Examination of Age Differences in Incentive Motivation and Impulsivity as Possible Contributing Factors to a Susceptibility to the Effects of Drugs of Abuse during Adolescence

by

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A thesis submitted in conformity with the requirements for the degree of Doctorate of Philosophy
Psychology
University of Toronto

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Abstract

Rationale. Adolescence may be a period of susceptibility to the effects of drugs of abuse. This vulnerability may result from a convergence of psychological processes that contribute to drug addiction including impulsive action and incentive motivation during adolescence. Objectives. I examined age differences in incentive motivation, as measured by responding for a conditioned reinforcer (CR) previously paired with natural or drug rewards, and age and sex differences in impulsive action, as measured by responding on a differential reinforcement of low rates of responding (DRL) schedule or premature responding on the 2-Choice Serial Reaction Time Test (2-CSRTT), in Sprague-Dawley rats. The effects of drugs that affect these behaviours in adulthood and that act on neurochemical systems still developing during adolescence were also examined. Methods. In a first set of experiments (Chapter 3), I compared male adolescents and adults on responding for a CR previously paired with sucrose and the effect of amphetamine on this behaviour. In a second set of experiments (Chapter 4), I examined age differences in responding for a CR previously paired with passive or self-administered intravenous (IV) nicotine infusions. Subsequently, I investigated age and sex differences in responding on a DRL schedule in response to amphetamine (Chapter 5) and 2-CSRTT performance in response to
amphetamine, nicotine and Ro 63-1908 (a glutamate N-Methyl-D-aspartic acid [NMDA] receptor subunit antagonist; Chapter 6). Results. Compared to adults, adolescents responded more for a CR previously paired with sucrose or passive, but not self-administered, IV nicotine infusions. Amphetamine only enhanced responding for a CR previously paired with sucrose. Adolescents responded more than adults on a DRL schedule, while adolescents made the most premature responses in the 2-CSRTT. No consistent sex differences were observed during the acquisition of either behaviour. Amphetamine increased premature responding most in adolescent males and adult females in the 2-CSRTT but not in responding on the DRL schedule. No consistent age or sex differences were observed for Ro 63-1908 or nicotine. Conclusions. Adolescents show enhanced impulsivity and incentive motivation than adults depending on the behavioural measure. Dopamine may contribute to age and sex differences in these behaviours.
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<th>Description</th>
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<tbody>
<tr>
<td>AMPA</td>
<td>(2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl)propanoic acid)</td>
</tr>
<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
</tr>
<tr>
<td>BLA</td>
<td>basolateral amygdala</td>
</tr>
<tr>
<td>cAMP</td>
<td>cyclic adenosine monophosphate</td>
</tr>
<tr>
<td>CCAC</td>
<td>Canadian Council on Animal Care</td>
</tr>
<tr>
<td>CPA</td>
<td>conditioned place aversion</td>
</tr>
<tr>
<td>CPP</td>
<td>conditioned place preference</td>
</tr>
<tr>
<td>CRF</td>
<td>continuous reinforcement</td>
</tr>
<tr>
<td>CS</td>
<td>conditioned stimulus</td>
</tr>
<tr>
<td>CTA</td>
<td>conditioned taste aversion</td>
</tr>
<tr>
<td>DAT</td>
<td>dopamine transporter</td>
</tr>
<tr>
<td>DOPAC</td>
<td>3,4-dihydroxyphenylacetic acid</td>
</tr>
<tr>
<td>DRL</td>
<td>differential reinforcement of low rates of responding</td>
</tr>
<tr>
<td>DSM-IV</td>
<td>Diagnostic and statistical manual of mental disorders, 4th edition</td>
</tr>
<tr>
<td>FR</td>
<td>fixed ratio</td>
</tr>
<tr>
<td>FSH</td>
<td>follicle-stimulating hormone</td>
</tr>
<tr>
<td>GABA</td>
<td>gamma-aminobutyric acid</td>
</tr>
<tr>
<td>GnRH</td>
<td>gonadotropin-releasing hormone</td>
</tr>
<tr>
<td>HPG</td>
<td>hypothalamic-pituitary-gonadal</td>
</tr>
<tr>
<td>IP</td>
<td>intraperitoneal</td>
</tr>
<tr>
<td>IRT</td>
<td>inter-response time</td>
</tr>
<tr>
<td>ITI</td>
<td>inter-trial interval</td>
</tr>
</tbody>
</table>
IV intravenous
LH luteinizing hormone
m-IRT mean inter-response interval
nAChR nicotinic acetylcholine receptors
NMAD N-methyl-D-aspartic acid
NET norepinephrine transporter
OFC orbitofrontal cortex
PFC prefrontal cortex
PLC phospholipase C
PND postnatal day
PR progressive ratio
RR random ratio
RT random time
SAMHSA the substance abuse and mental health services administration
SC subcutaneous
SEM standard error of the mean
SSRT stop-signal reaction time
T time
US unconditioned stimulus
VTA ventral tegmental area
2-CSRTT 2-Choice Serial Reaction Time Test
5-CSRTT 5-Choice Serial Reaction Time Test
5-HT serotonin (5-hydroxytryptamine)
Chapter 1: General Introduction

Adolescents may be more susceptible than adults to some of the effects of drugs abuse. In humans, recreational drug use typically begins during adolescence (Degenhardt et al., 2008; Kandel, Yamaguchi, & Chen, 1992; SAMHSA, 2007; Wagner & Anthony, 2002) and adolescents may progress from drug use to drug abuse more rapidly than adults (Deas, Riggs, Langenbucher, Goldman, & Brown, 2000; Estroff, Schwartz, & Hoffmann, 1989). However, the factors that contribute to age differences in the effects of drugs of abuse are still unclear. Adolescence is a developmental period when many psychological, biological and social factors that are linked to drug abuse and addiction converge. For example, adolescents are described as impulsive and prone to risky behaviours (Spear, 2000; Steinberg, 2004), including experimenting with drugs of abuse, which may result from age differences in reward processing, aspects of motivation and response inhibition. Behaviour during adolescence is likely shaped by the continued development of neuronal circuits that mediate these behaviours (see Chambers, Taylor, & Potenza, 2003; Ernst, Romeo, & Andersen, 2009 for review). Both motivation and impulsivity play important roles in drug addiction (Dalley, Everitt, & Robbins, 2011; Robinson & Berridge, 1993; Winstanley, Olausson, Taylor, & Jentsch, 2010), thus, these two psychological processes (among others) may contribute to a susceptibility to the onset of drug use and abuse during adolescence. However, relatively few empirical studies have directly compared adolescents and adults on measures of motivation and impulsivity. Therefore, the focus of my doctoral work was to examine age differences in impulsivity and aspects of motivation in rats as a stepping stone to understand how these behavioural processes may contribute to the onset and progression of recreational drug use during adolescence in humans.

Adolescence

Adolescence is the developmental period between childhood and adulthood, generally accepted to occur from the ages of 12-18 in humans (Spear, 2000). Although some believe that
adolescence is unique to humans (Bogin, 1994), a similar developmental period appears to exist in other mammals such as non-human primates and rodents. Generally, the adolescent period in non-human primates spans from years two to four (Lewis, 1997). In rodents, no absolute consensus exists on the specific age range that constitutes the adolescent period. Some use the more conservative age range of postnatal days (PNDs) 28-42 which encompasses the period immediately prior to and just after the onset puberty (Collins, Montano, & Izenwasser, 2004; Spear, 2000; Spear & Brake, 1983) while others have divided the adolescent period into early (PND 24-35), middle (PND 36-48) and late adolescence (PND 49-60, e.g., Adriani, Macri, Pacifici, & Laviola, 2002; Tirelli, Laviola, & Adriani, 2003). The broadest age range is from PND 21, the typical age of weaning, to PND 60 when rats reach young adulthood (Spear, 2000; Tirelli et al., 2003).

Across species, one hallmark of the adolescent period is puberty. Although the terms adolescence and puberty are often used interchangeably, they are not synonymous (Sisk & Zehr, 2005; Spear, 2000). Puberty refers to a period of sexual development while adolescence encompasses puberty and also includes psychological and social maturation (Sisk & Zehr, 2005). Physical markers are typically used to denote the onset of puberty. During puberty there is a rise in gonadal sex hormones including estrogen and progesterone primarily in females and testosterone primarily in males. Puberty is mediated by the system that regulates the release of these sex hormones: the hypothalamic-pituitary-gonadal (HPG) axis. The onset of puberty is thought to be triggered by nocturnal pulses of gonadotropin-releasing hormone (GnRH) from the preoptic area of the hypothalamus which act on the anterior pituitary to trigger the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH). These hormones elicit the release of testosterone from the testes and estrogen and progesterone from the ovaries (Harris & Levine, 2003; Matsumoto, Karpas, Southworth, Dorsa, & Bremner, 1986; Sisk, Richardson, Chappell, & Levine, 2001; Urbanski & Ojeda, 1987).
Sex hormones produced and released from the gonads activate several biological changes associated with puberty. These include the onset of secondary sexual characteristics such as growth of body hair, the onset of menarche and enlargement of breasts and in females and growth of the larynx in males. During puberty, adolescents undergo a growth spurt which may result, in part, from hormonal changes (Wieland, Chen, Zorn, & Hallberg, 1971; Zachmann et al., 1986). Adolescents also consume more calories as a function of body weight compared to adults (Post & Kemper, 1993) which may play a role in the pubertal growth spurt. In addition to height and body weight, body composition changes during puberty. Males typically gain lean muscle mass and lose body fat, while females gain body fat which is re-distributed to areas including the breasts and the hips. Sex hormone secretion during puberty also affects brain development as discussed below in section 4.1.1.

Adolescence is a developmental stage that is not only biologically, but psychologically and socially distinct from childhood and adulthood (Spear, 2000). Humans transition from childhood, a period characterized by dependence on parents and family, into adolescence, a period of increased independence when individuals become sexually mature and begin to adopt adult roles. Many aspects of behaviour change during adolescence including emotionality, social interactions, cognitive abilities, and sleeping patterns (Burnett, Sebastian, Cohen Kadosh, & Blakemore, 2011; Burnett, Thompson, Bird, & Blakemore, 2011; Colrain & Baker, 2011; Spear, 2000; Steinberg, 2005). Several behaviours also often occur for the first time including, but not limited to, obtaining employment, seeking romantic relationships, and learning to drive. Another important behavioural change that occurs during the adolescent period in humans is the onset of recreational drug use. A main purpose of this thesis was to investigate behavioural processes which may contribute to a susceptibility to the onset of drug use and abuse during this period. Therefore, drug-taking and the effects of drugs of abuse during adolescence will be a focus of the following literature review.
2. Recreational Drug Use during Adolescence in Humans

Adolescents and young adults report the highest prevalence of illicit drug use (SAMHSA, 2007; Warner, Kessler, Hughes, Anthony, & Nelson, 1995). In the past year, 37% of Ontario students from grades 7-12 reported the use of at least one illicit drug (Paglia-Boak, 2011). Recreational drug use typically occurs before the age of 21 (Degenhardt et al., 2008; Kandel et al., 1992; SAMHSA, 2007; Wagner & Anthony, 2002). Conversely, the onset of drug or alcohol use is rare over the age of 24 (Anthony & Petronis, 1995; SAMHSA, 2007). Smoking during adolescence is particularly well-documented. The majority of adult smokers began using cigarettes before the age of 18 (Centers for Disease Control and Prevention, 1994; Eissenberg & Balster, 2000). Together these findings clearly show that the onset of the majority of drug use begins during the adolescent period.

Recreational drug use during adolescence influences the progression of drug use across an individual’s lifespan. First, adolescents escalate their drug use, or shift from drug use to drug abuse and dependence, more rapidly than adults (Deas et al., 2000; Estroff et al., 1989). Second, the age of initiation of recreational drug use inversely predicts subsequent alcohol and drug consumption (Dawson, Goldstein, Chou, Ruan, & Grant, 2008; Taioli & Wynder, 1991), risk of drug use, abuse and dependence (Anthony & Petronis, 1995; Chen, Storr, & Anthony, 2009; Sintov, Kendler, Walsh, Patterson, & Prescott, 2009) and negatively predicts the likelihood of drug use cessation (Breslau & Peterson, 1996; Khuder, Dayal, & Mutgi, 1999). Specifically, individuals that report using recreational drugs prior to the age of 15 are more likely to receive a subsequent diagnosis of drug dependence compared to individuals that report the initiation of recreational drug use after the age of 15 (National Institute on Drug Abuse, 1985; Robins & Przybeck, 1985). Similar relationships between the early onset of drug use and later drug dependence have been documented for alcohol (Dawson et al., 2008; Hingson, Heeren, & Winter, 2006), cocaine (Windle & Windle, 2011) and nicotine (Breslau, Fenn, & Peterson, 1993; Breslau & Peterson, 1996; Chassin, Presson, Sherman, & Edwards, 1990). Thus, recreational
drug use during adolescence, compared to other stages of development, appears to increase the risk of developing a subsequent substance dependence.

3. The Effects of Drugs of Abuse during Adolescence in Animals

Although evidence from human studies indicates that age of drug use onset inversely correlates with a subsequent diagnosis of drug dependence, a causal relationship has yet to be established. Studies using animals have begun to address this issue. Experiments using animal subjects show that compared to adults, adolescents may be differentially affected by drugs of abuse, although the direction and magnitude of this effect depends on the drug of abuse and the behavioural measure. Below I will describe age differences in the effects of drugs of abuse after a discussion of the neurochemical mechanisms of these drugs.

3.1 Neurochemical Mechanisms of Drugs of Abuse

Each drug of abuse has a different primary neurochemical mechanism of action. However, one commonality between drugs of abuse is that they all interact with the mesolimbic dopamine system (Pierce & Kumaresan, 2006; Sulzer, 2011). This system also mediates, in part, the stimulant, rewarding and reinforcing properties of drugs of abuse (Acquas, Carboni, Leone, & Di Chiara, 1989; Spyraki, Fibiger, & Phillips, 1983; Yokel & Wise, 1976). Amphetamine directly enhances dopamine neurotransmission via an action on pre-synaptic dopamine vesicles to release dopamine and blockade of the dopamine transporter (DAT) to inhibit dopamine reuptake (Azzaro & Rutledge, 1973; Besson, Cheramy, & Glowinski, 1969). Amphetamine also increases norepinephrine release (to a lesser extent than dopamine; Florin, Kuczenski, & Segal, 1994) and serotonin (5-hydroxytryptamine - 5-HT) release but only in response to doses greater than 2 mg/kg (Kuczenski & Segal, 1989). Cocaine and methylphenidate block the re-uptake of dopamine and norepinephrine via blockade of the DAT and the norepinephrine transporter (NET) respectively (Ferris, Tang, & Maxwell, 1972; Ritz & Kuhar, 1989; Volkow, Fowler, Wang, Ding, & Gatley, 2002; Volkow et al., 2001), while cocaine also blocks the reuptake of 5-HT (Blackburn, French, & Merrills, 1967; Kuczenski & Segal, 1997). Nicotine enhances
acetylcholine neurotransmission through stimulation of acetylcholine nicotinic receptors (nAChR; Dale, 1914). Nicotine also indirectly increases dopamine release via stimulation of nAChRs on dopaminergic cell bodies in the ventral tegmental area (VTA; Nisell, Nomikos, & Svensson, 1994). Ethanol increases gamma-aminobutyric acid (GABA) neurotransmission via an action on GABA receptors and blocks N-methyl-D-aspartic acid (NMDA) receptors (Hakkinen & Kulonen, 1959; Larsson & Engel, 2004; Lovinger, White, & Weight, 1989). Ethanol also indirectly increases dopamine neurotransmission (Di Chiara & Imperato, 1985) however the mechanism underlying this effect is still unclear (Sulzer, 2011). Notably, the neurochemical systems that mediate the effects of drugs of abuse continue to develop throughout adolescence as discussed below in section 4.1.2.

3.2 Drug-Induced Locomotor Activity

Many drugs of abuse alter locomotor activity, either increasing or decreasing this behaviour depending on the drug and the dose (e.g., Masur & dos Santos, 1988; Steketee & Braswell, 1997). Thus, locomotor activity is often used to assess age differences in the effects of drugs of abuse. Locomotion after a single injection of a drug (acute treatment) is a measure of the ability of the drug to stimulate or depress locomotor activity. Repeated injections of a drug can induce tolerance: a decreased effect of a drug in response to the same dose (e.g., Morrison & Stephenson, 1972). For example, initially nicotine injections decrease locomotor activity but after multiple injections this depressant effect dissipates (Morrison & Stephenson, 1972). Alternatively, multiple injections of a drug can induce sensitization, an increased effect of the drug in response to the same dose (e.g., Shuster, Yu, & Bates, 1977). Psychomotor stimulants such as amphetamine or cocaine often induce greater locomotor responses after repeated treatment compared to acute treatment with the same dose (Pierce & Kalivas, 1997; Robinson & Berridge, 1993). A sensitized locomotor response is considered to be a functional measure of the degree of neuroplastic changes induced by the drug (Stewart & Badiani, 1993) in particular, changes in the function of the mesolimbic dopamine system (Kalivas, 1995; Vanderschuren &
Kalivas, 2000). Therefore, examining age differences in the effects of drugs of abuse on locomotor activity provides information about the behavioural effects of drugs of abuse but may also suggest possible changes in some aspects of brain function.

3.2.1 Acute Treatment

The most consistently observed age difference in drug-induced locomotor activity is that acute systemic injections of amphetamine and its derivatives, methamphetamine and methylenedioxymethamphetamine (MDMA), increase locomotor activity to a greater extent in adults compared to adolescent rats (Aberg, Wade, Wall, & Izenwasser, 2007; Bolanos, Glatt, & Jackson, 1998; Lanier & Isaacson, 1977; Laviola, Adriani, Terranova, & Gerra, 1999; Mathews & McCormick, 2007; Wiley, Evans, Grainger, & Nicholson, 2008). However, recent evidence suggests that when amphetamine is injected into the nucleus accumbens core, rats in early adolescence (PND 30) show more amphetamine-stimulated locomotor activity than adult rats (Mathews, Brudzynski, & McCormick, 2011). The locomotor stimulating effects of systemic treatment with cocaine, nicotine and ethanol are also the most pronounced during the early adolescent period (Acevedo, Molina, Nizhnikov, Spear, & Pautassi, 2010; Belluzzi, Lee, Oliff, & Leslie, 2004; Cao et al., 2010; Cao et al., 2007; Caster, Walker, & Kuhn, 2005; Cruz, Delucia, & Planeta, 2005; Dow-Edwards & Izenwasser, 2012; Frantz, O'Dell, & Parsons, 2006; Laviola, Wood, Kuhn, Francis, & Spear, 1995; Lopez, White, & Randall, 2001; McQuown, Dao, Belluzzi, & Leslie, 2009; Quoilin, Didone, Tirelli, & Quertemont; Schochet, Kelley, & Landry, 2004; Stevenson, Besheer, & Hodge, 2008; Vastola, Douglas, Varlinskaya, & Spear, 2002). This trend was not observed in all experiments (Balda, Anderson, & Itzhak, 2009; Caster et al., 2005; Collins et al., 2004; Faraday, Elliott, Phillips, & Grunberg, 2003; Parylak, Caster, Walker, & Kuhn, 2008; Rezvani & Levin, 2004) but in general suggests that young adolescents may be the most sensitive to the locomotor stimulating effects of several drugs of abuse, with the exception of systemically injected amphetamine.
3.2.2 Repeated Treatments

Most studies report that compared to adults, adolescents are less or equally susceptible to the locomotor sensitizing effects of many drugs of abuse including methamphetamine, cocaine, ethanol and nicotine (Balda et al., 2009; Carrara-Nascimento, Griffin, Pastrello, Olive, & Camarini, 2011; Collins & Izenwasser, 2002; Cruz et al., 2005; Faraday et al., 2003; Frantz et al., 2006; King, Dafny, Yang, & Swann, 2009; Laviola et al., 1995; McQuown et al., 2009; Schochet et al., 2004; Stevenson et al., 2008; Vastola et al., 2002; Zakharova, Leoni, Kichko, & Izenwasser, 2009 but see Belluzzi et al., 2004; Caster et al., 2005; Caster, Walker, & Kuhn, 2007; Schramm-Sapyta, Pratt, & Winder, 2004). Similarly, no age differences were observed in the locomotor effects of repeated treatment with amphetamine in male rats although adolescent females showed a more pronounced sensitized locomotor response compared to adult females (Mathews & McCormick, 2007). Generally, adolescents show fewer stereotyped behaviours than adults in response to repeated amphetamine injections (Adriani, Chiarotti, & Laviola, 1998; Laviola et al., 1999). Thus, in contrast to the effects of acute drug treatment, adolescents may be less sensitive than adults to the locomotor stimulating effects of repeated treatment of many drugs of abuse. One implication of these findings is that adolescents may be more sensitive to some, but not all, the behavioural effects of drugs of abuse.

3.3 Drug Reward and Reinforcement

Drugs of abuse are rewarding and potent reinforcers of behaviour. Reward can be defined as the appetitive or hedonic aspect of a stimulus although some argue that reward represents a broader concept that includes three dissociable components: the learned aspect, the hedonic aspect or “liking”, and the incentive salience aspect or “wanting” (see Berridge & Robinson, 1998 for review). Incentive salience refers to the ‘attractiveness’ of a stimulus which can motivate approach to that stimulus. Thus, rewards can act as incentives in that they elicit approach behaviour. A reinforcer can be defined as a stimulus that increases the probability of
the contingent behaviour (Skinner, 1938). Rewards can also act as reinforcers if they increase the probability of the contingent behaviour.

The rewarding and reinforcing properties of a drug can be assessed in animals by examining whether the drug is self-administered. Most drugs abused in humans are self-administered by animals (Collins, Weeks, Cooper, Good, & Russell, 1984; Howell & Fantegrossi, 2009). If a drug is rewarding and reinforcing, animals will learn to make a response to receive an intravenous (IV) infusion (or oral delivery) of the drug often in the presence of a predictive cue. Similarly to humans, the animal is able to control the amount and rate of drug intake which adds face validity to the drug self-administration measure. Patterns and rates of drug self-administration reflect the reinforcing properties of a substance (Weeks, 1962). On a schedule of reinforcement that delivers one infusion of a reinforcing drug per response (a fixed ratio 1 [FR1] schedule of reinforcement), an animal will show a stable and steady pattern of responding across the session (e.g., Roberts, Koob, Klonoff, & Fibiger, 1980; Weeks, 1962). When the drug is replaced with its vehicle, responding typically extinguishes which demonstrates the reinforcing properties of that drug. Thus, patterns and rates of drug self-administration can be used to evaluate quantitative and qualitative differences in the reinforcing effects of drugs across development.

Few consistent age differences have been observed in drug self-administration studies. Adolescents acquire amphetamine self-administration more rapidly and earn more infusions than adults (Shahbazi, Moffett, Williams, & Frantz, 2008). For nicotine, some studies report enhanced self-administration in adolescents compared to adults (Adriani et al., 2002; Chen, Matta, & Sharp, 2007; Levin et al., 2007; Levin, Rezvani, Montoya, Rose, & Swartzwelder, 2003) while other studies report no age differences (Belluzzi, Wang, & Leslie, 2005; Shram, Funk, Li, & Le, 2008; Shram, Li, & Le, 2008). Variability in the strain or the method of self-administration may contribute to these divergent results. For ethanol, species and strain may also affect age differences in self-administration. Adolescent mice or Sprague-Dawley rats consume more
ethanol compared to adults (Brunell & Spear, 2005; Doremus, Brunell, Rajendran, & Spear, 2005; Tambour, Brown, & Crabbe, 2008; Vetter, Doremus-Fitzwater, & Spear, 2007) while adolescent Wistar rats consume equal or less ethanol compared to adults (Bell et al., 2006; Fullgrabe, Vengeliene, & Spanagel, 2007; Siegmund, Vengeliene, Singer, & Spanagel, 2005). Sex differences in ethanol self-administration appear to emerge after adolescence in rats (Bell et al., 2006; Doremus et al., 2005) although in mice both adolescent and adult females self-administer more ethanol than their male counterparts (Tambour et al., 2008). Most studies report no age differences in cocaine self-administration (Belluzzi et al., 2005; Frantz et al., 2006; Harvey, Dembro, Rajagopalan, Mutebi, & Kantak, 2009; Kantak, Goodrich, & Uribe, 2007; Kerstetter & Kantak, 2007; Leslie et al., 2004) with the exception of two experiments that utilized more complex predictive visual stimuli (e.g., a flashing stimulus light; Anker & Carroll, 2010; Schramm-Sapyta et al., 2011). In summary, these findings suggest that adolescents may self-administer more drug than adults in some but not all cases. When age differences in self-administration do exist, adolescents often self-administer more drug than adults. Procedural and strain differences may significantly affect the expression of age differences in drug self-administration.

3.4 Reinstatement of Responding for a Drug Reinforcer

In human drug users, relapse is a common feature of addiction. After a period of abstinence and treatment, drug users often re-initiate drug use within a year (Hunt, Barnett, & Branch, 1971). Relapse is an important contributor to the chronic nature of addiction (Koob & Volkow, 2010) and one of the most pressing issues in the treatment of this disorder. This stage of the addiction process can be modeled in animals by examining reinstatement of responding for a drug reinforcer. Responding for a drug can be extinguished when the operant response delivering the drug (i.e., responding on a lever) no longer has a programmed consequence (i.e., delivers the drug reinforcer). Subsequently, exposure to the drug reinforcer, cues previously associated with the drug, or stress can reinstate responding on the lever previously associated with drug delivery.

Relatively few studies have examined age differences in reinstatement of responding for a drug. Adolescent and adults show similar nicotine-induced reinstatement of responding (Shram, Funk, et al., 2008; Shram, Li, et al., 2008). Compared to adults, adolescents also exhibit enhanced cocaine- and stress-induced, but reduced cue-induced reinstatement of responding (Anker & Carroll, 2010). Reliable conclusions about age differences in reinstatement of responding cannot be drawn from this limited number of published studies.

3.5 The Conditioned Effects of Drugs of Abuse

Animals can learn to associate the effects of primary rewards with stimuli paired with those rewards. When a drug (unconditioned stimulus – US) is administered in the presence of a neutral environmental stimulus (conditioned stimulus – CS, e.g. a location, visual cue, etc.), the effects of that drug become associated with that stimulus through Pavlovian conditioning. The drug-paired stimulus (CS) is presumed to elicit the rewarding or aversive effects of the drug (US) in the absence of the drug through the process of incentive motivation (Berridge, 2001; see Section 4.2). Approach to, or avoidance of, drug-paired stimuli is assumed to reflect the rewarding or aversive properties of that drug. These conditioned drug-paired stimuli are believed to play an important role in the onset and progression of drug use (Childress et al., 1993; Grant et al., 1996; Robinson & Berridge, 1993).

Two commonly used measures of the conditioned effects of drugs of abuse are tests of conditioned place preference (CPP; Rossi, 1976) or conditioned place aversion (CPA). Both procedures involve several pairings of the effects of a drug with a distinct environment and the effects of a vehicle with another distinct environment. Subsequently in a drug-free state, the animal is allowed to explore both environments. Approach to the drug-paired environment
reflects the rewarding properties of the drug while avoidance of the drug-paired environment reflects the aversive properties of the drug (Bardo & Bevins, 2000). In practice, the two distinct environments are two different compartments of a 2 chamber apparatus (e.g., compartment A: vertical stripes on the walls, perforated floors, and compartment B: horizontal stripes on the walls, smooth floors). Aversion can also be assessed using conditioned taste aversion (CTA) which is also based on Pavlovian conditioning principles (Cappell, LeBlanc, & Endrenyi, 1973; Wise, Yokel, & DeWit, 1976). CTA involves pairing a palatable substance (e.g., sucrose) with the effects of a passively-administered drug (US). Subsequently, avoiding the substance (CS) in the absence of the drug reflects the aversive properties of that drug.

Findings from studies that examined age differences in the conditioned rewarding and conditioned aversive effects of drugs of abuse are mixed. Compared to adults, adolescents are reliably more sensitive to the conditioned rewarding effects of nicotine as measured by CPP (Belluzzi et al., 2004; Brielmaier, McDonald, & Smith, 2007; Shram, Funk, Li, & Le, 2006; Shram & Le, 2010; Vastola et al., 2002). Compared to adults, adolescents show either reduced (Adriani & Laviola, 2003) or equal amphetamine-induced CPP (Mathews & McCormick, 2007; Torres, Tejeda, Natividad, & O'Dell, 2008) but require fewer training sessions to acquire methamphetamine-induced CPP (Zakharova, Leoni, et al., 2009). Age differences in cocaine-induced CPP depend on the stage of adolescence that testing occurs. Rats and mice during early adolescence (PND 28-35; Badanich, Adler, & Kirstein, 2006; Brenhouse & Andersen, 2008; Zakharova, Wade, & Izenwasser, 2009) are more sensitive than their adult or late adolescent counterparts (> PND 45) to the conditioned rewarding effects of cocaine (Aberg et al., 2007; Balda, Anderson, & Itzhak, 2006; Schramm-Sapyta et al., 2004). Adolescents also show more resistance to extinction and enhanced reinstatement of cocaine-induced CPP compared to adults (Brenhouse & Andersen, 2008). Age differences in ethanol-induced CPP appear to be species dependent. In mice, only adults show ethanol-induced CPP (Song et al., 2007) but in rats, adolescents show an enhanced ethanol-induced CPP compared to adults (Philpot, Badanich, &
Kirstein, 2003). In the context of aversion, adolescents are more resistant than adults to acquiring nicotine- (Shram et al., 2006), cocaine- (Schramm-Sapyta, Morris, & Kuhn, 2006), amphetamine- (Infurna & Spear, 1979) or ethanol-induced CTA (Philpot et al., 2003). Thus, adolescents are consistently less susceptible to the conditioned aversive effects of drugs of abuse and may be more susceptible to the conditioned rewarding effects of some drugs.

3.6 The Role of Pharmacokinetics

Several studies have examined the possibility that age differences in pharmacokinetics may contribute to age differences in the effects of drugs of abuse. After equivalent doses of cocaine, nicotine or ethanol, adolescents show lower levels of the respective drugs or its metabolite in the blood compared to adults (Caster et al., 2005; Lopez, Simpson, White, & Randall, 2003; McCarthy et al., 2004; Slotkin, 2002; Trauth, Seidler, & Slotkin, 2000) with the exception of one experiment that reported no age differences (Pautassi, Myers, Spear, Molina, & Spear, 2011). Conversely, after treatment with cocaine, amphetamine or ethanol, levels of these respective drugs in the brain were equal in adolescents and adults (Pautassi et al., 2011; Spear & Brake, 1983; Zombeck, Gupta, & Rhodes, 2009). Some evidence suggests that levels of cocaine in the brain may be lower in adolescents compared to adults immediately after the injection of the drug (Caster et al., 2005). However, in several studies, levels of drug in the blood or brain did not account for age differences in behaviour (Lopez et al., 2003; Spear & Brake, 1983; Zombeck et al., 2009). Thus, age differences in pharmacokinetics may not necessarily contribute to age differences in the effects of drugs of abuse on behaviour.

4. Possible Contributing Factors to Age Differences in the Effects of Drugs of Abuse

Biological and psychological development during adolescence may contribute to the differential effects of drugs of abuse in adolescents compared to adults (Laviola et al., 1999; Spear, 2000). First, the neurocircuitry underlying the effects of drugs of abuse undergoes significant changes during adolescence (Crews, He, & Hodge, 2007; Ernst et al., 2009). Second, this neurocircuitry also mediates two psychological processes that play important roles in drug
addiction: incentive motivation and impulsivity (Dalley et al., 2011; Everitt et al., 1999). Thus, the pattern of brain development during adolescence may lead to changes in incentive motivation and impulsivity, and in turn, contribute to the onset and progression of drug use and abuse during this period.

4.1 Brain Development during Adolescence

The brain continues to develop throughout childhood and into adulthood. In particular, the connections between regions of the frontal cortex and various sub-cortical structures including the ventral striatum, dorsal striatum, amygdala and hippocampus are refined during adolescence (see below). Several neurotransmitter systems, including the mesocorticolimbic dopamine and glutamate systems, also undergo many changes. The brain regions and neurochemical systems that continue to develop during adolescence are also involved in mediating the effects of drugs of abuse and drug addiction (Everitt et al., 2008; Kalivas, Volkow, & Seamans, 2005; Robinson & Berridge, 2000). Therefore the continued development of brain likely contributes to age differences in the effects of drugs of abuse.

4.1.1 Brain Structure

Although the basic size and structure of the brain are already developed before the onset of adolescence (Armstrong, Schleicher, Omran, Curtis, & Zilles, 1995; Dobbing & Sands, 1973; Giedd et al., 1996; Reiss, Abrams, Singer, Ross, & Denckla, 1996), the synaptic and neural connections formed during prenatal and childhood development continue to be refined into adulthood (Geier & Luna, 2009). These connections are modified through the same processes that shape the brain during early development such as neurogenesis, synaptogenesis, myelination, dendritic arborization, apoptosis and synapse elimination (Andersen, Thompson, Rutstein, Hostetter, & Teicher, 2000; Benes, Turtle, Khan, & Farol, 1994; Bourgeois, Goldman-Rakic, & Rakic, 1994; Huttenlocher & Dabholkar, 1997; Meyer, Ferres-Torres, & Mas, 1978; Nunez, Lauschke, & Juraska, 2001; Rankin, Partlow, McCurdy, Giles, & Fisher, 2003).
White and gray matter volumes change during adolescence in humans. White matter volume increases in a linear fashion during this period (Giedd, 2004; Giedd et al., 1999; Paus et al., 2001; Paus et al., 1999). This white matter volume increase is presumed to reflect increased myelination and axon calibre (Paus, 2010; Wahlstrom, White, & Luciana, 2010). Gray matter volume also changes across development but according to a non-linear U-shaped function: gray matter volume peaks before the onset of puberty and then decreases to adult levels during adolescence (Giedd et al., 1999; Gogtay et al., 2004; Paus et al., 1999). Adult levels of gray matter volume are attained more slowly in regions of the frontal cortex compared to other cortical regions (Giedd et al., 1999; Gogtay et al., 2004). The structural development of the brain is also sexually dimorphic. Males show a steeper increase in white matter volume compared to females (Giedd et al., 1999; Lenroot et al., 2007), while the peak in gray matter occurs a year earlier for girls than for boys (Giedd, 2004). Testosterone is linked to the development of white and gray matter volumes during puberty (Paus et al., 2010; Perrin et al., 2008).

Changes in gray matter volume in adolescence likely reflect changes in synaptic density (Bourgeois et al., 1994; Gogtay et al., 2004; Huttenlocher, 1979). Synaptic connections are overproduced during childhood and then undergo massive pruning during adolescence (Bourgeois et al., 1994; Huttenlocher, 1979, 1984; Mrzljak, Uylings, Van Eden, & Judas, 1990; Zecevic, Bourgeois, & Rakic, 1989). This U-shaped pattern of synapse production and elimination is believed to follow Jacobson’s theory of neuronal modification (Jacobson, 1973) which states that synapse over-production is genetically determined and synapse elimination is guided by the degree of cellular activity at the synapses. Thus, synaptic pruning is thought to specifically eliminate weak or unused synapses (Huttenlocher, 1984). Overproduction and elimination of synapses is evident across species including rodents, non-human primates and humans (Aghajanian & Bloom, 1967; Andersen, 2003; Huttenlocher, 1979; Kalsbeek, Voorn, Buijs, Pool, & Uylings, 1988; Lewis, 1997). Selective pruning of neural connections, in concert
with increases in myelination, reflect the refinement of brain structure during adolescence with the presumed end result of more efficient information processing in adulthood.

Connections between the various brain structures, particularly between regions of the frontal cortex and subcortical structures, also change throughout adolescence based on evidence from studies with animals. Relay areas between the frontal cortex and the hippocampus, including the presubiculum and subiculum, show increased myelination during adolescence (Benes, 1989). At the same time, inhibitory projections from the basolateral amygdala (BLA) to the prefrontal cortex (PFC) are strengthened (Cunningham, Bhattacharyya, & Benes, 2002, 2008) while the reciprocal connections from the PFC to the BLA are pruned (Cressman et al., 2010). Dopaminergic projections to the PFC peak (Kalsbeek et al., 1988), while excitatory projections to the PFC are pruned, during this period (Rakic, Bourgeois, & Goldman-Rakic, 1994). The PFC, BLA and dopamine and glutamate systems all play important roles in mediating the effects of drugs of abuse and related cues (see Everitt et al., 1999; Everitt & Robbins, 2005; Kalivas et al., 2005; Perry et al., 2011; Robinson & Berridge, 2000), thus changes in connectivity between these structures and within neurochemical systems likely contribute to the differential effects of drugs of abuse during adolescence compared to adulthood.

4.1.2 Neurotransmitters

Neurotransmitters are the primary means of synaptic transmission and the primary substrate for drugs of abuse. Although neurotransmitters are present during embryonic development (Kalsbeek et al., 1988; Lidov & Molliver, 1982), many neurotransmitter systems continue to develop over the course of adolescence, including the mesocorticoclimbic dopamine, glutamate, and GABA systems (see Crews et al., 2007; Ernst et al., 2009; O'Dell, 2009; Spear, 2000) for review). Relatively few studies have focused on the development of the 5-HT and endogenous opioid systems (Daval et al., 1987; Ellgren et al., 2008; Lidov & Molliver, 1982; Morilak & Ciaranello, 1993; Talbot, Happe, & Murrin, 2005; Winzer-Serhan, Chen, & Leslie, 2003).
4.1.2.1 Dopamine. Of all the neurotransmitter systems, the development of the mesocorticolimbic dopamine system is the most thoroughly documented in animals. This system is comprised of projections from dopamine cell bodies in the VTA to the nucleus accumbens, the PFC and the amygdala. Dopamine released by VTA neurons acts on multiple subtypes of dopamine receptors. Two classes of dopamine receptors exist; both types are metabotropic although they act through different postsynaptic molecular pathways. The D₁-like family of dopamine receptors, including the D₁ and D₅ receptors, is found exclusively on postsynaptic cells and is excitatory through action on adenylate cyclase which increases cyclic adenosine monophosphate (cAMP; Missale, Nash, Robinson, Jaber, & Caron, 1998; Monsma, Mahan, McVittie, Gerfen, & Sibley, 1990). The D₂-like family of dopamine receptors, including the D₂, D₃ and D₄ receptors, is primarily inhibitory via inhibition of adenylate cyclase (Missale et al., 1998; Onali, Olianas, & Gessa, 1985). On the pre-synaptic terminal, the activation of D₂ dopamine autoreceptors inhibits dopamine release (Wolf & Roth, 1990). On the post-synaptic cell, activation of the D₂ dopamine receptor can be excitatory if paired with D₁ receptor stimulation (Wachtel, Hu, Galloway, & White, 1989; Walters, Bergstrom, Carlson, Chase, & Braun, 1987) or through activation of Gβγ proteins which acts on phospholipase C (PLC) to increase cytoplasmic calcium (Beaulieu & Gainetdinov, 2011).

The mesocorticolimbic dopamine system is hypothesized to be over-active during adolescence compared to adulthood (Chambers et al., 2003; O'Dell, 2009; Spear, 2000). In support of this hypothesis, PFC dopaminergic input and dopamine fibre density increases and peaks during adolescence (Benes, Taylor, & Cunningham, 2000; Kalsbeek et al., 1988). However, baseline extracellular dopamine in the PFC and striatum, as measured by voltammetry and microdialysis, is reduced or not significantly different in adolescent animals compared to adults (Staiti et al., 2011; Stamford, 1989). In the nucleus accumbens, findings on baseline extracellular dopamine conflict; one study reported no age differences (Frantz, O'Dell, & Parsons, 2007) while another showed higher extracellular dopamine release in adolescents.
compared to adults (Badanich et al., 2006). Together these findings imply that age differences in dopamine system function may stem from differences in post-synaptic and synapse clearing mechanisms.

Many studies report changes in dopamine receptor expression across development in animals (Andersen et al., 2000; Tarazi & Baldessarini, 2000; Teicher, Andersen, & Hostetter, 1995). In the dorsal striatum D₁ and D₂ dopamine receptor densities peak before PND 40 and then decline to adult levels by PND 60 (Andersen et al., 2000; Tarazi & Baldessarini, 2000; Teicher et al., 1995). Conversely, in the ventral striatum, dopamine receptor densities increase from PND 20-40 but remain relatively elevated until adulthood. D₂ dopamine receptors reach adult levels more quickly than D₁ dopamine receptors in this region (Andersen et al., 2000; Tarazi & Baldessarini, 2000; Teicher et al., 1995). In the PFC, both D₁ and D₂ dopamine receptors increase over the course of adolescence and remain elevated until PND 60-80 (Andersen et al., 2000; Tarazi & Baldessarini, 2000). Specifically, D₁ dopamine receptor expression on PFC glutamate projections to the nucleus accumbens peaks during adolescence (Brenhouse, Sonntag, & Andersen, 2008). Thus dopamine receptor densities appear to reach adult levels more quickly in the striatum than in the PFC (Andersen et al., 2000). Notably, females do not exhibit the same degree of overproduction and pruning of dopamine receptors compared to males (Andersen, Rutstein, Benzo, Hostetter, & Teicher, 1997; Andersen, Thompson, Krenzel, & Teicher, 2002), a sex difference that occurs independently from sex hormones (Andersen et al., 2002). DAT density reaches adult level by approximately PND 35 or mid adolescence (Coulter, Happe, & Murrin, 1996). In summary, age differences in aspects of the mesocorticolimbic dopamine system reflect an increased potential for dopamine signalling during adolescence, particularly in sub-cortical structures (Wahlstrom, Collins, White, & Luciana, 2010), which may enhance the effects of drugs of abuse.

4.1.2.2 Serotonin. Compared to dopamine, relatively less research has focused on the development of the 5-HT system during adolescence in animals. 5-HT neurons that originate
from cell bodies in the raphe nuclei extend to many subcortical and cortical regions. The wide range of physiological and behavioural effects of 5-HT are mediated by 15 subtypes of 5-HT 7-transmembrane-spanning receptors belonging to seven receptor families. Cortical 5-HT innervation is reported to reach adult levels by PND 21 (D'Amato et al., 1987; Lidov & Molliver, 1982), although, no adolescent rats older than PND 21 were included in these studies. Expression of the 5-HT$_{1A}$ and 5-HT$_2$ receptors reaches adult levels before PND 28 (Daval et al., 1987; Morilak & Ciaranello, 1993). Thus, based on the relatively limited evidence, the 5-HT system appears to be largely developed before the adolescent period begins (Lidov & Molliver, 1982; Spear, 2000).

### 4.1.2.3 Opioids

Endogenous opioids act on various types of opioid receptors. The most prominent subtypes include μ, δ, and κ opioid receptors (Lord, Waterfield, Hughes, & Kosterlitz, 1977; Martin, Eades, Thompson, Huppler, & Gilbert, 1976). Opioids are commonly associated with pain regulation but are also important for mediating reward (Barbano & Cador, 2007; Berridge, 1996, 2009; Petrovic et al., 2008; Schneider, Heise, & Spanagel, 2010; Smith & Berridge, 2007). Few studies exist on the development of the endogenous opioid system during adolescence in animals. Opioid receptor mRNA expression appears to peak during the second postnatal week and then decline until PND 60 (Winzer-Serhan et al., 2003) although μ opioid receptors appear to be relatively stable across the adolescent period (Ellgren et al., 2008; Talbot et al., 2005). Further study into the development of the opioid system is warranted as activity of this system may influence age differences in the effects of rewards.

### 4.1.2.4 Glutamate

The largest excitatory neurotransmitter system, glutamate, undergoes many changes throughout adolescence in animals. Reports show that extracellular glutamate release is either reduced or similar in adolescents compared to adults (Camarini, Marcourakis, Teodorov, Yonamine, & Calil, 2011; Carrara-Nascimento et al., 2011). More consistent developmental changes have been observed for glutamate receptors. Glutamate acts on NMDA and (2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-y1)propanoic acid) AMPA receptors. Mirroring
the development of gray matter, NMDA receptors are over-produced prior to adolescence and then a third of these receptors are pruned from PND 28-60 (Insel, Miller, & Gelhard, 1990; Miller, Johnson, Gelhard, & Insel, 1990). The NMDA receptor is composed of three subunits which belong to the NR1, NR2 or NR3 families. The configuration of NMDA receptor subunits affects the ability of ligands to bind to NMDA receptors. Densities of the various NMDA receptor subunits, particularly the NR2A and NR2B subunits, also fluctuate across development. During the early postnatal period, the NR2B subunit shows higher expression than in adulthood and is more commonly expressed than the NR2A subunit (Liu, Murray, & Jones, 2004; Sheng, Cummings, Roldan, Jan, & Jan, 1994). Later in postnatal development, the NR2A subunit becomes more highly expressed compared to the NR2B subunit which is pruned to adult levels (Sheng et al., 1994). NR2B subunit levels may be lower during adolescence compared to adulthood (Pian, Criado, Milner, & Ehlers, 2010). Although NMDA receptors are generally pruned across the adolescent period, compared to adults, adolescents show enhanced NMDA-mediated long-term potentiation in the nucleus accumbens (Schramm, Egli, & Winder, 2002). Together these results suggest that NMDA receptor dependent functions are enhanced during adolescence compared to adulthood. Plasticity in the glutamate system plays an important role in the effects of drugs of abuse (Kalivas, 2000; Schmidt & Pierce, 2010), thus, age differences in glutamate function may contribute to the differential effects of drugs of abuse across development.

4.1.2.5 GABA. The main inhibitory neurotransmitter system, GABA, also develops throughout adolescence in animals. GABA acts on two main classes of receptors: ionotropic GABA_A receptors and metabotropic GABA_B receptors. The distribution of GABA receptors changes during adolescence. Compared to adults, adolescents show higher GABA_A receptor densities and chloride uptake in the cortex (Kellogg, Taylor, Rodriguez-Zafra, & Pleger, 1993; Verdurand, Dalton, & Zavitsanou, 2010). The expression of various subtypes of the GABA_A receptor also changes across adolescence (Yu, Wang, Fritschy, Witte, & Redecker, 2006) which
may have implications for behavioural responses to drugs that act on these receptors such as anxiolytics and ethanol (Crews et al., 2007).

4.2 Incentive Motivation

Incentive motivation is the process through which stimuli associated with primary rewards can acquire the reinforcing and motivational properties of these rewards (Berridge & Robinson, 1998; Bindra, 1978; Toates, 1986). Specifically, when an initially neutral environmental stimulus is repeatedly paired with a reward (US; e.g., food, water, sex, a drug), the stimulus (CS) becomes salient and reinforcing as the result of Pavlovian or classical conditioning. The CS becomes a conditioned reinforcer which is a learned incentive that has acquired the motivational properties of the US. Thus a conditioned reinforcer can elicit approach or operant behaviour in a similar fashion to a primary reward (Berridge, 2004; Lovibond, 1983). Incentive motivation is a process considered to be a fundamental aspect of motivation in general, and may play a crucial role in addiction and drug-seeking behaviour as discussed in section 4.2.4.

4.2.1 Conditioned Reinforcement: A Measure of Incentive Motivation

One method of assessing incentive motivation in animals is to measure responding for a conditioned reinforcer (Robbins, 1976). First, in a Pavlovian conditioning phase, animals learn to associate a passively delivered or self-administered reward with a CS (e.g., a light and/or tone). Second, animals are given the opportunity to respond for the initially neutral environmental stimulus that now functions as a conditioned reinforcer in the absence of the primary reward. Responding for a conditioned reinforcer is an ideal measure of incentive motivation because it satisfies the necessary conditions to demonstrate conditioned reinforcement developed by (Mackintosh, 1974). These conditions include demonstrating that 1) responding for the conditioned reinforcer depends upon the explicit pairing of the CS and the reward and 2) that the response for the conditioned reinforcer is novel (i.e., different from any response that was reinforced during the conditioning phase).
As discussed previously, CPP can also be used to assess the conditioned rewarding and incentive motivational properties of natural- and drug-paired stimuli in animals. Although informative, CPP is a relatively passive measure of conditioned reward. Unlike responding for a conditioned reinforcer where the outcome measure is an operant response, CPP measures approach to a location previously paired with a reward which requires relatively little effort on behalf of the subject. One advantage of using an operant-based measure of incentive motivation is the ability to quantify how much an animal is willing to work for the conditioned reinforcer—a well-established measure of motivation. Thus responding for a conditioned reinforcer can potentially provide additional and important information about incentive motivation over-and-above that acquired from CPP experiments.

4.2.2 Neurocircuitry of Incentive Motivation

Most of the work on delineating the neurocircuitry of incentive motivation as measured by responding for a conditioned reinforcer has involved pairing of a CS with natural primary rewards (e.g., food or water). Inactivation of the VTA blocks responding for a conditioned reinforcer (Murschall & Hauber, 2006). Excitotoxic lesions of brain regions that receive projections from the VTA such as the BLA, and to a lesser degree the nucleus accumbens, also block responding for a conditioned reinforcer (Burns, Robbins, & Everitt, 1993; Everitt et al., 1999; Parkinson, Olmstead, Burns, Robbins, & Everitt, 1999). Although the PFC also receives projections from the VTA, excitotoxic lesions of this region do not affect the acquisition of responding for a conditioned reinforcer suggesting that the PFC is not critical for this behaviour (Burns et al., 1993; Everitt et al., 1999; Pears, Parkinson, Hopewell, Everitt, & Roberts, 2003). However, excitotoxic lesions of the orbitofrontal cortex (OFC) block responding for a conditioned reinforcer (Everitt et al., 1999; Pears et al., 2003). Dopamine also plays an important role in responding for a conditioned reinforcer (Everitt et al., 1999; Sutton & Beninger, 1999). This behaviour is enhanced by dopamine agonists such as amphetamine and blocked by dopamine antagonists (Beninger & Ranaldi, 1992; Fletcher & Higgins, 1997; Fletcher, Korth,
Dopamine, specifically in the nucleus accumbens shell, also mediates the amphetamine-induced potentiation of responding for a conditioned reinforcer (Taylor & Robbins, 1984). Therefore in adult rats, connections between the nucleus accumbens, the BLA and OFC, combined with dopaminergic innervation from the VTA, are involved in responding for a conditioned reinforcer.

In addition to dopamine, other neurotransmitters have been implicated in mediating responding for a conditioned reinforcer including 5-HT which is known to modulate dopamine activity (Alex & Pehek, 2007; Kapur & Remington, 1996). Depleting 5-HT throughout the brain enhances responding for a conditioned reinforcer in response to saline or amphetamine (Fletcher, Korth, & Chambers, 1999). Conversely, elevating 5-HT via systemic injection of a 5-HT releaser and reuptake inhibitor or micro-injections of 5-HT into the nucleus accumbens decreases responding for a conditioned reinforcer and the ability of amphetamine to potentiate this effect (Fletcher, 1995, 1996). The opioid system, which is known to mediate some of the effects of rewards, may also contribute to the expression of responding for a conditioned reinforcer (Barbano & Cador, 2007; Berridge, 1996, 2009; Petrovic et al., 2008; Robbins et al., 1983; Schneider et al., 2010; Smith & Berridge, 2007). Morphine, a µ opioid receptor agonist increases responding for a conditioned reinforcer (Robbins et al., 1983). Together these findings suggest that decreased 5-HT and increased opioid activity enhance responding for a conditioned reinforcer.

**4.2.3 Incentive Motivation during Adolescence**

The majority of the work on the age differences in incentive motivational processes in animals stems from studies of drug- and natural reward-induced CPP. As discussed above in section 3.5, compared to adults, adolescent rats often exhibit enhanced drug-induced CPP, require lower doses of these drugs to produce CPP, and show reduced drug-induced CTA (see
Chambers et al., 2003; Schramm-Sapyta, Walker, Caster, Levin, & Kuhn, 2009; Spear, 2000 for review). Adolescents are also more sensitive than adults to the conditioned rewarding effects of natural rewards such as novel objects or social interactions, although these age differences were not observed in females (Douglas, Varlinskaya, & Spear, 2003, 2004). Together these findings suggest that adolescents may show enhanced incentive motivation for natural and drug reward-paired stimuli. No published studies have compared adolescents and adults on responding for a conditioned reinforcer.

4.2.4 Incentive Motivation, Drug use and Abuse

The process of incentive motivation plays a central role in one of the prominent theories of drug addiction (Robinson & Berridge, 1993). According to the incentive salience theory of addiction, dopamine is associated with attributing incentive salience to stimuli, such that those stimuli become more “attractive”. Enhancement of the activity of this dopamine system through repeated drug use can lead to attribution of maladaptively high incentive salience to the primary reward (e.g., drug) as well as to the stimuli associated with the reward such that these environmental stimuli become increasingly ‘wanted’ (Robinson & Berridge, 1993, 2001). An enhancement of the incentive salience or ‘wanting’ of the drug or drug-paired stimuli may trigger cravings. For example, when cocaine-users view a video of someone purchasing or preparing cocaine for self-administration, these visual stimuli induce drug craving (Childress et al., 1999; Grant et al., 1996). Cravings may lead to relapse in abstinent individuals (see Heinz, Beck, Grusser, Grace, & Wrase, 2009 for review). Thus, drug-paired cues, which acquired motivational and reinforcing properties through the process of incentive motivation, may contribute to the progression and maintenance of drug abuse. One implication of this theory is that humans with enhanced dopamine function may be more prone than others to attribute incentive salience to drug rewards and stimuli paired with these rewards, which in turn increases the probability of drug addiction.
Several lines of converging evidence discussed above support the hypothesis that incentive motivation may play a role in the progression from drug use to abuse in adolescents. First, incentive motivation is an important process involved in drug addiction. Second, the neurocircuitry underlying incentive motivation and the effects of drugs of abuse continues to develop during adolescence. Third, evidence from animal studies suggests that adolescents may show enhanced incentive motivation compared to adults based on CPP studies. However, no studies have examined an operant measure of incentive motivation for natural or drug-paired stimuli. This information is needed before the relationship between incentive motivation and age differences in the effects of drugs of abuse can be examined.

4.3 Impulsivity

Impulsivity is a complex construct that covers a spectrum of behaviours but generally refers to acting without considering, or simply ignoring, possible negative consequences. Several discrete aspects of impulsivity have been recognized (Evenden, 1999b; Winstanley, Dalley, Theobald, & Robbins, 2004), including two major sub-types: impulsive choice and impulsive action. Impulsive choice reflects poor decision-making which manifests as an inability to delay gratification and/or an increased tolerance to risk (Cardinal, 2006; Winstanley, Eagle, & Robbins, 2006). An example of an impulsive choice would be to spend a whole paycheck on an expensive item on a whim instead of saving the money. Impulsive action can be defined as an inability to withhold a motor response until the correct time or prior to receiving all the necessary information (i.e., waiting or action restraint; Dalley, Mar, Economidou, & Robbins, 2008; Winstanley, Eagle, et al., 2006). Another recognized form of impulsive action is a reduced ability to inhibit a motor response, either planned or in progress, in the face of changing internal or external demands (i.e., action cancellation or stopping; Dalley et al., 2008; Schachar et al., 2007; Winstanley, Eagle, et al., 2006). Impulsive action is also referred to as inhibitory control or response inhibition. An example of an impulsive action would be to interrupt a person who is speaking in mid-sentence (action restraint) or continue driving through an intersection when the
light turns from yellow to red (action cancellation). Both impulsive choice and impulsive action occur in normally functioning individuals (Evenden, 1999a) but when these behaviours occur repeatedly and consistently they may impair daily function. Accordingly, impulsivity is a core feature of many disorders listed in the *Diagnostic and Statistical Manual of Mental Disorders* (4th Edition [text revision] - DSM-IV-TR) such as problem gambling, attention-deficit/hyperactivity disorder, borderline personality disorder and substance abuse and dependence disorders (American Psychiatric Association, 2000; Rogers, Moeller, Swann, & Clark, 2010).

Impulsivity is often used interchangeably with the terms risk-taking and sensation-seeking, although each term represents different behaviours that are interconnected (Arnett, 1992; Evenden, 1999a; Forbes & Dahl, 2010; Kelly et al., 2006). Establishing the distinction between these constructs is important for understanding risky or reckless behaviour during adolescence. Adolescents are often labeled as impulsive based on the presence of risky behaviours during this period (Chambers et al., 2003; Spear, 2000; Steinberg, 2004) such as activities with a high potential of a negative consequences including criminal activity, unsafe sex, and dangerous driving (Arnett, 1992). Sensation-seeking and impulsivity are thought to be important contributors to risky behaviour (Arnett, 1992; Cooper, Agocha, & Sheldon, 2000) but the presence of risky behaviour does not necessarily imply increased impulsivity and sensation-seeking. Sensation-seeking is defined as the pursuit of highly stimulating, exciting, and intense experiences (Zuckerman, 1979, 1984). Examples include skydiving, racing, and drug use. It is important to note that sensation-seeking is not synonymous with impulsivity. Many acts of sensation-seeking are often well-planned and involve many safety measures (e.g., sky-diving) and thus participating in these activities may not result from impulsive decision making (Arnett, 1992).
4.3.1 Measures of Impulsivity

Mirroring the complex and multifaceted nature of impulsivity, several measures have been developed to quantify this psychological construct. In humans, researchers routinely use self-report and laboratory based measures. Many of the measures used to assess impulsivity in humans have also been adapted for use in non-human primates and rodents (e.g., Eagle & Robbins, 2003; Robbins, 2002).

4.3.1.1 Impulsive Choice. The most commonly quantified aspect of impulsive choice is delay discounting, a process where increasing the delay to obtain a reinforcer decreases the subjective value of that reinforcer (Ainslie, 1975; Cardinal, 2006; Rachlin, Logue, Gibbon, & Frankel, 1986; Stevenson, 1986). The rate at which the reinforcer is discounted as the delay increases is a common measure of ability to delay gratification. Delay discounting can be measured in humans and animals (see Winstanley, Eagle, et al., 2006 for review). The procedure involves the subject choosing between two outcomes: an immediate but small reward or a delayed but large reward. The delay to receive the large reward is gradually increased within or across sessions (e.g., Adriani & Laviola, 2003; Winstanley, Dalley, Theobald, & Robbins, 2003). Switching to the smaller, immediate reward rapidly (i.e., enhanced delay discounting) is a measure of impulsive choice. In experiments with human participants, rewards may be either hypothetical or real and often include money and cigarettes (e.g., Green, Myerson, & Ostaszewski, 1999; Mitchell, 2004; Olson, Hooper, Collins, & Luciana, 2007). In experiments using animal subjects, the reward is typically food (e.g., 1 vs. 4 pellets; Winstanley et al., 2003).

Impulsive choice may also manifest as an increased tolerance for risk. Similar to delay discounting, probability discounting is a process in which a reinforcer loses value as the probability of receiving that reinforcer decreases (Green & Myerson, 2004; Ho, Mobini, Chiang, Bradshaw, & Szabadi, 1999; Rachlin et al., 1986). The procedure for measuring probability discounting involves the choice between a small but certain reward and a large but uncertain reward. The probability of receiving the large reward is gradually decreased across the session. A
more rapid shift in preference from the small, certain reward to a large uncertain reward reflects impulsive choice. In humans, risk-taking and decision-making are also assessed using other computer based measures such as the Iowa Gambling task (Bechara, Damasio, Damasio, & Anderson, 1994) and Balloon Analogue Risk Task (Lejuez et al., 2002). Both tasks generally involve making choices between a safe or risky action to maximize real or hypothetical monetary gains. In both measures, impulsive choice is quantified by the number of risky decisions or the proportion of risky to advantageous choices.

4.3.1.2 Impulsive Action.

4.3.1.2.1 Stop-Signal Reaction Time test. Measures of impulsive action, specifically action cancellation, examine the ability of subjects to inhibit a pre-potent motor behaviour in response to an unexpected signal or stimulus. One example is the Stop-Signal Reaction Time test (Logan, 1997) which assesses how quickly a person can inhibit a pre-potent response already in progress. The participant learns to respond after the presentation of a ‘go’ stimulus (e.g., a cue light). During a sub-set of trials, a ‘stop’ signal (e.g., a tone) is presented immediately after the cue light indicating that the participant should not respond. The delay between the onset of the stimulus and the ‘stop’ signal are varied until the participant successfully inhibits responding on 50% of the ‘stop’ signal trials. The stop-signal reaction time (SSRT) is calculated based on the difference between the delay required to inhibit responding on 50% of the trials and the mean reaction time in response to the ‘go’ stimulus. Higher SSRTs reflect enhanced impulsive action. Action cancellation is also measured using the Stroop test (Stroop, 1935) which involves inhibiting the natural reaction to read the content of a word in order to name the colour of the text.

4.3.1.2.2 Go/No-Go task. Measures of action restraint assess the ability to withhold a pre-potent response until the appropriate time. One example is performance on the Go/No-Go task (Newman, Widom, & Nathan, 1985) used in humans, non-human primates and rodents. Participants learn to respond to a ‘go’ stimulus (e.g., yellow light) to receive a reinforcer and
inhibit a response after a ‘no-go’ stimulus (e.g., blue light). ‘Go’ trials occur more frequently so that responding becomes pre-potent. Successful performance on the Go/No-Go test requires the participant to inhibit responding of the pre-potent response during ‘no-go’ trials. Increased responses or false alarms during ‘no-go’ trials reflect increased impulsive action.

4.3.1.2.3 Responding on a DRL Schedule of Reinforcement. In rodents, action restraint can be measured using responding on a DRL schedule of reinforcement (differential reinforcement of low rates of responding; Sidman, 1955). Performance on this schedule examines the ability of the animal to withhold responding for a reinforcer until after an un-signalled waiting period. A reduced ability to wait to respond may reflect impulsive action. On a DRL schedule the animals must learn to respond for a reinforcer. To receive a subsequent reinforcer, the animal must withhold from responding until a pre-specified amount of time (T) has passed. Inter-responses times (IRTs) shorter than T reset the clock and thus do not result in the delivery of a reinforcer. Thus, to maximize the number of earned reinforcers, the animal must inhibit responding until the appropriate time (i.e., IRT>T; Gonzalez & Newlin, 1976). A high number of responses and short IRTs when responding on a DRL schedule may reflect impulsive action. Since the waiting time is un-signalled in responding on the DRL schedule of reinforcement, this behaviour also requires the ability to assess the passage of time and thus is not necessarily a pure measure of impulsivity.

4.3.1.3.4 The 5-Choice Serial Reaction Time test (5-CSRTT). The 5-CSRTT also assesses action restraint (Bari, Dalley, & Robbins, 2008; Carli, Robbins, Evenden, & Everitt, 1983; Robbins, 2002). This measure of attention and response inhibition used in animals was developed based on the continuous performance test of attention and vigilance in humans (Rosvold, Mirsky, Sarason, Bransome, & Beck, 1956). The 5-CSRTT involves training the animal to respond in one of 5 possible briefly lit apertures (light cue) to receive a reward. If the animal responds prior to the onset of the light cue (premature response), the animal is punished by receiving a time-out and no reinforcer. Premature responses are the primary measure of
impulsive action on the 5-CSRTT. To receive the maximum number of reinforcers, animals must 1) focus their attention on the apertures to observe the brief predictive stimulus in order to respond accurately (i.e., requires visual attention) and 2) wait to respond until the onset of this visual stimulus (i.e., requires response inhibition). The potential confound of the ability to assess the passage of time is removed because the end of the waiting period is signalled by the presentation of a stimulus light. Another strength of the 5-CSRTT is that performance on this test provides measures of reaction time to respond to the light and the reinforcer and therefore inferences can be made about general motivation and performance (Bari et al., 2008; Robbins, 2002).

4.3.2 Neurocircuitry of Impulsivity

4.3.2.1 Impulsive Choice. Studies examining the neurocircuitry of impulsive choice have focused on the reciprocally connected OFC, the nucleus accumbens core and the BLA. In rats, excitotoxic lesions of the OFC reduce (Winstanley, Theobald, Cardinal, & Robbins, 2004) or increase impulsive choice (Mobini et al., 2002). Differences in the timing of the lesion in relation to training and procedural differences may account for these contrasting findings (Winstanley, Theobald, Dalley, & Robbins, 2005; Zeeb, Floresco, & Winstanley, 2010). While excitotoxic lesions of the OFC affect impulsive choice, excitotoxic lesions of the prefrontal or anterior cingulate cortices do not affect this behaviour (Cardinal, Pennicott, Sugathapala, Robbins, & Everitt, 2001). Excitotoxic lesions of the BLA (Winstanley, Theobald, et al., 2004) and nucleus accumbens core, but not the shell, precipitate a switch to a small, more immediate reward (i.e., increase impulsivity (Basar et al., 2010; Cardinal et al., 2001; Pothuizen, Jongen-Reilo, Feldon, & Yee, 2005). A role for the OFC and amygdala in impulsive choice has also been demonstrated in humans. Humans with lesions of these brain regions also exhibit enhanced impulsive choice (Bechara, Damasio, Tranel, & Anderson, 1998; Becker, 1999). Unlike the findings from animal studies, functional magnetic resonance imaging (fMRI) studies in humans do not strongly support a role for the ventral striatum, which includes the nucleus accumbens, in impulsive
choice (Basar et al., 2010). The reason for the divergent findings between human and animal studies on the involvement of the nucleus accumbens in impulsive action is unclear. Some have theorized that the role of the nucleus accumbens in impulsive action may depend on the type of behavioural measure used and whether activation is measured prior to, or after, the receipt of the reward (Basar et al., 2010). Together these results strongly indicate a role for the BLA and OFC in impulsive choice although the role of the ventral striatum is less clear.

Neurotransmitters in cortical and subcortical brain regions mediate impulsive choice. Findings on the role of dopamine in delay discounting in animals are mixed. In the OFC, the dopamine metabolite, 3,4-dihydroxyphenylacetic acid (DOPAC) is increased during delay discounting sessions (Winstanley, Theobald, Dalley, Cardinal, & Robbins, 2006) suggesting increased dopamine utilization in this region. However, depletion of dopamine in the nucleus accumbens does not affect delay discounting (Winstanley et al., 2005). Amphetamine enhances delay discounting in some studies (Cardinal, Robbins, & Everitt, 2000; Evenden & Ryan, 1996; Isles, Humby, & Wilkinson, 2003) but decreases this behaviour in other studies (Baarendse & Vanderschuren, 2012; Floresco, Tse, & Ghods-Sharifi, 2008; Isles et al., 2003; Wade, de Wit, & Richards, 2000; Winstanley et al., 2003). Dopamine receptors are involved in impulsive choice (Cardinal et al., 2000; Floresco et al., 2008) although the degree of involvement of D₁ compared to the D₂ dopamine receptors is debated (Koffarnus, Newman, Grundt, Rice, & Woods; Wade et al., 2000). The role of 5-HT is also unclear for impulsive choice. Depleting forebrain 5-HT either increases (Bizot, Le Bihan, Puech, Hamon, & Thiebot, 1999; Mobini, Chiang, Ho, Bradshaw, & Szabadi, 2000; Wogar, Bradshaw, & Szabadi, 1993) or has no impact on delay discounting (Winstanley et al., 2003; Winstanley, Dalley, et al., 2004). Glutamate is also involved in this behaviour; blocking NMDA receptors with ketamine enhances delay discounting (Floresco et al., 2008). Thus dopamine, 5-HT and glutamate all likely interact and contribute to the expression of impulsive choice.
4.3.2.2 Impulsive Action. Some of the same brain structures have been implicated in the expression of impulsive action across species. Human studies indicate a role for the PFC and dorsal, but not the ventral, striatum in response inhibition (Aron & Poldrack, 2005; Basar et al., 2010; Dalley et al., 2011; Zandbelt & Vink, 2010). In non-human primates, waiting to respond activates the PFC (Narayanan & Laubach, 2006; Niki & Watanabe, 1979). For premature responding on the 5-CSRTT in rats, excitotoxic lesions or inactivation of the PFC increase impulsive action (Chudasama et al., 2003; Narayanan & Laubach, 2006) while lesions of the nucleus accumbens core only increase premature responding if the previous trial did not result in the delivery of a reinforcer (Basar et al., 2010; Christakou, Robbins, & Everitt, 2004; Murphy, Robinson, Theobald, Dalley, & Robbins, 2008) or if the waiting period to make a response was increased (i.e., a longer inter-trial-interval – ITI; Christakou et al., 2004; but see Murphy et al., 2008). However, connections between the PFC and the nucleus accumbens may play a critical role in impulsive action. Disconnecting the PFC and nucleus accumbens core increases premature responding in the 5-CSRTT (Christakou et al., 2004). Learning to inhibit a pre-potent response also results in enhanced excitatory neuroplasticity in the projections from the PFC to the nucleus accumbens (Hayton, Lovett-Barron, Dumont, & Olmstead, 2010). For responding on a DRL schedule, excitotoxic lesions of the nucleus accumbens core (Pothuizen et al., 2005), but not the PFC (see Markowitsch & Pritzel, 1977 for review), increase impulsive action. The differences in the effects of lesions on premature responding in the 5-CSRTT and responding on a DRL schedule of reinforcement highlight that these measures may capture different aspects of impulsive action and behaviour.

Several neurotransmitter systems contribute to the expression of impulsive action. One example is 5-HT. Global depletion of central 5-HT increases premature responding on the 5-CSRTT and performance on the Go/No-Go task (Harrison, Everitt, & Robbins, 1997, 1999). Systemic or intra-accumbens injections of 5-HT_{2A} or 5-HT_{2C} receptor antagonists result in opposing effects on premature responding in the 5-CSRTT (decreasing and increasing premature
responding, respectively; Fletcher, Tampakeras, Sinyard, & Higgins, 2007; Robinson et al., 2008). The ability of 5-HT to affect impulsive action may also be mediated by interactions between the 5-HT and dopamine systems (see Dalley et al., 2008 for review). The mesocorticolimbic dopamine system plays an important role in this behaviour. Amphetamine increases impulsive action (Blackburn & Hevenor, 1996; Cole & Robbins, 1989; Murphy et al., 2008; Pattij, Janssen, Vanderschuren, Schoffelmeer, & van Gaalen, 2007) and antagonism of D2 dopamine receptors or depletion of dopamine in the nucleus accumbens blocks this effect (Cole & Robbins, 1989; Pattij et al., 2007). In humans, increased amphetamine-induced striatal dopamine release correlates with increased self-reported impulsivity (Buckholtz et al., 2010). Glutamate also plays a role in impulsive action. Systemic and intra-PFC injections of non-selective NMDA receptor antagonists (e.g., MK-801), and more selective NR2B subunit antagonists, increase premature responding (Calcagno, Carli, Baviera, & Invernizzi, 2009; Carli, Baviera, Invernizzi, & Balducci, 2006; Fletcher, Rizos, Noble, & Higgins, 2011; Higgins, Ballard, Huwyler, Kemp, & Gill, 2003; Murphy, Dalley, & Robbins, 2005; Paine & Carlezon, 2009). Increased extracellular glutamate and dopamine in the PFC resulting from NMDA receptor antagonism may mediate these effects (Bonaventure et al., 2011) although recent evidence implicates GABA, and not glutamate, in the effects of NMDA receptor antagonism on impulsive action (Murphy et al., 2012). The brain regions and neurochemical systems involved in impulsive action continue to develop during adolescence, which suggests that expression of this behaviour may be altered during this specific developmental period.

4.3.3 Impulsivity, Risk-Taking and Sensation Seeking during Adolescence

Adolescents engage in many risky behaviours including unsafe sex, dangerous driving, minor criminal activity and illicit substance abuse (Arnett, 1992; Centers for Disease Control and Prevention, 2006; Maggs, Almeida, & Galambos, 1995). Risky behaviour is believed to contribute to the health paradox of adolescence: adolescence is a period of peak physical health but also of high rates of mortality and morbidity (Forbes & Dahl, 2010; Resnick et al., 1997;
Accidents are the leading cause of death in adolescents in the United States (approximately 50%), followed by homicides and suicides (Centers for Disease Control and Prevention, 2008). The majority of accidental deaths in adolescents are related to motor vehicle accidents (Sleet, Ballesteros, & Borse, 2010) which arguably may be related to unsafe and inexperienced driving (Steinberg, 2004) combined with the influence of peers (Chein, Albert, O'Brien, Uckert, & Steinberg, 2011; Steinberg, 2004). In the presence of their respective peers, adolescents make more risky decisions than adults in a simulated driving test (Chein et al., 2011; Gardner & Steinberg, 2005; Steinberg, 2004). The prevalence of reckless or risky behaviour in adolescence compared to adulthood may have contributed to the perception that adolescents are more impulsive than adults.

Relatively few studies exist on age differences in impulsivity in humans. Using self-report measures, adolescents respond similarly to adults about their hypothetical reactions in risky situations but report more sensation seeking (Steinberg, 2004; Zuckerman, Eysenck, & Eysenck, 1978). For laboratory measures of impulsive choice, adolescents consistently discount rewards less steeply than children but more steeply than adults (Green, Fry, & Myerson, 1994; Green et al., 1999; Scheres et al., 2006; Steinberg et al., 2009). Across the adolescent period, discounting also becomes less steep with age (Olson et al., 2007). Adolescents are not consistently more impulsive in other laboratory measures of impulsive choice. Adolescents and adults perform similarly on the Wheel of Fortune test (Eshel, Nelson, Blair, Pine, & Ernst, 2007), although findings on age differences in performance on the Iowa Gambling Task are mixed (Ernst et al., 2003; Overman et al., 2004). For laboratory measures of impulsive action, adolescents show enhanced (Eigsti et al., 2006; Rubia et al., 2006; Williams, Ponesse, Schachar, Logan, & Tannock, 1999) or similar impulsivity compared to adults as measured by performance on the Go/No-Go or Stop-Signal tests (Galvan, Poldrack, Baker, McGlennen, & London, 2011; Stevens, Kiehl, Pearlson, & Calhoun, 2007). Thus, for humans empirical evidence suggests that
observed age differences in impulsivity depend on the type of impulsivity and how it is measured.

Even fewer studies have examined age differences in impulsivity in animals. Adolescent mice discount rewards at shorter delays than adult mice (Adriani & Laviola, 2006) although this effect may depend on strain (Pinkston & Lamb, 2011). Adolescent rats show adult-typical patterns of delay discounting (Adriani & Laviola, 2006), however no adults were included in that study thus it is not clear if age differences in delay discounting exist in rats. Two previous studies have reported that adolescents show enhanced impulsive action as measured by responding on a DRL schedule of reinforcement (Andrzejewski et al., 2011; Lejeune & Jasselette, 1987). No studies have compared adolescents and adults on premature responding in the 5-CSRTT or an equivalent measure.

4.3.4 Sex Differences in Impulsivity

Males outnumber females in a number of risky behaviours including violent and most non-violent crimes, substance use, dangerous driving and extreme sports (Degenhardt et al., 2008; Harris & Jenkins, 2006; Norris, Matthews, & Riad, 2000; Pampel, 2001; United States Department of Justice, 2008; Wymer, Self, & Findley, 2008). Although these findings may suggest that males are more impulsive than females, results from empirical human studies on sex differences in impulsivity are mixed and vary as a function of the type of impulsivity measured. Males consistently self-report higher levels of sensation-seeking (Rosenblitt, Soler, Johnson, & Quadagno, 2001; Zuckerman et al., 1978; Zuckerman & Kuhlman, 2000) although sex differences are not reliably observed with other self-report measures of impulsivity (Hunt, Hopko, Bare, Lejuez, & Robinson, 2005; Macapagal, Janssen, Fridberg, Finn, & Heiman, 2011; Skinner, Aubin, & Berlin, 2004). For behavioural measures of impulsivity, males generally show enhanced impulsive choice compared to females in most (Hunt et al., 2005; Kirby & Marakovic, 1996; Petry, Kirby, & Kranzler, 2002) but not all experiments (Reynolds, Richards, & de Wit, 2006), while most studies report no sex differences in measures of impulsive action (Fillmore &
Weafer, 2004; Li, Huang, Constable, & Sinha, 2006; Reynolds et al., 2006). A recent meta-
alysis concluded that males and females show similar delay discounting and performance on
measures of impulsive action (Cross, Copping, & Campbell, 2011).

The findings on sex differences in impulsivity in animals, from a limited number of
studies, are also mixed. For impulsive choice, one study reported that females showed enhanced
delayed discounting compared to males (Koot, van den Bos, Adriani, & Laviola, 2009) but no
sex differences were reported in another study (Perry, Nelson, Anderson, Morgan, & Carroll,
2007). Males showed more impulsive action than females as measured by premature responses in
a modified 5-CSRTT and responding on a DRL schedule of reinforcement (Beatty, 1973; Jentsch
& Taylor, 2003; van Hest, van Haaren, & van de Poll, 1987) although no sex differences were
observed in performance on a Go/Go-No task (Anker, Gliddon, & Carroll, 2008). Strain
differences may partially account for these divergent findings on the effects of sex on impulsivity
but the procedural differences between experiments such as the role of timing, the presence of
cues and the type of reward used also likely play a role.

Similar to adults, young adult males are more likely than young adult females to engage
in illicit drug use, gambling and dangerous driving (Zuckerman & Kuhlman, 2000). However,
findings on sex differences in human adolescents from empirical studies using behavioural
measures of impulsivity are mixed (Cross et al., 2011; Fields, Collins, Leraas, & Reynolds, 2009;
Olson et al., 2007; Overman et al., 2004; Silveri et al., 2006). In adolescents, no sex differences
are reported for delay discounting (Fields et al., 2009; Olson et al., 2007) although females may
be more impulsive than males as measured by performance on the Iowa Gambling task
(Overman et al., 2004). Compared to adolescent females, adolescent males show enhanced
impulsive action on the Go/No-Go test but not the Continuous Performance test (Fields et al.,
2009). Adolescent males and females also self-report similar levels of impulsivity (Overman et
al., 2004; Silveri et al., 2006). A recent meta-analysis of studies with human participants
concluded that sex differences in impulsivity may emerge after adolescence (Cross et al., 2011)
although no published studies have examined sex differences in impulsivity across development in animals.

4.3.5 Impulsivity, Drug Use and Abuse

Impulsivity is often associated with drug use and addiction (Dalley et al., 2011; Rogers et al., 2010; Winstanley et al., 2010). In fact, the DSM-IV-TR criteria for substance abuse and dependence suggest that important diagnostic features of substance abuse and substance dependence disorders include a lack of self-control and the inability to stop a pre-potent behaviour (i.e., taking the drug) despite negative consequences (American Psychiatric Association, 2000) which reflects both impulsive choice and action. Several empirical studies indicate a relationship between impulsivity, drug use and addiction. In some studies, impulsivity and sensation-seeking positively correlates with the effects of drugs of abuse. For example in humans, sensation-seeking scores positively correlates with the self-reported ‘high’ and ‘rush’ derived from amphetamine (Kelly et al., 2006; Stoops et al., 2007) and how much an individual will work to self-administer the drug (Stoops et al., 2007). Drug users and addicts also score higher on measures of impulsivity. Adult smokers show enhanced discounting of hypothetical monetary rewards compared to non-smokers (Bickel, Odum, & Madden, 1999; Businelle, McVay, Kendzor, & Copeland; Mitchell, 1999). A similar relationship between impulsivity and drug use status has been reported for other types of drugs (Businelle et al.; Ersche et al., 2011; Kirby & Petry, 2004; Robles, Huang, Simpson, & McMillan, 2011). Impulsivity predicts other aspects of drug use and abuse including the degree of cravings in cocaine and methamphetamine users (Tziortzis, Mahoney, Kalechstein, Newton, & De la Garza, 2011) and the age of recreational drug use onset (Kollins, 2003). The correlation of impulsivity with the effects of drugs of abuse is not surprising given that similar brain regions and neurotransmitter systems mediate both types of behaviour (Dalley et al., 2011; Winstanley et al., 2010).

Relatively fewer studies have focused on establishing a relationship between impulsivity and drug use in human adolescents. Adolescent males who reported heavy drinking subsequently
self-reported higher levels of impulsivity during a one year follow up (White et al., 2011). Adolescent drug, alcohol and nicotine users also report increased delay discounting (Fields et al., 2009; Peters et al.; Reynolds & Fields, 2011; Reynolds, Patak, & Shroff, 2007). During adolescence, impulsivity predicted substance use disorders in some (von Diemen, Bassani, Fuchs, Szobot, & Pechansky, 2008) but not all studies (Malmberg et al., 2010). Thus, a relationship between impulsivity and drug use may exist in human adolescents.

Recently, several studies have examined how impulsivity and measures of drug taking influence each other in animals (Winstanley et al., 2010). Rats that rapidly shift from a large delayed reward to a small immediate reward also acquire cocaine self-administration more rapidly, escalate drug-taking during extended access sessions and show enhanced reinstatement of nicotine and cocaine seeking (Anker, Perry, Gliddon, & Carroll, 2009; Economidou, Pelloux, Robbins, Dalley, & Everitt, 2009; Perry, Nelson, & Carroll, 2008). However, rats that showed high or low impulsive choice as measured by delay discounting showed comparable levels of heroin self-administration (McNamara, Dalley, Robbins, Everitt, & Belin, 2010; Schippers, Binnekade, Schoffelmeer, Pattij, & De Vries, 2011). Impulsive action also affects measures of drug-taking and seeking in rodents. High premature responders in the 5-CSRTT also self-administer more cocaine and nicotine (Dalley et al., 2007; Diergaarde et al., 2008). Less research has focused on whether drug self-administration increases the propensity for impulsive behaviour. Cocaine and heroin self-administration did not affect impulsive action as measured by premature responding in the 5-CSRTT (Dalley et al., 2005) but heroin self-administration may enhance impulsive choice (Schippers et al., 2011). Together these findings suggest that high levels of impulsivity may result in increased psychostimulant self-administration but that psychostimulant self-administration may not increase impulsivity. The possible reciprocal relationship between impulsivity and the effects of drugs of abuse has not been examined in adolescent rats to my knowledge. However, one implication from the results from studies using adult rats is that if impulsivity is enhanced during adolescence compared to adulthood, then
adolescents may be more prone to drug use. Before this hypothesis can be tested, age differences in measures of impulsivity should be investigated.

5. Objectives & Hypotheses

Based on the reviewed literature, it can be concluded that 1) drug use typically commences during adolescence and adolescents respond differently than adults to some of the effects of drugs of abuse, 2) incentive motivation and impulsivity play important roles in the onset and progression of drug use and abuse 3) the neurocircuitry involved in the effects of drugs of abuse, incentive motivation and impulsivity continue to develop during adolescence. One implication of these conclusions is that the expression of incentive motivation and impulsivity may differ between adolescents and adults which may contribute to a susceptibility to the effects of drugs of abuse during the adolescent period. However, relatively little empirical research has examined the expression of impulsivity, in particular impulsive action, and incentive motivation during adolescence using animal models. Even fewer published reports exist on sex differences in these behaviours across development. The purpose of the work described in this thesis was to examine age differences in impulsive action and incentive motivation. I chose to focus on impulsive action instead of impulsive choice because when I began my thesis work, fewer studies had examined this behaviour in adolescent rats and evidence suggests that impulsive action and incentive motivation may interact to contribute to drug addiction (Jentsch & Taylor, 1999; Papachristou, Nederkoorn, Havermans, van der Horst, & Jansen, 2012; Verdejo-Garcia et al., 2012; Wiers et al., 2007). To help elucidate the possible role of neurotransmitters in age differences in these behaviours, I used pharmacological challenges that targeted the dopamine, glutamate and opioid neurotransmitter systems which continue to develop during adolescence (Crews et al., 2007; O'Dell, 2009; Spear, 2000).
5.1 Aim 1: Examine Possible Age Differences in Incentive Motivation for Natural Reward-Paired Stimuli as Measured by Responding for a Conditioned Reinforcer

To examine age differences in incentive motivation for natural reward-paired cues, I compared adolescents and adults on responding for a conditioned reinforcer previously paired with sucrose (Chapter 3). I also examined the effects of amphetamine (a dopamine releaser), SCH 23390 (a D₁ dopamine receptor antagonist), eticlopride (a D₂ dopamine receptor antagonist) and naltrexone (a non-selective opioid receptor antagonist) on responding for a conditioned reinforcer. My hypotheses were that adolescents would respond more than adults for a conditioned reinforcer based on previous studies on age differences in natural-reward induced CPP (Douglas et al., 2003, 2004). I also hypothesized that amphetamine would enhance, and SCH 23390, eticlopride, and naltrexone, would block responding for a conditioned reinforcer in adolescent rats based on previous studies using adult rats (Beninger & Ranaldi, 1992; Ranaldi & Beninger, 1993; Robbins et al., 1983).

5.2 Aim 2: Investigate Possible Age Differences in Incentive Motivation for Drug-Paired Stimuli as Measured by Responding for a Conditioned Reinforcer

To investigate age differences in incentive motivation for drug-paired cues, I compared adolescents and adults on responding for a conditioned reinforcer previously associated with passive IV nicotine infusions or self-administered IV nicotine infusions (Chapter 4). To assess whether IV nicotine exposure during conditioning would affect other aspects of behaviour (indicating a functional consequence of IV nicotine exposure), I examined age differences in the effects of a systemic nicotine challenge on locomotor activity. I hypothesized that adolescents would respond more than adults for a conditioned reinforcer previously paired with passive or self-administered IV nicotine infusions based on the findings from Chapter 3 and previous experiments on age differences in nicotine-induced CPP (Belluzzi et al., 2004; Brielmaier et al., 2007; Shram et al., 2006; Shram & Le, 2010; Vastola et al., 2002). I also hypothesized that both adolescents and adults with IV nicotine exposure would show a sensitized response to a systemic
nicotine challenge (Cruz et al., 2005; Faraday et al., 2003; Schochet et al., 2004; Vastola et al., 2002).

5.3 Aim 3: Examine Possible Age and Sex Differences in Impulsive Action as Measured by Responding on a DRL Schedule of Reinforcement

Age and sex differences in impulsive action were assessed by comparing male and female adolescents and adults during acquisition of responding on a DRL schedule of reinforcement for food and the effects of amphetamine on this behaviour (Chapter 5). I hypothesized that adolescents would respond more than adults during acquisition of responding on a DRL schedule of reinforcement based on two previous experiments (Andrzejewski et al., 2011; Lejeune & Jasselette, 1987). Adolescents and females may be the most sensitive to some effects of amphetamine thus I hypothesized that adolescents and adult females would show the most amphetamine-induced responses on a DRL schedule (Becker, Molenda, & Hummer, 2001; Laviola et al., 1999; Schramm-Sapyta et al., 2009).

5.4 Aim 4: Investigate Possible Age and Sex Differences in Impulsive Action as Measured by Premature Responding on a Modified Version of the 5-CSRTT

Finally, age and sex differences in impulsive action were investigated by comparing male and female adolescents and adults on premature responding on a modified version of the 5-CSRTT with only two choices (Chapter 6). Age and sex differences in the effects of amphetamine, Ro 63-1908 (an NMDA receptor NR2B subunit antagonist) and nicotine (a nAChR antagonist and indirect dopamine releaser) on premature responding were also examined. I hypothesized that adolescents would make more premature responses than adults based on previous studies suggesting that adolescents may show enhanced impulsive action (Andrzejewski et al., 2011; Eigsti et al., 2006; Lejeune & Jasselette, 1987; Rubia et al., 2006; Williams et al., 1999). I also hypothesized that adolescents and adults would respond differently to the effects of amphetamine, Ro 63-1908 and nicotine on premature responding (Pian et al., 2010; Ramirez, Varlinskaya, & Spear, 2011; Schramm-Sapyta et al., 2009). Finally, I hypothesized that adult
males would make more premature responses than adult females (Jentsch & Taylor, 2003) but that sex differences may not be observed in adolescent rats (Cross et al., 2011).
Chapter 2: General Methods

1. General Methods

The experiments described in this thesis involved the use of a variety of behavioural measures and procedures. The specifics of each measure are discussed in the individual chapters. Below I describe the subjects and their care, surgery, the various apparatuses and the general statistical approach.

1.1 Subjects

Pregnant Sprague-Dawley dams (gestational day 13) and male and female adult rats (>PND 70) were obtained from Charles River Farms (St. Constant, Quebec, Canada). For each experiment, dams and pair-housed males and females were brought into the colony on the same day. Adult male and non-pregnant female rats were pair-housed with a same-sex rat upon arrival to the colony and were left undisturbed until behavioural testing commenced. On PND 4, litters were culled to five male and five female rats. The colony was maintained on a 12 hr reverse light–dark cycle (09:00 to 21:00) in a room maintained at ~22 °C and humidity ~50–60%. Experimental procedures conformed to the guidelines of the Canadian Council on Animal Care (CCAC) and were approved by the Centre for Addiction and Mental Health Animal Care Committee.

1.2 Weaning

On PND 21, each adolescent rat was pair-housed with a rat of the same sex from a different litter. A maximum of 2 rats per litter per experimental group was used. For experiments that used food or sucrose rewards, all rats were given access to the respective reward in the home cage for 3 days prior to training (Adolescents PND 21-23 and adults > PND 70).
1.3 Food and Water Restriction

For experiments requiring food and water restriction, rats were deprived daily throughout training and testing for the 6 hr period (8:00 to 14:00) immediately prior to behavioural testing. We found that rats exposed to this food and restriction regimen gained weight across adolescence similarly to control rats (see Appendix 1).

1.4 Implantation of Intravenous Jugular Catheter

Rats were anaesthetized with Ketamine hydrochloride (Ketalean - 70 mg/kg; Vetoquinol, Lavaltrie, QC) and Xylazine (Rompun - 10 mg/kg; Bayer, Toronto, ON) administered intraperitoneally (IP). Silastic catheters with a 22 g guide cannulae on the terminal end (2 cm for adolescents, 3 cm for adults) were implanted into the right jugular vein and secured between the shoulder blades on the dorsal surface of the rat. Immediately after surgery rats were administered penicillin (Derapen, 0.1 ml, IM; Ayerst Veterinary Laboratories, Guelph, ON) and ketoprofen, an analgesic (Anafen, 0.03 mg/kg, SC; Merial Canada Inc, Baie d’Urfe, QC). Commencing on the day following surgery, catheters were flushed daily with heparin (0.1 ml of 30 i.u./ml, IV, LEO Pharma, Thornhill, ON) to minimize clotting within the catheter. Rats were allowed at least 5 days for recovery. Cather patency was tested at the end of IV drug experiments by infusing 0.1 ml of methohexital sodium IV (Brevital Sodium; 10mg/ml; Monarch Pharmaceuticals, Bristol, TN). If rats lost muscle tone, their catheters were considered patent.

1.5 Apparatus

1.5.1 Operant Conditioning Chambers

Various operant conditioning chambers were used for different experiments as described in the subsequent chapters. Each of these chambers was illuminated by a houselight and housed in a sound-attenuating box equipped with a ventilating fan. All of these chambers were supplied
by Med Associates (Med. Associates Inc, St Albans, VT, USA). Experiments were conducted using custom designed computer programs run on Med-PC IV.

1.5.2 Locomotor Activity

Locomotor activity was measured in a custom-built locomotor activity monitor. This system consisted of 16 clear polycarbonate cages, measuring 25 cm wide, 20 cm high, and 45 cm long. An array of six infrared photocells was attached outside the longer sides of the cages. The photocells were spaced 7.5 cm apart and 2 cm above the floor of the cage. The number of photocell disruptions was used as the measure of locomotor activity.

1.6 Drugs

D-amphetamine sulphate (U.S. Pharmacopeia, Rockville, MD) was dissolved in saline and administered IP immediately before testing. Nicotine bitartrate (Sigma, St. Louis, MO) was dissolved in saline, its pH was adjusted to 7.2 ± 0.2, and injected subcutaneously (SC) 15 min prior to (Chapter 6) or immediately before (Chapter 4) testing. Ro 63-1908 (Tocris Bioscience, Ellisville, MO) was dissolved in 0.3% Tween80 saline solution and injected SC 30 min prior to testing. SCH 39166 (Tocris Bioscience, Missouri, US) and eticlopride (Sigma, St. Louis, MI) were dissolved in saline and administered subcutaneously 15 min prior to testing. Naltrexone (Sigma, St. Louis, MI) was dissolved in saline and injected SC 30 min prior to testing.

1.7 Statistical Analyses

Statistical analyses were conducted using Statistica 7.0. Parametric statistics were used including analysis of variance (ANOVAs) and Student’s t-tests. Post-hoc analyses were conducted using the Bonferroni correction or student t-tests as necessary.
Chapter 3: Age Differences in Responding for a Conditioned Reinforcer Previously Paired with Sucrose: Possible Roles for Dopamine and Opioid Systems*

Abstract

Vulnerability to the effects of drugs of abuse during adolescence may be related to altered incentive motivation, a process believed to be important in addiction. Incentive motivation can be seen when a neutral stimulus acquires motivational properties through repeated association with a primary reinforcer. We compared adolescent (postnatal day – PND 24-50) and adult (> PND 70) rats on a measure of incentive motivation: responding for a conditioned reinforcer (CR). Rats learned to associate the delivery of 0.1 ml of 10% sucrose with a conditioned stimulus (CS; light and tone); 30 pairings per day were given over 14 days. Then, we measured responding on a lever delivering the CS (now a CR) after injections of amphetamine (0, 0.25 or 0.5 mg/kg). We also examined responding for a CR when the CS and sucrose were paired or unpaired during conditioning and responding for the primary reward (10% sucrose) in control experiments. Finally, we examined the effects of D₁ and D₂ dopamine receptor antagonists (SCH 39166 and eticlopride respectively) and an opioid receptor antagonist (naltrexone) on responding for a CR in adolescent rats. Adolescents but not adults acquired responding for a CR but adolescents responded less than adults for the primary reward. Responding for a CR depended upon the pairing of the CS and sucrose during conditioning. Both dopamine and opioid receptor antagonists reduced responding for the CR. Therefore, incentive motivation may be enhanced in adolescents compared to adults and incentive motivation may be mediated in part by both dopamine and opioid systems.

1. Introduction

Adolescents may be more vulnerable to the effects of drugs abuse but the issue of what confers this vulnerability remains unresolved (Schramm-Sapyta et al., 2009; Spear, 2000). During this developmental period, many factors linked to drug abuse and addiction converge. Adolescents are considered to be prone to sensation-seeking and risky behavior which may be, in part, the result of altered reward processing (Doremus-Fitzwater, Varlinskaya, & Spear, 2010; Ernst et al., 2009; Spear, 2000). Although understanding how rewards themselves are processed during adolescence is important, another critical question is how do adolescents respond to reward paired-stimuli, particularly in the context of substance abuse. According to the incentive salience theory of addiction (Robinson & Berridge, 1993, 2001), cues paired with drugs contribute prominently to the development, maintenance and reinstatement of drug abuse. One process that plays an important role in attributing salience to such drug-paired cues is incentive motivation.

Incentive motivation is, in part, the process whereby initially neutral environmental stimuli, such as lights and tones, acquire motivational properties by association with primary reinforcers (Robbins, 1976; Taylor & Robbins, 1984). This process can be measured in a test of operant responding for a conditioned reinforcer. In this procedure, rats learn to associate, through Pavlovian conditioning, an environmental stimulus (e.g., light and a tone) with a reinforcer (e.g., sucrose or drug). During a subsequent operant phase, rats learn to respond for the initially neutral stimulus that now functions as a conditioned reinforcer. Incentive motivation is considered to be a fundamental aspect of motivation in general, and may play a crucial role in addiction and drug-seeking behavior. For example, when cocaine-users view a video of someone purchasing or preparing cocaine for self-administration, these cocaine-associated stimuli can induce craving (Childress et al., 1999). In adult rats, psychostimulants such as amphetamine and cocaine can enhance incentive motivation as measured by increased responding for conditioned reinforcers that were previously paired with natural reinforcers (Beninger & Ranaldi, 1992; Fletcher et al.,
1998; Ranaldi & Beninger, 1993; Smith et al., 1997; Taylor & Robbins, 1984). This effect is mediated in part by increased activity of the mesocorticolimbic dopamine system upon which these psychostimulants act. One function of this pathway is to attribute incentive salience to stimuli, such that those stimuli become more ‘attractive’. Augmented activity of this dopamine system can lead to attribution of maladaptively high incentive salience to the primary reinforcer (e.g., drug) as well as to the stimuli associated with the reinforcer (Berridge & Robinson, 1998). This change in dopamine system function may result in increased potential for the attribution of salience to environmental stimuli, such that these stimuli become increasingly ‘wanted’. Alterations of incentive motivation have been suggested to increase drug seeking and drug taking, and therefore addictive behaviors, through enhancement of ‘wanting’ (Berridge & Robinson, 1998).

Incentive motivation may contribute to a vulnerability to the effects of drugs of abuse during adolescence (Geier & Luna, 2009). Results of some studies suggest that adolescents do show increased incentive motivation for drug-paired cues. These studies using the CPP procedure showed that compared to adults, adolescent rats prefer an environment that was previously paired with a cocaine, amphetamine or nicotine (Badanich et al., 2006; Laviola et al., 1999; Shram & Le, 2010; Torres et al., 2008; Zakharova, Leoni, et al., 2009).

Our aim was to extend these findings by comparing adolescents and adults on responding for a conditioned reinforcer previously paired with sucrose in adolescents and adults in a food and water deprived or non-deprived state. Having found an age-related difference and in light of the findings of Olsen and Winder (2009), we further characterized this behavior by examining whether pairing the CS and sucrose during conditioning was critical for rats to respond for a conditioned reinforcer. To examine whether unconditioned motivation for sucrose, the primary reward, might contribute to the age-related difference in responding for a conditioned reinforcer, we also examined responding for sucrose on a progressive ratio (PR) schedule of reinforcement. Finally, we investigated the role of the dopamine and opioid receptors in responding for a
conditioned reinforcer in adolescent rats given their respective roles in `wanting’ and `liking’ as put forth by the incentive salience model (Berridge & Robinson, 1998).

2. Methods

2.1 Group Assignment

Only male rats were used for these experiments. For experiments 1, 2 and 4, separate cohorts of 12 adolescents and 12 adults per experiment were used. In experiment 3, 24 adolescents and 12 adults were divided equally into 2 groups each (Paired and unpaired). In experiment 5, 36 rats were equally divided into the following three groups: SCH 39166, eticlopride and naltrexone. For details on weaning, please see Chapter 2.

2.2 Apparatus

Twelve operant conditioning boxes were used (28 cm long x 21 cm wide x 21 cm high). Two levers 4.5 cm wide and 7 cm above the floor with stimulus lights positioned directly above were located 6.5 cm on either side of a central, recessed magazine with a solenoid valve positioned 3 cm from the floor of the chamber.

2.3 Procedure

2.3.1 Experiment 1: Age Differences in Responding for a Conditioned Reinforcer with Food and Water Restriction

Adolescent (PND 24) and adult rats (> PND 70) were placed in the chambers for one 15 min session with 2 ml of sucrose in the magazine receptacle; response levers were retracted. The following day food and water was removed from the cages daily for 6 hr (8:00 to 14:00) prior to behavioral testing until the end of the experiment 1a. We found that rats exposed to this food restriction regimen gained weight across adolescence similarly to control rats (see Appendix 1).
2.3.1.1 Experiment 1a: Responding for a Conditioned Reinforcer

2.3.1.1.1 Pavlovian Conditioning. Conditioning occurred in 14 daily 30 min sessions. With the levers retracted, adolescent (PND 25-38) and adult rats (> PND 70) learned to associate the delivery of 0.1 ml of 10% sucrose with the CS: two red stimulus lights were illuminated and the houselight was turned off for 5 s and during the last 0.5 s a tone sounded (2900 Hz; 85 dB). At the end of the tone, sucrose was delivered. The CS and reinforcer were presented on a random time 60 s (RT60) schedule (30 paired CS and sucrose presentations per session). To assess discriminated approach we measured nose pokes in the magazine during the 5 s CS periods (CSR), and during the 5 s periods immediately prior to CS periods (PCSR).

2.3.1.1.2 Operant Responding for a Conditioned Reinforcer. During this phase responding was never reinforced with sucrose. Adolescent (PND 39) and adult rats (> PND 70) were habituated to the levers in the chamber in one session. A response on the left lever delivered the CS (now the conditioned reinforcer) according to a random ratio 2 (RR2) schedule (CR - active lever) and responses on the right lever had no programmed consequence (NCR – inactive lever). After 10 CR lever presses the session terminated. Next we measured responding for conditioned reinforcer on a RR2 schedule in adolescent (PND 40-44) and adult rats (> PND 70) in three 40 min sessions following IP injections of saline, 0.25 or 0.5 mg/kg amphetamine immediately before the session. The order of the doses was counterbalanced and sessions occurred 48 hr apart. Rats were left undisturbed during these 48 hr periods. We measured responses on the CR and NCR levers.

2.3.1.2 Experiment 1b: Amphetamine-Induced Locomotion. Next, locomotor activity was assessed in a randomly selected subset of adolescent (PND 54) and adult rats (> PND 70; n = 8 for both groups). First rats were exposed to the locomotor chambers for 2 hr. The following day, locomotion was measured for 2 hr and then for another 1 hr after a saline injection (1 ml/kg). Rats were then injected IP with 0.5 mg/kg of amphetamine and activity was measured for another hour.
2.3.2 Experiment 2: Age Differences in Responding for a Conditioned Reinforcer with No Food and Water Restriction

2.3.2.1 Experiment 2a: Responding for a Conditioned Reinforcer. Procedures for experiment 2a were the same as for experiment 1a except that rats were allowed free access to food and water throughout the entire experiment.

2.3.2.2 Experiment 2b: Amphetamine-Induced Locomotion. Adolescent (PND 46-47) and adult (> PND 70) rats from experiment 2a were used. Procedures for experiment 2b were the same as experiment 1b.

2.3.2.3 Experiment 2c: Extinction of Responding for a Conditioned Reinforcer. We examined whether responding on the lever that delivered the conditioned reinforcer (CR lever) would extinguish across multiple daily 40 min sessions in adolescent (PND 48-54) and adult (> PND 70) rats. No sucrose was available during these sessions.

2.3.3 Experiment 3: Role of Pairing the CS and US during Pavlovian Conditioning on Age Differences in Responding for a Conditioned Reinforcer

The Pavlovian phase of the experiment was conducted as for experiment 2a for adolescent and adult rats in the paired group. For rats in the unpaired group, both the CS and US (sucrose) were presented 30 times each but never at the same time. Tests of operant responding for a conditioned reinforcer were conducted as for experiment 2a. We then examined whether responding on the CR lever extinguished after multiple sessions in adolescent (PND 48-56) and adult (> PND 70) rats as described for experiment 2c.

2.3.4 Experiment 4: Age Differences in Responding for Sucrose on a PR Schedule

Adolescent (PND 24 – 31) and adult (> PND 70) rats were trained to respond for 10% sucrose on a FR1 schedule of reinforcement. Each lever press delivered 0.1 ml of sucrose and a white stimulus light was illuminated for 6 s. During this 6 s, lever presses were recorded but had no programmed consequences. For the first three days of training only, rats were food restricted
to facilitate learning to respond for sucrose. Adolescent rats were given approximately 8 g and adults were given approximately 16 g of Purina Rat chow per day. Starting on the fourth day, all rats were fed ad lib. After responding stabilized, a PR schedule of reinforcement was introduced. The progression was derived from the equation: ratio = \[5 \times e^{(0.1 \times \text{infusion no.})} - 5\] yielding response ratios of 1, 1, 2, 2, 3, 4, 5, 6, 7, 9, 10, 12, 13, 15, 17, 20, etc. Thus the number of responses required for 0.1 ml of sucrose increased with successive infusions. Sessions lasted until a period of 20 min without the delivery of a reinforcer, or were a maximum of 2 hr in length. The number of reinforcers earned before this breaking point was recorded. On PND 38 for adolescents, food was removed from all rats in the afternoon after testing and the next day responding for sucrose on the PR schedule was examined. Afterwards, food was available ad lib for the rest of the experiment. Responding was re-stabilized the following day.

2.3.5 Experiment 5: The Effect of SCH 39166, Eticlopride or Naltrexone on Responding for a Conditioned Reinforcer

2.3.5.1 Experiment 5a: Responding for a Conditioned Reinforcer. Only adolescent rats were used in this experiment. Pavlovian conditioning and lever habituation was conducted as described in experiment 1a except rats were allowed free access to food and water. Rats were assigned to receive injections of either SCH 39166, eticlopride or naltrexone. On PND 42, 44 and 46 responding on the CR and NCR lever under the influence of either SCH 39166 (0, 0.03 or 0.06 mg/kg), eticlopride (0, 0.05 or 0.1 mg/kg) or naltrexone (0, 1.0 or 2.0 mg/kg) was measured in 40 min sessions. Each rat received each dose of their respective drugs and the order of the doses was counterbalanced across the sessions. Rats were left undisturbed during the 48 hr periods between test sessions. SCH 39166 and eticlopride were injected SC 15 min prior and naltrexone was injected SC 30 min prior to the beginning of the session.

2.3.5.2 Experiment 5b: The Effect of SCH 39166, Eticlopride or Naltrexone on Responding for Water. Next, rats were trained to respond for 0.01 ml delivery of tap water on a RR2 schedule of reinforcement in 30 min sessions (PND 51-54). Prior to each session, rats were
deprived of water for 15 hr. Once responding was stable (4 sessions), rats from each respective group were injected with either saline or SCH 39166 (0.6 mg/kg), eticlopride (0.05 mg/kg) or naltrexone (2 mg/kg) over PND 55-57. The doses were counterbalanced across sessions and the pre-treatment times were as described in experiment 5a. Sessions were 48 hr apart.

2.4 Statistical Analyses

For experiment 1 and 2, data from the Pavlovian phases of the conditioned reinforcer experiments were analyzed using a 3 way ANOVA with session and discrimination (CSR vs. PCSR) as within subjects factors and age as the between subjects factor. Data from the operant phase of the conditioned reinforcer experiments were analyzed using a 3 way ANOVA with drug (amphetamine or saline) and lever (CR or NCR) as within subjects factors and age as the between subject factor. Responding on the CR and NCR lever during extinction was analyzed with a 3 way ANOVA (age x lever x session). For experiment 3, we analyzed the conditioning data with a 4 way ANOVA (age x discrimination x session x contingency: CS and US paired or unpaired). We found a contingency x discrimination x session interaction and so to simplify analyses for this experiment we analyzed the paired and unpaired groups separately. The effects of saline and amphetamine on locomotor activity were analyzed separately with 2 way ANOVAs with age as the between subject factor and time as the within subjects factor. For experiment 4, the number of earned reinforcers was analyzed with age as the between subjects factor. The effects of overnight food deprivation on reinforcers earned was analyzed with a repeated measures analyses with age as the between subjects factor and session (session before food deprivation; session after food deprivation) as the within subjects factor. For experiment 6, responding during the Pavlovian phase was analyzed with group (SCH 39166, eticlopride or naltrexone) as the between subject factor. For responding for a conditioned reinforcer and water, these groups were analyzed separately with repeated measures analyses. Bonferroni post hoc tests were used when appropriate.
3. Results

3.1 Experiment 1: Age Differences in Responding for a Conditioned Reinforcer with Food and Water Restriction

3.1.1 Experiment 1a: Responding for a Conditioned Reinforcer

3.1.1.1 Pavlovian Conditioning. As shown in Figure 1a, rats responded in the magazine more during the CS periods than in the 5 s periods prior to the CS [main effect of discrimination; \( F_{(1,22)} = 74.9, p < 0.001 \)] and this difference increased across sessions [session x discrimination; \( F_{(13,286)} = 18.7, p < 0.001 \)]. There was no main effect of age [\( F_{(1, 22)} = 0.7, \text{ ns} \)] and no significant interactions with age (\( p > 0.05 \)).

3.1.1.2 Operant Responding for a Conditioned Reinforcer. The results of the 3 way ANOVA confirmed that rats responded more on the CR than NCR lever [main effect; \( F_{(1, 22)} = 26.6, p < 0.001 \)] and amphetamine increased responding [\( F_{(2, 44)} = 7.1, p = 0.002 \)]. Significant lever x age and lever x dose interactions [\( F_{(2, 44)} = 6.5, p = 0.003 \) and \( F_{(1, 22)} = 8.6, p = 0.008 \) respectively] were also found. As shown in Figure 1, adolescent rats, but not adult rats, responded more on the CR lever than the NCR lever after saline and amphetamine injections (\( p < 0.05 \)). Both doses of amphetamine increased CR responding compared to saline in adolescents (\( p < 0.05 \); Figure 1b and c), but not in adults. Following saline injection, adolescent rats made 46.2 ± 6.2 responses and adult rats made 20.2 ± 6.2 responses on the CR lever. Using the Bonferroni test in the context of the overall ANOVA, this specific difference was not significant. However, to examine this apparent difference further we used a 2 way ANOVA to analyze CR and NCR lever responding as a function of age under saline treatment. This analysis revealed a significant lever x age interaction [\( F_{(1, 22)} = 10.8, p = 0.03 \)]. With this more restricted analysis of the data, Bonferroni post hoc analyses showed that adolescents responded more than adults on the CR lever after a saline injection (\( p = 0.015 \)).
Figure 1: Responding for a Conditioned Reinforcer Previously Paired with Sucrose in Food and Water Deprived Rats. Panel A shows that approach behaviour during conditioning was similar in adolescents and adults. The number of nose pokes during the conditioned stimulus periods (CSR) as compared to the responses in the 5 s periods immediately prior to the conditioned stimulus (PCSR) increased across sessions ($p < 0.05$). Adolescents (Panel B) responded more for on the CR than NCR lever while responding on the levers was not significantly different in adult rats (Panel C). $$p < 0.01; \ast p < 0.05$$ CR vs. NCR responding. Amphetamine increased CR responding in adolescents rats only ($+ p < 0.05$, amphetamine CR responding compared to baseline). Values are means ± SEM. CR = lever delivering light and tone. NCR = lever with no programmed consequence. Adolescents $n = 12$, adults $n = 12$. 
3.1.2 Experiment 1b: Amphetamine-Induced Locomotion

Adolescent and adult rats previously deprived of food and water did not show significantly different levels of locomotor activity during the habituation phase \[F_{(1, 14)} = 0.9, \text{ns}\;\text{; data not shown}\], or after a saline injection \[F_{(1, 14)} = 0.4, \text{ns}\]. As shown in Figure 3, amphetamine increased locomotor activity, although adolescents and adults did not significantly differ in their response \[F_{(1, 14)} = 1.9, \text{ns}\]. The apparent reduction in amphetamine-induced locomotor activity in adolescent versus adult rats was not significant \((p = 0.2)\). No main effect of time and no time x age interaction was observed \[F_{(11, 154)} = 0.9, \text{ns}\;\text{and } F_{(11, 154)} = 1.1, \text{ns}\] respectively).

3.2 Experiment 2: Age Differences in Responding for a Conditioned Reinforcer with No Food and Water Restriction

3.2.1 Experiment 2a: Responding for a Conditioned Reinforcer

3.2.1.1 Pavlovian Conditioning. As shown in Figure 2a, rats responded in the magazine more during the CS periods than in the 5 s periods prior to the CS [main effect of discrimination; \(F_{(1, 22)} = 32.2, \text{< 0.001}\)] and this difference increased across sessions [session x discrimination; \(F_{(13, 86)} = 18.6, \text{p < 0.001}\)]. There was no main effect of age \[F_{(1, 22)} = 0.3, \text{ns}\] and no significant interactions with age \((p > 0.05)\).

3.2.1.2 Operant Responding for a Conditioned Reinforcer. The results of the 3 way ANOVA confirmed that rats responded more on the CR than NCR lever [main effect; \(F_{(1, 22)} = 31.0, \text{p < 0.001}\)] and adolescents responded more than adults \[F_{(1, 22)} = 7.3, \text{p = 0.01}\]. A lever x age interaction \[F_{(1, 22)} = 11.1, \text{p = 0.003}\] was also observed. As shown in Figure 2, adolescent rats, but not adult rats, responded more on the CR lever than NCR lever after saline and amphetamine injections \((p < 0.05)\). Following a saline injection, adolescent rats made \(64.4 \pm 12.4\) responses and adult rats made \(16.8 \pm 5.1\) responses on the CR lever. Using the Bonferroni test in the context of the overall ANOVA, this specific difference was not significant.
Figure 2: Responding for a Conditioned Reinforcer Previously Paired with Sucrose in Non Food and Water Deprived Rats. Panel A shows that approach behaviour during conditioning was similar in adolescents and adults. Adolescents (Panel B) responded more for on the CR than NCR lever at baseline and with amphetamine, while adults did not acquire responding for the CR. ** p < 0.01; CR vs. NCR responding. Amphetamine produced a non-significant increase in CR responding in adolescent rats. Values are means ± sem. CSR = nose pokes during conditioned stimulus. PCSR = nose pokes in the 5 s prior to the onset of the conditioned stimulus. CR = lever delivering light and tone. NCR = lever with no programmed consequence. Adolescents n = 12, adults n = 12.
**Figure 3: Amphetamine-Induced Locomotor Activity.** For rats previously deprived of food and water (Panel A; experiment 1) and rats fed ad lib (Panel B; experiment 2), locomotor activity was not significantly different between adolescent and adult rats after a saline or amphetamine injection. However, amphetamine appeared to stimulate locomotor activity less in adolescents. Values are means ± SEM. AMPH = amphetamine. For Panel A: adolescents n = 8, adults n = 8. For Panel B: adolescents n = 12, adults n = 12.
However, to examine this apparent difference further we used a 2 way ANOVA to analyze CR and NCR lever responding as a function of age under saline treatment. This analysis revealed a significant lever x age interaction [$F_{(1, 22)} = 15.2, p < 0.001$]. With this more restricted analysis of the data, Bonferroni post hoc analyses showed that adolescents responded more than adults on the CR lever after a saline injection ($p = 0.03$). As seen in Figure 2b, the 0.5 mg/kg of amphetamine appeared to increase responding in adolescent rats ($p = 0.14$) but this effect was not significant with the Bonferroni correction. In adult rats CR and NCR lever responding was not significantly different after saline or amphetamine injections.

3.2.2 Experiment 2b: Amphetamine-Induced Locomotion

Adolescent and adult rats not previously deprived of food and water did not show significantly different levels of locomotor activity during the habituation phase [$F_{(1, 22)} = 1.9, ns$; data not shown], or after a saline injection [$F_{(1, 22)} = 0.1, ns$; Figure 3b]. After an amphetamine injection, locomotor activity was increased similarly in both age groups [$F_{(1, 22)} = 3.0, ns$]. There was also no time x age interaction [$F_{(11, 242)} = 1.4, ns$].

3.2.3 Experiment 2c: Extinction

As shown in Figure 4, overall responding decreased across repeated sessions [main effect; $F_{(6, 132)} = 8.3, p < 0.001$], adolescents responded more than adults [main effect; $F_{(1, 22)} = 8.5, p = 0.008$] and rats responded more on the CR than NCR lever [main effect; $F_{(1, 22)} = 41.3, p < 0.001$]. Further, there was an age x lever x session interaction [$F_{(6, 132)} = 3.7 p = 0.002$]. Bonferroni post hoc tests showed that adolescent rats responded more on the CR than NCR lever during the first four sessions only ($p < 0.05$). During the remaining sessions responding on both the CR and NCR lever was low and stable. Adults did not respond significantly more on the CR than NCR lever during any session ($p > 0.05$). By the last session there were no significant effects of age or lever.
Figure 4: Extinction of Responding for a Conditioned Reinforcer Previously Paired with Sucrose. Adolescent rats responded more on the CR than NCR lever during the first four sessions ($p < 0.05$) although CR responding decreased across sessions until responding was not significantly different between levers. Responding on both the CR and NCR lever was low and stable in adult rats. Values = mean $+$ SEM. CR = lever delivering light and tone. NCR = lever with no programmed consequence. Adolescents $n = 12$, adults $n = 12$. 
3.3 Experiment 3: Role of Pairing the CS and UCS during Pavlovian Conditioning on Responding for a Conditioned Reinforcer

3.3.1 Experiment 3a: Responding for a Conditioned Reinforcer

3.3.1.1 Pavlovian Conditioning. A three way ANOVA revealed a significant main effect of contingency \( [F_{(1,3)} = 16.9, p < 0.001] \) and a contingency x discrimination x session interaction \( [F_{(13,416)} = 5.8, p < 0.001] \) so the paired and unpaired groups were analyzed separately. Paired rats of both ages responded in the magazine more during the CS periods than in the 5 s periods prior to the CS and this difference increased across sessions [main effect of discrimination: \( F_{(1,16)} = 25.7, p < 0.001 \); discrimination x session interaction: \( F_{(13,208)} = 8.1, p < 0.001 \)]. Adolescents also responded in the magazine more overall than adults [main effect of age; \( F_{(1,16)} = 5.4, p = 0.034 \)] but there were no significant interactions with age and discrimination or session (ns; Figure 5a). Conversely as shown in Figure 5b, in the unpaired group rats of both ages responded more during the 5 s periods prior to the CS than during the CS periods although responding was low and stable during both periods [main effect of discrimination: \( F_{(1,16)} = 46.0, p < 0.001 \)]. There was no discrimination x session interaction \( [F_{(13,208)} = 0.8, ns] \).

3.3.1.2 Operant Responding for a Conditioned Reinforcer. We analyzed the effects of a paired or unpaired CS-US on operant responding for the conditioned reinforcer. As shown in Figures 6a and b for rats in the paired group, adolescents responded more than adults after saline and amphetamine injections [main effect of age; \( F_{(1,16)} = 4.2, p = 0.05 \)] and rats responded more on the CR than NCR lever [main effect of lever; \( F_{(1,16)} = 9.9, p = 0.006 \)] with adolescents responding more on the CR lever than adults [age x lever interaction; \( F_{(2,32)} = 3.5, p = 0.05 \)]. As shown in Figures 6c and d for rats in the unpaired groups, rats responded more on the CR than NCR lever although generally responding was low [main effect of lever; \( F_{(1,16)} = 8.9, p = 0.008 \)]. There was also a lever x age interaction \( [F_{(1,16)} = 7.3, p = 0.016] \). Although it appeared that adolescents in the unpaired group responded more on the CR than the NCR lever, this difference was not significant \( (p = 0.3) \).
Figure 5: Approach Behaviour (Paired vs. Unpaired CS and Sucrose Experiment). As shown in Panel A, both adolescent and adult rats that had paired delivery of the conditioned stimulus (CS) and sucrose showed discriminated approach towards the magazine during the CS compared to during the 5 s prior to the CS. As shown in Panel B, both adolescent and adult rats that received both CS and sucrose delivery but never together did not show discriminated approach to the magazine during the CS. Values are means ± SEM. CSR = nose pokes during conditioned stimulus. PCSR = nose pokes in the 5 s prior to the onset of the conditioned stimulus. CR = lever delivering light and tone. NCR = lever with no programmed consequence. Paired adolescents n = 12, unpaired adolescents n = 12, paired adults n = 6, unpaired adults n = 6.
Figure 6: Responding for a Conditioned Reinforcer (Paired vs. Unpaired CS and Sucrose Experiment). Adolescent rats that had the CS and sucrose delivery paired during Pavlovian conditioning responded more on the CR compared to the NCR lever at baseline and in response to amphetamine (Panel A and B; ** $p < 0.01$ CR vs. NCR responding). Amphetamine (0.5 mg/kg) increased responding on the CR lever compared to baseline (+ $p < 0.05$). Adult rats and adolescent rats that did not have the conditioned stimulus (CS) and sucrose delivery paired during Pavlovian conditioning responded more on the CR than the NCR lever and amphetamine did not increase responding (Panel C and D). Values are means ± SEM. CR = lever delivering light and tone. NCR = lever with no programmed consequence. Paired adolescents n = 12, unpaired adolescents n = 12, paired adults n = 6, unpaired adults n = 6.
3.3.2 Experiment 3b: Extinction of Responding for a Conditioned Reinforcer

In the paired group, overall responding decreased across sessions \( F_{(15,240)} = 4.1, p < 0.001 \), although adolescents responded more than adults \( F_{(1, 16)} = 10.9, p < 0.001 \), and rats responded more on the CR than NCR lever \( F_{(1, 16)} = 27.1, p < 0.001 \). There was also a marginal but non-significant age x session x lever interaction \( F_{(15,240)} = 1.6, p = 0.06 \). Bonferroni post hoc tests showed that in adolescent rats responding on the CR lever was significantly greater than on the NCR lever from sessions 1-9 \( p < 0.05 \) but not during the remaining sessions. In adult rats responding on the CR and NCR lever was not significantly different during any session \( p > 0.1 \); see Table 1 for values). In the unpaired group, responding decreased across sessions \( F_{(15, 240)} = 4.1, p < 0.001 \) and rats responded more on the CR than NCR lever \( F_{(1, 16)} = 8.6, p = 0.01 \). However, responding on the CR and NCR lever was low across all sessions \(< 15 \) responses). There were no significant interactions \( ns \).

3.4 Experiment 4: Age Differences in Responding for Sucrose on a PR Schedule

As shown in Figure 7a, adolescents and adult rats readily acquired responding for sucrose on a PR schedule. Adults earned more reinforcers than adolescents [main effect of age: \( F_{(1, 22)} = 14.9, p = 0.001 \)]. There was no main effect of session and no session x age interaction for reinforcers earned. As shown in Figure 7 b, adolescents consumed more sucrose as a function of body weight (g/kg) \( F_{(1, 22)} = 103.9, p < 0.001 \), however the amount of sucrose consumed decreased over time for adolescent rats while adult sucrose consumption remained stable [age x session interaction: \( F_{(6,132)} = 2.5, p = 0.028 \)].

After responding was stable under the PR schedule, we examined the effects of overnight food deprivation on responding for sucrose. Food deprivation increased the number of reinforcers earned [main effect of session: \( F_{(1, 22)} = 6.6, p < 0.001 \) and generally adults earned more reinforcers than adolescents [main effect of age: \( F_{(1, 22)} = 14.9, p = 0.02 \).
Table 1: Extinction of Responding for a Conditioned Reinforcer (Paired vs. Unpaired Conditioned Stimulus and Sucrose Experiment). During the first sessions after responding for a conditioned reward was observed, only adolescent paired rats responded more on the CR than NCR lever (**p < 0.003: CR vs. NCR lever). By the final session, CR and NCR responding was not significantly different in all groups. Values are means ± SEM. CR = lever delivering light and tone. NCR = lever with no programmed consequence. Paired adolescents n = 12, unpaired adolescents n = 12; paired adults n = 6; unpaired adults n = 6.

<table>
<thead>
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<th>Group</th>
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<th>First Session</th>
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<th>Last Session</th>
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<td>NCR</td>
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<td>NCR</td>
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<td>16.8 (± 2.0)</td>
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<td>8.7 (± 4.4)</td>
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<tr>
<td>Adult Unpaired</td>
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<td>7.8 (± 2.8)</td>
<td>4.3 (± 1.0)</td>
<td>4.6 (± 0.8)</td>
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Figure 7: Responding for Sucrose on a PR Schedule. Panel A shows that adults earned more reinforcers than adolescent rats at baseline. Adolescents consumed more g/kg of sucrose than adults although this difference diminished across sessions (Panel B). Panel C shows that food deprivation increased the number of reinforcers earned in both adolescent and adult rats, although the increase was greater in adolescent rats (* $p < 0.05$; non-deprived vs. deprived; + $p < 0.05$; adults vs. adolescents). Food deprivation only increased g/kg of sucrose consumed in adolescent rats (Panel D, * $p < 0.05$; non-deprived vs. deprived). Values are means ± SEM. Adolescents n = 12, adults n = 12.
There was a significant age x session interaction [$F_{(1, 22)} = 6.3, p = 0.02$]. As shown in Figure 7c, compared to the previous session adolescents showed increased responding after food deprivation whereas adults did not. Food deprivation also increased the g/kg of sucrose consumed [main effect of session: $F_{(1, 22)} = 60.4, p < 0.001$] and compared to adults, adolescents consumed more g/kg of sucrose [main effect of age: $F_{(1, 22)} = 51.4, p < 0.001$]. There was also an age x session interaction [$F_{(1, 22)} = 27.3, p < 0.001$]. Bonferroni post hoc tests showed that food deprivation increased the g/kg of sucrose consumed in adolescents but not adults ($p < 0.05$; Figure 7d).

3.5 Experiment 5: The Effect of SCH 39166, Eticlopride and Naltrexone on Responding for a Conditioned reinforcer in Adolescent rats.

3.5.1 Experiment 5a: Responding for a Conditioned reinforcer

3.5.1.1 Pavlovian Conditioning. Rats responded more in the magazine during the CS periods than in the 5 s periods prior to the CS [main effect of discrimination; $F_{(13,429)} = 29.8, p < 0.001$] and this difference increased across sessions [session x discrimination; $F_{(13,429)} = 57.4, p < 0.001$]. The three drug groups were not significantly different [main effect: $F_{(1, 33)} = 0.2, ns$] and there were no significant interactions with age ($p > 0.05$; data not shown).

3.5.1.2 Operant Responding for a Conditioned Reinforcer. As seen in Figure 8a, for the SCH 39166 group rats responded more on the CR than NCR lever [main effect; $F_{(1,11)} = 22.0, p < 0.001$] and SCH39166 reduced responding [$F_{(2,22)} = 7.0, p = 0.0041$]. There was also a lever x dose interaction [$F_{(2,22)} = 4.4, p = 0.02$]. Post hoc analyses showed that compared to the saline condition, CR responding was only decreased after the highest dose (0.06 mg/kg) of SCH 39166 ($p < 0.05$). Responding on the NCR lever was not significantly reduced by either dose of SCH39166 (ns).
Figure 8: The Effect of SCH 39166, Eticlopride or Naltrexone on Responding for a Conditioned Reinforcer in Adolescent Rats. SCH 39166 (0.06 mg/kg; D₁ dopamine receptor antagonist), eticlopride (0.05 and 0.1 mg/kg; D₂ dopamine receptor antagonist) and naltrexone (2.0 mg/kg; opioid receptor antagonist) decreased CR lever responding compared to saline as shown in Panels A, B, and C respectively. (** p < 0.01; CR saline vs. CR antagonist) Values are means ± SEM. CR = lever delivering light and tone. NCR = lever with no programmed consequence. SCH 39166 n = 12, eticlopride n = 12, naltrexone n = 12.
For the eticlopride group, rats responded more on the CR than NCR lever [main effect; \( F_{(1,11)} = 16.4, p < 0.001 \)] and eticlopride reduced responding \[ F_{(2, 22)} = 12.4, p = 0.001 \]. There was also a lever x dose interaction \[ F_{(2, 22)} = 6.2, p = 0.007 \]. Post hoc analyses showed that compared to the saline condition, CR responding was decreased by both doses of eticlopride \( p < 0.01 \); Figure 8b). Responding on the NCR lever was not significantly reduced by either dose of eticlopride \( (ns) \).

As shown in Figure 8c, rats in naltrexone group responded more on the CR than NCR lever [main effect; \( F_{(1,11)} = 23.0, p < 0.001 \)] and naltrexone reduced responding \[ F_{(2, 22)} = 4.7, p = 0.02 \]. There was no lever x dose interaction \[ F_{(2, 22)} = 2.9, ns \]. Post hoc analyses showed that compared to the saline condition, CR responding was decreased by the highest dose of naltrexone \( p < 0.01 \). Responding on the NCR lever was not significantly reduced by either dose of naltrexone \( (ns) \).

To examine whether a higher dose of naltrexone would reduce responding for the CR even further, we conducted an additional two test sessions with vehicle and 3 mg/kg of naltrexone. We found that responding for the conditioned reinforcer with 3 mg/kg was similar to responding with 2 mg/kg of naltrexone (2 mg/kg CR responses: 16.1 ± 2.7, NCR responses: 5.9 ± 1.3; 3mg/kg CR responses 18.5 ± 3.0; NCR responses: 7.9 ± 2.0; data not shown).

3.5.2 Experiment 5b: The Effect of SCH 39166, Eticlopride and Naltrexone on Responding for Water in Adolescent Rats.

All rats readily acquired responding for water on a RR2 schedule of reinforcement (data not shown). The average responses for each group during the 4 acquisition sessions were SCH 39166: 398.3 ± 56.6, eticlopride: 372.6 ± 44.5, and naltrexone: 399.7 ± 34.3. During testing, SCH 39166 and naltrexone significantly reduced responding for water compared to saline [main effect of dose; \( F_{(1,11)} = 56.7, p < 0.001 \) and \( F_{(1,11)} = 25.6, p < 0.001 \) respectively]. The highest dose of eticlopride (0.1 mg/kg) almost completely abolished responding (data not shown;
<table>
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<th>Group</th>
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<th>Antagonist Responses</th>
</tr>
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<tr>
<td>SCH 39166</td>
<td>486.4 (± 73.1)</td>
<td>91 (± 36.9) **</td>
</tr>
<tr>
<td>Eticlopride</td>
<td>402.5 (± 74.6)</td>
<td>72.8 (± 26.1) **</td>
</tr>
<tr>
<td>Naltrexone</td>
<td>495.2 (± 51.3)</td>
<td>305.6 (± 33.1) **</td>
</tr>
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**Table 2: The Effect of SCH 39166, Eticlopride or Naltrexone on Responding for Water.**

SCH 39166, eticlopride and naltrexone all significantly reduced responding for water on a RR2 schedule of reinforcement compared to saline (**p < 0.01). Values are means ± SEM. SCH 39166 n = 12, eticlopride n = 6, naltrexone n = 12.
average of 8.2 ± 5.8 responses; n = 6). We then tested the lower dose of eticlopride (0.05 mg/kg) in the remaining six rats. At this dose, eticlopride also significantly reduced responding for water compared to saline [main effect; \( F_{(1, 5)} = 15.6, p = 0.008 \)]. See Table 2 for values.

### 4. Discussion

Adolescent rats but not adults acquired responding for a conditioned reinforcer previously paired with sucrose, indicating enhanced incentive motivation for reward-paired cues in adolescence. This effect was unlikely the result of enhanced unconditioned motivation for the primary reinforcer in adolescence because adolescent rats did not respond more than adults for the 10% sucrose. Further, adolescent rats only responded for a conditioned reinforcer when it had been previously paired with sucrose suggesting that the enhanced responding on the CR lever was not driven by an increased unconditioned motivation for the light and tone alone (e.g., Olsen & Winder, 2009). Finally, responding for a conditioned reinforcer during adolescence appeared to be mediated in part by both the dopamine and opioid systems.

In the present study, the acquisition of responding for a conditioned reinforcer in only adolescent rats was a robust and reliable effect. This behavioral difference was observed in 3 separate experiments and under various conditions. Acquisition of responding for a conditioned reinforcer in adolescent but not adult rats was observed regardless of the level of food deprivation. Thus responding for a conditioned reinforcer in adolescent rats was not dependent on hunger and was more likely related to the acquired incentive value of the CS. Responding for the conditioned reinforcer also extinguished following multiple exposures in adolescent rats in two separate experiments. This finding likely indicates that rats had learned an association between the CS and US, and therefore when the sucrose was no longer delivered with the CS after multiple sessions, responding for that stimulus was reduced. Further evidence that the acquisition of responding for a conditioned reinforcer reflected a learned association between the CS and the reinforcer was that the light and tone stimulus only supported operant responding if it had been previously paired with sucrose. Thus rats not only learned that the light and tone
predicted the delivery of sucrose but also that the incentive value of the CS, and not the light and tone itself, supported responding for the conditioned reinforcer. This distinction is important because Olsen et al (2009) showed that adult rats respond for a flashing light and responding for this stimulus was resistant to extinction. In the present experiment, when the CS was not paired with sucrose during conditioning, rats responded less on the CR lever and responding on this lever remained low and stable throughout the extinction sessions. Together our findings that responding for a conditioned reinforcer was 1) enhanced in adolescent rats compared to adults in multiple experiments and regardless of their level of food deprivation, 2) extinguished with repeated exposure to the conditioned reinforcer without the primary reinforcer and 3) dependent on the pairing of the CS and sucrose, demonstrate that adolescents have enhanced incentive motivation for cues previously paired with a natural reinforcer.

Adults did not significantly respond for a conditioned reinforcer in any of the present experiments. This lack of effect may be because we used only mild or no food deprivation. In previous studies using more severe deprivation regimens adult rats responded for a conditioned reinforcer (Burton, Nobrega, & Fletcher, 2009; Fletcher et al., 1999; Taylor & Robbins, 1984). We found in pilot work that these more severe food deprivation regimens do not permit adolescents to gain weight normally. Given this potential confound we chose a less severe regimen that was sufficient to motivate rats to learn a discriminated approach behaviour and still permitted adolescent rats to gain weight comparably to non-restricted controls (see Appendix 1). Although in the present study adult rats did not acquire responding for a conditioned reinforcer, the same rats during the Pavlovian conditioning phase approached the site of reinforcer delivery during the presentations of the CS similarly to adolescent rats. This finding implies that adults learned that the CS predicted reinforcer delivery even though subsequently this CS did not support responding (i.e., did not acquire incentive salience) in the absence of delivery of the primary reinforcer.
Free-fed adolescent rats did not respond more than adults for 10% sucrose on a PR schedule of reinforcement. Adolescents only consumed on average 1 ml of sucrose during each of the 1 hr sessions thus reduced responding in the adolescents is unlikely related to satiation. Food deprivation increased the number of earned sucrose reinforcers to a greater extent in adolescents compared to adults which may be interpreted as increased general motivation for sucrose under those conditions. However, adolescent rats responded more for a conditioned reinforcer regardless of their level of food deprivation in experiments 1 and 2. Also, mildly deprived adolescent rats (~40 responses) responded less on the CR lever than non-deprived rats (~ 60 responses) which implies that level of deprivation was not directly related to the amount of responding on the CR lever. Together these data imply that enhanced responding for the conditioned reinforcer in adolescent compared to adult rats was unlikely driven by enhanced inherent motivation for the sucrose reinforcer itself. We also found that compared to adults, adolescent rats consumed more sucrose as a function of their body weight (g/kg) although this difference decreased with age. Although in adolescents the amount of sucrose consumed as a function of body weight decreased across sessions, the number of reinforcers earned did not vary as a function of body weight. Thus although compared to adults, adolescent rats consumed more sucrose as a function of body weight, they were not more willing than adults to work for sucrose. Further evidence that adolescents were seemingly not more motivated than adults for sucrose in the present experiments is that discriminated approach behavior during the Pavlovian conditioning phase was similar between age groups in most of the experiments. Adolescents also showed enhanced responding for a conditioned reinforcer regardless of their state of satiety. Some previous studies suggest that compared to adults, adolescents may find sweet liquids more rewarding. Adolescent rats show increased hedonic responses to 10% sucrose and consumed more 1% sucrose as a function of body weight than adults (Wilmouth & Spear, 2009). In self-administration studies using the PR schedule of reinforcement, compared to adults, adolescents worked harder for condensed milk (Friemel, Spanagel, & Schneider, 2010) but not for saccharin
(Shram, Funk, et al., 2008). These discrepant findings, combined with our data, indicate that adolescents are not consistently more motivated than adults by sweet tasting rewards.

Our finding that adolescents show enhanced incentive motivation compared to adults is consistent with previous studies. Adolescent male rats demonstrated enhanced cocaine and amphetamine CPP compared to adult male rats (Badanich et al., 2006; Zakharova, Leoni, et al., 2009; Zakharova, Wade, et al., 2009), although this age difference was not consistently shown in all studies (e.g., Aberg et al., 2007; Mathews & McCormick, 2007). Adolescents also show enhanced CPP compared to adults for nicotine (Shram & Le, 2010; Torres et al., 2008), methamphetamine (Zakharova, Leoni, et al., 2009) and for natural reinforcers, such as a novel object (Douglas et al., 2003). Compared to adults adolescent rats also show enhanced c-fos protein expression in the nucleus accumbens core and dorsal striatum in response to an odor cue previously paired with a sweet reward (Friemel et al., 2010). These findings indicate that adolescents show enhanced incentive motivation and neuronal activation for reward-paired cues - our data now demonstrate that adolescent rats will work more than adults for the same type of cues.

Amphetamine, a dopamine releaser, enhanced responding for a conditioned reinforcer in adolescent more than in adult rats. This behavioural difference is unlikely the result of differential amphetamine-induced locomotor activity. The same adolescent rats that showed enhanced amphetamine-induced responding for a conditioned reinforcer tended to be hyporesponsive to the locomotor-stimulating effects of amphetamine compared to adults, which supports previous findings (Bolanos et al., 1998; Lanier & Isaacson, 1977; Laviola et al., 1999). A more likely interpretation for the differential effect of amphetamine between adolescents and adults is the differential drug-free responding for a conditioned reinforcer. Amphetamine is known to potentiate a pre-existing response for a conditioned reinforcer (Beninger & Ranaldi, 1992; Fletcher et al., 1998; Ranaldi & Beninger, 1993; Smith et al., 1997; Taylor & Robbins, 1984). Since adults did not respond for a conditioned reinforcer after a saline injection in any
experiment, amphetamine likely only selectively increased responding for a conditioned reinforcer in adolescent rats compared to adults because this response was only observed in adolescent rats.

Complementary to our results that amphetamine enhanced responding for a conditioned reinforcer in adolescents, the D₁ dopamine receptor antagonist SCH 39166 and the D₂ dopamine receptor antagonist eticlopride significantly reduced responding for a conditioned reinforcer. These findings are consistent with several previous experiments in adult rats demonstrating the role of dopamine receptors in responding for a conditioned reinforcer (Beninger & Ranaldi, 1992; Fletcher & Higgins, 1997; Fletcher et al., 1998; Ranaldi & Beninger, 1993; Robbins, 1976; Robbins et al., 1983; Smith et al., 1997; Sutton & Beninger, 1999; Taylor & Robbins, 1984). Given that dopamine receptor antagonists can also disrupt motor function, the potential role of the motor-depressing effects of these drugs on responding for a conditioned reinforcer must be considered. Both SCH 39166 and eticlopride reduced responding for water on a RR2 schedule. However, doses of eticlopride (0.05 mg/kg) and SCH 39166 (0.06 mg/kg) that significantly reduced responding for a conditioned reinforcer, also permitted rats to physically make more responses than the rats had previously made for a conditioned reinforcer after a saline injection (> 50 responses). Together these findings suggest a role for dopamine in incentive motivation, and motivation in general, as argued by others (Berridge, 2007; Berridge & Robinson, 1998; Everitt et al., 1999) although the potential contribution of reduced motor effects cannot be completely ruled out.

The opioid system also plays a role in mediating reward (Barbano & Cador, 2007; Berridge, 1996, 2009; Petrovic et al., 2008; Schneider et al., 2010; Smith & Berridge, 2007). We showed that naltrexone, a non-selective opioid receptor antagonist also reduced responding for a conditioned reinforcer in adolescent rats. This effect is unlikely to be the result of motor impairments because rats made on average over 300 responses for water after a naltrexone injection. Our data are consistent with a previous study that reported that morphine increased
responding for a conditioned reinforcer (Robbins et al., 1983). Together, these findings suggest a role of the opioid system in responding for a conditioned reinforcer and incentive motivation.

Responding for a conditioned reinforcer in adult rats is known to be mediated in part by limbic-striatal circuitry and the mesocorticolimbic dopamine pathway (Everitt et al., 1999). The acquisition of a response for a conditioned reinforcer is dependent on the BLA and the nucleus accumbens core, which receives many afferent projections from the BLA (Burns et al., 1993; Everitt et al., 1999; Taylor & Robbins, 1984). The psychostimulant potentiation of responding for a conditioned reinforcer is mediated by dopamine in the nucleus accumbens shell (Taylor & Robbins, 1984). Both the core and shell regions of the nucleus accumbens are richly innervated by dopaminergic projections from the VTA. Inactivation of the VTA blocks responding for a conditioned reinforcer (Murschall & Hauber, 2006). Although it is part of the mesocorticolimbic pathway, the PFC is not believed to be critical for acquiring responding for a conditioned reinforcer in adults (Burns et al., 1993; Everitt et al., 1999; Pears et al., 2003). Therefore in adult rats, connections between the nucleus accumbens and the BLA combined with dopaminergic innervation from the VTA mediate responding for a conditioned reinforcer.

This limbic-striatal circuit is still developing during adolescence. In particular, connections between the striatum, the amygdala and the PFC undergo many changes throughout adolescence, and thus the PFC may play a role in the enhanced responding for a conditioned reinforcer observed in adolescent rats compared to adults. Reciprocal projections between the BLA and the PFC continue to develop during adolescence (Cressman et al., 2010; Cunningham et al., 2002, 2008). PFC dopamine fiber density and dopaminergic input from the striatum increases and peaks during adolescence (Kalsbeek et al., 1988; Lewis, 1997). In the PFC both D₁ and D₂ dopamine receptor densities remain elevated until early adulthood (Andersen et al., 2000; Teicher et al., 1995) while in the ventral striatum, there is greater D₁ compared to D₂ dopamine receptor binding and receptor density during periadolescence. Increased D₁ dopamine receptor expression in the nucleus accumbens and PFC may be related to enhanced incentive motivation.
during adolescence. This hypothesis is supported by the report that increased D₁ dopamine receptor expression in the PFC-accumbal pathway mediates increased cocaine CPP in adolescent rats (Brenhouse et al., 2008). These data suggest, as discussed in Ernst’s triadic model (2006), that during adolescence an immature PFC supervisory system contributes to an overactive striatal reward system. The dynamic state of the mesocorticolimbic dopamine system during adolescence may mediate in part the enhanced responding for conditioned reinforcer and incentive motivation.

Relatively less is known about the development of the opioid system during adolescence. Some report that opioid receptors appear to peak during the second postnatal week and decline until adulthood (Winzer-Serhan et al., 2003) while others reported that µ opioid receptors are relatively stable across the adolescent period (Ellgren et al., 2008; Talbot et al., 2005). Therefore although previous studies imply that the immature state of the mesocorticolimbic system may be involved in the enhanced incentive motivation during adolescence, the role of the opioid system is less clear.

In conclusion, this study indicates that adolescent rats show enhanced incentive motivation for sucrose-paired cues compared to adults. This might contribute to an enhanced vulnerability to the effects of drugs of abuse through increasing the probability of incentive salience attribution to stimuli paired with reinforcers and in turn may increase ‘wanting’ and cravings more easily than in adults. Although these findings may suggest that adolescents are more prone to develop ‘wanting’ of reinforcers and thus may be more vulnerable to some of the effects of drugs of abuse, the interpretations from this study are limited to natural rewards. To investigate if this age difference in incentive motivation would also be observed for drug-paired cues, the work described in the next chapter examined possible age differences in responding for a conditioned reinforcer previously paired with IV drugs.
Chapter 4: Age Differences in Responding for a Conditioned Reinforcer Previously Paired with IV Nicotine Infusions

Abstract

Adolescents respond more than adults for a conditioned reinforcer previously paired with sucrose (Chapter 3). To examine if this age difference would be also observed in the conditioned reinforcing effects of drug-associated stimuli, adolescent and adult rats were compared on responding for a conditioned reinforcer previously paired with passive infusions of IV nicotine (experiment 1) or self-administered IV nicotine (experiment 2). I also examined whether this IV nicotine exposure would differentially produce a sensitized locomotor response to a systemic nicotine challenge in adolescent and adults. Adolescent and adult Sprague-Dawley rats with indwelling IV jugular catheters were used. During a conditioning phase, rats received either passive or self-administered IV infusions of nicotine (0.0, 0.003 or 0.03 mg/kg/infusion) paired with a light (CS). Next, responding on a lever that delivered the CS (now a conditioned reinforcer) was measured. Subsequently, the effects of an acute SC nicotine injection (0.4 mg/kg) on locomotor activity were examined. Adolescents self-administered more nicotine than adults. Compared to adults, adolescents responded more for a stimulus previously associated with passive IV nicotine infusions but less for a stimulus previously paired with self-administered IV nicotine infusions. Adolescent and adult rats previously exposed to passive or self-administered IV nicotine infusions, but not IV saline, showed a sensitized locomotor response to a nicotine challenge. This finding shows that IV nicotine infusions in both experiments affected behaviour, but this drug exposure did not induce responding for a conditioned reinforcer in adults in experiment 1 and adolescents in experiment 2. The results from this study suggest that control over the delivery of the drug may play an important role in the effects of nicotine across development.
1. Introduction

As described in chapter 3, adolescents responded more than adults for a conditioned reinforcer previously associated with a natural reward. Although this finding suggests that compared to adults, adolescents show evidence of enhanced incentive motivation for stimuli associated with natural rewards, it is still unclear whether this process is relevant to age differences in susceptibility to the effects of drugs of abuse. To investigate whether the age differences observed in chapter 3 would extend to responding for a conditioned reinforcer previously paired with drug cues, I examined age difference in responding for stimuli previously associated with IV nicotine infusions. Nicotine was chosen for the present experiments because of the particular importance of cues for the rewarding effects of nicotine (Caggiula, Donny, Chaudhri, et al., 2002; Caggiula et al., 2009; Caggiula, Donny, White, et al., 2002; Chaudhri et al., 2007; Chaudhri et al., 2006; Chiamulera, 2005; Donny et al., 2003) and because the onset of cigarette smoking typically occurs during adolescence (Centers for Disease Control and Prevention, 1994; Eissenberg & Balster, 2000). I hypothesized that, similar to the previous finding with sucrose-associated cues in chapter 3, adolescents would respond more than adults for a conditioned reinforcer previously paired with IV nicotine infusions.

2. Experiment 1: Responding for a Conditioned Reinforcer Previously Paired with Passive IV Nicotine Infusions

2.1 Rationale

I examined the effect of age on responding for a conditioned reinforcer previously associated with passive infusions of IV nicotine. I also investigated if amphetamine would enhance responding for the conditioned reinforcer as previously shown in chapter 3 (Burton, Noble, & Fletcher, 2011). Passive infusions were chosen initially for consistency with the previous work using a sucrose reward (Chapter 3). Adult rats have been shown to respond for a conditioned reinforcer previously paired with passively-administered IV infusions of heroin and amphetamine (Davis, Smith, & Khalsa, 1975). Based on the findings from Chapter 3, I
hypothesized that adolescents would respond more than adults for a conditioned reinforcer previously paired with passive IV nicotine infusions and that amphetamine would enhance this effect.

During the passive IV nicotine administration phase of the experiment, no behaviours were measured thus there was no behavioural indicator that nicotine was having a functional effect in the rats. To determine whether the passive IV nicotine infusions had some functional effect, I also examined nicotine-induced locomotion in the same rats. In adult rats, nicotine initially decreases locomotor activity, but after repeated injections, nicotine stimulates locomotor activity (Clarke & Kumar, 1983). Therefore, I hypothesized that rats receiving passive IV nicotine infusions during conditioning would show increased locomotor activity in response to a subsequent nicotine challenge compared to rats that had received IV passive saline infusions during conditioning. I also hypothesized that adolescents would show an enhanced nicotine-induced sensitized locomotor response compared to adults based on previous work (Belluzzi et al., 2004; Cruz et al., 2005; Faraday et al., 2003).

2.2 Experiment 1: Method

2.2.1 Apparatus

Experiments using IV drug administration and examining responding for a conditioned reinforcer were conducted in 12 operant boxes measuring 28 cm long, 21 cm wide and 21 cm high. Each chamber contained two response levers 4.5 cm wide and 7 cm above the floor with stimulus lights located 6.5 cm on either side of a central, recessed magazine. Response levers were inserted into the chambers only during tests of responding for a conditioned reinforcer and never during drug administration sessions. A counterbalanced arm held a fluid swivel above the ceiling of the chamber. The swivel was attached at one end by Tygon tubing to a syringe mounted on a motor-driven pump (Razel) located outside the chamber. At the other end of the swivel, a length of Tygon tubing, encased by a stainless-steel tether, allowed an animal’s catheter
to be connected to the syringe via the swivel. The apparatus used to measure locomotor activity is described in Chapter 3.

2.2.2 Procedure

2.2.2.1 Subjects. Rats had free access to food and water throughout the experiment.

2.2.2.2 Group Assignment. Adolescent rats from 8 litters, and adults, were assigned to six groups: Saline (adolescent n = 8, adult n = 8), nicotine 0.003 mg/kg/infusion (adolescent n = 6, adult n = 7) or nicotine 0.03 mg/kg/infusion (adolescent n = 7, adult n = 8).

2.2.2.3 Experiment 1a: Pavlovian Conditioning with Passively-Administered IV Nicotine Infusions. IV jugular catheters were implanted in adolescent (PND 26-27) and adult rats (> PND 70). Before conditioning, adolescents (PND 31) and adults (> PND 70) were habituated to the chamber and the tether in a single 60 min session. After another four recovery days, adolescents (PND 36-47) and adults (> PND 70) began daily conditioning sessions. Concentrations of nicotine were calculated daily based on each group’s respective average body weight (g). Nicotine (0.003 – 0.03 mg/kg/infusion) was dissolved in sterile saline and its pH was adjusted to 7.2 ± 0.2. Eighteen passive 0.1 ml IV infusions of nicotine (0.003 or 0.03 mg/kg/infusion) over 2 s were delivered per session on a random time (RT240) schedule. Each infusion was paired with a conditioned stimulus (CS). At the onset of each infusion, the house light was extinguished and two white stimulus lights were illuminated for 5 s. During the final 0.1 s of the light stimulus, a tone sounded. The parameters of the CS were the same as the previous study with a sucrose reward (Chapter 3). Each session lasted approximately 76 min. Each rat received a total of 216 pairings of either IV nicotine or saline infusions and the CS.

2.2.2.4 Experiment 1b: Responding for a Conditioned Reinforcer. During this phase, responding was never reinforced with nicotine. Adolescent (PND 48) and adult rats (> PND 70) were habituated to the levers in the chamber in one session. A response on the left lever (CR – the active lever) delivered the CS previously associated with nicotine infusions (now the
conditioned reinforcer) according to a RR2 schedule. Responses on the right lever had no programmed consequence (NCR – the inactive lever). After 10 CR lever presses or 40 min the session terminated. Next responding for conditioned reinforcer on a RR2 schedule was examined in adolescent (PND 49-53) and adult rats (> PND 70) in three 40 min sessions following IP injections of saline, 0.25 or 0.5 mg/kg amphetamine immediately before the session. The order of the drug doses was counterbalanced across sessions and a 48 hr washout period was given between test sessions. Rats were left undisturbed during the washout period.

2.2.2.5 Experiment 1c: Nicotine-Induced Locomotion. Locomotor activity was assessed in adolescent (PND 55) and adult rats (> PND 70). The number of photocell interruptions (beam breaks) was the measure of locomotor activity. Rats were exposed to the locomotor chambers for 2 hr per session during three daily sessions. Then, nicotine-induced locomotion was assessed in two sessions separated by a 48 hr washout period. During each session, locomotion was measured for 30 min and then for another 1 hr after a saline injection (1 ml/kg). Rats were then injected with either 0.4 mg/kg of nicotine SC or saline and activity was measured for another hour. The order of doses was counterbalanced across sessions and rats were left undisturbed during the washout period.

2.2.3 Statistical Analyses

No data was collected during Pavlovian conditioning (experiment 1a) therefore no statistical analyses were performed. Responses on the CR and NCR lever were analyzed with a 4 way ANOVA with age (adolescent vs. adult) and group (0, 0.003 or 0.03 mg/kg/infusion IV nicotine) as between subjects factors and lever (CR vs. NCR) and amphetamine dose (0, 0.25 vs. 0.5 mg/kg) as within subjects factors. Locomotor activity (beam breaks) during the 3 habituation sessions was analyzed with a 3 way ANOVA with age and group as the between subjects factors and session (1-3) as the within subjects factors. Locomotor activity totals during the test sessions were analyzed with a 3 way ANOVA with age and group as between subjects factors and drug challenge (saline vs. nicotine) as the within subjects factor. To investigate the time course of the
effects of the nicotine challenge, a 4 way ANOVA was conducted with the additional within
subjects factor of time (twelve 5 min bins)

2.3 Experiment 1: Results

2.3.1 Experiment 1b: Responding for a Conditioned Reinforcer

Generally, rats responded more on the CR than the NCR lever [main effect of lever;
$F_{(1,38)} = 4.1, p = 0.05$] and amphetamine decreased overall responding [main effect of dose; $F_{(2,76)}$
$= 4.5, p = 0.01$]. As shown in Figure 9, there was a non-significant trend for adolescents to
respond more than adults [main effect of age; $F_{(1,38)} = 3.5, p = 0.06$]. There was also an age x
lever x group interaction [$F_{(2,38)} = 3.2, p = 0.05$]. Separate repeated measures analyses for each
age and group showed that only adolescent rats that had received 0.03 mg/kg/infusion infusions
of IV nicotine responded more on the CR than NCR lever [main effect of lever; $F_{(1,6)} = 11.0, p =$
$0.02$].

2.3.2 Experiment 1c: Nicotine-Induced Locomotor Activity

There were no group differences during the first exposure to the locomotor chambers
(data not shown). Analysis of locomotor activity totals revealed drug challenge x group and drug
challenge x age interactions [$F_{(2,38)} = 4.4, p = 0.04$ and $F_{(1,38)} = 6.3, p = 0.01$ respectively]. To
understand these interactions, Student’s t-tests comparing saline and nicotine locomotor activity
total for each group showed that systemic nicotine significantly increased locomotor activity
compared to saline in both adolescent and adult rats that had previously received the 0.03
mg/kg/infusion dose of IV nicotine ($t_{(7)} = 1.4, p = 0.004$ and $t_{(7)} = 2.5, p = 0.04$ respectively;
Figure 10) and adolescents that previously received the 0.003 mg/kg/infusion dose of IV nicotine
($t_{(5)} = 5.4, p = 0.003$)

For the time course of the effects of systemic nicotine, there was also a drug x time x age
interaction [$F_{(11,418)} = 2.8, p = 0.001$]. Locomotor activity in response to the saline challenge and
nicotine challenges were analyzed separately. For saline, there were no main effects of age or sex
Figure 9: Responding for a Conditioned Reinforcer Previously Paired with Passive IV Nicotine Infusions. Only adolescents that had previously received pairings of the CS with passive IV nicotine infusions (0.03 mg/kg) responded for the conditioned reinforcer regardless of the acute dose of amphetamine (Panel C; * $p < 0.05$; main effect of lever: CR vs. NCR). Values are means + SEM. CR = lever that delivered conditioned reinforcer, NCR = lever with no programmed consequence, AMPH = amphetamine. Adolescent saline $n = 8$, adult saline $n = 8$, adolescent nicotine 0.003 mg/kg/infusion $n = 6$, adult nicotine 0.003 mg/kg/infusion $n = 7$, adolescent nicotine 0.03 mg/kg/infusion $n = 7$, adult nicotine 0.03 mg/kg/infusion $n = 8$. 
and no significant interactions (data not shown). For nicotine, there was a significant time x age interaction \([F_{(11,418)} = 4.9, p < .0001]\). As shown in Figure 10, previous exposure to passive IV infusions of nicotine increased locomotor activity in response to a systemic nicotine challenge in adolescents compared to adults at 5 and 10 min post-injection. This result was confirmed with Student’s t-tests \((t_{(13)} = 2.8, p = 0.02 \text{ and } t_{(13)} = 2.9, p = 0.01\) respectively).

### 2.4 Experiment 1: Discussion

Only adolescents with previous IV 0.03 mg/kg/infusion nicotine exposure responded significantly more on the CR than NCR lever which suggests that only this group responded for a conditioned reinforcer. This IV nicotine exposure produced a sensitized locomotor response to a subsequent systemic nicotine challenge in all animals. Together these findings suggest that adolescents may be more susceptible than adults to some of the effects of passive infusions of IV nicotine.

This study is the first to my knowledge demonstrating age differences in acquiring a novel operant response for a conditioned reinforcer previously associated with a drug. Only adolescents responded for a conditioned reinforcer previously paired with IV nicotine infusions. Complementary to these findings, previous studies examining age differences in CPP also suggest that adolescents may be more sensitive than adults to the conditioned rewarding effects of experimenter-delivered nicotine (Belluzzi et al., 2004; Brielmaier et al., 2007; Shram et al., 2006; Shram & Le, 2010; Vastola et al., 2002). The lack of responding for a conditioned reinforcer in adults is unlikely because the IV nicotine exposure was insufficient to induce a behavioural change in these animals. Both adolescents and adults also showed a sensitized locomotor response to a systemic nicotine challenge. This finding suggests that the previous IV nicotine exposure in adults resulted in behavioural sensitization but not responding for a conditioned reinforcer.
Figure 10: Nicotine-Induced Locomotor Activity after Previous Exposure to Passive IV Nicotine Infusions. Adolescents that previously received passive IV infusions of either dose of nicotine and adults that received the 0.03 mg/kg/infusion dose of nicotine showed enhanced nicotine-induced locomotor activity (0.4 mg/kg) (* \( p < 0.05 \) saline vs. nicotine; Panel A). Adolescents that received passive IV infusions of the highest dose of nicotine also showed enhanced nicotine-induced locomotor activity at 10 and 15 min post-injection (Panel B; * \( p < 0.05 \)) compared to adults (Panel C). Values are means ± SEM. IV = intravenous. Sal = saline. Adolescent saline n = 8, adult saline n = 8, adolescent nicotine 0.003 mg/kg/infusion n = 6, adult nicotine 0.003 mg/kg/infusion n = 7, adolescent nicotine 0.03 mg/kg/infusion n = 7, adult nicotine 0.03 mg/kg/infusion n = 8.
A nicotine challenge induced differential locomotor activity in adolescents and adults with previous IV nicotine exposure. Adolescents, but not adults, that previously received IV infusions of 0.003 mg/kg/infusion of nicotine showed a sensitized locomotor response. Additionally, although adolescents and adults that received previous infusions of IV 0.03 mg/kg/infusion of nicotine showed behavioural sensitization to a nicotine challenge, there were age differences in the pattern of nicotine-induced locomotor activity. In adults, nicotine induced a small peak in locomotor activity and then activity levels remained relatively low and stable throughout the session. This pattern of activity is similar to that of adult rats in previous studies (Schochet et al., 2004). Conversely, in adolescents, nicotine induced a significantly larger peak in locomotor activity during the first 15 minutes of the session before stabilizing to a relatively low and stable level. Together these findings show that adolescents may be more sensitive than adults to the locomotor sensitizing effects of nicotine. Previous studies also suggest that adolescents may be more susceptible to the locomotor sensitizing effects of nicotine (Belluzzi et al., 2004; Cruz et al., 2005; Faraday et al., 2003) although some have found no age differences (Schochet et al., 2004; Vastola et al., 2002). Enhanced susceptibility to the locomotor sensitizing effects of systemic nicotine in adolescents compared to adults with previous IV nicotine exposure suggests increased behavioural and neural plasticity in response to passive IV infusions of nicotine during this period.

3. Experiment 2: Responding for a Conditioned Reinforcer Previously Paired with Self-Administered IV Nicotine Infusions

3.1 Rationale

The results of experiment 1 showed that adolescents responded more than adults for a conditioned reinforcer associated with passive IV nicotine infusions. However, the level of responding for a conditioned reinforcer previously paired with passive IV nicotine infusions in the present experiment was less than the level of responding for a conditioned reinforcer previously paired with sucrose as reported in Chapter 3. One possible factor to account for the
relatively lower levels of responding in the current study is the ability to control the delivery of nicotine infusions. Nicotine may have depressant and aversive effects especially after the initial exposure, particularly in adults (Belluzzi et al., 2004; Clarke & Kumar, 1983; Shram et al., 2006). If passive IV nicotine infusions produced aversive effects during the initial phases of conditioning, then the attribution of incentive motivational properties to the CS may have been affected, thus resulting in reduced responding on the CR lever. I hypothesized that responding for a conditioned reinforcer may be enhanced if the primary reward was self-administered IV nicotine infusions as opposed to passive IV nicotine infusions because drug self-administration allows the rat to determine the amount and rate of drug intake. Previous studies have demonstrated that rats will respond for a conditioned reinforcer previously associated with self-administered IV nicotine infusions (Palmatier et al., 2008; Palmatier et al., 2007). To extend these results, age differences in this behaviour and nicotine self-administration were examined. Findings from previous research on age differences in nicotine self-administration are mixed (Adriani et al., 2002; Belluzzi et al., 2005; Chen et al., 2007; Levin et al., 2007; Levin et al., 2003; Shram, Funk, et al., 2008; Shram, Li, et al., 2008), therefore the direction of this effect was difficult to predict.

In experiment 1, amphetamine did not potentiate responding for a conditioned reinforcer previously paired with passive IV nicotine infusion as previously observed in chapter 3 when sucrose was the primary reinforcer. Given that nicotine is the primary reinforcer in the present studies, I hypothesized that systemic nicotine, instead of amphetamine, may enhance responding for a conditioned reinforcer previously paired with IV nicotine infusions. Therefore, in experiment 2, the effects of systemic nicotine challenge on responding for a conditioned reinforcer previously associated with self-administered IV nicotine infusions were assessed. In a similar fashion to experiment 1, the effects of a subsequent systemic nicotine challenge on locomotor activity were examined. Based on the findings from experiment 1, I hypothesized that adolescents would show an enhanced sensitized locomotor response compared to adults.
3.2 Experiment 2: Method

3.2.1 Apparatus

Same as described for experiment 1.

3.2.2 Procedure

3.2.2.1 Subjects. Rats had free access to food and water throughout the experiment.

3.2.2.2 Group Assignment. Adolescent rats from 5 litters, and adults, were assigned to six groups: Nicotine + CS (adolescent n = 8, adult n = 8), saline + CS (adolescent n = 6, adult n = 8) or yoked (adolescent n = 6, adult n = 8).

3.2.2.3 Experiment 2a: IV Nicotine Self-Administration. IV jugular catheters were implanted in adolescent (PND 27-28) and adult rats (> PND 70). Next, adolescent (PND 34-45) and adult rats (> PND 70) in the nicotine + CS and saline + CS groups learned to nose poke in the central recessed magazine on a FR1 for one infusion per response in daily 1 hr sessions. Separate concentrations of nicotine were calculated daily for the adolescent and adult groups based on each group’s respective average body weight (g). Sessions commenced with a priming infusion of 0.1 ml over 2 s of either nicotine (0.03 mg/kg/infusion) or sterile saline. Infusions were paired with a CS: at the onset of each infusion the house light was extinguished and a white stimulus light was illuminated for 15 s. During the 60 s following the onset of the infusion and the CS, responses had no programmed consequences. Levers were always retracted during the self-administration phase of the experiment.

Rats in the yoked group received passive infusions of IV nicotine (0.03 mg/kg/infusion). The number of infusions each yoked rat received was based on the number of earned infusions of a ‘master’ rat in the nicotine + CS group. In the yoked group, each infusion also resulted in the presentation of the CS but the CS was always presented 70-110 s before or after the infusion to prevent any direct pairing of the CS and the effects of the drug. The order that the rats received the CS and infusion was also random. The magazine was covered during the entire experiment.
for the yoked group in order to prevent the formation of any unintended associations with nose poking and the delivery of the drug (i.e., false sense of control over of drug taking). Sessions lasted approximately 60 min. The procedures from this experiment were based on Palmatier et al. (2007).

3.2.2.4 Experiment 2b: Responding for a Conditioned Reinforcer. Responding for a conditioned reinforcer was measured in adolescent (PND 49-53) and adult rats (> PND 70) in three 1 hr sessions on consecutive days. A response on the left (CR) lever delivered the conditioned reinforcer according to a RR2 schedule and responses on the right (NCR) lever had no programmed consequence.

3.2.2.5 Experiment 2c: The Effect of Nicotine on Responding for a Conditioned Reinforcer. Subsequently, responding for the conditioned reinforcer was measured following a SC injection of nicotine (0 or 0.3 mg/kg) 5 min prior to the session. The order of the drug doses were counterbalanced across sessions and a 48 hr washout period was given between test sessions. Rats were left undisturbed during the washout period.

3.2.2.6 Experiment 2d: Nicotine-Induced Locomotion. Locomotor activity was assessed in previously adolescent (PND 60) and adult rats (> PND 70) as described for experiment 1c.

3.2.3 Statistical Analyses

The total number of infusions and nose pokes during the self-administration phase were analyzed separately with a 2 way ANOVA with age and group as between subjects factors. These behaviours were also analyzed across session with 3 way ANOVAs with age and group as between subjects factors and sessions (1-12) as the within subjects factor. The yoked group was not included in these analyses because these rats had no access to the magazine and therefore made no recorded nose pokes. From the responding for a conditioned reinforcer phase of the experiment, responses on the CR and NCR levers during the three consecutive test sessions were collapsed across session and analyzed with a 3 way ANOVA with age and group as between
subjects factors and lever (CR vs. NCR) as the within subjects factor. The effect of systemic nicotine on responding for a conditioned reinforcer was analyzed with a 4 way ANOVA with age (adolescent vs. adult) and group (0 or 0.03 mg/kg/infusion IV nicotine) as between subjects factors and lever (CR vs. NCR) and dose (0 or 0.3 mg/kg SC nicotine) as within subjects factors. Locomotor activity totals were analyzed with a 2 way ANOVA with drug challenge as the within subjects factor (saline or nicotine) and age and group (IV nicotine vs. IV saline) as between subject factors.

3.3 Experiment 2: Results

3.3.1 Experiment 2a: IV Nicotine Self-Administration

As shown in Figure 11a and b, adolescents made more nose pokes in total [main effect of age: $F_{(1,27)} = 13.0, p = 0.001$] and earned more IV nicotine infusions in total than adults [main effect of age: $F_{(1,27)} = 4.9, p = 0.04$]. There was a non-significant trend for rats receiving IV nicotine to earn more infusions than rats receiving IV saline ($p = 0.1$). Analysis of nose pokes and infusions across sessions revealed a similar pattern (Figure 11c and d). Adolescents made more nose pokes [$F_{(1,24)} = 16.8, p = 0.001$] and earned more infusions than adults [$F_{(1,24)} = 7.6, p = 0.01$]. There were also session x age interactions for nose pokes and infusions ($[F_{(11,264)} = 2.2, p = 0.01$] and $[F_{(11,264)} = 2.0, p = 0.03$] respectively). Post hoc analyses did not reveal any significant differences for either measure. However, as shown in Figure 11c and d, adolescents made more nose pokes and earned more infusions than adults until approximately session 7. For the number of infusions, there was also a session x group interactions $[F_{(11,264)} = 2.4, p = 0.01]$. Separate analyses were conducted for each age. As shown in Figure 11d, in adult rats, infusions earned did not vary across sessions and there was no effect of group (ns). In adolescent rats, there was a time x group interaction $[F_{(11,132)} = 1.8, p = 0.05]$. Although post hoc analyses did not reveal any significant differences, as shown in Figure 11d, infusions earned by the nicotine group remained high and stable while in the saline group, the number of earned infusions decreased after the first session.
**Figure 11: IV Nicotine Self-Administration.** Adolescents made more nose pokes (Panel A) and earned more infusions than adult rats (Panel B). IV nicotine and IV saline rats made a similar number of nose pokes but there was a non-significant trend for rats receiving IV nicotine to earn more infusions than rats receiving IV saline. Across sessions, adolescents also nose poked more and earned more infusions than adults during the first 6 sessions (Panel C and D; * p < 0.05). As shown in Panel D, adult rats in the nicotine and saline groups earned a similar number of infusions across sessions. In adolescent rats, the number of earned IV nicotine infusions remained high and stable across sessions while the number of IV saline infusions decreased after the first session. Values are means ± SEM. CS = conditioned stimulus. Adolescent nicotine + CS adolescent n = 8, adult nicotine + CS n = 8, adolescent saline + CS n = 6, adult saline + CS n = 8, or adolescent yoked adolescent n = 6, adult yoked n = 8.
Figure 12: Patterns of Earned Infusions of IV Nicotine and Saline. Compared to adolescent rats receiving IV saline, adolescents receiving IV nicotine earned more infusions in a more consistent pattern across the duration of the 60 min session. A similar trend was observed in adult rats. Data represents the number of earned infusions from session 11 as shown in Figure 11. Each vertical mark denotes an infusion. Each horizontal line represents an individual rat and the numbers on the right hand side of each line correspond to the total number of infusions taken during the 60 min self-administration session. CS = conditioned stimulus
I also examined the pattern of earned infusions for each rat over the course of a representative 60 min self-administration session (session 11; Figure 12). Adolescent rats in the IV nicotine + CS group not only earned more infusions than adolescent rats in the IV saline + CS group, but also earned infusions more consistently across the course of the session. In fact, many adolescent rats in the IV saline + CS stopped earning infusions after the first 25 min of the session. For adults, rats in the IV nicotine + CS group also appeared to earn infusions more consistently across the 60 min session than adult rats in the IV saline + CS group.

3.3.2 Experiment 2b: Responding for a Conditioned Reinforcer

Rats responded more on the CR than NCR lever [main effect of lever; $F_{(1,38)} = 12.2, p = 0.001$]. There was also a significant lever x age x group interaction [$F_{(2,38)} = 3.9, p = 0.03$]. As shown in Figure 13, adult rats that previously self-administered nicotine and CS and rats previously that self-administered saline and CS responded significantly more on the CR than NCR lever. Post hoc analyses with independent t-tests supported these findings ($t_{(7)} = 2.8, p = 0.03, t_{(7)} = 3.8, p = 0.007$ and $t_{(5)} = 4.1, p = 0.01$ respectively).

3.3.3 Experiment 2c: The Effects of Nicotine on Responding for a Conditioned Reinforcer

There was no main effect of the nicotine challenge ($ns$). Rats responded more on the CR than NCR lever [main effect of lever; $F_{(1,38)} = 10.97, p = 0.002$] and there was a significant lever x age x group interaction [$F_{(2,38)} = 6.4, p = 0.004$]. Separate repeated measures analyses for each group showed that only adult rats that previously self-administered IV nicotine and the CS responded more on the CR than NCR lever after saline and acute nicotine [main effect of lever; $F_{(1,7)} = 6.5, p = 0.04$; Figure 14] although there was a non-significant trend for adolescent rats in the yoked nicotine group to respond more on the CR than NCR lever.
Figure 13: Responding for a Conditioned Reinforcer Previously Paired with Self-Administered IV Nicotine. Only adult rats responded for a conditioned reinforcer previously paired with self-administered IV infusions (Panel B; * $p < 0.05$; CR vs. NCR). Adolescents (Panel A) and adults that self-administered IV saline also responded for the conditioned reinforcer (* $p < 0.05$; CR vs. NCR). Values are means + SEM. CR = lever that delivered conditioned reinforcer, NCR = lever with no programmed consequence, Nic = nicotine, Sal = saline. Adolescent nicotine + CS adolescent n = 8, adult nicotine + CS n = 8, adolescent saline + CS n = 6, adult saline + CS n = 8, or adolescent yoked adolescent n = 6, adult yoked n = 8.
Figure 14: The Effects of Acute Systemic Nicotine on Responding for a Conditioned Reinforcer Previously Paired with Self-Administered IV Nicotine

Only adults that self-administered IV nicotine + CS responded significantly for the conditioned reinforcer (* \( p < 0.05 \); main effect of lever in nicotine + CS group). Values are means ± SEM. CR = lever that delivered conditioned reinforcer, NCR = lever with no programmed consequence, CS = conditioned stimulus. Adolescent nicotine + CS adolescent n = 8, adult nicotine + CS n = 8, adolescent saline + CS n = 6, adult saline + CS n = 8, or adolescent yoked adolescent n = 6, adult yoked n = 8.
3.3.4 Experiment 2d: Nicotine-Induced Locomotion

There were no group differences during the first exposure to the locomotor chambers (data not shown). To analyze saline- and nicotine-induced locomotor activity, I compared the two groups that had received previous IV nicotine exposure (IV nicotine + CS and IV nicotine yoked groups) using a 2 way ANOVA. There were no significant main effects of group or interactions (ns) therefore these groups were collapsed into one group referred to as IV nicotine and compared against the IV saline group in subsequent analyses. As shown in Figure 15, compared to saline, systemic nicotine increased locomotor activity [main effect of drug; \( F_{(1,40)} = 23.7, p < 0.001 \)]. There was a non-significant drug x group interaction \( [F_{(1,40)} = 2.2, p = 0.1] \).

Bonferroni post hoc analyses revealed that both adolescent and adult rats with previous IV nicotine exposure were significantly more active in response to a nicotine challenge compared to saline \( (p < 0.01) \). Rats that previously received IV saline responded similarly to the nicotine and saline challenges (ns).

3.4 Experiment 2: Discussion

Adolescents self-administered more nicotine than adults, but only adults responded significantly more on the CR than NCR lever for a conditioned reinforcer previously paired with IV nicotine infusions. Initially, both adolescent and adult rats also responded more on the CR than NCR lever for a conditioned reinforcer previously paired with IV saline infusions. A subsequent nicotine challenge produced a sensitized locomotor response in adolescent and adult rats with previous IV nicotine but not IV saline exposure.

This experiment was designed to examine responding for a conditioned reinforcer previously paired with self-administered IV nicotine infusions. Accordingly, it is important to establish that rats in fact self-administered nicotine. Evidence from the present study generally suggests that rats truly self-administered nicotine, although this effect was clearer in adolescent than adult rats. Adolescent rats in the IV nicotine + CS group earned more infusions than adolescent rats in the IV saline + CS group although this effect was not statistically significant.
Figure 15: Nicotine-Induced Locomotor Activity after Previous Exposure to Self-Administered IV Nicotine Infusions. An acute nicotine injection increased locomotor activity in adolescent (Panel A) and adult rats (Panel B) that previously received IV nicotine only (* $p < 0.05$, saline vs. nicotine challenge). IV = intravenous. Values are means + SEM. Adolescent IV nicotine n = 14, adolescent IV saline n = 6, adult IV nicotine n = 16, adult IV saline n = 8.
A similar trend was observed in adult rats. However, the pattern of infusions across the self-administration sessions suggests that rats self-administered nicotine. Adolescent, and to a lesser extent adult, rats in the IV nicotine + CS group generally earned infusions in a steady and paced fashion similar to adult rats self-administering nicotine as shown in previous studies (Corrigall & Coen, 1989). In contrast, many rats in the IV saline + CS group only earned infusions during the first 25 min of the 1 hr session. This finding suggests that IV saline and the CS were not reinforcing enough to sustain responding across the session, particularly in adolescent rats.

Although the pattern of infusions suggests that nicotine was self-administered, rats responded to a similar degree for IV nicotine and saline. This finding may be interpreted as evidence that rats did not self-administer nicotine, however, a similar number of responses in both IV saline and IV nicotine groups may be related to the relatively easy nature of the response operandum, nose-poking, as compared to lever-pressing. For rats, a species that is naturally exploratory, nose-poking in a magazine likely involves little effort and thus this measure may reflect exploration and not purely goal-directed behaviour. Therefore, based on the pattern of infusions, adolescent and perhaps adult rats, likely self-administered nicotine.

Adolescents self-administered more nicotine than adults, however, only adults responding for a conditioned reinforcer previously associated with self-administered IV nicotine infusions. Adult rats, that earned relatively few IV nicotine infusions (approximately 5/1 hr), responded significantly more on the CR than NCR lever during the initial test sessions and after a systemic nicotine challenge, while adolescents that earned more IV nicotine infusions (approximately 15/1 hr) responded equally on CR and NCR levers. This finding suggests that responding for a conditioned reinforcer may not be directly correlated with the number of previous drug infusion and CS pairings.

Rats that received explicit pairings of IV nicotine infusions and the CS were not the only group that responded for a conditioned reinforcer. Both adolescent and adult rats in the IV saline + CS groups also acquired a novel response to receive the CS, although to a lesser degree than
adults that self-administered nicotine. This finding is similar to the results of a previous study using adult rats (Palmatier et al., 2007). One possible implication of this finding is that the cue alone may have been mildly reinforcing as shown in previous studies (e.g., Olsen & Winder, 2009) although rats only responded more on the CR than NCR lever initially and this effect was no longer significant during the nicotine challenge sessions. Another possible implication is that in adolescent rats, self-administered IV nicotine reduced the reinforcing properties of the cue. Further evidence for this hypothesis is that adolescent rats that previously received unpaired yoked nicotine infusions and the CS, but not rats that received explicit pairings of self-administered IV nicotine infusions and the CS, responded for a conditioned reinforcer in response to a systemic nicotine challenge. Thus, the CS alone (saline + CS group), or when explicitly unpaired with IV nicotine infusions during conditioning (yoked group), was reinforcing enough for adolescent rats to acquire a novel operant response. However, some aspect of the pairings of the CS with self-administered IV nicotine infusions appeared to prevent the attribution of incentive salience to the CS.

One possible hypothesis for the age differences observed in responding for a conditioned reinforcer in the present study is that adolescents may be more sensitive than adults to the conditioned locomotor activity effects of nicotine during adolescence compared to adulthood. If adolescents are more sensitive to the conditioned locomotor effects of nicotine, then this hypothesis could explain why adolescent rats in the self-administered IV nicotine group responded similarly on both levers while rats in the IV saline group selectively responded on the CR lever.

Importantly, both adolescent and adults that received previous IV nicotine infusions showed a sensitized locomotor response to a systemic nicotine challenge. This finding suggests that IV nicotine exposure had long-term neuroplastic effects on the brain resulting in at least one behavioural difference in both adolescents and adults. Therefore, self-administration of IV
nicotine was sufficient to induced behavioural sensitization but not responding for a conditioned reinforcer in adolescent rats.

4. General Discussion

The main purpose of the experiments in this chapter was to test the hypothesis that adolescents are more sensitive than adults to the conditioned reinforcing effects of nicotine-paired stimuli. These experiments built on previous work using the CPP paradigm by examining age differences in the acquisition a new operant response for a CS previously associated with IV nicotine infusions. Based on the findings from Chapter 3 that adolescents show enhanced incentive motivation for a stimulus previously paired with a sucrose reward (Burton et al., 2011), I hypothesized this age difference would be observed in the responding for a CS previously paired with IV nicotine infusions. In the present study, only adolescent rats responded for a conditioned reinforcer previously paired with passive IV nicotine infusions. However, adults, but not adolescents, responded for a conditioned reinforcer previously associated with self-administered IV nicotine infusions even though adolescents seemingly self-administered more nicotine than adults. Together these results suggest that sensitivity to the conditioned reinforcing effects of nicotine-associated stimuli during adolescence depends on the ability to control the delivery of IV drug infusions.

Control over the delivery of IV nicotine infusions appears to play an important role in age differences in the conditioned reinforcing effects of nicotine-associated stimuli. Adolescents are more sensitive than adults to the conditioned rewarding effects of passively administered systemic nicotine based on CPP studies (Belluzzi et al., 2004; Brielmaier et al., 2007; Shram et al., 2006; Shram & Le, 2010; Vastola et al., 2002) and passively administered sucrose (Chapter 3; Burton et al., 2011). However, adults appear to be more sensitive to the conditioned reinforcing properties of stimuli previously-associated with self-administered compared to passively administered IV nicotine infusions based on the present study and a previous study using similar methodology (Palmatier et al., 2007). Together, this evidence suggests that control
over the delivery of nicotine is crucial to the acquisition of incentive motivation to nicotine-associated stimuli.

Age differences in the aversive properties of nicotine and number of pairings of the unconditioned stimulus and CS may potentially explain the opposing age differences in responding for a conditioned reinforcer previously paired with IV nicotine infusions. In experiment 1, adolescents and adults received an equivalent number of pairings of passive IV nicotine infusions and the CS (a total of 216 pairings). These nicotine infusions may have been less aversive to adolescents than adults, as suggested by previous work (Shram et al., 2006), which may explain why adolescents, and not adults, responded for a conditioned reinforcer. However, in experiment 2 adults earned fewer pairings of self-administered IV nicotine infusions and the CS (approximately 95 total pairings) which may have produced less aversive effects and thus resulted in responding for a conditioned reinforcer. Compared to experiment 1, adolescents also earned fewer pairings of self-administered IV nicotine infusions and the CS (approximately 135 total pairings). Although adolescents may have self-administered more nicotine than adults, the number of pairings of IV nicotine infusions and the CS may have been insufficient for the CS to acquire the motivational properties of the CS. Thus, the difference in the number of IV nicotine infusions may explain the lack of responding for a conditioned reinforcer in experiment 2 as compared to experiment 1.

4.1 Possible Mechanisms

Age differences in responding for a conditioned reinforcer and behavioural sensitization in experiment 1 may be mediated by the effects of nicotine on the brain regions involved in these behaviours across development. Dopamine is known to mediate the conditioned reinforcing effects of drugs, drug-paired cues and behavioural sensitization (Everitt, Dickinson, & Robbins, 2001; Everitt et al., 1999; Kalivas, Striplin, Steketee, Klitenick, & Duffy, 1992; Robinson & Becker, 1986). Nicotine indirectly stimulates dopamine release via its actions on nicotinic receptors on VTA neurons (Nisell et al., 1994). The ability of repeated nicotine treatment to
enhance extracellular dopamine release and post-mortem dopamine concentrations in areas of the mesolimbic dopamine system is augmented in adolescent compared to adult rats (Dao, McQuown, Loughlin, Belluzzi, & Leslie; Shearman, Fallon, Sershen, & Lajtha, 2008). Age differences in the effects of nicotine on dopamine system function may result from enhanced glutamate system function and up-regulation of various nicotinic receptors in the VTA in adolescents compared to adults (Adriani & Laviola, 2003; Dwyer, Broide, & Leslie, 2008; Huttenlocher, 1984; Insel et al., 1990; Miller et al., 1990; Spear, 2000). However, it should be noted that in experiment 1 amphetamine did not enhance responding for a conditioned reinforcer in adolescent rats which suggests that this effect may not be mediated exclusively by dopamine. 5-HT is also involved in responding for a conditioned reinforcer since decreasing brain 5-HT levels increases this behaviour (Fletcher, Korth & Chambers, 1999). Nicotine decreases 5-HT in dorsal hippocampus more in adolescents than adults (Shearman et al., 2008). Thus age differences in the ability of nicotine to modulate 5-HT, more than dopamine function, could contribute to age differences in responding for a conditioned reinforcer previously associated with passive IV nicotine infusions.

4.2 Limitations

Some limitations of the present experiments must be considered in the interpretation of the results. First, based on the present findings, it is difficult to conclude whether adult rats self-administered nicotine. Although there was a trend for adults to earn more infusions of nicotine than saline and the pattern of infusions suggests that adult rats self-administered nicotine, the results are not conclusive. Regardless of whether adults self-administered IV nicotine, these rats responded for a conditioned reinforcer and showed a sensitized locomotor response to a subsequent systemic nicotine challenge. This finding demonstrates that the pairing of IV nicotine infusions and the CS were sufficient to induce behavioural changes in adults. Second, the group sizes in both experiments were relatively small (n = 6-8 rats per group). Adding additional rats to both experiments may help clarify the results by reducing within group variability. Finally, one
possibility raised by the findings in the present study is that the number of pairings of IV nicotine infusions and the CS was insufficient for the CS to obtain incentive motivational properties. Therefore, a procedural difference and not necessarily a lack of incentive motivation for self-administered IV nicotine paired cues may account for the results from this study.

4.3 Conclusions & Implications

Adolescents and adults differentially responded for a conditioned reinforcer previously associated with IV nicotine infusions. However, the direction of effects observed appeared to depend on the degree of control over the delivery of the infusions. Age differences in the aversive properties of nicotine and the difference in the number of pairings between experiments may partially account for the divergent findings across experiments. IV nicotine exposure in adolescents and adults was sufficient to induce behavioural sensitization, but not necessarily to induce responding for a conditioned reinforcer previously paired with IV nicotine infusions.

Incentive motivation, as measured by responding for a conditioned reinforcer previously paired with a drug, was hypothesized to mediate age differences in the effects of drugs of abuse during adolescence. The process of incentive motivation is believed to play an integral role in the development, maintenance and relapse to drug addiction via the acquisition of incentive motivational properties to stimuli experienced during drug use (Robinson & Berridge, 2000). The findings from the previous and current chapter suggest that adolescents may exhibit enhanced incentive motivation for cues previously associated with passively administered natural rewards and drugs but not self-administered drugs. Given that in humans nicotine is self-administered through smoking cigarettes, the findings from the present study imply that cues associated with smoking may acquire less incentive salience in adolescents than adults. Notably, the experiments in this chapter only examined age differences in the conditioned reinforcing effects of nicotine-associated stimuli and thus the findings from this study may not necessarily be generalizable to other drug-paired stimuli. Future research should focus on whether age
differences in responding for a conditioned reinforcer previously paired with IV nicotine is a robust effect and whether this effect extends to other drugs of abuse.

Another possible implication from these findings is that onset and progression of cigarette smoking during adolescence may be mediated by another mechanism other than enhanced incentive motivation for nicotine-associated cues. One alternative risk factor may be impulsivity. Impulsivity has been linked to drug addiction in many studies (Anker et al., 2009; Bickel et al., 1999; Dalley et al., 2007; Diergaarde et al., 2008; Economidou et al., 2009; Kelly et al., 2006; Kollins, 2003; Mitchell, 1999; Perry, Larson, German, Madden, & Carroll, 2005; Stoops et al., 2007) and impulsivity may be more pronounced during the adolescence compared to adulthood (Arnett, 1992; Spear, 2000). However, relatively few published empirical reports on age differences in impulsivity exist, particularly for impulsive action. To examine if adolescents show more impulsive action than adults, I explored age differences in this behaviour as measured by responding on a DRL schedule of reinforcement (Chapter 5) and premature responding in a modified version of the 5-CSRTT with only two choices (Chapter 6).
Chapter 5: Age and Sex Differences in Impulsive Action as Measured by Responding on a DRL Schedule of Reinforcement

Abstract

Adolescents engage in more risky behaviour than adults. However, little experimental work has examined age differences in impulsivity and even fewer studies have considered sex differences. The present study examined age and sex differences in responding on a DRL-18 schedule of reinforcement, a measure of impulsive action. The effects of amphetamine on responding on a DRL-18 schedule were also examined. Male and female adolescent and adult Sprague-Dawley rats were trained to respond for a food reinforcer and to withhold subsequent responding for 18 s to receive another food reinforcer. Responding before 18 s had elapsed reset the inter-response timer. Impulsive action was reflected by increased responses and decreased efficiency of responding (number of responses/number of reinforcers). The distribution of IRTs was also examined. After responding was stable, the effects of amphetamine (0, 0.5, 1.0 mg/kg) on DRL-18 performance were examined. Adolescents responded less on a DRL schedule than adults. There was a non-significant trend for adult males to be the least efficient group. Unlike adult rats, adolescents did not exhibit burst responding. Amphetamine generally decreased responses and efficiency of responding but adolescents were not more sensitive to the effects of this drug on these behaviours. The results from this study suggest that adolescents do not show enhanced impulsive action or increased sensitivity to the impulsivity-inducing effects of amphetamine compared to adults in the context of responding on a DRL schedule.
1. Introduction

Adolescence may be a period of enhanced risk-taking and impulsivity (Arnett, 1992; Spear, 2000). Adolescents are more likely than adults to engage in a variety of reckless behaviours including unsafe sex, dangerous driving, minor criminal activity and recreational drug use (Arnett, 1992; Romer, 2010; Spear, 2000; Steinberg, 2004). Although adolescents are commonly described as “risk-takers” (Spear, 2000), very little research has empirically compared adolescents and adults on established, empirical measures of impulsivity. Compared to adult mice, adolescent mice show enhanced delay discounting (Adriani & Laviola, 2003). Age differences in impulsive action have been examined using responding on a DRL schedule of reinforcement (Andrzejewski et al., 2011; Lejeune & Jasselette, 1987). As described in chapter 1, on a DRL schedule, animals must learn to withhold responding until the appropriate time to receive a reinforcer. Compared to adults, adolescents respond more on the DRL schedule which may reflect increased impulsive action (Andrzejewski et al., 2011; Lejeune & Jasselette, 1987). Sex differences in impulsive action across development were not examined in these studies although adult males have been shown to respond more than adult females on a DRL schedule of reinforcement (Beatty, 1973; van Hest et al., 1987).

To examine age and sex differences in impulsive action, I compared male and female adolescent and adult rats on responding on a DRL-18 schedule of reinforcement or IRT>18 s schedule. This study had three aims. First, to replicate previous studies (Andrzejewski et al., 2011; Lejeune & Jasselette, 1987), I examined age differences in responding on a DRL schedule. Second, to extend the findings from previous work (Beatty, 1973; van Hest et al., 1987), I investigated sex differences in responding on a DRL schedule in both adolescents and adults. Third, I assessed age and sex differences in the effects of amphetamine on responding on a DRL schedule. Amphetamine enhances impulsive action as measured by responding on a DRL schedule and premature responding on the 5-CSRTT (Blackburn & Hevenor, 1996; Cole & Robbins, 1989; Murphy et al., 2008; Pattij et al., 2007). Amphetamine also acts on the
mesolimbic dopamine system which continues to develop during adolescence (Crews et al., 2007; Ernst et al., 2009; Spear, 2000). I hypothesized that adolescents would respond more than adults on a DRL-18 schedule of reinforcement both during acquisition and in response to amphetamine. Based on previous work in humans and rats (Beatty, 1973; van Hest et al., 1987), I also predicted that males would respond more than females on a DRL-18 schedule (i.e., males would show enhanced impulsive action) and that this sex difference would appear after adolescence (Cross et al., 2011).

2. Methods

2.1 Group Assignment

Ten male and 10 female adolescent rats from 5 litters and 10 adult male and 10 adult female rats were used. All adult rats were at least 10 weeks old at the beginning of training. For details about weaning, please see Chapter 2.

2.2 Apparatus

Training on the DRL schedule was conducted in 12 operant conditioning chambers (28 cm long x 21 cm wide x 21 cm high). One lever, 4.5 cm wide and 7 cm above the floor, was located 6.5 cm on the left side of a central, recessed magazine that was attached to a pellet dispenser.

2.3 Procedure

2.3.1 Experiment 1a: Responding on a DRL schedule of Reinforcement

Training began on PND 24 for adolescent rats. For the first three sessions, rats responded on a lever for a food pellet (45mg: Bio-Serv Frenchtown, NJ, USA) on a continuous reinforcement (CRF) schedule. Each session lasted 30 min, or until a total of 50 responses was made. Next, food pellets were delivered according to a DRL-6 schedule of reinforcement (DRL-6 phase from PND 27-31 in adolescent rats). The first response of each session was always reinforced and began the inter-response timer. To receive a subsequent reinforcer, rats had to
withhold responding for at least 6 s. Responses occurring earlier than 6 s reset the inter-response
timer. Each session lasted 30 min. Therefore to receive a reinforcer, the inter response time (IRT)
needed to be at least 6 s. The schedule of reinforcement was increased to DRL-12 after 5
sessions (DRL-12 phase from PND 32-38 in adolescent rats) where the rat received a reinforcer
when the IRT for each trial was 12 s or greater. Then the reinforcement requirements were
changed to a DRL-18 schedule, where the rat received a reinforcer when the IRT was equal or
greater to 18 s. Acquisition of responding on this schedule was assessed for 7 sessions (DRL-18
phase from PND 40-46 in adolescent rats). The reinforcement requirements were increased from
a DRL-6 to a DRL-12 schedule and a DRL-12 to a DRL-18 schedule when the average number
of responses was stable for 3 sessions (less than 15% variation in the means) and the mean time
in between responses (m-IRT) was at least the amount of time required to withhold a response (6
s or 12 s respectively).

The measures recorded were the number of responses, the number of reinforcers and
efficiency of responding ([number of reinforcers/number of responses] x 100). The time between
each response (IRT) was also recorded and the percentage of IRTs falling into 2 s bins from 0 to
38 s was calculated (frequency of IRTs for any given 2 s bin/total number of IRTs x 100). These
percent IRTs were used to examine the distribution of IRTs in order to establish how long each
group of rats typically waited in between responses. Less impulsive rats may have a distribution
of percent IRTs with a peak around the reinforcement requirement (e.g., 6, 12 or 18 s) while
more impulsive rats typically show a distribution that is shifted to the left (i.e., cannot wait to
respond until the appropriate time). The m-IRT was calculated based on all IRTs except those in
the 0-2 s bin which reflect burst responding. IRTs often follow a bimodal distribution with one
peak for responses separated by 2 s or less and another peak for responses around the
reinforcement criterion (e.g., Fletcher, Chambers, Rizos, & Chintoh, 2009; Lovic, Keen,
Fletcher, & Fleming, 2011; Uslaner & Robinson, 2006). Burst responding can be considered a
separate behaviour and is often analyzed separately from the remaining responses (e.g., Evenden,
Ryan, & Palejko, 1995; Sokolowski & Seiden, 1999). Thus, calculating the m-IRT without burst responding allows for the assessment of the average IRT around the reinforcement criterion without the potential influence of burst responding on the mean.

2.3.2 Experiment 1b: The Effects of Amphetamine on Responding on a DRL-18 schedule of Reinforcement

Starting on PND 47, rats were injected with amphetamine (0.5 or 1.0 mg/kg) or vehicle (saline) immediately before responding on a DRL-18 schedule was examined. The doses of each drug were counterbalanced across sessions and a 48 hr washout period was given between test sessions. During the washout period, responding on a DRL-18 schedule was assessed. In all cases, performance on this task returned to baseline prior to the subsequent test session.

2.4 Statistical Analyses

For all analyses, age (adolescent vs. adult) and sex (male vs. female) were the between subjects factors. Each measure from the DRL-12 and DRL-18 phases were analyzed separately with 3 way ANOVAs with session as the within subjects factor (1-7). An average of the percentage of IRTs in 2 s bins from the last four DRL-12 sessions and an average of the percentage of IRTs in 2 s bins from the last four DRL-18 sessions was calculated and analyzed separately with 3 way ANOVAs with time (nineteen 2 s bins; 0-38s) as the within subject factor. The effects of amphetamine on each measure were analyzed separately using 3 way ANOVAs with drug dose (0, 0.5 and 1.0 mg/kg) as the within subjects factor. The relative frequency of responding after amphetamine treatment was analyzed with a 4 way ANOVA with time (nineteen 2 s bins; 0-38s) and drug dose as the within subjects factors. Post hoc analyses were conducted using the Bonferroni test.
3. Results

3.1 Experiment 2a: Acquisition.

As shown in Figure 16, during acquisition of responding on the DRL-12 schedule, generally the number of responses decreased [main effect of session; $F_{(6,216)} = 14.1, p < 0.001$] while the number of reinforcers and efficiency of responding increased across sessions [$F_{(6,216)} = 22.3, p < 0.001$ and $F_{(6,216)} = 21.5, p < 0.001$ respectively]. There were also session x age and session x sex interactions for both responses and efficiency of responding [responses: $F_{(6,216)} = 3.1, p = 0.01$ and $F_{(6,216)} = 4.1, p = 0.001$; efficiency: $F_{(6,216)} = 2.7, p = 0.02$ and $F_{(6,216)} = 2.5, p = 0.02$]. Post hoc analyses did not reveal any significant differences in age or sex across session ($p > 0.05$). Adult females earned more reinforcers than adolescent females ($p = 0.03$) but no age differences were observed in males [age x sex interaction; $F_{(1,36)} = 4.8, p = 0.04$]. There were no significant main effects or interactions for m-IRTs.

For the distribution of the IRTs on the DRL-12 schedule there was a significant time x age interaction [$F_{(18,648)} = 2.3, p = 0.01$]. As shown in Figure 17, in adolescent rats, the highest percentage of IRTs occurred between 12-16 s, while in the adult rats, there was a peak in the percentage of IRTs in the 0-2 s category and another smaller peak in the 12-14 s category. Post hoc analyses showed that adults made proportionally more responses than adults within 2 s or less of a previous response ($p < 0.05$) while adolescents made proportionally more responses than adults within 8-10 s or 10-12 s of a previous response ($p’s < 0.05$).

During acquisition of responding on the DRL-18 schedule, adults made more responses than adolescents [main effect; $F_{(1,36)} = 5.9, p = 0.02$, see Figure 18a]. For both the number of responses and efficiency of responding there were significant session x age interactions [$F_{(6,216)} = 7.1, p < 0.01$ and $F_{(6,216)} = 3.9 = 0.001$ respectively]. Post hoc analyses showed that adults made more responses than adolescents during the last 4 sessions ($p < 0.05$), and although there
Figure 16: Acquisition of Responding on a DRL-12 Schedule. Across sessions, the number of responses decreased (Panel A) while the number of reinforcers (Panel B) and efficiency of responding (Panel C) increased. m-IRTs remained stable around 12s (Panel D – Dashed line represents 12 s). Overall, adult females earned more reinforcers than adolescent females (* * p < 0.05) but no age differences were observed in males. Values represent mean ± SEM. m-IRT = mean inter-response times. Each group had an n = 10.
Figure 17: Distribution of IRTs on a DRL-12 Schedule. Adults made proportionally more responses than adolescents in the first 2 s after a response (*p < 0.05) while adolescents made proportionally more responses than adults in the first 8-10 s or 10-12 s after a response (#p < 0.05). IRT = inter-response time. Values represent mean ± SEM. Each group had an n = 10.
was a trend for adults to be less efficient during the last few sessions, this effect was only significant for the last session (Figure 18a; \( p < 0.05 \)). The number of reinforcers earned increased across sessions [main effect of session; \( F_{(1,36)} = 5.6, p = 0.001 \), see Figure 18b]. There were no significant main effects or interactions with sex for any measure although as shown in Figure 18c, males appeared to be the least efficient group. There were no significant main effects or interactions for m-IRTs (see Figure 18d).

For the distribution of the IRTs on the DRL-18 schedule there was a significant time x age interaction \([F_{(21,756)} = 5.8, p = 0.001]\). As shown in Figure 19, in adolescent rats, the percentage of IRTs was generally increased in the range of 12-16 s, while in the adult rats, there was a peak in the percentage of IRTs in the 0-2 s category and another smaller peak in the 12-14 s category. Adults made proportionally more responses within 2 s or less after a response compared to adolescents \((p < 0.05)\).

3.2 Experiment 2b: The Effects of Amphetamine on Responding on a DRL-18 schedule of Reinforcement.

Amphetamine decreased the number of reinforcers earned and efficiency of responding [main effect of drug dose; \( F_{(2,72)} = 8.1, p = 0.001 \) and \( F_{(2,72)} = 3.5, p = 0.04 \) respectively]. As shown in Figure 20, there was a non-significant trend towards adolescents being more efficient than adults, particularly in the males. Adolescents made fewer responses than adults, and females made fewer responses than males [main effect of age; \( F_{(1,36)} = 4.5, p = 0.04 \) and main effect of sex: \( F_{(1,36)} = 5.6, p = 0.02 \) respectively]. Females also showed longer m-IRTs than males [main effect of sex; \( F_{(1,36)} = 6.8, p = 0.01 \)] and there was a trend for adult females to show the longest amphetamine-induced m-IRTs. There were no significant interactions for drug dose for any measure.
Figure 18: Acquisition of Responding on a DRL-18 Schedule. Adults made more responses than adolescents in the last four sessions (Panel A; *p < 0.05; adolescents vs. adults). Adults were also significantly less efficient than adolescents during the last session (Panel C; *p < 0.05; adolescents vs. adults) and this same trend was also apparent during the last few sessions. Mean inter-response time (m-IRTs) showed a trend to increase across sessions (dashed line represents 18 s; Panel D). The number of reinforcers did not vary by age or sex (Panel B). Values represent mean ± SEM. Each group had an n = 10.
Figure 19: Distribution of IRTs on a DRL-18 Schedule. Adults made proportionally more responses than adolescents in the first 2 s after a response (*$p < 0.05$). There was a non-significant trend for adolescents to make proportionally more responses than adults during the 12-16 s periods after a response. IRT = inter-response time. Values represent mean ± SEM. Each group had an $n = 10$. 
Figure 20: Effects of Amphetamine on Responding on a DRL-18 Schedule. Amphetamine (AMPH) decreased the number of reinforcers (Panel B) and efficiency (Panel C). Males made more responses than females (Panel A; $^* p < 0.05$) and adolescents responded less than adults (* $p < 0.05$). Females also showed higher m-IRTs than males (Panel D; $^\wedge p < 0.05$). Values represent mean ± SEM. m-IRT = mean inter-response time. Each group had an n = 10.
For the distribution of IRTs, a 4 way ANOVA showed that there were time x age \(F(18,648) = 6.1, p = 0.001\) and time x dose interactions \(F(36,1296) = 4.0, p = 0.001\). As shown in Figure 21, adults showed the highest percentage of IRTs in the 0-2 s category regardless of dose. Additionally, the percentage of IRTs was also increased in the range of 8-16 s and amphetamine treatment shifted this peak to the left. In adolescents treated with saline, the percentage of IRTs was the highest from 14-16 s and this peak was also shifted to the left by amphetamine treatment. To examine burst responding, the percentage of responding in the 0-2 s category was analyzed with a 3 way ANOVA with age and sex as between subjects factors and dose as the within subjects factor. Adolescents made significantly fewer responses than adults [main effect of age; \(F(1,36) = 10.7, p = 0.002\); Figure 21]. There were no significant main effects of sex or dose or significant interactions.

4. Discussion

Adolescents made fewer responses than adults on a DRL-18 schedule of reinforcement. Generally, there were no significant effects of age or sex in the effects of amphetamine but adult females appeared to show the longest amphetamine-induced m-IRTs. When responding on a DRL-12 or DRL-18 schedule, adults also showed a greater proportion of responses within 2 s of the previous response compared to adolescents. This ‘burst-responding’ is reported in many other studies on responding on a DRL schedule adult rats (Fletcher et al., 2009; Lovic, Keen, et al.; Uslaner & Robinson, 2006). Together the findings from the present study suggest that adolescents may be less impulsive as measured by responding on the DRL schedule and are equally sensitive to the effects of amphetamine in this context. Although there were no consistent significant sex differences during acquisition, adult males appeared to be the least efficient group.
Figure 21: Effects of Amphetamine on the Distribution of IRTs. Adults made significantly more responses than adolescents in the first 2 s after a response regardless of dose (* $p < 0.05$; main effect of age). Generally, amphetamine shifted the distribution of the IRTs to the left. Values represent mean ± SEM. Each group had an n = 10.
4.1 The Role of Age in Impulsive Action

Contrary to my hypothesis, adolescents were not more impulsive than adults. In fact, adolescents made fewer responses and tended to be more efficient than adults. Age differences in performance became most apparent during responding on the DRL-18 schedule. These findings are in contrast to a recent report showing that adolescents made more responses and showed shorter m-IRTs than adults on a DRL-15 schedule of reinforcement (Andrzejewski et al., 2011). However the findings from that study have more than one interpretation given an important procedural difference. In that study, rats were previously trained on a random ratio (RR) 4 schedule for a sweet liquid reinforcer (Ensure®). On this schedule, each response had a 25% probability of being reinforced which increased general levels of responding (Andrzejewski et al., 2011). When rats were subsequently trained to respond on a DRL-15 schedule of reinforcement, high levels of responding persisted. As suggested by the authors of that study, this effect may be explained by a reduced ability for adolescents to adapt their behaviour to changing reinforcer contingencies compared to adults, particularly when the previous contingency was intermittent (Andrzejewski et al., 2011). In support of this hypothesis, adolescents also took longer than adults to extinguish responding for Ensure® on a random interval 15 s schedule of reinforcement in the same study (Andrzejewski et al., 2011) but no age differences were observed in extinction of responding for sucrose, or saccharin on a FR1 or FR3 schedule (Li & Frantz, 2010; Shram, Funk, et al., 2008). Thus increased responding in adolescents compared to adults in the Andrzejewski study (2011) may be explained by age differences in the ability to adapt to intermittent reinforcer contingencies as opposed to impulsive action.

My findings also contrast with those of another previous study showing that early adolescent rats (PND 24-33) responded more and showed shorter m-IRTs than adult rats on a DRL-20 schedule (Lejeune & Jasselette, 1987). One important procedural difference between that study and the present experiment is the degree of food restriction. In the present study rats were deprived of food and water for 6 hr before each session and adolescents gained weight
similarly to free-fed controls (see Appendix 1). In the Lejeune & Jasselette study (1987) rats were kept at 75% of free-fed controls. Adolescent rats have higher caloric needs than adults (Post & Kemper, 1993) and rats double their body weight from the beginning of adolescence to adulthood (see Appendix 1). Thus food restriction regimens that affect weight gain may differentially affect hunger across ages. Hunger may be an important factor in responding on a DRL schedule of reinforcement because increased motivation for the reinforcer enhances impulsive behaviour as shown in previous studies in adult rats (e.g., Bizarro, Patel, & Stolerman, 2003; Grottick & Higgins, 2002; Harrison et al., 1997). Thus in the Lejeune and Jasselette study, increased hunger in adolescents compared to adult rats may have contributed to enhanced responding on the DRL-20 schedule. Further, the previous study examined early adolescent rats (PND 24-33; Lejeune & Jasselette, 1987) while in the present study, rats were in mid-adolescence (PND 32-46) during acquisition of responding on a DRL-18 schedule of reinforcement. Thus, degree of hunger and developmental stage during the adolescent period might explain differences in the results between the present and the previous study (Lejeune & Jasselette, 1987).

Unlike adults in the present and previous studies, adolescents did not exhibit burst responding (e.g., Fletcher et al., 2009; Lovic, Keen, et al.; Uslaner & Robinson, 2006). This finding is consistent with a previous report comparing adolescents and adults on a DRL-15 schedule (Andrzejewski et al., 2011). Burst responding has been described as representing frustration, impulsivity and behavioural reactivity (Cheng, Scott, Penney, Williams, & Meck, 2008). Given that adolescent rats also made fewer and less efficient responses than adult rats, the absence of burst responding in adolescent rats may be interpreted as reduced impulsive action. However, there is an alternative interpretation of the lack of burst responding in adolescents in the present study. Adolescents may consume food pellets more slowly than adults given the substantial difference in body size between age groups. Thus, adolescent rats may have been ready to make a subsequent response less rapidly than adult rats.
In the present study, amphetamine generally increased impulsivity across groups. Although adolescents are commonly considered to be more sensitive than adults to the effects of drugs of abuse (Laviola et al., 1999; Spear, 2000), amphetamine did not enhance impulsivity in adolescents. This finding could be explained by the stage of adolescence during testing. Adolescent rats were on the cusp of young adulthood during the amphetamine challenges (PND 47-51) so perhaps if the amphetamine was administered earlier, age difference may have possibly emerged. Further, if adolescents are more sensitive to the effects of amphetamine than adults, a lower sub-threshold dose of amphetamine such as 0.25 mg/kg might have elicited more responding on a DRL schedule in adolescents compared to adults. Adolescents may not be more sensitive to the impulsivity-inducing effects of amphetamine and whether this effect applies to other measures of impulsive action is explored in the next chapter.

4.2 The Role of Sex in Impulsive Action

Although no significant main effects of sex were observed, adult males appeared to be the least efficient group during acquisition of responding on a DRL-18 schedule. The direction of this effect is consistent with previous studies showing that males made significantly more responses on a DRL schedule than females (Beatty, 1973; Cheng et al., 2008; van Hest et al., 1987). One factor that might contribute to the magnitude of the sex difference between these previous and the present studies is the degree of food restriction. Rats in the present study were deprived of food and water for 6 hr prior to training and their body weight was comparable to free fed controls (see Appendix 1). In most studies using adult rats, the animals are kept at 85% of ad lib body weight. As discussed previously, degree of satiety can affect impulsive behaviour (Bizarro et al., 2003; Grottick & Higgins, 2002; Harrison et al., 1997). Thus, degree of hunger may explain the reduced magnitude of the sex difference in the present study.

Additional behavioural factors may partially account for the observed sex differences in responding on a DRL schedule. Females may respond more efficiently on a DRL schedule because they also exhibit increased exploratory behaviour compared to males. This hypothesis is
supported by the finding that when rats are trained to hold down a lever for a pre-determined amount of time instead of withholding a response, females spend less time than males in contact with the lever (van Hest et al., 1987). Exploratory behaviour may also partially account for the finding that adult females appeared to show longer m-IRTs after amphetamine treatment than males. Adolescent and adult females have been reported to be more sensitive than males to some effects of amphetamine such as increased amphetamine-induced locomotion (Becker et al., 2001; Hensleigh, Smedley, & Pritchard, 2011; Mathews & McCormick, 2007). Therefore, sex differences in responding on a DRL schedule may reflect sex differences in exploratory behaviour and not necessarily impulsive action.

4.3 Conclusions

Adolescents were less prone to impulsive action than adults as measured by responding on the DRL schedule of reinforcement. Adult males appeared to be the most impulsive group as measured by responding on a DRL schedule of reinforcement. The effects of amphetamine were most pronounced in adult females as reflected in longer m-IRTs. The conflicting findings from the present and previous studies demonstrate the importance of examining age differences in different measures of impulsivity and the importance of considering the role of sex in these behaviours. Whether the age differences in impulsive action found in the present study are consistent across other measures of impulsive action is examined in the following chapter.
Chapter 6: Age and Sex Differences in Impulsive Action as Measured by Performance on a 2-CSRTT: The Role of Dopamine and Glutamate*

Abstract

Although impulsive behaviour is considered to peak during adolescence, relatively little empirical work has examined this issue. Sex differences in impulsivity across development are also poorly understood. We examined age and sex differences in impulsive action with a simplified version of the 5-CSRTT, using only 2 choices. Adolescent and adult male and female Sprague-Dawley rats were trained to respond to one of two possible brief light stimuli to receive a food reinforcer. Responding before the onset of the light stimulus (a premature response) was considered a measure of impulsive action. We also investigated age and sex differences in the impulsivity-inducing effects of drugs that 1) target neurotransmitter systems still developing during adolescence and 2) increase premature responding in adult rats. To this end, we examined the effects of increasing the ITI (9s) and amphetamine (dopamine releaser; 0, 0.25, 0.5 mg/kg; Experiment 1), Ro 63-1908 (glutamate NMDA receptor NR2B subunit antagonist; 0, 0.3, 1.0 mg/kg; Experiment 2 and 3), and nicotine (nicotinic receptor agonist and indirect dopamine releaser; 0, 0.15, 0.3 mg/kg; Experiment 3) on premature responding. Adolescent rats were more impulsive than adults. In response to a long ITI and amphetamine, adolescent males and adult females also made more premature responses compared to adult males. No consistent age or sex differences were observed for Ro 63-1908 or nicotine. These findings suggest that impulsive action is heightened in adolescents compared to adults. Further, age and sex differences in impulsive action may be mediated by dopamine.

1. Introduction

Adolescents are often described as impulsive (Arnett, 1992; Romer, 2010; Spear, 2000). In humans, this description derives mostly from the findings that adolescents are more likely than adults to engage in a variety of reckless behaviours including sensation-seeking, unsafe sex, dangerous driving, minor criminal activity and recreational drug use (see Arnett, 1992; Spear, 2000; Sturman & Moghaddam, 2011 for review). In fact progression from drug use to abuse and addiction may occur more rapidly during adolescence (Deas et al., 2000; Estroff et al., 1989) and findings from animal models suggest that adolescents may have an increased vulnerability to the effects of recreational drugs such as the psychostimulants cocaine, amphetamine and nicotine (Laviola et al., 1999; Schramm-Sapyta et al., 2009; Spear, 2000). Reckless behaviours during adolescence, including drug use, have been attributed to impulsivity (Chambers et al., 2003) although few empirical studies have directly examined age differences in this behaviour.

Impulsivity is a complex construct that covers a spectrum of behaviours but generally refers to acting without considering, or simply ignoring, possible negative consequences of behaviour. More than one form of impulsivity has been recognized (Evenden, 1999b; Winstanley, Dalley, et al., 2004) and impulsivity is often divided into two major types: poor decision-making, resulting in the choice of small immediate rewards over larger delayed rewards (i.e., impulsive choice; Dalley et al., 2008; Winstanley, Eagle, et al., 2006) and poor inhibitory control of motor responses (i.e., impulsive action Winstanley, Eagle, et al., 2006). Impulsive action is an inability to withhold a prepotent response until the appropriate time or prior to receiving all necessary information (Winstanley, Eagle, et al., 2006). Impulsivity may be maladaptive in many situations and is a common feature in several disorders such as attention-deficit/hyperactivity disorder, problem gambling, and substance abuse (American Psychiatric Association, 2000; Rogers et al., 2010).

Although adolescents are commonly thought of as impulsive (Spear, 2000), relatively little research has empirically compared adolescents and adults on established measures of
impulsivity. In humans, performance on laboratory tests measuring impulsivity generally improves from childhood to adulthood (Green et al., 1994; Overman et al., 2004; Sinopoli, Schachar, & Dennis, 2011; Williams et al., 1999). Adolescents appear to be more impulsive than adults in many (Eigsti et al., 2006; Green et al., 1994; Green et al., 1999; Rubia et al., 2006; Steinberg et al., 2009) but not all studies (Ernst et al., 2003; Eshel et al., 2007; Stevens et al., 2007). More consistent results have been reported from a few studies using rodents. Compared to adults, adolescent mice preferred a small immediate reinforcer to a larger delayed reinforcer, indicating increased impulsive choice (Adriani & Laviola, 2003). However this effect may also depend on strain (Pinkston & Lamb, 2011). Even fewer data are available on age differences in impulsive action. One recent report suggests that adolescents show more impulsive action as measured by responding on the DRL schedule of reinforcement (Andrzejewski et al., 2011) although the findings from Chapter 5 suggest that adolescents may be less impulsive than adults using a similar behavioural measure. Given the lack of data on this issue, further research is required to fully understand age differences in impulsivity, particularly impulsive action.

Similarly, relatively little is known about the role of sex in impulsivity, particularly during adolescence. In adult rodents, males show more impulsive action than females as measured by performance in a modified version of the 5-CSRTT (Jentsch & Taylor, 2003) and responding on a DRL schedule of reinforcement (Beatty, 1973; van Hest et al., 1987) but not a Go/No-Go test (Anker et al., 2008). In humans, findings on sex differences in impulsive action from empirical studies are mixed (Cross et al., 2011; Li et al., 2006; Perry & Carroll, 2008), particularly during adolescence (Cross et al., 2011; Fields et al., 2009; Silveri et al., 2006). Some findings suggest that sex differences are more likely to appear in adulthood (Cross et al., 2011) although to our knowledge sex differences in impulsive action across development have not be examined in animal models.

To investigate age and sex differences in impulsive action, we compared the performance of male and female adolescent and adult rats on a simplified version of the 5-CSRTT with only 2
choices. The 5-CSRTT has been extensively used to explore the neuropsychopharmacology of impulsive action (Robbins, 2002). The typical length of the training period of this test (~2 months) makes it impossible to use in adolescent rats because this developmental period is brief (approximately 1 month). Therefore we used the 2-choice serial reaction time test (2-CSRTT) with a longer stimulus duration (2.5 s) in order to train and test the rats within the adolescent period. Similar to the 5-CSRTT, successful performance partially depends upon the ability of the rat to withhold responding until the target stimulus is presented (Robbins, 2002). Responses occurring before this stimulus are termed premature responses and were used as a measure of impulsive action (e.g., Fletcher et al., 2009; Robbins, 2002).

First, our study examined age differences in impulsive action as measured by premature responding in the 2-CSRTT. Second, we investigated the role of sex in the expression of impulsive action. Third, we tested age and sex differences in the effects of drugs that are known to enhance impulsive action in adults such as amphetamine, nicotine and Ro 63-1908 (Higgins et al., 2003; Mirza & Stolerman, 1998; Pattij et al., 2007; van Gaalen, Brueggeman, Bronius, Schoffelmeer, & Vanderschuren, 2006). Amphetamine is a dopamine releaser (Azzaro & Rutledge, 1973; Besson et al., 1969; Nielsen, Chapin, & Moore, 1983), while nicotine indirectly stimulates dopamine release via activation of nicotinic acetylcholine receptors (Nisell et al., 1994). Ro 63-1908 acts on the glutamate system by antagonising the glutamate NMDA receptor NR2B subunit (Gill et al., 2002). Dopamine and glutamate are known to mediate impulsive action in adults (Dalley et al., 2008) and these neurotransmitter systems continue to develop during adolescence (see Spear, 2000 for review). For example, the mesocorticolimbic dopamine system, including the PFC and the nucleus accumbens, undergo dramatic changes during adolescence (Andersen et al., 2000; Ernst et al., 2009; Kalsbeek et al., 1988; Lewis, 1997). The glutamate system also continues to develop during this period as reflected in changes in expression and distribution of NMDA receptors and receptor subunits such as the NR2A and NR2B (Insel et al., 1990; Liu et al., 2004; Pian et al., 2010; Sheng et al., 1994). These brain
regions and neurotransmitter systems also play a major role in both drug abuse and impulsivity (Dalley et al., 2011; Kalivas, 1995; Robinson & Berridge, 1993; Volkow et al., 2006). Evidence also suggests that adolescents are differentially sensitive to the effects of Ro 63-1908 and the drugs of abuse amphetamine and nicotine (Ramirez et al., 2011; Schramm-Sapyta et al., 2009). Based on previous work, we hypothesized that 1) adolescents would be more impulsive than adults, 2) adolescents would be differentially responsive to the impulsivity-inducing effects of amphetamine, nicotine and Ro 63-1908 and 3) if sex differences were observed in impulsive action that this difference would increase with age.

2. Method

2.1 Subjects

Adult male and non-pregnant female rats were pair-housed with a same-sex rat upon arrival to the colony and were left undisturbed until behavioural testing commenced. In rats, the broadest accepted age range of adolescence is PND 21-59 (Spear, 2000; Tirelli et al., 2003) and in our study all adults were at least PND 70 at the beginning of training. For details about weaning, please see Chapter 2.

2.2 Apparatus

The 2-CSRTT was conducted in eight operant conditioning boxes (Med Associates, St Albans, VT) measuring 33 x 31 x 29 cm³. The rear stainless-steel wall of the chamber was curved and contained an array of five 2.5 cm square apertures located 2.5 cm above the floor and 2.5 cm apart. Only the apertures on either side of the middle aperture were used. An infrared photo-detector was located 1 cm from the front of the entrance to each aperture. A 3-W yellow stimulus light, 6.4 mm in diameter, was centered at the back of each aperture. A 5 cm square reinforcer magazine was centered in the opposite wall 2.5 cm above the floor. The magazine contained an infrared photo-detector at the entrance, and a light mounted in the roof. A pellet dispenser was connected to the magazine and food pellets were delivered to the magazine floor.
Each box was illuminated by a houselight, and enclosed in a sound-attenuating chamber equipped with a ventilation fan. The boxes were controlled by an IBM-compatible computer running Med-PC IV.

2.3 Procedure

For all experiments, rats were deprived of food and water for 6 hr (08:00 – 14:00) prior to any training or testing. This regimen was designed to ensure that adolescents gained weight normally (see Appendix 1) but were still motivated to respond for a food reinforcer.

2.3.1 Experiment 1: The Effects of Amphetamine and a Long ITI on 2-CSRTT Performance

2.3.1.1 Experiment 1a: Acquisition. In experiment 1, 12 male and 12 female adolescent rats and 11 male and 12 female adult rats were used. For the first two 30 min training sessions (PND 25-26 in adolescent rats, > PND 70 for adults), rats received food pellets (45 mg, Noyes) on a random time 30 s schedule. Next, rats were trained to respond in one of two lit apertures on the back wall to receive a food pellet. A response in the lit aperture extinguished the stimulus light, illuminated the magazine light and resulted in the delivery of a food pellet. These sessions lasted until 60 trials were completed, or for 30 min. When each animal had successfully acquired this response (approximately 2 days) training on the 2-CSRTT began.

At the beginning of each session, the house and magazine lights were illuminated and a food pellet was delivered. A nose poke in the magazine started the first trial. After 5 s (the ITI), one of two light apertures was illuminated for a brief period and a response in that hole while the light was on, or during a 5 s limited hold period afterwards, resulted in the delivery of a food pellet and illumination of the magazine light. A nose-poke into the magazine to collect the reinforcer initiated the ITI to the next trial. Incorrect responses in any of the other four holes were not reinforced and were followed by a 5 s time out period of darkness. Failures to respond during the periods when the stimulus light was illuminated or during the limited hold periods (omissions) were also followed by a 5 s time out. At the end of the time out periods, the
magazine light was turned on and a nose-poke in the magazine began the next trial. Responses in any aperture during the ITI were recorded as premature responses, and were followed by a 5 s time out. Magazine responses at the end of these time out periods restarted the same trial. Sessions lasted for 30 min, or until the rats had completed 80 trials. Each stimulus was presented 40 times in a semi-random order. Training began with a stimulus duration of 60 s and a limited hold of 5 s. The stimulus duration was gradually decreased depending upon performance until the final stimulus duration of 2.5 s was reached. Timeouts were always 5 s and the ITI was held constant at 5 s except for one challenge session (described below). Training criteria were reached when rats responded consistently with an accuracy of > 80% and < 20% omissions with a stimulus duration of 2.5 s. Acquisition of performance on the 2-CSRTT took 14 sessions.

Several behavioural outcome measures were observed during performance on the 2-CSRTT. Number of premature responses was the measure of impulsive action; higher premature responding indicated enhanced impulsive action. Accuracy ([correct responses/correct + incorrect responses] x 100) was a measure of visual attention and the rat’s ability to perform the 2-CSRTT. Latency to collect the food reinforcer was a measure of general motivation for that reinforcer. Percent of trials when no response was made (percent omissions) and latency to make a correct response reflected upon sensory, motor or general motivational aspects of performance depending on the profile of the other behavioural measures (Robbins, 2002).

2.3.1.2 Experiment 1b: Effects of a Long ITI on Performance. On PND 45, rats performed the 2-CSRTT with the ITI increased from 5 to 9 s and the session length was extended to 45 min. This manipulation increases premature responding on the 5-CSRTT (e.g., Cole & Robbins, 1989; Fletcher et al., 2009).

2.3.1.3 Experiment 1c: Effects of Amphetamine on Performance. The ITI was returned to 5 s for these sessions and the remainder of the experiment. Starting on PND 47, rats were injected with amphetamine (0.25 or 0.5 mg/kg IP) or vehicle (saline) immediately before being placed in the operant conditioning chambers.
2.3.2 Experiment 2: Effects of Ro 63-1908 on 2-CSRTT Performance

In experiment 2, 12 male and 12 female adolescent rats and 9 male and 11 female adult rats were used. The ITI remained at 5 s throughout all experiments.

2.3.2.1 Experiment 2a: Acquisition. Rats were trained on the 2-CSRTT in the same fashion as experiment 1. Acquisition of performance on the 2-CSRTT took 13 sessions.

2.3.2.2 Experiment 2b: Effects of Ro 63-1908 on Performance. Adolescent (PND 42-46) and adult rats (> PND 70) were injected with vehicle or Ro 63-1908 (0.3 and 1.0 mg/kg) 30 min prior to 2-CSRTT sessions.

2.3.3 Experiment 3: Effects of Ro 63-1908 and Nicotine on 2-CSRTT Performance

In experiment 3, 11 male and 11 female adolescent rats and 11 male and 12 female adult rats were used. The original purpose of this experiment was to examine the effects of age and sex on nicotine-induced premature responding. However, after acquisition we did not observe the same magnitude of age difference compared to the two previous experiments. Therefore we also examined whether the effects of Ro 63-1908 were dependent on baseline premature responding. A 5 s ITI was used throughout all experiments.

2.3.3.1 Experiment 3a: Acquisition. Rats were trained on the 2-CSRTT in the same fashion as experiment 1. Acquisition of the 2-CSRTT performance task took 12 sessions.

2.3.3.2 Experiment 3b: The Effects of Ro 63-1908 on Performance. Adolescent (PND 42-50) and adult rats (> PND 70) were injected with vehicle or Ro 63-1908 (0.1, 0.3 and 1.0 mg/kg) 30 min prior to 2-CSRTT sessions.

2.3.3.3 Experiment 3c: The Effects of Nicotine on Performance. Adolescent (PND 52-56) and adult rats (> PND 70) were injected with vehicle or nicotine (0.15 and 0.3 mg/kg) 15 min prior to 2-CSRTT sessions.
2.4 Statistical Analyses

We analyzed the following behavioural measures: premature responses, accuracy, percent omissions, correct response latency and latency to collect the food reinforcer. During acquisition of performance on the 2-CSRTT, these measures were averaged across sessions and analyzed using a 2 way ANOVA with age and sex as between subjects variables. Data from the long ITI experiment were analyzed in a similar fashion. For the effects of drugs on 2-CSRTT performance, data were analyzed with 3 way ANOVAs with age and sex as the between subject variables and drug dose (amphetamine, Ro 63-1908 or nicotine) as the within subject variable.

Finally, to more closely examine age differences in the acquisition of performance on the 2-CSRTT, data from the training phases of all three experiments were pooled and analyzed using a 2 way ANOVA with age and sex as between subjects factors. Because no consistent sex differences were observed on any measure during acquisition, male and female groups were combined for each age group (adolescent n = 70, adult n = 66). Measures of performance from each experiment were averaged and analyzed with a 1 way ANOVA with age as the between subjects factor. Premature responding was also analyzed across session with a 2 way ANOVA with age as the between subject factor and session (1-12) as the within subject factor. Only the first 12 sessions from each experiment were used for this analysis because all experiments had at least this number of acquisition sessions. Additionally, rats were assigned to high and low responders based on their average premature responses during acquisition relative to the mean ± 1 standard deviation (mean = 9, standard deviation = 6). Rats with mean premature responses equal to 3 or less were considered low responders and rats with mean premature responses equal to 15 or more were considered high responders. A chi-squared test was performed on these data. Post hoc analyses were conducted with the Bonferroni test or student t-tests as needed.
3. Results

The primary dependent variable in these experiments was the number of premature responses, which is the measure of impulsive action. However each experiment also generated several other performance measures. The results section is arranged so that for each experiment we first report the results of statistical analyses for the effects of all experimental manipulations on premature responses. This section is organized according to main effect of age, main effect of sex, main effect of drug (where appropriate) and interactions between factors. We then report the results for the other measures of performance when they are important for interpretation of effects on premature responding, according to the same sequence of main effects and interactions.

Overall, there were no consistent significant effects of any factor on latency to respond to the light stimulus and so these data are not shown.

3.1 Experiment 1: Age and Sex Differences in the Effects of Amphetamine and a Long ITI on 2-CSRTT Performance

3.1.1 Experiment 1a: Acquisition

As shown in Figure 22, compared to adults, adolescent rats made significantly more premature responses [main effect of age; \(F_{(1,43)} = 10.9, p = 0.002\)] which suggests adolescents were more impulsive than adults. There were no main effects of sex or significant interactions.

On other performance measures adolescents were significantly less accurate than adults in responding [main effect of age; \(F_{(1,43)} = 9.0, p = 0.004\)] and took longer than adults to retrieve the reinforcer [\(F_{(1,43)} = 15.6, p = 0.0001\)]. There were no main effects of sex. In terms of interactions, adolescent females showed a lower percentage of omissions than adult females (\(p < 0.05\); age x sex interaction [\(F_{(1,43)} = 7.3, p = 0.01\)].
Figure 22: Experiment 1a – Acquisition of 2-CSRTT Performance. Adolescents made significantly more premature responses (Panel A: * $p < 0.05$), were less accurate (Panel B: * $p < 0.05$) and took longer to retrieve the reinforcer than adults (Panel D: * $p < 0.05$). Adolescent females also showed a greater percent of omissions than adult females (Panel C: # $p < 0.05$). Values represent the mean ± SEM. Adolescent male n = 12, adolescent female n = 12, adult male n = 11, adult female n = 12.
3.1.2 Experiment 1b: The Effects of a Long ITI on Performance

The results are shown in Figure 23. There were no main effects of age or sex on premature responding but there was a significant age x sex interaction \( [F_{(1,43)} = 12.6, p = 0.001] \). When the ITI was set at 9 s, adult females made significantly more premature responses than adult males \( (p = 0.02) \) and there was a non-significant increase in premature responding in adolescent compared to adult male rats \( (p = 0.07) \).

There were no main effects of age or sex for any other measures of performance. Adolescent females took the longest to retrieve the reinforcer \( (p < 0.05; \text{age x sex interaction } [F_{(1,43)} = 4.5, p = 0.04]) \). There were no significant effects for accuracy or percent omissions (Figure 23) suggesting general performance was similar across groups.

3.1.3 Experiment 1c: The Effects of Amphetamine on Performance

The results are shown in Figure 24. For premature responding, there were no main effects of age or sex. Amphetamine increased premature responding \( \text{[main effect of drug; } F_{(2,86)} = 18.3, p = 0.0001]\). There was a significant drug x age x sex interaction for premature responses \( [F_{(2,86)} = 9.3, p = 0.004] \). Separate repeated measures for each group with drug as the between subjects factor showed that amphetamine significantly increased premature responding in adolescent males \( [F_{(2,22)} = 5.2, p = 0.01] \) and adult females \( [F_{(2,22)} = 10.5, p = 0.001] \). A Student’s t-test revealed that adolescent males also made more premature responses than adult males after 0.25 mg/kg of amphetamine \( [t_{(21)} = 3.1, p = 0.005] \). Adult female rats made more premature responses than adolescent female rats after 0.5 mg/kg of amphetamine \( [t_{(22)} = 2.7, p = 0.01] \) and adult males after both doses of amphetamine \( [0.25: t_{(21)} = 2.3, p = 0.01 \text{ and } 0.5: t_{(21)} = 3.3, p = 0.003] \). No age or sex differences were observed for premature responding after saline \( (ns) \).

There were no main effects of age or sex for any other measure of performance. The highest dose of amphetamine decreased accuracy selectively in adolescent males \( (p < 0.05) \) and adult females \( (p < 0.05; \text{drug x age x sex interaction } [F_{(2,86)} = 3.5, p = 0.03]) \). The highest dose
Figure 23: Experiment 1b – Effects of Long ITI on 2-CSRTT Performance. As shown in Panel A, adult females made more premature responses than adult males when the inter-trial interval (ITI) was increased to 9s (# p < 0.05) and there was a non-significant trend for adolescent males to make more premature responses with a 9s ITI than adult males ($ p = 0.07). Adolescent females also took the longest to retrieve the reinforcer (Panel D: * p < 0.05; adolescent females vs. all groups). There were no significant effects for accuracy (Panel B) or percent omissions (Panel C). M = male, F = female. Values represent the mean ± SEM. Adolescent male n = 12, adolescent female n = 12, adult male n = 11, adult female n = 12.
Figure 24: Experiment 1c – Effects of Amphetamine on 2-CSRTT Performance.

Amphetamine significantly increased premature responding in adolescent males and adult females (Panel A; * p < 0.05; saline vs. amphetamine). Adolescent males and adult females also made more premature responses than adult males in response to amphetamine (# p < 0.05). Adult females also made more premature responses than adolescent females in response to 0.5 mg/kg of amphetamine ($ p < 0.05). Amphetamine decreased accuracy in adolescent males and adult females only (^ p = 0.05, Panel B; saline vs. 0.5mg/kg). The highest dose of amphetamine increased reinforcer latencies only in adolescent females (Panel D, ^ p < 0.001; drug x age x sex interaction). There were no significant effects for percent omissions (Panel C). M = male, F = female. Values represent the mean ± SEM. Adolescent male n = 12, adolescent female n = 12, adult male n = 11, adult female n = 12.
of amphetamine increased reinforcer latencies only in adolescent females (drug x age x sex interaction for reinforcer latency [$F_{(2,86)} = 6.7, p = 0.002$]).

### 3.2 Experiment 2: Age and Sex Differences in the Effects of Ro 63-1908 on 2-CSRTT Performance

#### 3.2.1 Experiment 2a: Acquisition

The results are shown in Figure 25. Compared to adults, adolescents made more premature responses, suggesting that they were more impulsive, than adults [main effect of age: $F_{(1,40)} = 6.3, p = 0.02$]. There were no main effects of sex or significant interactions.

Adolescents also showed reduced percent omissions [main effect of age; $F_{(1,40)} = 9.1, p = 0.004$] and longer reinforcer latencies than adults [$F_{(1,40)} = 9.0, p < 0.01$]. There were no main effects of sex and no significant interactions for other measures of performance.

#### 3.2.2 Experiment 2b: The Effects of Ro 63-1908 on Performance

The results are shown in Figure 26. For premature responding, there were no main effects of age or sex. Ro 63-1908 increased premature responding [$F_{(2,80)} = 67.1, p < 0.001$]. There was also an age x sex interaction [$F_{(1,40)} = 4.1, p = 0.04$]. Separate 2 way ANOVAs with age as the between subjects factor and drug as the within subjects factor were conducted for male and female rats. In female rats, Ro 63-1908 significantly increased premature responding [main effect of drug: $F_{(2,42)} = 29.9, p < 0.001$]. In male rats, adolescents made more premature responses than adults [$F_{(1,19)} = 5.5, p = 0.03$] and both doses of Ro 63-1908 increased premature responding compared to saline in adolescent rats ($p < 0.001$) but only the 1.0 mg/kg dose increased premature responding in adults ($p = 0.005$; drug x age interaction [$F_{(2,38)} = 4.2, p = 0.02$]). Premature responding after administration of saline was not significantly different between adolescent and adult male rats (ns).
Figure 25: Experiment 2a – Acquisition of 2-CSRTT Performance. Adolescents made more premature responses (Panel A: * $p < 0.05$), fewer percent omissions (Panel C: * $p < 0.05$) and took longer to retrieve the reinforcer than adults (Panel D: * $p < 0.05$). There were no significant effects for accuracy (Panel B). Values represent the mean ± SEM. Adolescent male n = 12, adolescent female n = 12, adult male n = 9, adult female n = 11.
Figure 26: Experiment 2b – Effects of Ro 63-1908 on 2-CSRTT Performance. The highest dose of Ro 63-2908 increased premature responding in all groups (* *p < 0.05; 0.0 vs. 1.0mg/kg), but only adolescent males showed increased premature responses after the 0.3 mg/kg dose of Ro 63-1908 ($ *p < 0.05; Ro 63-1908 vs. vehicle). Ro 63-1908 decreased accuracy (Panel B, * *p < 0.05, main effect of drug) and also selectively decreased percent omissions and reinforcer latencies in adolescent rats compared to adults ( ^ *p < 0.05; drug x age interaction). M = male, F = female. Values represent the mean ± SEM. Adolescent male n = 12, adolescent female n = 12, adult male n = 9, adult female n = 11.
In terms of other measures of performance, Ro 63-1908 decreased accuracy \( F_{(2,80)} = 5.7, p = 0.005 \) and also selectively decreased percent omissions and reinforcer latencies in adolescent compared to adult rats (drug x age interactions: \( F_{(2,40)} = 3.5, p = 0.03 \) and \( F_{(2,80)} = 3.4, p = 0.04 \) respectively).

3.3 Experiment 3: Age and Sex Differences in the Effects of Ro 63-1908 and Nicotine on 2-CSRTT Performance

3.3.1 Experiment 3a: Acquisition

The results are shown in Figure 27. Adolescents made significantly more premature responses than adults, although this effect was modest [main effect of age; \( F_{(1,41)} = 4.2, p = 0.055 \)]. There were no significant main effects of sex or interactions.

There were no main effects of age or sex for other measures of performance. Adolescent males took the longest to retrieve the reinforcer \( (p < 0.004; \) age x sex interaction \( F_{(1,41)} = 4.6, p = 0.04 \)) suggesting this group was the least motivated by the reinforcer.

3.3.2 Experiment 3b: The Effects of Ro 63-1908 on Performance

The results are shown in Figure 28. There were no main effects of age or sex on premature responding. Ro 63-1908 increased premature responding \( F_{(3,123)} = 88.3, p < 0.001 \). There were no significant interactions for this measure but planned comparisons showed that premature responding was not significantly different between adolescents and adults in response to saline treatment \( (ns) \) suggesting similar baseline levels of impulsive action between groups.

There were no main effects of age or sex for any other measure of performance. Ro 63-1908 decreased accuracy \( F_{(3,123)} = 6.4, p < 0.001 \) and percent omissions \( F_{(3,123)} = 7.8, p < 0.001 \). All doses of Ro 63-1908 decreased reinforcer latency in adolescents compared to vehicle \( (p < 0.05) \) but had no effect in adults (drug x age interaction \( F_{(3,123)} = 3.8, p = 0.01 \); data not shown).
Figure 27: Experiment 3a – Acquisition of 2-CSRTT Performance. Adolescents made modestly more premature responses than adults (Panel A: * $p = 0.055$). Adolescent males showed the longest reinforcer latencies (Panel D: # $p < 0.05$ adolescent males vs. all groups). There were no significant main effects for accuracy (Panel B) or percent omissions (Panel C). Values represent the mean ± SEM. Adolescent male $n = 11$, adolescent female $n = 11$, adult male $n = 11$, adult female $n = 12$. 
Figure 28: Experiment 3b – The Effects of Ro 63-1908 on 2-CSRTT Performance. All doses of Ro 63-1908 increased premature responding compared to vehicle (Panel A, * $p < 0.05$). Ro 63-1908 decreased accuracy (Panel B, # $p < 0.05$, main effect of drug) and omissions (Panel C, # $p < 0.05$; main effect of drug). All doses of Ro 63-1908 decreased reinforcer latencies compared to vehicle in adolescents (Panel D, # $p < 0.05$) but no effect of this drug in adult rats. M = male, F = female. Values represent the mean ± SEM. Adolescent male n = 11, adolescent female n = 11, adult male n = 11, adult female n = 12.
3.3.3 Experiment 3c: The Effects of Nicotine on Performance

The results are shown in Figure 29. There were no main effects of age or sex on premature responding. Nicotine increased premature responding \([F_{(2,82)} = 32.5, p < 0.001]\). Although there were no significant interactions, planned comparisons showed that premature responding was not significantly different between adolescents and adults in response to saline treatment \((ns)\).

For other measures of performance, adolescents showed longer reinforcer latencies than adults \([\text{main effect of age; } F_{(1,A1)} = 6.0, p = 0.02]\). Nicotine also decreased percent omissions \([F_{(2,82)} = 24.2, p < 0.00]\), accuracy \([F_{(2,82)} = 6.3, p = 0.002]\) and reinforcer latency \([F_{(2,82)} = 3.6, p = 0.03]\). There were no significant main effects of sex or interactions.

3.4 Age Differences in Acquisition of 2-CSRTT Performance across all Experiments

For the final analysis, to further examine the apparent age difference in premature responding during acquisition of 2-CSRTT performance the data from all three experiments were pooled. As shown in Figure 30a adolescents made more premature responses than adults \([F_{(1,134)} = 19.4, p < 0.001]\) throughout the first 12 days of the acquisition period. Analyses of premature responding across sessions also showed that overall, compared to adults, adolescents made more premature responses \([\text{main effect of age; } F_{(1,120)} = 15.9, p < 0.001]\). As shown in Figure 30b, age differences in impulsivity were evident starting with the 4th session \([\text{session x age interaction; } F_{(11,1320)} = 2.5, p = 0.004]\). This observation was supported by t-tests at each time point with the Bonferroni correction applied for multiple comparisons \((t \text{ values ranged from 2.5-4.2, } df = 134, p’s < 0.001)\). For other measures of performance (data not shown) compared to adults, adolescents also omitted fewer responses \([F_{(1,134)} = 7.6, p = 0.01]\) and took longer to collect the reinforcer \([F_{(1,134)} = 31.1, p < 0.01]\). No age differences were observed in accuracy of responding \((ns)\).
Figure 29: Experiment 3c – The Effects of Nicotine on 2-CSRTT Performance. Nicotine generally increased premature responding (Panel A: # $p < 0.05$) and decreased accuracy (Panel B: # $p < 0.05$), percent omissions (Panel C: # $p < 0.05$) and reinforcer latencies (Panel D: # $p < 0.05$) in all groups. M = male, F = female. Values represent the mean ± SEM. Adolescent male n = 11, adolescent female n = 11, adult male n = 11, adult female n = 12.
Figure 30: Age Differences in Premature Responding during Acquisition of 2-CSRTT Performance. Adolescents made more premature responses than adults on average (Panel A, * $p < 0.001$). Over the course of acquisition, this age difference appeared after the third acquisition session (Panel B* $p < 0.001$). Values represent the mean ± SEM. Rats were assigned to low and high responder groups based on the average premature responses relative to the mean ± 1 standard deviation (mean = 9, standard deviation = 6). Low responders made fewer than 3 (dark grey) and high responders made more than 15 responses on average (light grey). A greater proportion of adolescents (Panel C) were high premature responders compared to adults (Panel D) and vice versa. Adolescent low: $n = 4$; adolescent high $n = 15$, adult low $n = 13$, adult high $n = 3$. 
Figure 30 also shows frequency distributions of rats according to their average number of premature responses. Based on the criterion of number of premature responses relative to the mean ± 1 standard deviation (as described in section 2.9), rats were divided into high and low responders resulting in the following groups: Adolescent low: n = 4; adolescent high n = 15, adult low n = 13, adult high n = 3. Notably, two of the three adults in the high group were from experiment 3 when a more modest age difference was observed. The chi-squared test revealed that the distribution of high and low responders between adolescents and adults was significantly different \[X^2_{(1, N = 35)} = 12.6, p = 0.001\]. As shown in Figure 30, there was a greater proportion of high responders and smaller proportion of low responders among adolescent rats compared to adults.

4. Discussion

Impulsive action was generally increased in adolescent compared to adult rats. Adolescent males were more sensitive than adult males to the impulsivity-inducing effects of amphetamine and Ro 63-1908, although the effects of the latter drug may have been baseline-dependent. Adolescents were not differentially sensitive to the effects of nicotine. No consistent sex differences were observed across manipulations although adult females were more impulsive than adult males in response to amphetamine and a long ITI (9 s). Thus, adolescents and adult females may be most impulsive under certain conditions.

4.1 The 2-CSRTT

Impulsive action was measured by premature responding in the 2-CSRTT similarly to previous studies (Dillon et al., 2009; Lovic, Saunders, Yager, & Robinson, 2011). The 2-CSRTT in the present experiment follows the same procedure as the standard 5-CSRTT, with a few modifications to make it amenable for use in adolescent rats (i.e., the number of possible spatial locations, food and water restriction regimen, and stimulus duration length). These procedural changes seemingly did not affect the expression of premature responding, or other measures of
performance, compared to what is normally seen in the 5-CSRTT. Further, drug and parameter challenges that increase premature responding in adults in the 5-CSRTT (i.e., long ITI, amphetamine, Ro 63-1908 and nicotine; Cole & Robbins, 1989; Fletcher et al., 2009; Higgins et al., 2003; Mirza & Stolerman, 1998) also increased premature responding in the present study. Therefore, impulsive action measured as premature responses on the 2-CSRTT appears to be comparable to that observed in the well-established version of the 5-CSRTT.

4.2 The Role of Age in Impulsive Action

Adolescence is often viewed as a period of heightened impulsivity (Arnett, 1992; Chambers et al., 2003; Spear, 2000). Human adolescents perform worse than adults on most (Eigsti et al., 2006; Rubia et al., 2006) but not all response inhibition tasks (Galvan et al., 2011). Consistent with the few studies conducted using rodents (Adriani & Laviola, 2003; Andrzejewski et al., 2011; Pinkston & Lamb, 2011; Sturman, Mandell, & Moghaddam, 2010) although not with the findings from the previous chapter, we found that adolescent rats were more impulsive than adults. Based on an examination of the distribution of premature responses, significantly more adolescents than adults were also defined as high impulsive responders and fewer as low responders compared to adults. Age differences in premature responding were unlikely related to age differences in motor function or visual attention given that there were no consistent age-related effects on accuracy of responding or percent omissions. Adolescents consistently took longer than adults to collect the reinforcer suggesting that age differences in impulsivity were not driven by an increased inherent motivation to seek the reinforcer. Together these findings indicate that adolescents are more impulsive than adults, as measured by premature responding. Inconsistencies between the findings from the present and the previous chapter will be explored in the general discussion (Chapter 7).

This age difference in impulsive action appeared to be short-lived. Adolescents made more premature responses than adults during acquisition of the 2-CSRTT however no age differences were observed in response to vehicle injections during the drug-testing phases of all
experiments. One possible explanation for these findings is that impulsivity decreases with age during adolescence as result of the continued development of the brain. Alternatively, it is possible that after approximately two weeks of training, adolescents learned to suppress premature responding. However whether the disappearance of the age difference reflects a development of the brain systems underlying impulsivity, or simply learning of appropriate inhibition with extended training, is not clear.

4.3 The Role of Sex in Impulsive Action

We did not observe any consistent sex differences in premature responding during acquisition of the 2-CSRTT. A previous study reported that males showed more impulsive action than females in a modified version of the 5-CSRTT and that gonadectomy prevented the expression of sex differences (Jentsch & Taylor, 2003). This modified test required rats to make a sustained nose poke in the middle aperture prior to responding in one of two target apertures (either the far left or far right aperture) when they were briefly illuminated. Responding in the target aperture before completing the sustained nose poke and the onset of the target stimulus light was considered a premature response. Notably, males made more premature responses than females when the target stimulus light duration was 1 s but not 2.5 s (comparable to the stimulus duration used in the present experiment). Thus parametric factors may play a role in the expression of sex differences in premature responding. Sex differences in impulsive action appear to vary as a function of the task used to measure the behaviour (Anker et al., 2008; Beatty, 1973; van Hest et al., 1987). Similarly, in empirical studies with human participants, reports on sex differences are equivocal (Cross et al., 2011; Li et al., 2006; Perry et al., 2011). These results indicate that age, but not sex, appears to be an important factor contributing to the expression of impulsive action during acquisition of responding on the 2-CSRTT.

4.4 Age and Sex Differences in the Effects of Drugs on Impulsive Action

Amphetamine increased premature responding similarly to previous studies (Cole & Robbins, 1989; Pattij et al., 2007) and adolescent males were more responsive than adult males
to this effect. This age difference is unlikely related to increased locomotor activity or altered inherent motivation for the reinforcer because adolescents are hypo-responsive to the locomotor stimulating effects of systemic amphetamine (Burton et al., 2011; Lanier & Isaacson, 1977; Laviola et al., 1999) and the average latency to collect the reinforcer was greatest in adolescents. Baseline differences in premature responding also unlikely contributed to age differences in the effects of amphetamine because adolescents and adults responded similarly to vehicle injections.

Females are generally more responsive than males to the behavioural effects of amphetamine (Becker et al., 2001; Mathews & McCormick, 2007) and nicotine (Carroll & Anker, 2010). In the present study adult females made more amphetamine-induced premature responses than adult males although no sex differences were observed for nicotine. Amphetamine also may have decreased visual attention to a greater extent in adult females compared to males as evidenced by sex differences in accuracy. However, a general deficit in 2-CSRTT performance unlikely mediated sex differences in the effects of amphetamine on impulsivity because females made relatively fewer percent omissions than males and no sex differences in reinforcer latency was observed. Sex differences in premature responding may have been mediated, in part, by enhanced amphetamine-induced locomotion in adult females than adult males (Becker et al., 2001; Mathews & McCormick, 2007). However, adolescent females, who are also more sensitive than adolescent males to the locomotor-stimulating effects of this drug (Mathews & McCormick, 2007), performed similarly to adolescent males in response to amphetamine. Our data suggest that females may be more susceptible than males to the impulsivity-inducing effects of amphetamine, but not nicotine, and that this sex effect appears after adolescence.

Although the expression of the glutamate NMDA receptor NR2B subunit across development has been characterized (Liu et al., 2004; Sheng et al., 1994), few experiments have examined the possible behavioural outcomes of age differences in NR2B subunit expression (Ramirez et al., 2011). In the present study, the glutamate NMDA receptor NR2B subunit
antagonist Ro 63-1908 increased premature responding as shown in previous work using adult rats (Higgins et al., 2003), although, age differences in the effects of this drug on premature responding were inconsistent as they were observed in experiment 2 but not 3. Adolescent males showed a similar number of premature responses in both experiments during acquisition and drug testing. However, adult males made fewer premature responses during acquisition and in response to Ro 63-1908 in experiment 2 compared to 3. These findings suggest that the effects of Ro 63-1908 may be partially dependent on baseline premature responding, in this case, of the adult male group. Further work on the role of the NR2B subunit in age differences in impulsive action may be warranted.

Adolescents are more sensitive to some but not all effects of nicotine (Belluzzi et al., 2004; Schramm-Sapyta et al., 2009; Shram, Funk, et al., 2008; Vastola et al., 2002). We found that nicotine injections increased premature responding in all rats which is consistent with previous work in adult rats (Mirza & Stolerman, 1998; Stolerman, Mirza, Hahn, & Shoaib, 2000). These results suggest that adolescents may be more sensitive to some effects of nicotine, but not in the context of impulsive action as measured by premature responding in the 2-CSRTT. Another possible explanation is that the time point during adolescence when nicotine was tested may have affected the results. Testing of the effects of nicotine on 2-CSRTT performance occurred during late adolescence (PND 52-56) and some studies suggest that some of the effects of nicotine during adolescence may be the most pronounced in early adolescence (Adriani et al., 2002; Dao et al., 2011). Therefore, our results suggest that late adolescent rats perform similarly to adults in response to nicotine.

4.5 Possible Mechanisms

Altered impulsivity during adolescence is likely related to the developing state of the brain during this period. The PFC is important for impulsive action, as shown by the fact that lesions or inactivation of this structure increase premature responding (Chudasama et al., 2003; Murphy et al., 2005). The PFC undergoes many changes during adolescence (Eshel et al., 2007;
and develops later than many subcortical structures like the striatum (Chambers et al., 2003; Eshel et al., 2007; Galvan et al., 2006; Steinberg, 2010). Thus the protracted development of the PFC compared to the striatum, and the immature connections between these regions, during adolescence may mediate increased impulsivity in adolescent rats via altered prefrontal control over sub-cortically driven approach behaviour (Chambers et al., 2003; Ernst et al., 2006).

Age differences in dopamine system function may affect the expression of amphetamine-induced impulsive action. Premature responding in the 5-CSRTT depends upon accumbal dopamine release (Cole & Robbins, 1989; Pattij et al., 2007) and the subsequent action of dopamine on post-synaptic D1 and D2 receptors in this region (van Gaalen et al., 2006). The striatum, a region innervated by dopaminergic projections, is also involved in mediating premature responding (Rogers, Baunez, Everitt, & Robbins, 2001). Compared to adults, rats in mid to late adolescence (~40-45; approximately when testing of the effects of amphetamine occurred during the present study) show more extracellular baseline dopamine in the nucleus accumbens (Badanich et al., 2006), and enhanced expression of D1 and D2 receptors in the striatum and nucleus accumbens (Andersen et al., 1997; Andersen et al., 2000). Extracellular dopamine release in response to amphetamine is similar in both age groups in the nucleus accumbens in response to an acute injection (Silvagni, Barros, Mura, Antonelli, & Carboni, 2008). However, in response to repeated amphetamine injections, adolescents show greater extracellular dopamine release than adults in the dorsal striatum (Laviola, Pascucci, & Pieretti, 2001). Thus, age differences in the effects of amphetamine on premature responding in male rats may be mediated by age-related differences in dorsal striatum dopamine release or increased dopamine signalling potential in the nucleus accumbens.

Dopamine system function may also contribute to sex differences in amphetamine-induced impulsive action. Adult females showed the largest response to amphetamine and to increasing the ITI on premature responding. The effects of both manipulations involve, in part,
dopamine in the nucleus accumbens (Cole & Robbins, 1989). Dopamine function is modulated by female sex hormones; estrogen increases dopamine release (Becker, 1999; Carroll & Anker, 2010; Thompson & Moss, 1994), while progesterone decreases dopamine in the nucleus accumbens (Carroll & Anker, 2010; Laconi, Reggiani, Penissi, Yunes, & Cabrera, 2007). Thus higher estrogen levels in adult females compared to adult males may enhance the effects of a long ITI and amphetamine on premature responding in adult females by increasing nucleus accumbens dopamine release. Thus the interaction of female gonadal hormones and mesolimbic dopamine may be important for sex differences in impulsive action.

Although dopamine is the primary neurotransmitter mediating the effects of amphetamine (Azzaro & Rutledge, 1973; Besson et al., 1969), this drug also acts on other neurotransmitter systems including 5-HT which plays a role in impulsive action (Dalley et al., 2008; Soubrie, 1986). Amphetamine increases 5-HT release at doses greater than 2 mg/kg (Kuczenski & Segal, 1989), which is much higher than the dose required to increase premature responding. Premature responding induced by amphetamine is also not affected by 5-HT depletion (Harrison et al., 1997). Thus, 5-HT is unlikely to be involved in mediating the effects of age or sex on amphetamine-induced premature responding in the current study.

One potential caveat to the hypothesis that dopamine contributes to age and sex differences in impulsive action is that these differences were observed following amphetamine but not nicotine treatment. The differential effect of these two drugs on premature responding may be accounted for by variations in their mechanism of action of dopamine release and the extent and pattern of dopamine release induced by these two drugs (Azzaro & Rutledge, 1973; Besson et al., 1969; Nisell et al., 1994; Sulzer). Additionally, unlike amphetamine, nicotine-induced premature responding involves nAChRs (Grottick & Higgins, 2000; Kirshenbaum et al., 2011). Thus, several mechanistic differences between the two drugs could account for their different interactions with age and sex in affecting impulsive action.
4.6 Conclusions & Implications

Our findings corroborate the general view that adolescents are more impulsive than adults but not that males are more impulsive than females. Sex differences, particularly in response to amphetamine, only appeared in adults. The varying magnitude of age differences in impulsive action across experiments observed in the present study suggests that adolescents, or at least some adolescents, may be more prone to behave impulsively. This propensity to behave impulsively, particularly in males, may be mediated in part the mesocorticolimbic dopamine system, including the nucleus accumbens and PFC, which continue to develop throughout adolescence.
Chapter 7: General Discussion

1. Summary of Findings

The experiments described in this thesis compared adolescents and adults on measures of incentive motivation and impulsive action, two processes which may contribute to age differences in the effects of drugs of abuse during adolescence. Adolescents have been labeled ‘impulsive’ and ‘reward-driven’ (Chambers et al., 2003; Ernst et al., 2006; Spear, 2000), however few empirical studies have documented differences in incentive motivation and impulsivity across development. Evidence from a limited number of experiments suggests that compared to adults, adolescents may show elevated altered reward processing and impulsivity (Adriani & Laviola, 2003; Ernst et al., 2009; Schramm-Sapyta et al., 2009; Steinberg et al., 2009), potentially because of the pattern of brain development during adolescence (Chambers et al., 2003; Crews et al., 2007; Ernst et al., 2009).

The first aim of the thesis was to examine possible age differences in incentive motivation for natural reward-paired cues as measured by responding for a conditioned reinforcer previously paired with sucrose (Chapter 3). I hypothesized that adolescent males would respond more than adult males for a conditioned reinforcer and that amphetamine would enhance responding more in adolescents than adults (Douglas et al., 2003, 2004; Schramm-Sapyta et al., 2009). These hypotheses were supported in multiple experiments. Adolescents also made fewer responses than adults for sucrose on a PR schedule of reinforcement suggesting that the increased responding for the conditioned reinforcer in adolescents compared to adults was unrelated to increased unconditioned motivation for the primary reward. The effects of D₁ and D₂ dopamine receptor antagonists and an opioid receptor antagonist on responding for a conditioned reinforcer were also examined because these receptors play roles in incentive motivation and reward (Berridge, 1996; Berridge & Robinson, 1998). In adolescent males, blockade of D₁, and D₂ dopamine receptors and opioid receptors decreased responding for a stimulus previously paired with sucrose, which suggests a role for these receptors in incentive motivation.
The second aim of this thesis was to investigate possible age differences in incentive motivation for drug-paired stimuli as measured by responding for a conditioned reinforcer previously paired with IV nicotine infusions (Chapter 4). Based on the reliable age difference in incentive motivation for sucrose-paired cues and previous studies examining age differences in nicotine-induced CPP (Belluzzi et al., 2004; Brielmaier et al., 2007; Shram et al., 2006; Shram & Le, 2010; Vastola et al., 2002), I hypothesized that adolescent males would respond more than adult males for a conditioned reinforcer previously paired with IV nicotine infusions. This hypothesis was supported in the experiment that paired the CS with passive IV nicotine infusions but only adults responded for a conditioned reinforcer previously associated with self-administered IV nicotine infusions. Subsequently, adolescents and adults showed a sensitized locomotor response to a systemic nicotine challenge after passively-administered or self-administered IV nicotine infusions. This latter finding suggests that IV nicotine reached the brain in sufficient concentrations to induce a functional behavioural change in all groups (i.e., behavioural sensitization) although the IV nicotine exposure was insufficient to induce responding for a stimulus previously paired with the IV nicotine infusions in some groups.

The third aim of this thesis was to investigate possible age and sex differences in impulsive action as measured by responding on a DRL schedule and the effect of amphetamine on this behaviour (Chapter 5). I hypothesized that adolescents would respond more than adults on this schedule based on two previous studies (Andrzejewski et al., 2011; Lejeune & Jasselette, 1987). Sex differences in responding on a DRL schedule were expected in adults only (Beatty, 1973; Cross et al., 2011; van Hest et al., 1987). I also hypothesized that adolescents and females may be most sensitive to the effects of amphetamine on a DRL schedule based on previous studies (Becker et al., 2001; Laviola et al., 1999; Schramm-Sapyta et al., 2009). The results showed that adolescents responded less than adults on a DRL schedule of reinforcement which implies reduced impulsive action in adolescents. Adult males appeared to make the most responses on this schedule. No age differences in the effects of amphetamine on responding on a
DRL schedule were observed although amphetamine appeared to increase m-IRTs in females compared to males.

The final aim of the thesis was to examine possible age and sex differences in impulsive action as measured by premature responding on a 2-CSRTT (Chapter 6). Based on previous studies (Andrzejewski et al., 2011; Eigsti et al., 2006; Lejeune & Jasselette, 1987; Rubia et al., 2006; Williams et al., 1999), I hypothesized that adolescents would make more premature responses than adults. I also hypothesized that sex differences in performance in the 2-CSRTT would appear after adolescence (Cross et al., 2011). Possible age and sex differences in the effects of amphetamine, Ro 63-1908 and nicotine on this behaviour were also assessed. These drugs increase premature responding in adults (Cole & Robbins, 1989; Higgins et al., 2003; Stolerman et al., 2000) and act on neurochemical systems continuing to develop during adolescence (Crews et al., 2007; Pian et al., 2010). Therefore, I hypothesized adolescents would be differentially responsive to the impulsivity-inducing effects of these drugs. Adolescents made more premature responses than adults in the 2-CSRTT in several experiments. No sex differences were observed in premature responding during acquisition of performance in the 2-CSRTT. Amphetamine selectively enhanced premature responding in adolescent males compared to adult males. In response to amphetamine, adult females also made more premature responses than adult males. No consistent age or sex differences in premature responding were observed after treatment with Ro 63-1908 or nicotine. These findings suggest that dopamine may be involved in age and sex differences observed in premature responding in the 2-CSRTT.

2. The Conditioned Reinforcing Effects of Stimuli Previously Paired with Natural versus Drug Rewards

One striking finding from the experiments that examined incentive motivation for stimuli previously associated with either sucrose or IV nicotine infusions was the difference in the magnitude of responding for the conditioned reinforcer. Rats generally responded less for a conditioned reinforcer previously paired with IV nicotine infusions as compared to a conditioned
reinforcer previously paired with sucrose. This finding was unexpected based on previous work showing that drug rewards, and cues that predict them, elicit greater activation of the mesolimbic dopamine system than natural rewards (Chen et al., 2008; Opris, Hampson, & Deadwyler, 2009; Willuhn, Wanat, Clark, & Phillips, 2009; Zhang, Kiyatkin, & Stein, 1994). In previous experiments using passively- or self-administered natural rewards (sucrose or water), deprived rats typically made approximately 40-60 responses on the CR lever and 5-20 responses on the NCR lever during 40 min test sessions (Burton et al., 2009; Di Ciano & Everitt, 2004; Fletcher et al., 1999; Fletcher, Korth, Robinson, & Baker, 2002; Smith et al., 1997). Conversely, in experiments using various self-administered IV drugs as the primary reinforcer (cocaine, heroin, nicotine), rats made an average of 20-40 responses on the CR lever and 10-15 responses on the NCR lever (Di Ciano & Everitt, 2004; Palmatier et al., 2007). One study reported a higher number of CR responses for a CS previous pairings with passively-administered IV morphine infusions (approximately 100 responses) but the test sessions were considerably longer than most studies (6 hr; Davis et al., 1975). Thus, across labs and experiments, rats consistently respond to a greater degree for conditioned reinforcers previously paired with natural versus drug rewards.

2.1 The Possible Role of Stimulus-Reward Pairing Frequency

An important difference between studies described in this thesis that examined responding for a conditioned reinforcer previously paired with a natural versus a drug reward is the number of stimulus-reward pairings. In the present studies using sucrose as the primary reward, rats received 420 CS-sucrose pairings (Chapter 3). This number of pairings is similar to previous studies using natural primary rewards (Burton et al., 2009; Fletcher et al., 1999; Fletcher et al., 2002). In the experiments using IV nicotine infusions as the primary reinforcer, the maximum number of CS-IV nicotine infusion pairings was fewer than 200 (Chapter 4). In a similar study using only adult rats (Palmatier et al., 2007), the number of stimulus-drug reward pairings was unreported. However, the number of responses for IV nicotine infusions in that study was similar to the number of responses for IV nicotine infusions made by adult males in
Chapter 4 and this group earned an average of 95 total CS-IV nicotine infusion pairings. Together these findings imply that the number of reward-stimulus pairings could influence the magnitude of responding for a conditioned reinforcer.

2.2 The Possible Role of Temporal Proximity of the CS and US

Another procedural difference between responding for a conditioned reinforcer previously paired with a natural versus a drug reward is the temporal proximity between the CS and the effects of the US. Studies of classical conditioning using animals report that the frequency of conditioned responses decreases as the interval between the CS and the US increase (Schneiderman & Gormezano, 1964; Smith, 1968). For example, delivery of a food pellet more than 10 s after the end of a CS presentation during classical conditioning trials significantly reduces the expression of a conditioned response to the food-paired CS (Holland, 1980). Therefore, a relatively short interval between the CS and the effects of the US appears to maximize classical conditioning.

The optimal CS-US interval is partially determined by the nature of the effects of the US. Evidence suggests that the onset and duration of the effects of natural rewards and drug rewards differ (Cheer et al., 2007; Day, Roitman, Wightman, & Carelli, 2007). For example, a previous study using fast-scan cyclic voltammetry showed that within 5 s of the delivery of a food reward, extracellular dopamine in the nucleus accumbens peaked and returned to baseline levels (Day et al., 2007). In contrast, after a passive IV infusion of nicotine, extracellular dopamine in the nucleus accumbens began to increase approximately 20 s after the infusion and the peak lasted approximately 25 s (Cheer et al., 2007). The difference between sucrose USs and drug USs was aptly summarized by Bormann and Cunningham: “In the case of drug-induced place conditioning, however, one must also consider the fact that the US is not a punctate event with a discrete onset and termination. Rather, it is a relatively long-lasting event whose intensity varies over time, presumably as some function of brain-drug level” (1998). Therefore, a short interval between the presentation of the CS and the delivery of a sucrose US interval facilitates
conditioning because the effects of the reward occur and dissipate quickly. However, in studies using IV drug infusions, if the CS and US are delivered at the same time, the presentation of the CS must be sufficiently long to ensure that the presentation occurs close enough to the onset of the effects of the US. Therefore, the optimal timing of the presentation of the CS in relation to the delivery and the onset of effects of the US will likely differ for natural versus drug rewards.

In the present studies, the CS-US interval may have been sub-optimal in the experiments using IV nicotine infusions. As described in Chapter 4, the beginning of the CS (experiment 1: 5 s and experiment 2: 15 s) co-occurred with a 2 s infusion of nicotine, thus the rewarding effects of nicotine may have occurred approximately 15 s (in experiment 1) or 5 s (in experiment 2) after the offset of the CS. Thus, the interval between the CS and the effects of the US may have been too long resulting in inadequate learning of the association between the CS and the effects of the US and relatively low levels of responding for a conditioned reinforcer. One implication of this hypothesis is that rats may have responded more for a conditioned reinforcer previously paired with IV drug infusions if the interval between the CS and the effects of the drug was minimized.

3. The Possible Role of Procedural Differences in the Conflicting Findings from Measures of Impulsive Action

Compared to adults, adolescents made more premature responses than adults in the 2-CSRTT (i.e., enhanced impulsive action) but fewer responses on the DRL schedule (i.e., reduced impulsive action). These opposing findings could be explained by procedural differences between these measures.

3.1 The Possible Effect of a Signaled versus an Un-signalled Waiting Period

One methodological difference between these two measures is the presence of a cue to signal the appropriate time to respond. In the 2-CSRTT, a brief light stimulus signals the end of the periods during which the rat must withhold a response. When responding on a DRL schedule, there is no event that indicates the end of similar periods, so the rat must independently assess
the amount of time elapsed from the delivery of the last reinforcer in order to respond appropriately. Therefore, age differences in timing abilities may affect responding on the DRL schedule to a greater extent than performance in the 2-CSRTT. To reduce the potential confound of timing, age and sex differences could be examined on responding on the DRL schedule of reinforcement using a visual cue to indicate the appropriate time to respond. If adolescents assess the passage of time differently than adults, the addition of a cue to this procedure may result in adolescents responding more than adults on this schedule.

3.2 The Possible Role of Cognitive Control

Performance in the 2-CSRTT and responding on a DRL schedule also differ in the degree of complexity of the procedure. Responding on a DRL schedule is a relatively simple behaviour: the rat must learn to withhold from responding for 18 s after the delivery of a reward. Only one lever is extended into the chamber during these sessions and thus a rat can only respond incorrectly if the response occurs before the appropriate time. In contrast, performance on the 2-CSRTT requires the rat to focus their visual attention to observe a brief stimulus, learn to respond in one of two possible apertures, and withhold responding during the 5 s ITI. Arguably, performance in the 2-CSRTT requires more cognitive control than responding on a DRL schedule. The degree of cognitive control required to execute behaviour may also differentially affect the expression of age differences in impulsive action in humans. In a previous experiment using a different measure, adolescents and adults performed similarly on a Go/No-Go task where the probability of the ‘go’ signal was 85% and the probability of the ‘no-go’ signal was 15% (Stevens et al., 2007). However, in a study using a Go/No-Go task where the probability of both signals varied as a function of the number of preceding ‘go’ trials (a task purposefully designed to require greater cognitive control), adolescents showed significantly more false alarms than adults (Eigsti et al., 2006). Similarly, adolescents made more false alarm responses than adults when the procedure involved a second ‘go’ signal that resembled the ‘no-go’ signal (Rubia et al., 2006). One hypothesis based on the findings from human studies and the present experiments is
that adolescents may show enhanced impulsive action compared to adults in situations with greater cognitive demands but reduced or similar impulsive action in situations with relatively few cognitive demands.

Age differences in PFC function may partially account for this effect. The development of the PFC occurs later than other sub-cortical structures (Chambers et al., 2003; Ernst et al., 2006; Geier & Luna, 2009; Giedd et al., 1999; Gogtay et al., 2004). This structure mediates response inhibition but also other aspects of executive function including attention, working memory, planning and behavioural monitoring (Dalley, Cardinal, & Robbins, 2004). In situations when the demands on multiple aspects of executive function are high, control over response inhibition may suffer compared to attention or working memory in adolescents compared to adults because the immature PFC may be unable to function properly in this context. Therefore, compared to adults, the protracted development of the PFC in adolescents may induce the expression of impulsive action in contexts requiring considerable executive function.

3.3 The Possible Effect of the Response Interval

Amphetamine produced different effects in adult females in the two measures of impulsive action. Adult females made more amphetamine-induced premature responses in the 2-CSRTT (i.e., increased impulsive action) but amphetamine increased m-IRTs in the adult females when responding on a DRL schedule (i.e., decreased impulsive action). One possible explanation for these opposing findings is that the amount time available to make an appropriate response in these two measures. When responding on the DRL-18 schedule, responses anytime after 18 s are rewarded and longer m-IRTs are interpreted as evidence of reduced impulsivity in a rat. Conversely, in the 2-CSRTT, a rat must sustain its attention to the back wall until a brief stimulus (2.5 s) signifies when to respond. Failing to respond during or briefly after the stimulus is punished by a time out, and no reward is delivered. Amphetamine-induced locomotor activity and exploratory behaviour, which may orient the rat away from the appropriate place to respond, may differentially affect performance on the 2-CSRTT and responding on the DRL schedule.
When responding on a DRL schedule, amphetamine-induced locomotor exploration may decrease the time focused on the lever and therefore may result in increased m-IRTs in responding on the DRL schedule (van Hest et al., 1987). However, in the 2-CSRTT, amphetamine-induced locomotor activity may increase the time spent exploring the wall with several apertures during the ITI and, in turn, result in more premature responses (because responses in any aperture prior to the onset of the stimulus light were considered a premature response). Females are more sensitive than males to the locomotor-stimulating effects of amphetamine (Becker et al., 2001; Mathews & McCormick, 2007). Therefore in the present studies, amphetamine-induced exploratory behaviour in adult females may have contributed to opposite outcomes in the two measures of impulsive action: longer m-IRTs on a DRL schedule and increased premature responses in the 2-CSRTT.

3.4 Evaluation of the Two Measures of Impulsive Action

In summary, although responding on a DRL schedule and premature responding in the 2-CSRTT are both considered measures of impulsive action in the rat, they may measure different aspects of behaviour. High levels of responding on a DRL schedule may reflect the expression of impulsive action or an altered ability to assess the passage of time. Conversely, high levels of premature responding in the 2-CSRTT is well-established measure of impulsive action (Bari et al., 2008). The potential confounds of other behavioural processes such as general motivation and learning can possibly be ruled out because these aspects of performance are captured in dissociable measures. Additionally, the ability to assess the passage of time likely plays less of a role in the 2-CSRTT compared to responding on a DRL schedule. Therefore, premature responding is a less ambiguous measure of impulsive action than responding on a DRL schedule. Further, given the difference in the cognitive demands required to respond on a DRL schedule and acquire performance in the 2-CSRTT, these behaviours may reflect response inhibition in different contexts. Therefore, the finding that adolescents made more premature responses in multiple experiments using the 2-CSRTT procedure strongly suggests that adolescents are more
prone than adults to exhibit impulsive action while reduced responding on a DRL schedule in adolescents compared to adults may have more than one interpretation and may not necessarily indicate that adolescents show less impulsive action than adults.

4. Possible Mechanisms

Evidence suggests that incentive motivation and impulsive action are mediated by different, but somewhat overlapping, neurocircuits (Dalley et al., 2011; Everitt et al., 1999). Brain structures involved in the expression of both behaviours include regions of the frontal cortex, the limbic-striatal circuit and the mesocorticolimbic dopamine, glutamate and GABA systems (Dalley et al., 2011; Dalley et al., 2008; Everitt et al., 1999; Pears et al., 2003; Robbins, 2002; Sutton & Beninger, 1999). These brain regions and neurochemical systems undergo many changes during the adolescent period (Chambers et al., 2003; Crews et al., 2007; Ernst et al., 2009; Spear, 2000) and thus may contribute to age differences in incentive motivation and impulsive action.

Dopamine and glutamate receptor expression and function may be enhanced in adolescents compared to adults (Crews et al., 2007; Ernst et al., 2009). The expression of D₁ dopamine receptors peaks in the ventral striatum, dorsal striatum and PFC around PND 40 (Andersen et al., 2000). Specifically, the peak in D₁ dopamine receptors in the PFC appears to occur preferentially on glutamate neurons projecting to the nucleus accumbens core. The percentage of D₁ dopamine receptors found on these neurons is increased in adolescent (44% on PND 44) compared to younger (2% on PND 21 rats) or adult rats (6% on PND 100) while the percentage of D₁ dopamine receptors on GABA interneurons remains relatively constant across developmental stages (Brenhouse et al., 2008). Some aspects of glutamate function are also enhanced in adolescents compared to adults. Adolescents show increased NMDA receptor densities and enhanced NMDA-mediated long-term potentiation in the nucleus accumbens compared to adults (Insel et al., 1990; Miller et al., 1990; Schramm et al., 2002). Thus, compared
to adults, adolescents show a peak in PFC and striatal D₁ dopamine receptor expression and NMDA-mediated function.

4.1 Incentive Motivation

The brain regions and neurotransmitter systems that mediate responding for a conditioned reinforcer also change over the course of adolescence (Everitt et al., 1999). The motivational salience of conditioned reinforcers may be mediated in part by dopamine release in the nucleus accumbens core (Berridge, 2007; Everitt et al., 1999; Horvitz, 2002). This dopamine signal requires activation of D₁ dopamine receptors (Sutton & Beninger, 1999). The BLA, a brain region involved in encoding information about reward value and contingencies, is also involved in the ability of conditioned cues to control behaviour (Everitt et al., 1999; Horvitz, 2002; Robbins, Cador, Taylor, & Everitt, 1989). Specifically, glutamate in the BLA modulates dopamine release in the nucleus accumbens core (Floresco, Blaha, Yang, & Phillips, 2001; Jones, Day, Aragona, et al., 2010; Jones, Day, Wheeler, & Carelli, 2010; Stuber et al., 2011), an effect also mediated by D₁ dopamine and NMDA receptors in this region (Floresco et al., 2001). The OFC, which shares some similar functionality with the BLA, also sends glutamate projections to the nucleus accumbens core and is required to respond for a conditioned reinforcer (Pears et al., 2003). Conversely, excitotoxic lesions of the PFC fail to alter responding for a conditioned reinforcer in adult rats (Burns et al., 1993; Pears et al., 2003). Although the PFC does not appear to be involved in responding for a conditioned reinforcer in adult rats, this brain structure may play a role in this behaviour in adolescent rats. D₁ dopamine receptor expression on glutamate projections from the PFC to the nucleus accumbens core peaks during adolescence (Brenhouse et al., 2008). Activation of D₁ receptors in the PFC also enhances the conditioned rewarding effect of cocaine (Brenhouse et al., 2008). Therefore, compared to adults, adolescents may exhibit enhanced incentive motivation because of enhanced dopamine signalling in the nucleus accumbens core resulting from 1) increased D₁ dopamine receptor density in this region,
2) increased glutamate input from the BLA, and 3) enhanced D₁ dopamine receptor-mediated glutamate input from the PFC.

### 4.2 Impulsive Action

Evidence suggests that the PFC and nucleus accumbens also play an important role in mediating premature responding in the 2-CSRTT (Dalley et al., 2011; Dalley et al., 2008). In adult rats, temporary inactivation of the PFC increases premature responding in the 5-CSRTT (Murphy et al., 2005). Excitotoxic lesions of the PFC or nucleus accumbens core, and disconnection of these brain regions produced a similar effect (Christakou et al., 2004; Chudasama et al., 2003). Deep brain stimulation of the nucleus accumbens core also increases premature responding in a reaction time test (Sesia et al., 2008). The seemingly contradictory findings from the lesion and stimulation studies suggest that appropriate response inhibition may require an optimal level of nucleus accumbens and PFC activation. In adult rats, stimulation of D₁ dopamine receptors in the PFC either reduces or has no effect on premature responding (Chudasama & Robbins, 2004; Fletcher, Tenn, Sinyard, Rizos, & Kapur, 2007). The role of PFC D₁ dopamine receptors in premature responding has not been investigated in adolescent rats but stimulation of these receptors may produce a differential effect in adolescents compared to adults. The density of D₁ dopamine receptors on PFC glutamate projections to the nucleus accumbens core is dramatically different in adolescents compared to adults (44% versus 6% respectively; Brenhouse et al., 2008). This age difference may result in increased dopamine-induced PFC stimulation of the nucleus accumbens and in turn increased premature responding in adolescents versus adults. Therefore, in adolescents, activation of D₁ dopamine receptors on glutamate projections from the PFC to the accumbens could increase premature responding in the 2-CSRTT during adolescence more than in adulthood.
4.3 The Effects of Amphetamine on Responding for a Conditioned Reinforcer Previously Paired with Sucrose and Premature Responding in the 2-CSRTT

Previous research shows that the effects of amphetamine to increase premature responding and responding for a conditioned reinforcer are mediated by the nucleus accumbens shell (Dalley et al., 2011; Everitt et al., 1999). The nucleus accumbens shell mediates unconditioned responses to external stimuli such as drugs of abuse or novelty (see Meredith, Baldo, Andrezjewski, & Kelley, 2008 for review). Responding for a conditioned reinforcer and premature responding in the 2-CSRTT are unaffected by excitotoxic lesions of the nucleus accumbens shell, but these lesions block the ability of amphetamine to potentiate both behaviours (Everitt et al., 1999; Murphy et al., 2008; Parkinson et al., 1999; Phillips, Setzu, & Hitchcott, 2003; Robbins et al., 1989). Blocking D₂ dopamine receptor also reduces amphetamine-induced increases in premature responding in the 2-CSRTT and responding for a conditioned reinforcer (Ranaldi & Beninger, 1993; van Gaalen et al., 2006). The expression of these receptors peaks in the nucleus accumbens around PND 40 (mid-adolescence; Andersen et al., 2000), which suggests that enhanced D₂ dopamine receptor expression in the nucleus accumbens shell during adolescence may contribute to age differences in the effects of amphetamine on these behaviours in male rats.

5. Future Research

5.1 Generalizability of Age Differences in Responding for a Conditioned Reinforcer Previously Paired with Self-Administered Drug

A general hypothesis in this thesis was that incentive motivation contributes to a susceptibility to the effects of drugs of abuse during adolescence. In support of this hypothesis, adolescents responded more than adults for a conditioned reinforcer previously paired with sucrose or passive IV nicotine infusions, although unexpectedly, adolescents failed to respond for a conditioned reinforcer previously paired with self-administered IV nicotine infusions. Drugs, including nicotine are self-administered in human adolescents, thus an implication of the
results from Chapter 4 is that self-administered drug use during adolescence may be unrelated to incentive motivation. However, one limitation of the work described in this thesis is that the conditioned reinforcing effects of only IV nicotine infusion-paired stimuli, and no other drug of abuse, were examined. Therefore, the findings from the present study on age differences in incentive motivation for drug-paired cues may not generalize to all drugs of abuse.

To investigate whether the age differences described in Chapter 4 are specific to nicotine, I would compare adolescents and adults on responding for conditioned reinforcers previously paired with other drugs of abuse such as cocaine, methamphetamine or ethanol. Adolescent rats may be more sensitive than adults to the conditioned rewarding effects of these drugs as measured by CPP and reinstatement of CPP (Badanich et al., 2006; Brenhouse & Andersen, 2008; Brenhouse et al., 2008; Philpot et al., 2003; Zakharova, Wade, et al., 2009). Thus, in combination with the finding that adolescents show enhanced incentive motivation for sucrose paired cues compared to adults, I hypothesize that adolescents may show enhanced responding for a conditioned reinforcer previously paired with cocaine, methamphetamine or ethanol cues. Regardless of the outcome of the experiment, the findings would help establish whether a lack of responding for a conditioned reinforcer previously paired with self-administered IV drug infusions in adolescent rats generalizes to all drugs of abuse, and therefore reflects a general lack of incentive motivation for drug-paired cues during adolescence. Understanding the nature of age differences to drug-paired cues could help inform drug prevention and drug treatment and may help elucidate possible mechanisms that contribute to drug use and abuse during adolescence.

5.2 The Role of Cognitive Control in Age Differences in the Expression of Impulsive Action

Compared to adults, adolescents showed less impulsive action as measured by responding on a DRL schedule and more impulsive action as measured by premature responding in the 2-CSRTT. One hypothesis to explain this finding is the difference in the degree of cognitive control required to perform these behaviours affects the expression of impulsive action during adolescence. Evidence from studies using human adolescent participants supports this hypothesis
However, no studies have directly examined the effect of cognitive demands on age differences in impulsive action. Identifying precipitating factors for the expression of impulsive action during adolescence may help clarify the conflicting findings from the present experiments and studies using human participants and further our understanding of the development of impulsivity.

To investigate whether decreasing cognitive demands reduces the expression of impulsive action in adolescents, age differences in premature responding could be compared in the 2-CSRTT versus a ‘1-Choice Serial Reaction Time Test’ (1-CSRTT) which involves only 1 possible lit aperture instead of two (Winstanley, Dalley, et al., 2004). Additionally, to simplify the procedure the unused apertures would be covered so that rats would only need to respond and focus their attention on one aperture. If cognitive demands increase premature responding then age differences in premature responding should be less pronounced in the ‘1-CSRTT’ as compared to the 2-CSRTT.

The effect of increasing cognitive demands on the expression of impulsive action could also be investigated. To extend the results of the current experiments, I could examine age differences in responding on a modified DRL schedule designed to increase the cognitive demands of the task or performance on a Go/No-Go test. However, although these types of experiment could generate useful information, it may be difficult to train rats within the adolescent period and using the 6 hr food and water restriction regimen used in the present experiments. Alternatively, these experiments could be conducted using human participants. Building on the work of Rubia and colleagues (2006), age differences in Go/No-Go performance could be examined with two versions of the task: one version with only one ‘go’ signal and a second version with a second ‘go’ signal that is similar to the ‘no-go’ signal. If adolescents show more false alarms than adults only in the more complex version of the task then evidence would suggest that cognitive demands increase the expression of age differences of impulsive action. Together, these direct comparisons of performance on tasks that differ on the degree of cognitive
control required in animals and rats would help identify if age differences exist in the ability of this factor to induce the expression of impulsive action.

5.3 Possible Mechanisms

One advantage of examining age differences in behaviour using animal subjects is the ability to investigate the possible corresponding neurobiological mechanisms of those differences. Although the present studies demonstrated age differences in the expression of impulsive action and incentive motivation, one limitation of this work is that the possible biological mechanisms that underlie these age differences were not addressed. To understand what biological factors contribute to age differences in the expression of incentive motivation and impulsive action, subsequent experiments should examine the possible role of brain structures and neurochemical systems that continue to develop during adolescence (Chambers et al., 2003; Crews et al., 2007; Ernst et al., 2009; Spear, 2000).

5.3.1 Incentive Motivation

As described above in Section 5.1, age differences in responding for a conditioned reinforcer previously paired with sucrose may be related, in part, to increased dopamine signalling in the nucleus accumbens core in adolescent rats. Increased dopamine signalling may result from enhanced D₁ dopamine receptor expression in this region and increased D₁ dopamine receptor-mediated glutamate input from the PFC (Brenhouse et al., 2008; Everitt et al., 1999; Pears et al., 2003; Sutton & Beninger, 1999). To test the hypothesis that enhanced D₁ dopamine receptor signalling in the PFC is involved in age differences in responding for a conditioned reinforcer previously associated with sucrose, I would examine the effect of injecting a D₁ dopamine antagonist into the PFC or nucleus accumbens core on responding for a conditioned reinforcer in adolescent rats (adults did not respond for a conditioned reinforcer therefore they would not be included in these experiments). If responding for a conditioned reinforcer in adolescents is mediated by D₁ dopamine receptor signalling in the nucleus accumbens core and the PFC, then blockade of D₁ dopamine receptors in these regions should attenuate this
behaviour in adolescents. The results from this experiment would demonstrate if D₁ dopamine receptors in the nucleus accumbens and the PFC play a role in responding for a conditioned reinforcer in adolescent rats.

5.3.2 Impulsive Action

Based on previous studies, age differences in premature responding in the 2-CSRTT may be mediated by differences in D₁ dopamine receptor signalling on PFC projections to the nucleus accumbens (see Section 5.2). Specifically, D₁ dopamine receptor activation in the PFC may stimulate premature responding in adolescents but have no effect in adults because of the difference in the expression of these receptors in the PFC during adolescence (Brenhouse & Andersen, 2008; Chudasama & Robbins, 2004; Fletcher, Tenn, et al., 2007). To test this hypothesis, I would examine the effects of micro-injections of a D₁ dopamine antagonist into the PFC in adolescent and adult rats. If D₁ dopamine receptor signalling in the PFC specifically enhances premature responding in adolescent rats, then blocking this receptor should reduce premature responding in adolescents and have no effect in adults. The results from this experiment would directly show that age difference in premature responding in the 2-CSRTT is mediated in part by D₁ dopamine receptor activation in the PFC.

5.4 The Role of Social Factors in Age Differences in the Expression of Incentive Motivation and Impulsive Action

Although beyond the scope of this thesis, social factors play an important role during adolescence (Spear, 2000). Social interactions are particularly rewarding during this period in both humans and animals (Csikszentmihalyi, 1977; Douglas et al., 2004) and may affect many aspects of development (Einon & Morgan, 1977; Fone & Porkess, 2008; Keremar, Budefeld, Grgurevic, Tobet, & Majdic, 2011; Schenk, Gorman, & Amit, 1990). The influence of peers, such as having friends that use drugs and peer pressure, contributes to the development of drug use during adolescence (Barber, Bolitho, & Bertrand, 1999; Bauman & Ennett, 1996; Ravishankar & Nagarajappa, 2009; Rumpold et al., 2006; Scherrer et al., 2011). The presence of
peers also enhances risky behaviour (Chein et al., 2011; Gardner & Steinberg, 2005; Steinberg, 2004). In humans, the ability of peers to influence behaviour presumably occurs through social expectations (Pearl, Bryan, & Herzog, 1990).

Age differences in the influence of peers on the effects of drugs of abuse, incentive motivation and impulsivity are inherently difficult to assess in rodents. In particular, the effects of peer pressure and a peer group that uses drugs cannot be examined in rodents. However, a few studies have shown that social interactions can influence the conditioned effects of drugs of abuse during adolescence. The presence of a same-sex conspecific during Pavlovian conditioning enhances cocaine- and nicotine-induced CPP (Thiel, Okun, & Neisewander, 2008; Thiel, Sanabria, & Neisewander, 2009). No adults were included in these studies so it is currently unknown whether adolescent rats are more sensitive than adults to the effects of peers on measure of incentive motivation. No published studies have examined the effect of peers on operant measures of impulsivity or incentive motivation in rats. Examining the influence of peers on these behaviours using the methods currently available would be difficult. Performance in operant-based measures such as the 2-CSRTT or responding for a conditioned reinforcer require sustained attention so the presence of a peer would likely prevent the rats from learning these behaviours. Therefore, the role of social factors in incentive motivation and impulsivity may be more easily studied in humans.

5.5 The Role of Incentive Motivation and Impulsive Action in a Vulnerability to the Effects of Drugs of Abuse during Adolescence

One overarching goal of this thesis was to examine whether incentive motivation and impulsive action contribute to a susceptibility to the effects of drugs of abuse during adolescence. Although my findings suggest that age differences exist in incentive motivation and impulsive action in rats, it is still unclear whether these processes play a role in age differences in the effects of drugs of abuse. Given that both incentive motivation and impulsive action play roles in addiction (Dalley et al., 2011; Everitt et al., 2008; Robinson & Berridge, 2000) and may be
enhanced during adolescence based on the findings from the present experiments, an examination of the role of these processes in age differences in the effects of drugs of abuse is warranted.

Future research should directly examine whether incentive motivation or impulsive action during adolescence contributes to a vulnerability to the effects of drugs of abuse. This line of research would help elucidate the factors that contribute to a susceptibility to the effects of drugs of abuse during adolescence. Despite the fact that age differences in the effects of drugs of abuse in animals are mixed (Schramm-Sapyta et al., 2009), the evidence from humans clear: most drug users begin to experiment with recreational drugs during adolescence and the age of onset of recreational drug use inversely predicts subsequent drug dependence (Anthony & Petronis, 1995; Chen et al., 2009; Degenhardt et al., 2008; Kandel et al., 1992; SAMHSA, 2007; Sintov et al., 2009; Wagner & Anthony, 2002). Understanding why adolescence may be a period of vulnerability to the effects of drugs of abuse could help inform prevention and treatment strategies for adolescents in the hopes of reducing the risk of drug addiction.

6. Implications and Conclusions

In line with a recent review (Schramm-Sapyta et al., 2009), the findings described in this thesis suggest that adolescents are vulnerable to some, but not all, of the effects of drugs of abuse. For example, adolescent males showed enhanced amphetamine-induced premature responding compared to adults in the 2-CSRTT but no consistent age differences in males were observed in responding on a DRL schedule. Amphetamine also potentiated responding for a conditioned reinforcer previously associated with a sucrose reward in adolescents but not adults, but no age differences were observed in the effects of amphetamine on responding for a conditioned reinforcer previously associated with passively-administered IV nicotine infusions. Thus, age differences in the effects of amphetamine depend upon the behavioural outcome measure. No consistent age or sex differences were observed in the effects of Ro 63-1908 or nicotine on 2-CSRTT performance. Thus, adolescents are vulnerable to some effects of drugs of
abuse but the type of drug, behaviour being measured and timing of drug delivery during adolescence may all contribute to age differences, or lack thereof, in the effects of drugs of abuse. One important implication from these results in combination with previous studies (Schramm-Sapyta et al., 2009) is that the propensity to use and abuse drugs during adolescence in humans may be related to age differences in non-pharmacological factors rather than the primary effects of drugs of abuse.

The existence of age differences in incentive motivation for reward-paired cues may have implications for a susceptibility to the effects of drugs of abuse during adolescence. Reward-paired cues play an important role in the onset and progression of drug-taking and drug-seeking (Robinson & Berridge, 2001). If reward-paired stimuli acquire more incentive salience in adolescence compared to adulthood, then these stimuli may trigger continued drug consumption resulting in drug abuse. Based on my findings it is unclear if adolescents are more motivated than adults for drug-paired cues. Adolescents showed enhanced incentive motivation for stimuli previously associated with passively-delivered sucrose or IV nicotine infusions but not self-administered IV nicotine infusions. Whether this latter effect extends to other drugs of abuse or is found in humans is still not known. However, the findings from this thesis strongly suggest that incentive motivation for stimuli paired natural rewards differs between adolescents and adults which may have implications for age differences in the effects of drugs of abuse. A recent study demonstrated that rats prone to attribute incentive salience to a stimuli that predicts a food reward show enhanced cocaine-induced CPP (Meyer, Ma, & Robinson, 2012). Therefore, incentive motivation for natural reward-paired cues during adolescence may contribute to the effects of drugs during this period.

Age differences in impulsive action also have implications for susceptibility to the effects of drugs of abuse. Adult rats characterized as impulsive, because of a high number of premature responses, show enhanced rates of drug self-administration (Dalley et al., 2007; Diergaarde et al., 2008) and increased reinstatement of cocaine-seeking after punishment compared to low
premature responders (Economidou et al., 2009). Whether such individual differences in impulsivity influence the effects of drugs of abuse during adolescence in rats is unknown, although a similar relationship likely exists during this period. Therefore, increased impulsive action during adolescence compared to adulthood may translate into a greater proportion of adolescents using, and in turn, abusing drugs.

In summary, from the results described in this thesis it can be concluded that 1) adolescents show enhanced incentive motivation for sucrose-paired cues, 2) adolescents may show enhanced incentive motivation for some but not all IV nicotine infusion-paired cues, 3) adolescents are more prone than adults to exhibit impulsive action as measured by performance on the 2-CSRTT and 4) adolescents are more sensitive than adults to some but not all the effects of drugs of abuse. Although incentive motivation and impulsive action are believed to play important roles in drug addiction in humans (Dalley et al., 2011; Robinson & Berridge, 2000), and the work from this thesis shows that age differences in these behaviours exist, it is still unclear whether age differences in these behaviours contribute to an enhanced susceptibility to drug-taking and drug-seeking during adolescence.
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Appendix 1: The Effect of 6 hr Food and Water Deprivation on Body Weight in Adolescents and Adults

1. Rationale

The experiments described in this thesis involved behavioural measures that require a degree of food or water restriction in order to motivate the rats to respond for a food, water or sucrose reinforcer (e.g., responding for a conditioned reinforcer, performance on the 2-CSRTT). Typically for experiments that involve these behaviours, adult rats are maintained at approximately 85% of their free-feeding body weight (e.g., Bari, Dalley, & Robbins, 2008; Beninger & Ranaldi, 1992). I was concerned that using a restriction regimen that did not permit adolescents to gain weight normally may produce differential effects on motivation for the reinforcer in adolescents versus adult rats. The reason for this concern is that previous experiments report that body weights can double during adolescence in rats (e.g., Lejeune & Jasselette, 1987), perhaps because adolescents have greater caloric requirements compared to adults (Post & Kemper, 1993). Pilot work showed that depriving adolescent and adult rats of food and water for 6 hr prior to training on a modified 2-CSRTT was sufficient to motivate the rats to respond comparably to adult rats maintained on 85% of their pre-restriction body weight on a 5-CSRTT (data not shown). Therefore, I investigated the effect of daily 6 hr of food and water deprivation prior to training sessions on two modified versions of the 5-CSRTT on body weight.

2. Method

To examine the effects of food and water restriction regimen on body weight gain, I weighed adolescents (postnatal day – PND 24-60) and adults daily at 8:00. Deprived rats were weighed immediately after both food and water were removed. Control rats were weighed and had ad lib access to food and water throughout the entire experiment. Every day at 14:00, deprived rats were trained to perform on a 2-CSRTT (as described in Chapter 6) or a ‘1-choice’
serial reaction time test (Winstanley, Dalley, Theobald, & Robbins, 2004) which involved the same procedure as the 2-CSRTT except only one aperture was illuminated (data not shown). At approximately 17:00 (approximately 9 hr of total food and water restriction), deprived rats were allowed access to food and water until the next morning at 8:00. Body weights were recorded daily for 27 days (PND 24 to 50 for adolescent rats). The group sizes were adolescent male deprived n = 18, adolescent male control n = 7, adolescent female deprived n = 22, adolescent female control n = 7, adult male deprived n = 20, adult male control n = 7, adult female deprived n = 22, adult female control n = 7.

Body weights for adolescents and adults were analyzed separately. For each age group, body weights were analyzed with a 3 way analysis of variance (ANOVA) with sex (male vs. female) and deprivation (deprived vs. control) as between subjects factors and day as the within subjects factor (1-27). Post hoc tests were conducted using the Bonferroni test.

3. Results

3.1 Adolescents

Body weight increased across days [main effect of day $F_{(26,1300)} = 1712.2, p < 0.001$] and overall males weighed more than females [main effect of sex $F_{(1,50)} = 46.2, p < 0.001$]. There was also a day x sex interaction $[F_{(26,1300)} = 60.7, p < 0.001]$. Although post hoc analyses did not reveal any significant differences, as shown in Figure A1a, males appear to weigh more than females beginning on PND 36. There was no main effect or interactions with deprivation

3.2 Adults

As shown in Figure A1b, body weight increased across days [main effect of day $F_{(26,1326)} = 46.8, p < 0.001$] and overall males weighed more than females [main effect of sex $F_{(1,50)} = 576.0, p < 0.001$]. There was also a day x sex interaction $[F_{(26,1326)} = 10.6, p < 0.001]$ although post hoc analyses did not reveal any significant differences. There was no main effect or interactions with deprivation
4. Discussion

Adolescents and adults deprived of food and water for 6 hr prior to daily training session daily gained weighed similarly to controls allowed free access to food and water. These results demonstrate that the food and water restriction regimen used in the experiments described in this thesis did not affect normal body weight gain in either age group.

References


**Figure A1: Body Weight.** Body weight increased across days for adolescents (Panel A) and adults (Panel B). In adolescents, males weighed more than females beginning on postnatal day (PND) 36 while in adults, males weighed more than females throughout the experiment. Values = mean ± SEM. Adolescent male deprived n = 18, adolescent male control n = 7, adolescent female deprived n = 22, adolescent female control n = 7, adult male deprived n = 20, adult male control n = 7, adult female deprived n = 22, adult female control n = 7.