after 2 hours or if there was more than 20min inactivity on the active lever, whichever came first. Breakpoint was determined by using the number of responses required to obtain the last infusion.

### 3.6 Stressors

#### 3.6.1 Yohimbine

Yohimbine hydrochloride solutions (Sigma-Aldrich, Oakville, ON, Canada) were prepared fresh in the morning of the testing days. Thirty minutes prior to the start of the operant sessions, yohimbine was injected intraperitoneally. The effect of 1.25 mg/kg and 0.625 mg/kg of yohimbine was tested on the 5\(^{th}\) and 7\(^{th}\) day of PR phase, respectively.

#### 3.6.2 Intermittent footshock

Intermittent footshock was applied to rats through the steel grid floor. Adult and adolescents may have different sensitivities to footshock, which can be inferred from a variety of behaviors, including audible vocalization. A pilot study was carried out to assign appropriate electric current to each age group. Eight adult (PD87) and 8 adolescent rats (PD38) were used for the pilot. On the test day, rats were brought to a holding area outside of the footshock chambers
room. One rat was tested at a time. The rat was placed in the footshock chamber and allowed 5 min to acclimate. Then, a mild footshock (0.05 mA, 0.5 s) was delivered to the rat. After every 30 s, another shock was delivered to the rat, with 0.05 mA increments to the previous one. The test was terminated when vocalization was heard by a technician standing within 0.5 m to the chamber. For the consistency of results, the same technician was used to detect all vocalizations. The average of the minimum footshock current need to elicit vocalization was 0.7 mA and 0.5 mA for the adult and adolescent rats, respectively (data not shown). These values were used in the footshock experiment.

The effect of footshock on nicotine reinforcing efficacy was determined on the 6th and 8th day of PR. On the 6th day, rats were placed in the chamber and allowed 5 min acclimation time. Footshock (0.5 s) was administered to the rats intermittently for 5 min, with an average of 40 s between each shock (9 shocks total). Nicotine self-administration session was started immediately after the last shock. The same procedure was used on the 8th of PR, with the exception that 10 min intermittent footshock was administered (18 shocks total).

3.6.3 Social Defeat

The social defeat paradigm applied in this experiment was based on studies conducted by other groups and our own lab (Funk et al. 2005; Tidey and Miczek 1997), which has been shown to affect drug self-administration behavior. Male Wistar rats (500-600 g) were housed with females to increase their aggression and territory over their homecage environment (Miczek 1979). After 10 days, the females were removed. The Wistar rats were selected as the “resident”
rats if they consistently displaying extensive aggressive behavior to a separate group of intruder rats introduced to their home cage.

On the days of social defeat, intruder rats were introduced to the home cage of the residents. The residents usually attacked within 2 min of confrontation (e.g. biting, threatening posture). After the intruder displayed definitive submissive posture, the supine posture, the rats were separated by placing a wire screen between the two rats for the next 30 min, so the intruder continued to receive threatening visual and olfactory cues, while being protected from physical injury. Four daily episodes of social defeat were given to the rats beginning 4 to 5 days after surgery. Nicotine self-administration on FR1 was started the day after. See Figure 6.1 for a timeline of the experiment.

3.7 Statistical analysis

Data were analyzed using analysis of variance (ANOVA), with Age and Nicotine dose as the between-subject factors, and Session as the within-subject factor. Post hoc test, the Student-Newmen-Keuls analysis, was used where appropriate.
CHAPTER 4:
THE EFFECT OF YOHIMBINE ON NICOTINE SELF-ADMINISTRATION IN ADOLESCENT AND ADULT RATS

4.1 Introduction

Clinical studies indicate that smoking behavior increases in response to stress (Colamussi et al. 2007; Pomerleau and Pomerleau 1991). Compared to other age groups, human adolescents experience more stressful life events (see Spear 2000 p. 429), which has been shown to be associated with initiation of cigarette smoking (Byrne et al. 1995). In animals, administration of stress stimulates cocaine, opioid and alcohol consumption (Matthews et al. 2008; Miczek and Mutschler 1996; Shaham and Stewart 1994). However, to our knowledge, there have been no studies investigating the effect of stress on nicotine self-administration using rodent models, let alone studies that focused on the sensitivity to nicotine in stressed adolescents.

In the current study, we therefore investigated age difference in nicotine taking in response to acute stress. The stressor we chose is yohimbine, a prototypical $\alpha_2$-adrenoceptor antagonist, which activates noradrenaline release by inhibiting the $\alpha_2$ autoreceptor on the cell bodies of noradrenergic neurons (Abercrombie et al. 1988; Aghajanian and VanderMaelen 1982). Yohimbine has been shown to produce stress-like effects in both human and animal subjects (File 1986; Holmberg and Gershon 1961). Unlike other stressors, studies examining the effect of yohimbine on drug taking did not emerge until recent years. However, its effect on drug taking and seeking behavior is potent and consistent across different drug class. For instance, yohimbine robustly reinstates cocaine- (Anker and Carroll 2010), opioid- (Shepard et al. 2004), and alcohol-seeking (Le et al. 2005). Moreover, yohimbine has been shown to reliably and
potently enhance alcohol self-administration behavior (Le et al. 2009; Le et al. 2005; Le et al. 2000; Marinelli et al. 2007), while results obtained with other stressors have been weak and inconsistent. The current study investigates the effect of yohimbine on the reinforcing efficacy of nicotine in adolescent and adult rats. Adolescent and adult rats were first trained to acquire nicotine self-administration on a FR schedule. Subsequently, the acute effect of yohimbine on the reinforcing efficacy of nicotine was assessed during self-administration under a PR schedule.

4.2 Material and Methods

4.2.1 Experimental design

Thirty adult and 36 adolescent Long Evans rats were used in this study. Adult (PD68) and adolescent animals (PD22-23) were first food-trained as described in Chapter 3. Then they received catheterization surgeries, followed by 3-5 days of recovery. Animals (adults PD82, adolescents PD33-34) were initially trained in the operant boxes to acquire 30 μg/kg nicotine infusion on a FR1 schedule for 4 days, followed by FR2 for another 4 days. On the last day of FR2 (adult PD89, adolescent PD40-41), rats were randomly assigned to three groups, and received different nicotine infusion dose from this point on. Each group contained 10 adults and 12 adolescents, and they were administered with either 15, 30, or 60μg/kg nicotine per infusion, respectively. Subsequently, the reinforcing efficacy of different doses of nicotine was examined using the PR schedule for the next 7 days. On the 5th day and 7th day of PR, the effect of 1.25mg/kg and 0.625mg/kg yohimbine injection was tested, respectively. A graphic representation of the experimental procedure is shown in Figure 4.1. The patency of catheters was tested at the end of PR phase.
4.2.2 Statistical analysis

On the last day of FR2 where animals were assigned to different dose groups, two-way ANOVA was used with Age and Dose of nicotine as between subject factors. PR phase was analyzed by two-way ANOVA as well. The effect of yohimbine was evaluated by comparing the data from the yohimbine test to those from the day preceding the test. Student-Newmen-Keuls post-hoc analysis was applied where appropriate. SPSS and Sigmastat statistic software (Jandel Scientific, San Rafael, CA) were used for data analysis.
Figure 4.1 Experimental timeline for the yohimbine experiment. Ages for adolescents were the calculated by taken the average of all the subjects as adolescents were born on two consecutive days.
4.3 Results

4.3.1 FR1 and FR2 schedule before assignment to different nicotine doses

The mean number (±sem) of infusions for adolescent rats on the last day under the FR1 and FR2 schedules prior to assigning them to the different nicotine dose conditions were 21.25±1.36 and 22.8±0.98, respectively. The mean number (±sem) of infusions for adult rats on the last day under the FR1 and FR2 schedules prior to assigning them to the different nicotine dose conditions were 17.58±1.28 and 21.87±1.01, respectively. ANOVA done on these data with the between factor of Age (adolescent, adult) and within factor of FR condition (FR1, FR2) showed a significant main effect of FR condition (F(1,64)=6.895, p<0.05), that resulted from a small, but significant decrease in the numbers of nicotine infusions received by the rats when under the FR2 schedule.

4.3.2 FR 2 schedule after assignment to different nicotine doses

Figure 4.2 presents the mean (±sem) number of infusions for the nicotine dose-response curve under the FR2 schedule (one session) after the animals were assigned to the different nicotine dose groups (15, 30, 60 µg/kg). The ANOVAs included the between-subjects factors of Nicotine Dose and Age. None of these parameters varied significantly as a function of age or nicotine dose.
4.3.3 PR schedule

Numbers of nicotine infusions received under the PR schedule was higher in adult rats than in adolescent rats at the lower nicotine doses (15 and 30 µg/kg) but did not differ at the highest dose (60 µg/kg). Numbers of active lever presses was higher in adults at the 30 µg/kg nicotine dose, but differences were not observed at the other two doses. There were no age differences in inactive responding at any dose of nicotine. Figure 4.3 presented the mean (±sem) number of infusions, total active lever presses, and inactive lever presses for the nicotine dose-response curve under the progressive ratio schedule (mean of 4 sessions). The statistical analyses included the between-subjects factors of Nicotine Dose and Age. Table 1 represented the median breakpoint achieved by adolescent and adult rats.

Infusions: The ANOVA revealed significant effects of Age (F(1,65)=18.51, p<0.05) and Nicotine dose (F(2,65)=9.91, p<0.05).
Active lever presses: There were significant effects of Age (F(1,65)=11.58, p<0.05) and Nicotine dose (F(2,65)=6.22, p<0.05).

Inactive lever presses: The ANOVA revealed significant effects of Nicotine dose (F(2,65)=9.79, p<0.05).

Post-hoc group differences within each nicotine dose are depicted in Figure 4.3.

Table 1. Breakpoint (median) achieved by adolescent and adult rats during PR. Data were collapsed over the four training days.

<table>
<thead>
<tr>
<th>Nicotine infusion dose</th>
<th>15µg/kg</th>
<th>30µg/kg</th>
<th>60µg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adolescent</td>
<td>9</td>
<td>21</td>
<td>75</td>
</tr>
<tr>
<td>Adult</td>
<td>46</td>
<td>72</td>
<td>76</td>
</tr>
</tbody>
</table>
Figure 4.3 Self-administration of nicotine by adolescent and adult rats at different nicotine infusion doses (15, 30, 60 μg/kg) on a progressive-ratio schedule. Data were collapsed over the four training days. In A, mean (±sem) of reinforcements was shown, while B and C show, respectively, mean (±sem) of active and inactive lever presses. n=10-12 per age and nicotine dose group. * Significantly different from 15 μg/kg dose. + Significant age difference
4.3.4 Effects of yohimbine on PR responding

Yohimbine increased nicotine intake under the PR schedule in both age groups, independent of the nicotine infusion dose. Irrespective of yohimbine dose, adult rats responded more than adolescents at the 15 and 30 µg/kg nicotine infusion doses, but did not differ at the 60 µg/kg dose. Figure 4.4 presents the mean (±sem) number of infusions, total active lever presses, and inactive lever presses for the yohimbine dose-response curve. Within each age group, both doses of yohimbine increased self-administration to a similar degree. The statistical analyses included the between-subjects factors of Nicotine Dose and Age, and the within-subjects factor of Yohimbine Dose. Table 2 presents the median breakpoint achieved at different doses of yohimbine administration.

Infusions: The ANOVA revealed significant effects of Yohimbine dose (F(2,120)=73.27, p<0.05), Age (F(1,60)=20.18, p<0.05), Nicotine Dose (F(2,60)=8.51, p<0.05) and a significant Age x Nicotine dose interaction (F(2,60)=4.44, p<0.05).

Active lever presses: There were significant effects of Yohimbine dose (F(2,120)=50.43, p<0.05), Age (F(1,60)=15.29, p<0.05), Nicotine dose (F(2,60)=3.99, p<0.05), and significant interactions of Yohimbine dose x Age (F(2,120)=5.71, p<0.05) and Age x Nicotine dose (F(2,60)=3.35, p<0.05).

Inactive lever presses: There were significant effects of Yohimbine dose (F(2,120)=15.56, p<0.05) and Nicotine dose (F(2,60)=4.62, p<0.05).

Post-hoc group differences within each yohimbine dose and nicotine dose are depicted in Figure 4.4.
As there was a significant effect of Yohimbine dose on inactive lever responding, an analysis of covariance (ANCOVA) was run on the infusion and active lever response data. For numbers of infusions, this revealed significant main effects of Age (F(1,57)=24.49, p<0.05) and Yohimbine dose (F(2,114)=18.19, p<0.05), and a significant Age x Nicotine dose interaction (F(2,57)=6.25, p<0.05). For active lever presses, there were significant main effects of Age (F(1,57)=17.28, p<0.05) and Yohimbine dose (F(2,114)=7.30, p<0.05), and significant Age x nicotine dose (F(2,57)=4.83, p<0.05) and Age x Yohimbine dose (F(2,114)=6.43, p<0.05) interactions.

Table 2. Breakpoint (median) achieved by adolescent and adult rats during yohimbine challenge under PR.

<table>
<thead>
<tr>
<th>Yohimbine dose</th>
<th>0 mg/kg</th>
<th>0.625 mg/kg</th>
<th>1.25 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15 μg/kg nicotine infusion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adolescent</td>
<td>6</td>
<td>32</td>
<td>36</td>
</tr>
<tr>
<td>Adult</td>
<td>36</td>
<td>95</td>
<td>86</td>
</tr>
<tr>
<td>30 μg/kg nicotine infusion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adolescent</td>
<td>15</td>
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<td>33</td>
</tr>
<tr>
<td>Adult</td>
<td>70</td>
<td>162</td>
<td>118</td>
</tr>
<tr>
<td>60 μg/kg nicotine infusion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adolescent</td>
<td>70</td>
<td>132</td>
<td>98</td>
</tr>
<tr>
<td>Adult</td>
<td>62</td>
<td>95</td>
<td>98</td>
</tr>
</tbody>
</table>
Figure 4.4 Effects of yohimbine on nicotine self-administration by adolescent and adult rats at different infusion doses of nicotine on a progressive-ratio schedule. A, B, and C show data collected at nicotine infusion dose of 15, 30 and 60 µg/kg, respectively. Left panel, mean (±sem) number of reinforcements; center panel, active lever presses; right panel, inactive lever presses. n = 10-12 per age and nicotine dose group. * Significantly different from vehicle. + Significant age difference.
4.4 Discussion

The primary goals of these studies were to determine the age difference in nicotine taking behavior in response to the acute effect of a pharmacological stressor, yohimbine, and whether such effect varies as a function of the self-administration dose of nicotine. Consistently with Shram’s findings, we observed that adolescents responded significantly less for nicotine on a PR schedule of reinforcement compared to adults (Shram et al. 2008a). Here, we extend these findings in showing that this age difference depends on nicotine infusion dose, in that adolescents responded significantly less at lower infusion doses than adults, but they did not differ at the highest dose of nicotine. Moreover, we found that yohimbine, an α-2 antagonist, increased the rewarding efficacy of nicotine depending on age. In other words, the magnitude of increases in active lever presses after administration of yohimbine is smaller in adolescent animals at the two lower doses of nicotine we used, but did not differ from adults at the highest dose (reflected by the significant Age×Yohimbine dose interaction in active lever presses). This suggests that adolescents are less susceptible to the potentiating effects of stress on the rewarding efficacy of nicotine. This speculation, however, seems to be weakened by a non-significant Age×Yohimbine dose interaction in number of infusions during the PR test. We think that this inconsistency comes from the nature of the number of infusions and active lever press escalates during PR session. Number of infusion increases numerically (e.g.1, 2, 3...20, 21), while number of active lever presses required to obtain the next nicotine infusion increases exponentially (e.g.3, 6, 10…492, 603). Statistical test would most likely detect a difference when the means of the samples are far from each other.
4.4.1 Inactive lever responding

We noted that yohimbine significantly increased responding on the inactive lever during PR tests. Even though this increase is much smaller than that seen in the active lever responses, it still complicates the interpretation of the results, as the specificity of the effects of yohimbine on nicotine-seeking are called into question. Therefore, we analyzed the infusion and active lever data in two ways, first, with ANOVAs, and secondly, using the inactive data as a covariate in the analyses described here; both of these strategies yielded similar results. Other groups have described high rates of inactive responding for drugs. For example, adolescent female Lewis rats trained to self-administer nicotine had comparatively high rates of inactive lever responding (Chen et al. 2007). Anker and Carroll (2010) also noticed that adolescent inactive lever responding was higher than adults during cocaine self-administration, and during yohimbine induced cocaine reinstatement, inactive lever presses increase in parallel with a much larger increase in the active lever responding in both ages. Therefore, yohimbine may moderately stimulate general motor responses; nevertheless, its potentiating effect on the rewarding efficacy of nicotine is clearly shown by its ability to increase responding on the lever associated with nicotine delivery.

4.4.2 Age differences in nicotine self-administration

With the exception of the studies done in our laboratory (Shram et al. 2008a; Shram et al. 2008b), little systematic work has been done on age differences in nicotine self-administration. More rapid acquisition of nicotine self-administration (Chen et al. 2007) and increased intake during maintenance in adolescents (Levin et al. 2003) have, however been shown by other laboratories. We extended this work using more elaborate designs, employing different infusion
doses of nicotine and reinforcement schedules. We found that adults earned significantly more nicotine infusions compared to adolescents under the PR schedule (Shram et al. 2008a). Our current findings with the PR schedule are consistent with this earlier work, and extend them to a wider range of nicotine infusion doses. Taken together, these results suggest that the motivation to seek nicotine is lower in adolescents.

Studies of age differences suggest that adolescents and adults do not differ in the acquisition or maintenance of cocaine self-administration (Frantz et al. 2007; Harvey et al. 2009; Li and Frantz 2009). Only one positive finding has been reported, in that adolescent rats bred for low saccharin intake acquired cocaine self-administration faster than adults bred for low saccharin intake (Perry et al. 2007). There were no age differences in acquisition of cocaine self-administration in rats bred for high intake.

Previous studies from our lab and others using CPP and CTA procedures have shown that adolescents find nicotine more rewarding and less aversive than adults (Belluzzi et al. 2004; Shram et al. 2006; Torrella et al. 2004; Vastola et al. 2002; Wilmouth and Spear 2004). Although the reasons for this discrepancy are not known, there are several potential explanations. In the CPP and CTA studies, nicotine is injected non-contingently, while in the self-administration studies, nicotine is injected voluntarily, contingent on lever pressing. There is evidence in adult rats that contingent and non-contingent drug exposure have different effects on brain and behavior (Dworkin et al. 1995; Jacobs et al. 2003; Wilson et al. 1994). Another difference is the route of administration. In the CPP studies, nicotine is injected subcutaneously, while in the self-administration studies it is injected intravenously. Intravenous injections may result in a more rapid increase in brain levels of nicotine than subcutaneous injections. More rapid increases in brain levels of nicotine or other drugs are associated with greater potential for self-administration
(Shoaib 1996). Nevertheless, previous studies in our lab demonstrated that adolescent rats developed CPP to intravenously injected nicotine while adults did not (Shram and Le 2010). This leads to another speculation that different anatomical substrates may mediate the effects of nicotine in CPP and operant self-administration procedures (see Bardo and Bevins 2000).

4.4.3 Effect of yohimbine on nicotine self-administration

Yohimbine significantly increased nicotine self-administration under PR conditions in adolescent and adult rats. These results are congruent with previous work showing that yohimbine can increase operant responding for alcohol, and extends them to nicotine. We observed that the effects of yohimbine were dependent on age and nicotine dose. The magnitude of responding induced by yohimbine was more prominent in adults compared to adolescents when the nicotine doses were low. In rats self-administering 15 and 30, but not 60 µg/kg infusion doses of nicotine, yohimbine produced markedly larger increases in active lever presses in adults compared to adolescents. These results suggest that adolescents are less sensitive to the potentiation effects of yohimbine on the rewarding efficacy of nicotine, and this age difference in sensitivity is detected when the nicotine dose is low.

To our knowledge, this experiment is the first attempt to study the effect of stress on the maintenance phase of nicotine self-administration and demonstrated that yohimbine increased nicotine taking during PR in both adolescents and adults. The mechanism of this phenomenon, however, is yet to be clarified. Previously, studies from our lab showed that yohimbine increase alcohol self-administration (Le et al. 2005; Marinelli et al. 2007), and this elevation of alcohol consumption by administration of yohimbine is attenuated by CRF receptor and 5-HT1A receptor antagonists, but not by drugs affecting the noradrenergic system (Le et al. 2009;
Marinelli et al. 2007). It is yet to be demonstrated whether the same elements contribute to yohimbine’s ability to increase nicotine taking.
CHAPTER 5:
The Effect of Intermittent Footshock Stress on Nicotine Self-Administration in Adolescent and Adult Rats

5.1 Introduction

Intermittent footshock is commonly used in studies of the relationship between stress and drug taking (Anisman and Waller 1974b; also see Lu et al. 2003; Myers and Holman 1967). Like other environmental stressors, footshock stress (FSS) induces significant hormonal and neurochemical changes in rodents. For example, acute mild footshock increases plasma corticosterone level in rats and mice (Friedman et al. 1967; Matthews et al. 2008). In rats, repeated intermittent footshock also triggers release of β-endorphin and N-acetylated β-endorphin, which are indicators of ACTH release from the anterior pituitary (Robinson et al. 1987). In addition, dopamine metabolism in several brain regions, including the medial frontal cortex, hypothalamus, dorsal striatum and nucleus accumbens, also increases after exposure to repeated intermittent footshock (Robinson et al. 1987).

Among various stressors, FSS has been shown to be a stressor that reliably elicits drug-seeking behavior using the reinstatement model. For instance, exposure to intermittent footshock reinstates heroin (Shaham and Stewart 1995), cocaine (Erb et al. 1996), alcohol (Le et al. 2000; Le et al. 1999) and nicotine seeking (Buczek et al. 1999; Zislis et al. 2007a). However, its effects on drug self-administration are more complicated. Exposure to footshock during food self-administration, increased acquisition of cocaine self-administration which immediately followed food-self-administration (Goeders and Guerin 1994). FSS also increases alcohol intake in rats.
(Funk et al. 2004; Vengeliene et al. 2003), and certain strain of mice (Matthews et al. 2008), but this effect is small and inconsistent from laboratory to laboratory. For instance, in rats, intermittent footshock increased alcohol drinking level only during ethanol deprivation periods (Funk et al. 2004; Vengeliene et al. 2003). And in mice, Matthews and colleagues showed that only one strain out of three had hormonal and behavioral responses to the effect of footshock; furthermore, in the strain of mice which did respond to footshock, alcohol taking level increased after FSS mainly due to a significant decrease in the control group (Matthews et al. 2008). So far, the only experiment that demonstrates footshock stimulates intravenous self-administration has been with heroin (Shaham and Stewart 1994). They showed that 10 minutes of intermittent footshock prior to self-administration session increased heroin taking. Although the magnitude of increment was small, the authors also reported that this effect was only observed during PR schedule, but not during the low ratio FR acquisition phase (Shaham and Stewart 1994). As PR is a model that quantifies the reinforcing effect of a drug, this result suggests that FSS may increase the motivation towards drug-seeking. This speculation leads to the design of the current study, which sets out to assess the effect of FSS on the reinforcing efficacy of nicotine in adolescent and adult rats. In this study, adolescent and adult rats were first trained to acquire nicotine self-administration on a FR schedule. Subsequently, the acute effect of intermittent footshock on the reinforcing efficacy of nicotine was assessed during self-administration under a PR schedule.
5.2 Material and Methods

5.2.1 Experimental design

Thirty adult and 36 adolescent animals were used initially. Through the experimental procedure, one adult rat died due to infection and 2 adolescent rats were excluded due to catheter blockage.

Adult (PD68) and adolescent rats (PD21) were first food-trained as described in chapter 3. Then they received catheterization surgeries, followed by 5 days of recovery. Animals (adults PD80, adolescents PD31) were initially trained in operant sessions to acquire 30μg/kg nicotine infusion on a FR schedule for 4 days, followed by FR2 for another 4 days. On the last day of FR2 (adult PD89, adolescent PD41), rats were randomly assigned to three groups, and each group received a different nicotine infusion dose from this point on. Ten adults and 11 adolescents received 15μg/kg nicotine per infusion. Another Ten adults and 11 adolescents continued to receive 30μg/kg nicotine per infusion. Nine adults and 12 adolescents received 60μg/kg nicotine per infusion. Subsequently, the reinforcing efficacy of different doses of nicotine was examined using the PR schedule for the next 7 days. On the 5th day and 7th of PR, the effect of 5 min and 10 min intermittent foot-shock was tested, respectively. In the adolescent rats, 0.5mA footshock intensity was used, and in the adults, 0.7mA footshock intensity was used. Footshock was applied in the operant box immediately before the start of the self-administration session. A graphical representation of the experiment procedure is shown in Figure 5.1. The patency of catheters was tested following the completion of PR phase.
5.2.2 Statistical analysis

In FR1 and the first 3 sessions of FR2 where all animals received 30 μg/kg nicotine infusion, two-way repeated ANOVA were used to determine statistical significance. On the last day of FR2 where animals were assigned to different dose groups, two-way ANOVA analysis was used. PR phase was analyzed by three-way repeated ANOVA, with PR sessions as within factor, and Age and Dose of nicotine as between factors. The effect of FSS was evaluated using 3-way repeated measures ANOVA, with Duration of Footshock as the within-subject factor. The baseline was set at the day prior to 5min footshock test. Student-Newmen-Keuls post-hoc analysis was applied where appropriate.
Figure 5.1 Experimental timeline for the footshock experiment. Ages for adolescents were the calculated by taken the average of all the subjects as adolescents were born on two consecutive days.
5.3 Results

5.3.1 Nicotine self-administration on fixed-ratio schedules

Figure 5.2 shows the average number of nicotine reinforcements (30μg/kg/infusion) obtained in each session on FR1 and FR2. Analysis of variance showed that there was no age difference in the number of nicotine reinforcers obtained during FR1 (F(1,62)=1.68, p=0.2), and this lack of age difference was observed across all four sessions (Age×Session, F(3,186)=0.949, p=0.4). However, the overall numbers of reinforcement differed among sessions (p<0.05). During FR2, adolescents obtained significantly fewer nicotine reinforcements compared to adults (F(1,61)=4.32, p<0.05). This age difference is not affected by session which was shown by a lack of age×session interaction (F(2,122)=0.41, p=0.7).

![Nicotine infusions on FR](image)

**Figure 5.2** Number of reinforcements obtained during self-administration of nicotine (30 μg/kg/infusion) by adolescent and adult rats on fixed-ratio schedules (FR1 and FR2). n=9-12 per age and nicotine dose group.

Figure 5.3 shows the average number of nicotine reinforcements obtained on a FR 2 schedule across three different doses, 15, 30, and 60μg/kg/infusion. Analysis of variance showed that
there was a significant effect of age \( (F(1,57)=5.717, p<0.05) \) and dose \( (F(2,57)=3.734, p<0.05) \). However, the effect of age and dose did not interact with each other \( (F(2,57)=1.25, p=0.3) \). Post-hoc analysis revealed that the significant overall age effect is mainly produced by age differences at \( 15 \mu g/kg/infusion \), because at the higher doses, no significant age difference was detected.

**Figure 5.3** Self-administration of nicotine by adolescent and adult rats at different infusion doses of nicotine (15, 30, 60 \( \mu g/kg \)) on an FR2 schedule. \( n = 9-12 \) per age and nicotine dose group. + Significant age difference.

5.3.2 Nicotine self-administration on progressive-ratio schedule

Figure 5.4 shows the average number of nicotine reinforcements obtained and the median break-point in each session on PR schedule in adult and adolescent rats. Panel 5.4A and B display nicotine taking at 15 and 30 \( \mu g/kg/infusion \), respectively. In 5.4A, analysis of variance showed that there was significant effect of age \( (F(1,19)=14.94, p<0.05) \) and session \( (F(4,76)=5.96, p<0.05) \) on number of nicotine reinforcements obtained. However, there was no age×session interaction \( (F(4,76)=2.33, p=0.06) \), which indicates that adults obtained more nicotine infusions than adolescents across all sessions. In 5.4B, similar statistics were observed. The effect of age \( (F(1,19)=18.82, p<0.05) \) and session \( (F(4,76)=9.73, p<0.05) \) on nicotine taking
was significant. The age×session interaction (F(4,76)=2.81, p<0.05) was also significant, which indicates that even though adults obtained more nicotine than adolescents, the age difference in nicotine taking varied with sessions. In 5.4C, which shows drug taking at 60μg/kg per nicotine infusion in adult and adolescents, there was no significant effect of age (F(1,18)=0.56, p=0.5), or age×session interaction (F(4,72)=0.534, p=0.7). However, nicotine taking varied with sessions, indicated by a significant effect of session (p<0.001).

Table 3. Breakpoint (median) achieved by adolescent and adult rats during PR.

<table>
<thead>
<tr>
<th>PR session</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
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</thead>
<tbody>
<tr>
<td>15 μg/kg nicotine infusion</td>
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<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Adolescent</td>
<td>18</td>
<td>6</td>
<td>10</td>
<td>11</td>
<td>13</td>
</tr>
<tr>
<td>Adult</td>
<td>25</td>
<td>15</td>
<td>32</td>
<td>32</td>
<td>40</td>
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<tr>
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<td>60 μg/kg nicotine infusion</td>
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<td>40</td>
<td>40</td>
<td>50</td>
<td>62</td>
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Figure 5.4 Self-administration of nicotine by adolescent and adult rats at different infusion doses of nicotine (15, 30, 60 μg/kg) on a progressive-ratio schedule over 5 sessions. A, B and C show number of reinforcements obtained at nicotine infusion dose of 15, 30 and 60μg/kg, respectively. n=9-12 per age and nicotine dose group. + Significant age difference
5.3.3 Effects of intermittent footshock on PR responding

Intermittent footshock decreased nicotine intake under the PR schedule in both age groups when infusion doses were low. Irrespective of footshock duration, adult rats responded more than adolescents at the 15 and 30 µg/kg nicotine infusion doses, but did not differ at the 60 µg/kg dose. Figure 5.5 presents the mean (±sem) number of infusions, total active lever presses, and inactive lever presses for the footshock dose-response curve. The statistical analyses included the between-subjects factors of Nicotine Dose and Age, and the within-subjects factor of Footshock Shock Duration. Table 4 presents the median breakpoint achieved by adolescent and adult rats after footshock challenge.

**Infusions:** The ANOVA revealed significant effects of Footshock (F(2,104)=16.34, p<0.05), Age (F(1,52)=23.54, p<0.05), Nicotine Dose (F(2,52)=7.85, p<0.05), Age x Nicotine dose interaction (F(2,60)=3.64, p<0.05), and a significant Footshock x Age x Nicotine dose interaction (F(4,104)=3.48, p<0.05).

Because of the significant three-way interaction, statistical analysis was done in each age group. Within adults, there was a significant Footshock x Nicotine dose interaction (F(4,80)=3.07, p<0.05). Post-hoc analysis revealed that 10min footshock, but not 5min decreases nicotine intake at 15 µg/kg dose only. Within adolescents, there was a significant effect of Footshock (F(2,92)=10.68, p<0.05), but no significant Footshock x Nicotine dose interaction (P=0.1)

**Active lever presses:** There were significant effects of Footshock (F(2,104)=11.2, p<0.05) and Age (F(1,52)=13.08, p<0.05).
**Inactive lever presses:** There were significant effects of Footshock (F(2,104)=5.28, p<0.05), Age (F(1,52)=8.84, p<0.05), Nicotine Dose (F(2.52)=3.65, p<0.05), and a significant Footshock x Age x Nicotine dose interaction (F(4,104)=3.13, p<0.05).

Post-hoc group differences within each Age groups and nicotine dose are depicted in Figure 5.5.

**Table 4. Breakpoint (median) achieved by adolescent and adult rats after intermittent footshock challenge under PR**

<table>
<thead>
<tr>
<th>Footshock</th>
<th>Baseline</th>
<th>5 minutes</th>
<th>10 minutes</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><strong>15 μg/kg nicotine infusion</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adolescent</td>
<td>13</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>Adult</td>
<td>40</td>
<td>40</td>
<td>10</td>
</tr>
<tr>
<td><strong>30 μg/kg nicotine infusion</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adolescent</td>
<td>15</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>Adult</td>
<td>77</td>
<td>77</td>
<td>62</td>
</tr>
<tr>
<td><strong>60 μg/kg nicotine infusion</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adolescent</td>
<td>50</td>
<td>50</td>
<td>40</td>
</tr>
<tr>
<td>Adult</td>
<td>50</td>
<td>50</td>
<td>62</td>
</tr>
</tbody>
</table>
Figure 5.5 Effects of intermittent footshock on nicotine self-administration by adolescent and adult rats at different infusion doses of nicotine on a progressive-ratio schedule. A, B, and C show data collected at nicotine infusion dose of 15, 30 and 60 µg/kg, respectively. Left panel, mean (±sem) of number of reinforcements; center panel, active lever presses; right panel, inactive lever presses. n = 9-12 per age and nicotine dose group. # Significantly different from baseline. + Significant age difference.
5.4 Discussion

The design of the current experiment is a partial replication of the previous yohimbine study, with a different stressor, as well as an extension of the Shram’s investigation on age difference of nicotine taking behavior (Shram et al. 2008a). Compared to the earlier experiments, we saw good reproducibility in the data we generated. First of all, in this study, we did not find an age-difference in the number of nicotine infusion obtained when the reinforcing requirement was low (FR1). However, when the reinforcing requirement increased, we observed that adult rats gained more nicotine infusions at lower doses than adolescents. This is consistent with the previous studies with yohimbine and Shram et al (Shram et al. 2008a), concerning the effect of increased workload or demand on motivation for nicotine between adult and adolescent rats.

The current experiment was conducted to investigate the age-differences in nicotine-taking behavior in response to intermittent footshock. Contrary to what we expected, exposure to 5 min intermittent footshock did not increase nicotine self-administration in either adolescent or adult rats as assessed with PR schedule of nicotine reinforcement, while 10 min shock significantly attenuated nicotine self-administration in adults at 15 μg/kg nicotine infusion dose and in adolescents at 30 μg/kg infusion dose during PR. This suppressive effect on nicotine taking thus indicates that duration and intensity of FSS, at least at 10 min, was effective in eliciting a stress effect in both age groups.

It is possible that the FSS at 10min decreased active lever responses and thereby the number of reinforcement obtained possibly because of its inhibitory effect on animals’ general activity. This speculation is supported by a significant decline in inactive lever responding in adult rats at 15 μg/kg nicotine infusion dose and in adolescents at 30 μg/kg infusion dose during PR. It is possible that at the 15 μg/kg dose of nicotine infusion, a suppressive effect of FSS also existed in
the adolescent group. However, as the baseline responding in adolescents was already low at this
dose during PR, it was hard to produce a further reduction on rate of lever presses. This
observation is consistent with the Yerkes-Dodson relationship (Yerkes and Dodson 1908), which
described an inverted U-shape function with stress levels and responses. Nevertheless, Shaham
and colleagues have previously showed that exposure to footshock delivered immediately prior
to the start of the session increased fentanyl oral consumption and intravenous heroin self-
administration during PR schedule in rats (Shaham et al. 1993; Shaham and Stewart 1994). In
addition, footshock stress with similar intensity (0.8mA) and duration (5min and 15min) has
been shown to successfully reinstate nicotine seeking in adult male Long-Evans rats (Buczek et
al. 1999). In the current experiment, the parameters of footshock (0.5mA and 0.7mA, 5min and
10min) were set within the range of those that had never been shown to elicit inhibitory effect on
general behavior; therefore, it is unlikely that the observed inhibition on nicotine self-
administration in both ages is due to a general suppression of behavior induced by the parameters
of the footshock employed in the present study.

Furthermore, if footshock at 10 minutes duration produced a general inhibitory effect on
ongoing behaviors, one would expect the effect to be generalized to various infusion dose
groups. It was, however, not the case: the inhibitory effect in both ages was restricted to lower
doses but not 60 µg/kg during PR. Nicotine has been repeatedly reported to influence anxiety
status in rodents using various models (e.g. Brioni et al. 1994; Cheeta et al. 2001; File et al. 1998;
Irvine et al. 1999). In the social interaction test, it was shown that effects of nicotine on anxiety
were dependent on dose and baseline level of anxiety (File et al. 1998). More specifically, the
acute effects of systematic injection of low dose of nicotine (0.01 and 0.1 mg/kg, i.p.) were
anxiolytic and higher doses (0.5 and 1.0 mg/kg) were anxiogenic under moderate anxiety
conditions; i.e. when anxiety level is either low or high, nicotine was without effect (File et al. 1998). This seems to contradict what we observed, because if high doses of nicotine are anxiogenic, one would predict an inhibition on nicotine intake at 60 μg/kg per infusion due to the potential additive or synergistic effect of footshock and the anxiogenic effect of nicotine. Nevertheless, in File’s study, nicotine was given in a bolus dose, whereas in the current experiment, smaller amounts of nicotine were self-administered by the animals over a 2-hour period. It is possible that at 60 μg/kg, each infusion of nicotine is sufficient to produce anxiolytic effects, which combat the inhibitory effect of footshock. At 15 and 30 μg/kg, however, infusion doses were too low to be anxiolytic. These findings concerning the effects of FSS on nicotine self-administration suggest a complex interaction between stress exposure and nicotine infusion doses.

My previous experiment showed that yohimbine, a pharmacological stressor, was able increase response on the active lever under PR schedule, whereas in the current experiment, an inhibition effect was seen following exposure to footshock. Nevertheless, others have observed that FSS could stimulate opioid self-administration. About 30 years ago, it was first found that rats would self-administer a lethal dose of morphine intravenously when each infusion was immediately following a brief mild shock (3 to 6V for 0.2msec duration or 5 to 13V for 0.02msec duration) (Beck and O'Brien 1980). While the authors themselves did not provide an answer, others have speculated that the steep elevation of morphine intake was caused by association between morphine infusion and release of pain that was learned by rats (Shaham et al. 2003). It is well documented that nicotine has analgesic effects as well (e.g. Damaj et al. 1998; Damaj et al. 1999). However, in the present experiment it is unlikely that the rats would modulate their response during PR session because footshock was delivered immediately prior to
the session but not preceding each infusion. Even though rats may experience the analgesic effect after the first nicotine infusion if they responded promptly enough after the shock, the chance of forming a learned association is low, because in the current experimental design, shock was not coincident with the SA session.

Shaham and colleagues have shown that FSS prior to test session increased heroin self-administration during PR (Shaham and Stewart 1994). We used a very similar design but observed opposite effects of FSS on nicotine taking. One recent study by Matthews and co-workers tested the effect of footshock on alcohol consumption in three different strains of male mice, and they found strong strain differences in response to footshock such that only the C57BL/6J mice but not the others showed increases in plasma corticosterone level and alcohol consumption after exposure to FSS (Matthews et al. 2008). Since Wistar rats were used in Shaham’s study and Long Evans were used in ours, the possibility of strain difference in producing these diverse effects on drug-taking behavior, therefore, cannot be ruled out. It is interesting to note that keeping the strain, the duration and intensity constant, FSS can reinstate nicotine taking (Buczek et al. 1999), which suggest a fundamental difference in the mechanism underlying drug-self administration and reinstatement.

This is the first study designed to investigate the age-difference in the nicotine-taking behavior in response to a physical stressor, inescapable intermittent footshock. By employing different infusion doses and different duration of footshock, we found that footshock, if anything, suppressed nicotine self-administration in a PR schedule at lower nicotine infusion doses while not affecting responding for the higher one. This is quite different from that observed for yohimbine under similar experimental conditions, which showed that yohimbine produced an increase in responding for nicotine across all infusion doses. The fact the FSS has been found to
reinstate responding for various drugs of abuse ranging from cocaine, heroin, alcohol as well as nicotine while having minimal effect on self-administration further pointed out that the mechanisms underlying the effect of stress on drug intake and relapse might be distinct from one another.
CHAPTER 6:
THE EFFECT OF REPEATED SOCIAL DEFEAT ON INITIATION OF NICOTINE SELF-ADMINISTRATION IN ADOLESCENT AND ADULT RATS

6.1 Introduction

In the two previous experiments, I have focused on the effect of acute exposure to stress on nicotine self-administration after stable nicotine self-administration was established; that is, the acute effect of different stressors was tested under a progressive-ratio schedule after the rats had learned to self-administer nicotine. It was found that the effect of intermittent footshock on drug taking behavior was minimal in adult and adolescent rats. On the other hand, the pharmacological stressor yohimbine, an alpha-2 adrenoceptor antagonist, significantly increased the reinforcing efficacy of nicotine in both ages in the same manner. The ability of stress to enhance nicotine taking thus appears to be stressor-specific.

Numerous studies have found that exposure to stress can also facilitate the acquisition of drug self-administration. For example, non-contingent exposure to footshock stress has been reported to increase the acquisition of cocaine self-administration in adult rats (Goeders and Guerin 1994). Stress in the form of social isolation introduced during adolescence has also been shown to facilitate the acquisition of amphetamine (Bardo et al. 2001), cocaine (Howes et al. 2000), and alcohol (Deehan et al. 2007; McCool and Chappell 2009) self-administration. Furthermore, rats subjected to 4 episodes of social defeat stress not only showed elevated rates of cocaine self-administration acquisition, but also achieved higher break-points for cocaine during a PR schedule (Quadros and Miczek 2009; Tidey and Miczek 1997).
The possibility that exposure to stress might enhance the reinforcing effect of nicotine and therefore might also facilitate the acquisition of nicotine has not been studied. In the present experiment, we explored the effects of stress induced by repeated exposure to social defeat (SD) on the initiation of nicotine self-administration in adolescent and adult rats. Social defeat stress was chosen for many reasons. Firstly, social defeat stress is chosen for its ecological and ethological validity (Miczek et al. 2008). Second of all, unlike other stressors, SD has been repeatedly shown to facilitate the acquisition as well as to increase cocaine self-administration (Quadros and Miczek 2009; Tidey and Miczek 1997). More importantly, exposure to only 4 discrete SD episodes over a period of 4 days has been shown to effectively influence cocaine self-administration in adult rats (Tidey and Miczek 1997). Such a condensed time frame is suitable for the purpose of the current experiment, which is set to examine the effect of stress on the initiation of nicotine taking during adolescence, a short period lasting only a few weeks.

6.2 Material and methods

6.2.1 Experimental design

Twenty-two adult and 22 adolescent animals were used. One adolescent and 2 adult rats were euthanized due to infection and 1 adolescent rat was excluded due to catheter blockage prior to the start of nicotine self-administration. Therefore, a total of 20 adult and 20 adolescent rats were included in the analysis.

Adult (PD68) and adolescent rats (PD21) were first food-trained as described in chapter 3, following which they received catheterization surgeries. After 3 to 4 days of recovery, rats were randomly assigned to either be control or to receive social defeat (SD), which generated four
experimental groups: adult control, adult SD, adolescent control, and adolescent SD, n=10 in each group. Then, adult (PD80 – 84) and adolescent rats (PD31 – 35) in the SD group received 4 daily episodes of social defeat followed 30min of social threat, as described above, over a 5-day period (rats did not receive SD on day 3).

On the next day of the last episode of social defeat, animals (adolescent PD36, adult PD85) were trained to acquire nicotine self-administration (30μg/kg/infusion) in operant boxes on a FR1 schedule (1hr session) for 4 days, followed by FR2 for 3 day. The reinforcing efficiency of the nicotine was then tested on PR schedule (2hr session) for the next 4 days. A graphic representation of the experiment procedure is shown in Figure 6.1. The patency of catheters was tested following the completion of PR phase.

6.2.2 Statistical analysis

Data in FR1, FR2 and PR were analyzed separated using 3-way repeated measures ANOVA with Age and SD condition as between-subject factors, and Session as the within-subject factor. Student-Newmen-Keuls post-hoc analysis was applied where appropriate. SPSS and Sigmastat statistical software were used for data analysis.
Figure 6.1 Experimental timeline for the social defeat experiment. Ages for adolescents were the calculated by taken the average of all the subjects as adolescents were born on two consecutive days.
6.3 Results

In every SD session, all intruders were successfully defeated, which was identified by display of submissive posture. However, the latency to defeat in adolescent group was longer compared to that in adults (10min in adolescents vs. less than 2min in adults). After removal from the resident cages, informal assessment revealed that all intruders showed hyperthermia and freezing behavior.

6.3.1 Effect of social defeat on nicotine self-administration on fixed-ratio schedule

Figure 6.2 shows the number of nicotine reinforcements obtained, active lever responses, and inactive lever responses in adult and adolescent rats during FR1.

*Number of infusions:* ANOVA showed that there was an overall effect of session ($F_{3,108}=6.93$, $P<0.05$) and age ($F_{1,36}=10.46$, $P<0.05$). SD stress was not a significant factor on number of infusions obtained ($F_{1,36}=2.064$, $P=0.2$). However, post-hoc analysis revealed significant effect of SD on number of nicotine infusions in adults in the 2\(^{nd}\), 3\(^{rd}\), and 4\(^{th}\) sessions. These results indicate that, overall, adult rats obtained higher number of nicotine reinforcements than adolescent rats; however, within adults, rats subjected to SD obtained lower number of reinforcement than controls.

*Active lever response:* similar result with number of reinforcement was observed with significant effect of session ($F_{3,108}=19.071$, $P<0.05$), and age ($F_{1,36}=5.722$, $P<0.05$), and stress being ineffective on active lever response ($F_{1,36}=3.004$, $P=0.1$). Post-hoc study revealed significant effect of SD on active lever response in adults in the 2\(^{nd}\), 3\(^{rd}\), and 4\(^{th}\) sessions.
**Inactive lever response:** analysis of variance showed that there was an overall effect of age (F$_{1.36}$=7.819, P<0.05) and stress (F$_{1.36}$=5.438, P<0.05) on inactive lever response.

Figure 6.3 shows the number of nicotine reinforcement, active lever response, and inactive lever response in adult and adolescent rats during FR2.

**Number of infusions:** analysis of variance showed that number of reinforcement differed with session (F$_{2.72}$=6.575, P<0.05) and age (F$_{1.36}$=39.489, P<0.05), but not stress (F$_{1.36}$=2.269, P=0.1). The result indicates that adult rats obtained more nicotine reinforcement during the FR2 schedule than adolescent rats.

**Active lever response:** there was an overall effect of session (F$_{2.72}$=3.961, P<0.05), and age (F$_{1.36}$=23.101, P<0.05). Again, stress was not a significant factor on active lever response (F$_{1.36}$=1.438, P=0.2).

**Inactive lever response:** analysis of variance showed that inactive lever response was not affected by age (F$_{1.36}$=0.657, P=0.4), stress (F$_{1.36}$=1.748, P=0.2), or session (F$_{2.72}$=1.481, P=0.2).
Figure 6.2 Self-administration of nicotine by adolescent and adult rats on an FR1 schedule over 4 sessions. Top to bottom: Means (±sem) of number of reinforcements, active lever response, and inactive lever response during self-administration of 30μg/kg/infusion nicotine. n=10 per age in each SD and control group. † Significantly effect of SD stress.
Figure 6.3 Self-administration of nicotine by adolescent and adult rats on an FR2 schedule over 3 sessions. Top to bottom: Means (±sem) of number of reinforcements, active lever response, and inactive lever response during self-administration of 30μg/kg/infusion nicotine. n=10 per age in each SD and control group.
5.3.2 Effect of social defeat on nicotine self-administration on progressive-ratio schedule

Figure 6.4 shows the average number of nicotine reinforcement obtained and the median break-point in each session on PR schedule in adult and adolescent rats. Analysis of variance showed that there was an overall effect of session (F$_{3, 108}=7.87$, P<0.05), and age (F$_{1, 36}=18.01$, P<0.05), but we did not observe an effect of social defeat stress on the number of reinforcement obtained during PR (F$_{1, 36}=3.6$, P=0.7). Table 5 presents median breakpoint achieved by adolescent and adult rats in the control and defeat group.

<table>
<thead>
<tr>
<th></th>
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<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
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<td><strong>Adolescent</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
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<td>18</td>
<td>18</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Defeat</td>
<td>20</td>
<td>18</td>
<td>13</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td><strong>Adult</strong></td>
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<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>59</td>
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<td>40</td>
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</table>
Figure 6.4 Self-administration of nicotine (30μg/kg/infusion) by adolescent and adult rats on a progressive-ratio schedule over 4 sessions. n=10 per age in each SD and control groups.
6.4 Discussion

In the present study, the effect of social defeat stress on nicotine taking behavior was tested in adult and adolescent rats. Unlike the previous experiments, where the acute effect of yohimbine and intermittent footshock on the reinforcing efficacy of nicotine was the measured, this time we focus on the initiation of nicotine taking following 4 episodes of social defeat.

The results are not consistent with our prediction. Instead of facilitating initiation of nicotine self-administration, we found that exposure to four episodes of SD prior to the initiation of nicotine self-administration slightly suppressed nicotine taking in adults rats at FR-1 and FR-2, while having no effect on adolescent rats. Similar to the findings from the previous two experiments, adult rats self-administered more nicotine than adolescent rats on a PR schedule at the 30 ug/kg/infusion. There were, however, no demonstrable effects of prior exposure to SD on nicotine self-administration under the PR schedule in either age group. In contrast to pervious studies, an age difference in number of infusions obtained appeared at FR1. During FR2 and PR, SD was largely ineffective in both ages, while the age difference in level of nicotine taking persisted.

Our findings are different from those that have been reported for cocaine self-administration (Quadros and Miczek 2009; Tidey and Miczek 1997). In their early studies, Miczek and colleagues demonstrated that rats with previous exposure to daily SD over 4 consecutive days acquired cocaine self-administration in about half the amount of the time required for non-defeated rats when cocaine access occurred in close temporal proximity with SD (Tidey and Miczek 1997). In a later study, the same group of investigators (Quadros and Miczek 2009) examined the effect of SD on cocaine self-administration 20 days after the last episode of SD, in which SD was given every 3 days for a total of 4 times. They found that the SD rats achieved
higher breakpoints for cocaine on a progressive ration schedule than the control. It appears that prior exposure to SD increases the reinforcing effect of cocaine which maintains the drug-taking behavior. In the present study, SD was conducted on alternating days and initiation of nicotine self-administration was carried out one day after the last episode of SD. Such a condensed schedule was used because we wanted to examine the effect of SD during the adolescent period. We observed a slight suppressive effect of SD on nicotine self-administration during FR1 and FR2, whereas SD did not alter PR responding in either age group. It is unlikely that such differences in SD exposure schedule would lead to the absence of facilitation or enhancing nicotine self-administration. Therefore, drug specificity might be a critical factor in determining the outcome of a particular self-administration behavior in response to prior exposure to SD defeat. Another possible explanation for the lack of enhancing effect of SD on the acquisition of nicotine-taking might be that maximal nicotine-taking was observed in control group; therefore, SD could not further increase intake level due to ceiling effect. This is, however, also unlikely, because in both age groups, nicotine intake level was the highest on the first day of FR1, and showed decreases from the 2nd day on. Social defeat could not maintain nor stimulate higher level of responses in either adult or adolescents.

To our knowledge, very few studies have examined the effect SD in adolescent rats. Watt et al showed that exposure to repeated-SD during adolescence (PD35) greatly impacted the behavioral and neurochemical responses in adulthood (Watt et al. 2009). Consistent with his work, we found that adolescent rats (PD31) received and responded to social defeat in a similar manner with adult rats, such that they were successfully defeated by aggressive rats and displayed submissive posture. Therefore, the absence of any observable effects of SD on nicotine self-administration in adolescent rats is rather surprising. It should be noted that in control groups, age difference in nicotine taking appeared during FR1, which was not observed in the
two previous studies. It might be possible that level of nicotine taking in adolescent control rats was exceptionally low, therefore, concealing the reduction in nicotine intake in adolescent SD group.

In summary, unlike cocaine, the present study demonstrated that exposure to defeat did not facilitate nicotine self-administration in either adolescent or adult rats. It is possible that the effect of SD on the acquisition of drug self-administration is dependent on the drug being examined. We did not observe an enhanced sensitivity to the effect of social stress in adolescents. Instead, prior exposure to social defeat slightly suppressed nicotine self-administration in adult rats, but did not change it in adolescents.
In the current studies, the effects of different types of stressors were examined on the acquisition and maintenance phases of nicotine self-administration behavior in adult and adolescent male rats. Through comparing and contrasting among current experiments and previous investigations on the effect of stress on drug self-administration, we found that factors such as stressor type, drug type and drug dose are all critical determinants of the effect of stress on drug taking and seeking behaviors. In the following section, the effect of each factor will be analyzed and elaborated upon by comparison with relevant studies.

7.1 Stressor-specificity in the acute effect of stress on drug self-administration

In the current studies, the acute effect of intermittent footshock and yohimbine on nicotine taking was tested after self-administration behavior was established. We found that only yohimbine profoundly increased nicotine self-administration while footshock slightly suppressed it. This divergence in the direction of their effects, however, is not surprising. The acute effect of stressors, such as intermittent footshock and social defeat, on drug self-administration has been examined across different drug types including psychostimulants, opioids, and alcohol, but the results are mixed. For example, in the case of social defeat, effects differ with the drugs being tested (Funk et al. 2005; Miczek and Mutschler 1996; van Erp and Miczek 2001). On the other hand, acute exposure to footshock generally increases opioid self-administration (Shaham et al. 1993; Shaham and Stewart 1994), but its effect on alcohol consumption is quite inconsistent using the two-choice paradigm (Ng Cheong Ton et al. 1983; Volpicelli et al. 1990). More
importantly, when footshock did increase drug self-administration, the effect was usually minimal (Matthews et al. 2008; Shaham and Stewart 1994). So far, yohimbine has been reported to be the only stressor that consistently and profoundly increases operant alcohol self-administration after acute exposure (Le et al. 2009; Le et al. 2005; Marinelli et al. 2007); the current experiment further extends the enhancing effect of yohimbine to nicotine self-administration. Thus, across different drug types, a stressor-specificity exists in the acute effect of stress on drug-self-administration.

This observation, however, is intriguing in a sense that all stressors induce behavioral signs of anxiety as well as sympathetic and adrenocortical activation in animals (Baldwin et al. 1989; Hajos-Korcsok et al. 2003; Haller and Bakos 2002; Haller et al. 1999; Hesketh et al. 2005; Korte and De Boer 2003). Glucocorticoid release has been proposed to be the mediator of stress induced change in the mesolimbic reward system (Dunn 1988; Kalivas and Duffy 1989; Piazza et al. 1996b; Thierry et al. 1976; Tidey and Miczek 1996), and therefore enhance the reinforcing property of drugs (see review by Piazza and Le Moal 1998). Therefore, if the ability to trigger glucocorticoid release is the sole requirement to enhance drug, in this case nicotine self-administration, one would predict that all stressors will have a stimulating effect. This is, however, not the case. A recent experiment by Marinelli et al (2007) pointed out the importance of the extrahypothalamic CRF system in the effect of yohimbine on alcohol taking, because systemic infusion of the CRF1 receptor antagonist, antalarmin, abolished yohimbine-induced increases in operant alcohol self-administration without changing yohimbine induced glucocorticoid release (Marinelli et al. 2007). Thus, it is very likely that yohimbine mediates its stimulating effect on alcohol self-administration through the extrahypothalamic CRF system, not the HPA axis. It is well known that extrahypothalamic CRF is crucially involved in footshock induced drug-reinstatement, because adrenalectomy has no effect on footshock stress induced
reinstatement of heroin (Shaham et al. 1997) and alcohol seeking (Le et al. 2000), but a CRF receptor antagonist attenuated footshock stress induced heroin (Shaham et al. 1997), cocaine (Erb et al. 1998), alcohol (Le et al. 2000) and nicotine (Zislis et al. 2007b) reinstatement. Therefore, if yohimbine increased nicotine self-administration through activation of extrahypothalamic CRF, the same phenomenon would likely be observed with footshock because of its effect on extrahypothalamic CRF system. Nevertheless, CRF-like activity is widely expressed in the brain (Charlton et al. 1987; Swanson et al. 1983). The areas responsible for stimulating drug self-administration might be different from that mediating the reinstatement. This highlights the differences in mechanisms underling stress enhanced self-administration vs. stress induced reinstatement, which will be further discussed in section 7.3. Furthermore, different stressors may elicit a unique activation pattern of extrahypothalamic CRF, which could be the reason that not all stressors could increase drug self-administration behavior.

Yohimbine could also influence nicotine self-administration through its modulatory effect on serotonin system transmission. There are many subtypes of serotonin receptor in the brain, such as 5-HT1A, 1B, 1D, 5-HT2A, 2B, 2C and 5-HT3. Yohimbine displays marked affinity to the 5-HT1A receptor (Millan et al. 2000), which is primarily expressed in the dorsal raphe nucleus (DRN) and the median raphe nucleus (MRN), functioning as somatodendritic autoreceptors that decreases serotonin release when activated (Marcinkiewicz et al. 1984; Verge et al. 1986). 5-HT1A receptors are also expresses post-synaptically in other brain areas such as hippocampus, septum, amygdala and cortical regions, although the density in these regions is low (Marcinkiewicz et al. 1984; Pompeiano et al. 1992; Verge et al. 1986). Yohimbine has agonistic effect on 5-HT1A receptor (Millan et al. 2000), and it was shown that blocking the 5-HT1A receptor attenuates yohimbine induced increases in alcohol self-administration in rats (Le et al. 2009), presumably because of its suppressive effect on serotonin release. The involvement of 5-
HT1A receptor in development of nicotine dependence has been suggested (see review Fletcher et al. 2008), but no studies have examined the role of serotonin in nicotine self-administration and reinstatement. The current study has demonstrated that yohimbine, a 5-HT1A agonist, profoundly increases nicotine self-administration in rats. This suggests a possible role of serotonin in controlling the sensitivity to the reinforcing effect of nicotine during self-administration.

Among all three stressors, yohimbine has been found to be the only one that significantly increased nicotine self-administration in rats. This raises the question of whether the effect of yohimbine on nicotine taking behavior is mediated via its effect on stress. As previously mentioned, yohimbine can bind to many neurotransmitter receptors with moderate affinity. Therefore, it is possible that other than its ability to induce stress state, yohimbine might stimulate nicotine taking by activating different neurotransmitter systems, such as the 5-HT system, or a combination of both.

7.2 Drug-specificity in self-administration behavior with prior exposure to repeated social stress

A lot of work has been done on interactions between chronic/repeated social stress and cocaine taking behavior. For example, it was shown that exposure to chronic social isolation during adolescence increased acquisition of cocaine self-administration in adult rats (Howes et al. 2000). Miczek’s group also showed that repeated exposure to social defeat reliably facilitates initiation and elevated breakpoint of cocaine self-administration (Covington et al. 2008; Quadros and Miczek 2009; Tidey and Miczek 1997). Using a very similar schedule of social defeat with Miczek’s studies, however, we did not find a stimulating effect of SD on nicotine self-
administration during initiation or maintenance. This discrepancy between studies suggests that the effect of prior exposure to repeated stress on behaviors maintained by reinforcing property of drug depends on the type of drug tested.

So far, it is not known how repeated stress increases self-administration of psychostimulants. However, possible mechanisms have been proposed. Repeated stress is suggested to impair glucocorticoid negative feedback which causes high basal glucocorticoid levels (Maccari et al. 1991; Maccari et al. 1995). Because administration of glucocorticoids could induce mesolimbic dopamine release (Piazza et al. 1996b), high levels of glucocorticoids enhance dopamine release, and therefore sensitize animals to the reinforcing properties of drugs (see review Piazza and Le Moal 1998). On the other hand, repeated social stress may also increase the rewarding effects of drugs by engendering neuroadaptations in the mesolimbic dopamine system in a glutamate-receptor dependent manner (Covington et al. 2008). No matter how repeated stress changes dopamine release, the involvement of the mesolimbic dopamine system in the repeated-stress-induced augmentation of drug-taking is likely. It is therefore surprising to see the inconsistency between Miczek’s and the current study, giving that the rewarding properties of cocaine and nicotine are both mediated through the mesolimbic dopamine pathway. However, it appears that repeated/chronic social stress facilitates the initiation of cocaine self-administration when the cocaine dose is low, decreased or does not change it when the dose was high (Howes et al. 2000; Tidey and Miczek 1997). A similar dose-response curve response to social defeat may also exist for nicotine self-administration, and it is possible that this dose-response curve is shifted between adult and adolescents. In the current experiment, we only examined the effect of social defeat on nicotine taking at 30μg/kg/infusion, which has been shown to be the optimal dose that sustains nicotine self-administration behavior in rats (Corrigall and Coen 1989). However, from previous
experiments and on-going studies in our lab, we know that nicotine self-administration behavior can also be sustained at much lower doses (15 or 7.5μg/kg/infusion). Therefore, it would be reasonable to further investigate the changes in nicotine self-administration in response to repeated social defeat on a spectrum of nicotine infusion dose.

7.3 Different mechanisms underlying stress enhanced self-administration and stress induced reinstatement

Previously, I have discussed that stress influences drug taking behavior differently depending on the specific stressor and drug used. However, even with the same stressor and drug, the effect of stress can still vary with the behavioral endpoint being measured. For instance, the ability of footshock to induce nicotine reinstatement has been repeatedly demonstrated (Buczek et al. 1999; Zislis et al. 2007b), but the current experiment showed that footshock did not enhance nicotine self-administration after acute exposure. This observation is consistent with studies on alcohol taking. When rats were given choice between water and alcohol, footshock has been shown to increase, decrease or not change the preference for alcohol (Ng Cheong Ton et al. 1983; Volpicelli et al. 1990). With the self-administration paradigm, one study showed that acute exposure to footshock elevates alcohol taking during maintenance in mice; however, the increase was minimal and mainly due to a significant decrease in alcohol taking in the control group (Matthews et al. 2008). On the other hand, footshock has been shown to reliably reinstate alcohol-seeking behavior after extinction (Le et al. 1999; Le et al. 1998). It appears that different mechanisms underlie the effect of stress on nicotine/alcohol-self administration and reinstatement. However, yohimbine was an exception to this; it can potently enhance nicotine and alcohol self-administration as well as induce nicotine and alcohol reinstatement.
7.4 Age difference in nicotine self-administration behavior in response to stress

As previously discussed, the stress system is a crucial component in mediating the reinforcing property of drugs of abuse. Some studies showed that chronic stress during adolescence impairs the adult stress system (Goliszek et al. 1996; Isgor et al. 2004; Le et al. 1999; Le et al. 1998; Pohl et al. 2007), and exposure to social stress during the adolescent period could enhance self-administration of psychostimulants in adulthood (Bardo et al. 2001; Howes et al. 2000). Responses to stress during adolescence have been examined as well. Some reported that after acute stressor exposure, corticosterone levels were higher and prolonged in adolescents compared to adults (Cruz et al. 2008; Goldman et al. 1973; Romeo et al. 2006b; Romeo et al. 2004a; Romeo et al. 2004b), but other studies reported a lower level of corticosterone in adolescents (Hodes and Shors 2005). After repeated restraint stress, high corticosterone level was maintained for a longer time in adult male rats than adolescents (Romeo et al. 2006a). It appears that acute response to stress does differ between ages.

The current experiment was designed to examine the effect of acute or repeated stress on different aspects of nicotine self-administration behavior in adult and adolescent rats. We have hypothesized that adolescents may be more sensitive to the reinforcing effect of nicotine after stress exposure compared to the adults. However, we found that yohimbine-induced increases in nicotine-taking were slightly, but significantly higher in adults compared to adolescents. In addition, we did not find any significant age differences in nicotine taking behaviors in response to intermittent footshock or social defeat. To our knowledge, these are the first studies that measure the effect of stress on rats during the adolescent period using a self-administration paradigm, while some other studies have looked at the long-term effect of stress on adolescents when they become adults (Howes et al. 2000; Moffett et al. 2006). This makes direct comparison between out results and other studies rather difficult.
7.4.1 Lack of age difference in the initiation of nicotine self-administration behavior in response to repeated social stress

In the social defeat study, both adolescent and adult rats were trained to acquire nicotine self-administration at 30μg/kg, and we did not observe that prior exposure to social defeat facilitates acquisition of nicotine taking in either age. It was previously shown that maximal nicotine responding occur at 30μg/kg in adult male rats (Corrigall and Coen 1989), which was also true in adolescent rats (Shram et al. 2008a). Therefore, it is possible that at this dose, both ages responded to nicotine maximally, so further increases were restricted by ceiling effects. The ceiling effect here most likely reflects the maximal total infusion of nicotine that a rat can tolerate within unit time, as we saw that adult rats can modulate their rate of nicotine intake according to infusion dose during FR schedule (Shram et al. 2008a). However, ceiling effect could also refer to that the positive and negative effects of nicotine during initial self-administration sessions is at its optimal at 30μg/kg/infusion; therefore, exposure to stress would not further improve this subjective feeling of nicotine or promote responding during initiation.

Another factor that may influence acquisition of nicotine self-administration after stress is the use of food-training procedure. Stress may sensitize the animals to the rewarding effect of nicotine, so rats exposed to stress would learn to associate active lever pressing with nicotine delivery at a faster rate than controls. However, in the current study, adolescent and adult rats have already learnt to lever press through food-training, which could conceal this stress-facilitated learning process.
7.4.2 Age difference in the effect of yohimbine on nicotine taking after establishment of self-administration behavior

It is interesting to note that yohimbine extensively elevates number of nicotine infusion during progressive-ratio schedule, but the level of increase did not differ with doses of yohimbine administered. Since the effect of yohimbine on nicotine taking was so profound, it is possible that active lever responses could not be increased further due to restriction of length of PR session or physical ability of rats. The latter could be especially true of adolescent rats. Because of their smaller body size, they might not be able to produce lever responses as well as the adults under the high-demand schedule, despite elevated sensitivity to the reinforcing effect of nicotine after stress exposure. It seems that at the two doses of yohimbine we chose, increases in nicotine self-administration behavior reached a plateau, and even if the yohimbine increased the reinforcing effect of nicotine differently with age, we would not be able to detect it using the current paradigm. Therefore, a lower dose of yohimbine is suggested to be used to further elucidate the effect of acute stress on nicotine self-administration in adolescent and adult rats.

7.4.3 Potential effect of single-housing on nicotine self-administration and nicotine self-administration in response to stress in adolescent and adult rats

In the current experiment, adolescent and adult rats were single-housed immediately after they received intravenous catheter implantation. This is to protect the plastic catheter from being damaged by other rats if they were to be kept group-housed. However, this precautionary measure could confound the results observed. Compared to adult rats, adolescents spend significantly more time in social interactions and play behaviors with their peers (Panksepp
This friendly play behavior during adolescence serves as practice for adult sexual and aggressive behavior (Thor and Holloway 1984). When exposed to an environment that lacks opportunity for social interaction, i.e. social isolation, during adolescence, stress responses were altered in adulthood (McCormick et al. 2008; van den Berg et al. 1999). Furthermore, chronic social isolation stress during adolescence also elevated sensitivity to the addictive properties of drugs (Kabbaj et al. 2002; McCormick et al. 2004), as well as increases psychostimulant self-administration as adults (Bardo et al. 2001; Howes et al. 2000). In the current experiment, rats were single housed prior and throughout the self-administration procedure. Therefore, it is not known how housing condition have affected the initiation and maintenance of nicotine self-administration behavior in adolescent and adult rats. Further, it is also not clear whether housing conditions interacted with the stressor that we introduced, and together, how they affect the nicotine self-administration behavior in both ages.

7.5 Summary and conclusion

Experiments discussed in the current thesis were designed to test the effect of acute and repeated stress on the initiation and maintenance phase of nicotine self-administration. A possible age-difference in nicotine taking behavior in response to stress was also examined. We found that the effect of stress on nicotine taking behavior differs depending on the stressor type as well as the specific behavioral endpoints being measured. Among all the stressors tested, yohimbine seems to be the only one that profoundly increased nicotine consumption at both ages. In addition, we did not find an elevated reinforcing effect of nicotine in adolescent rats compared to adults after stress exposure. This negative result, however, does not rule out the importance of stress in the development of addiction during adolescence.
As discussed in Chapter 1, human adolescents experience more stressful life events than other ages. The current study found that certain form of stress can increase the reinforcing effect of nicotine in both adult and adolescent rats. Therefore, an alternative explanation for the increased rate of smoking initiation during adolescence could be that, increased incidences of stressful life events, rather than increased sensitivity to stress, makes human adolescents more vulnerable to initiate smoking.
CHAPTER 8: FUTURE STUDIES

8.1 Effect of acute stress on level of nicotine self-administration during maintenance in adolescent and adult rats.

Stress has been shown to increase nicotine self-administration in both adolescent and adult rats, but as the current experiments demonstrated, the effect of footshock during the maintenance of nicotine taking is rather minimal. Therefore, to continue to investigate the effect of stress on nicotine self-administration, yohimbine may be a more appropriate acute stressor. However, to prevent the ceiling effects, a dose of yohimbine lower than 0.625mg/kg could be used. Ongoing experiments in our laboratory demonstrate that yohimbine at 0.3mg/kg increases breakpoints in nicotine self-administration in female, but not male adolescent rats. It would be interesting to see how yohimbine at this dose affects nicotine-taking behavior in adult female and male rats.

8.2 Effect of repeated social defeat on the initiation of nicotine self-administration in adolescent and adult rats

With previous experiments consistently demonstrating that repeated exposure to social defeat facilitates initiation of cocaine self-administration, it is a surprise to see that prior exposure to this stressor did not increase acquisition of nicotine taking in the current experiment. This may be because rats responded for nicotine at their maximal rate during the initiation phase even without prior exposure to stress. In order to reveal the effect of stress on initiation of nicotine self-administration, response rate could be lowered. This can be done by either decreasing the dose of nicotine infusion or removing the food-training procedure.
Lower nicotine dose: past studies showed that lower nicotine doses led to lower response rates during self-administration on a fixed-ratio schedule in adult rats. Ongoing experiments in our lab demonstrate that adolescents readily acquire nicotine self-administration behavior at 7.5\( \mu \text{g/kg/infusion} \), but the response rate is much lower than at the higher doses (15 and 30 \( \mu \text{g/kg/infusion} \)). Therefore, a lower nicotine infusion dose could be used to test how repeated social stress impacts initiation of nicotine taking in adolescent and adult rats.

No food training: in the current experiments, all rats were food-trained to learn bar-press behavior before nicotine self-administration started. Shram et al (2008b) have demonstrated that during spontaneous acquisition, a term that describes initiating self-administration without food-training, the number of nicotine infusions obtained in each session increases with session, whereas when animals were previously food-trained, number of nicotine infusions obtained was stable across session during initiation (Shram et al. 2008a). This shows that removal of the food-training procedure could lower the initial response rate, and therefore help to elucidate the effect of prior exposure to stress.

8.3 Effect of single-housing on nicotine self-administration in adult and adolescent rats

In the current studies, adolescent and adult rats were both single-housed starting after surgery until the end of the experiments. The housing condition is used to protect the catheters that were implanted. It could, however affect nicotine self-administration behavior, because social isolation is considered as a stressor in rats. As previously discussed, adolescent rats display more peer interactions and social play behavior; therefore, the effect of single housing is probably more prominent in this age group. To assess the effect of single-housing on subsequent nicotine
self-administration behavior in adult and adolescent rats, a group of rats that are double-housed should be included as control. Moreover, in order to prevent catheters from being damaged by another rat, metal protectors that shield the catheter would be used in all the rats.
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