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Age and Sex Differences in the Acquisition and Maintenance of Intravenous Amphetamine Self-Administration in Rats

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AGE AND SEX DIFFERENCES IN THE ACQUISITION AND MAINTENANCE OF INTRAVENOUS AMPHETAMINE SELF-ADMINISTRATION IN RATS

by

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Under the Direction of Kyle J. Frantz

ABSTRACT

Drug abuse peaks during adolescence, and exposure to drugs during adolescence predicts drug abuse in adulthood. Nevertheless, adolescence is not widely studied in animal models of drug intake. Moreover, few studies have investigated sex differences in drug-reinforced behavior during adolescence.

We studied age- and sex-differences in acquisition and maintenance of amphetamine self-administration in Sprague-Dawley rats. Adolescent males took more amphetamine than adult males, supporting the hypothesis that adolescents are more sensitive to amphetamine. A high rate of "inappropriate" active lever presses among periadolescent males suggests impulsive behavior.

In the maintenance phase of testing, young adult males failed to work as hard as adult males. In contrast, young adult females worked harder than adult females. Comparing sex groups, young adult females worked harder than age-matched males to obtain amphetamine. These results will ultimately help to form effective treatment and prevention programs for drug dependent individuals of all ages and both sexes.

INDEX WORDS: amphetamine, sex, age, adolescent, rat

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LIST OF ABBREVIATIONS

FR= fixed ratio schedule of reinforcement

FR1 = fixed ratio 1 schedule of reinforcement

i.v. = intravenous

PND = postnatal day

PR schedule = progressive ratio schedule of reinforcement

e.g. = for example

i.e. = that is

S.E.M = standard error of the mean

inf = infusion

GENERAL INTRODUCTION

Animal models of adolescence

One main goal of this thesis is to investigate differences in vulnerability to psychostimulant drugs in adolescent vs. adult animals. Therefore, this introduction first explores the adolescent phase of development and a rodent model of adolescence.

Adolescence is a developmental stage between youth and adulthood. This stage is a period of biological, psychological, and social transitions (Steinberg, 1999). Developing organisms of many species, especially mammals, undergo an ontogenetic transition from the dependence of youth to the independence of adulthood (Spear, 2000). Thus, adolescence is associated with physiological maturation such as adrenarche and gonadarche, as well as ethological factors such as leaving the early postnatal home environment and changing social companions from immediate family members to other age-mates (Campbell et al., 2000).

Adolescence in humans is considered to range from approximately 12 to 18 years of age (Steinberg, 1999; Spear, 2000). Human adolescents show particular behavioral characteristics such as increases in peer-directed social interactions (Steinberg, 1999; Spear, 2000) and elevations in novelty-seeking and risk-taking behaviors (Zuckerman, 1991; Adriani et al., 1998). Social interactions and affiliation with peers are very important during human adolescence (such as talking with peers or establishing new relationships). Human adolescents also show a disproportionate amount of reckless behavior (such as disobeying parents, school misconduct, or antisocial behaviors such as

theft or fighting). Related to these reckless behaviors, adolescence is associated with high drug use (Administration, 2003; Johnston, 2004), and unfortunately, drug exposure uring adolescence predicts drug dependence during adulthood (Administration, 2003; Spear, 2000).

Adolescence in non-human primates is loosely defined as the time between puberty and mature reproductive function (Pereira, 1993b). In non-human primate studies, the term "juvenile" (used more than "adolescence") refers to the age span from weaning until puberty (Pereira, 1993a), until sexual maturity (Crockett, 1993) or until growth slows (Janson, 1993).

Nevertheless, age-specific behavioral characteristics of adolescence in non-human primates include decreased "rough and tumble" play behavior (Fagen, 1993), increased levels of social interactions and affiliative behavior (huddling, grooming, and pair-sitting; de Waal, 1993), increased association with same-sex adults (Pereira, 1993a), engagement in aggressive behavior (Pereira, 1993), and novelty-seeking and risk-taking (Crockett, 1993; de Waal, 1993; Janson, 1993).

Therefore, non-human primate adolescence shares behavioral characteristics with human adolescence, making non-human primates good candidates for adolescence-associated drugs of abuse research. However, using non-human primates as an animal model of drug-related phenomena is not always cost effective, is subject to intense ethical scrutiny, and thereby is not necessarily the best model for adolescence experimentation.

Adolescence and high drug use can be explored effectively using rodent models. Periadolescence in rodents is about two weeks long, between approximately 35 and 50

days of age (postnatal days 35-50, PND 35-50; Spear and Brake, 1983; Spear, 2000). Others have reported that periadolescence is earlier in development by a week or so, such as PND 28-42 (Collins and Izenwasser, 2004; Collins et al., 2004).

Similar to primate adolescents, adolescent rodents spend more time in social interactions compared to adults and show high levels of play behavior such as play fighting, rough and tumble play, and wrestling (Panksepp, 1981; Brown, 1990). Periadolescent rodents also show general hyperactivity and hyperexploration of a novel environment (Spear and Brake, 1983; Bronstein, 1979; Caza and Spear, 1980). Periadolescent mice spend a significantly higher percentage of time in a novel compartment than adults, and a reduced habituation to a novel environment (Laviola et al., 1999), suggesting higher levels of risk-taking, sensation-seeking, or novelty-seeking (Adriani et al., 1998). Also, with regard to stress hormones, periadolescent male mice exhibit higher basal corticosterone levels than adults (Laviola et al., 2002; Adriani and Laviola, 2000), as well as different gonadal hormone levels (see below).

Differences in adolescent vs. adult behavior in multiple species may be explained by hormonal changes in the neuroendocrine system. Two particular types of hormonal changes are robust in adolescence: 1) adrenarche, increased output of adrenal hormones that occurs prior to other signs of impending adolescence; and 2) gonadarche, increased gonadal hormones associated with sexual maturation (Steinberg, 1999; Spear, 2000). Additional changes in brain neurocircuitry are likely to contribute to age-related behavioral changes.

The earliest sign of puberty in humans and chimpanzees is an increase in secretion of androgens from the adrenal gland which is regulated by the hypothalamus, the pituitary gland, and the adrenal gland (the HPA axis; Cutler1978; Parker 1991). In humans, increased adrenal androgens during adrenarche are associated with development of secondary sex characteristics and have occasionally been linked to behavior problems (Cutler, 1991; Steinberg, 1999). Adrenal androgens affect brain functions and are considered neuroactive steroids (neuroactive steroids are steroids that rapidly alter neural excitability through interaction with GABA receptors; Paul, 1992; Spear, 2000). Some of these neuroactive steroids increase overall brain excitability such as dehydroepiandrosterone (Paul, 1992; Spear, 2000). Therefore, developmental increases in neurosteroids may have significant effects on behavior during adolescence.

The onset of puberty is regulated by a feedback loop in the endocrine system, involving the hypothalamus, the pituitary gland, and the gonads (the HPG axis; Steinberg, 1999). Gonadarche involves pulsatile release of gonadotropin-releasing hormone from the hypothalamus which promotes increased release of both follicular-stimulating hormone and luteinizing hormone, which in turn stimulate release of gonadal hormones (e.g., testosterone in males and estrogen in females; Brooks-Gunn J, 1990). Increased gonadal hormones also stimulate many secondary sexual characteristics in human adolescence, while increases in both growth hormone and the sex steroids stimulate a growth spurt (Steinberg, 1999).

Finally, differences in adolescent vs. adult behavior may be explained by neuroanatomical and neurochemical reorganization of the brain. As reviewed by Spear

(2000), transition of the brain during adolescence seems highly conserved across species and may involve age-specific changes in neurotransmitters in the prefrontal cortex and other mesolimbic regions such as the nucleus accumbens, among other changes. Dopamine in mesocorticolimbic circuitry is integral to several age-specific behaviors of adolescence and has been implicated in the motor activating and reinforcing effects of psychomotor stimulants, such as amphetamine and cocaine (Koob, 1992). Therefore, understanding maturation in this system is important to understanding adolescent drug-related behavior in particular.

In humans (Seeman et al., 1987), nonhuman primates (Rosenberg and Lewis, 1995) and rodents (Rosenberg and Lewis, 1995; Andersen and Teicher, 2000), the number of dopamine D1 and D2 receptors in mesolimbic brain regions increases in early adolescence, and then decreases in early adulthood. Levels of basal or receptor-stimulated second messenger activities vary (Andersen, 2002), and voltammetric measures of dopamine release and uptake remain lower than in adults (Stamford, 1989). Dopamine input to the prefrontal cortex increases during adolescence in nonhuman primates and rodents. Dopamine fiber density and dopamine concentration in the prefrontal cortex increases during adolescence, but later is compensated by reduction in dopamine synthesis and / or turnover. In other words, dopamine concentration and fiber density in the prefrontal cortex are high in adolescence compared to adulthood and may cause differential responsivity to dopaminergic compounds. In all, neuroendocrine changes coupled with neuroanatomical and neurochemical maturation likely contribute to adolescent-specific behavioral profiles in multiple species.

Age differences in drug-related behavior

Almost all young people will use prescribed, over-the-counter, and /or illicit drugs during their teenage years (Hein, 1987; Administration, 2003; Johnston, 2004). Approximately 22% of 8th graders, 40% of 10th graders, and 51% of 12th graders have used *illicit* drugs (Johnston, 2004). Specific trends in annual prevalence of *amphetamine* use are 4.9% for 8th graders, 8.5% for 10th graders, and 10% for 12th graders (Johnston, 2004). In all, about 5 million people use amphetamine nationwide. Drug use during adolescence may lead to high rates of drug dependence in adulthood (Spear, 2000; Administration, 2003). This may be explained by reports like the one indicating that cocaine use among adolescents involves a rapid increase to high levels of drug intake (Estroff, 1989)**,** perhaps related to less intense acute euphoric and stimulatory effects of the drug in adolescents compared with adults (Koob, 1994)**.**

Age-differences exist in the motor effects of acute and repeated psychostimulant drug administration to rodents, as well as the reinforcing effects of psychostimulants. Locomotor effects of psychostimulants are important for our analysis because they share the same mesocorticolimbic dopamine circuitry with reinforcing effects.

Low-dose amphetamine administration results in less locomotor hyperactivity in periadolescent male rodents than adults (Spear and Brake, 1983). Moreover, although periadolescent rats show significant hyperactivity after a high dose of amphetamine, they exhibit lower levels of stereotyped behaviors such as licking and gnawing compared to adults (Adriani and Laviola, 2000; Adriani et al., 1998).

Another psychostimulant drug similar to amphetamine is cocaine, which has been studied broadly. [Approximately 2 million American adolescent and adults report current use of cocaine (Administration, 2003)]. Acute cocaine administered induces a less robust response profile in periadolescent relative to adult rodents (Laviola et al., 1995; Collins and Izenwasser, 2004; Collins et al., 2004). Periadolescent rats of both sexes show sensitization to the locomotor activating effects of cocaine, but consistent sensitization of stereotyped head scanning and focused sniffing is seen in adults but not periadolescent rats (Laviola et al., 1995). Repeated administration of cocaine also decreases body weight and food consumption in male adults, but not periadolescent rats of either sex (Laviola et al., 1995). In all, most studies show a lower sensitivity of periadolescent rodents to the acute motor effects of cocaine and a lesser degree of sensitization of motor activity after repeated cocaine injection (e.g. Collins and Izenwasser, 2004; Collins et al., 2004; Laviola et al., 1995; Snyder et al., 1998).

Nicotine is another common psychostimulant drug. [Almost 72 million American adolescents and adults report current use of a tobacco product (Administration, 2003)]. Nicotine also affects periadolescent and adults differently. For example, nicotine suppresses locomotor activity to a greater extent in periadolescent compared to adult rats (Rezvani and Levin, 2004). It is possible that adolescents are more sensitive to nicotine, but less sensitive to amphetamine and cocaine, compared with adults.

Age differences in drug-taking behavior have not been explored extensively. Regarding amphetamines, there are no studies on age differences in drug-taking behavior. With regard to cocaine, no robust difference between age groups are observed in the rate

of acquisition of i.v. cocaine self-administration (Frantz, 2000; Belluzzi et al., 2005). However, with regard to nicotine, periadolescent male rats take more nicotine and acetaldehyde (a major component of tobacco smoke) mixtures compared to adults (Belluzzi et al., 2005). Also, if rats begin nicotine self-administration during adolescence, then they self-administer more nicotine than rats that began during adulthood (Levin et al., 2003). This pattern of self-administration causes a substantially higher total nicotine intake even when the adolescent-onset rats reach adulthood. Therefore, adolescent vulnerability to drugs may be both behavior- and drug-specific.

Differences in vulnerability to psychostimulant drugs in adolescents vs. adults in both humans and rodents could be related to several factors such as: 1) drug pharmacokinetics including rates of distribution, metabolism, and excretion 2) hormonal changes associated with puberty, and 3) neuroanatomical and neurochemical reorganization of drug-related neural circuitry during adolescence.

Pharmacokinetic mechanisms are not likely to mediate periadolescent-specific behavioral effects of psychostimulants. For example, a post-mortem analysis shows that systemic injections of amphetamine produce a monotonic rise of amphetamine brain concentrations across ontogeny that do not correlate with the developmental course of behavioral responding (Spear and Brake, 1983). These results suggest that decreased amphetamine responsiveness during adolescence is not related to brain levels of amphetamine. Furthermore, after an intraperitoneal (i.p.) infusion of cocaine, no difference between periadolescent and adult brain levels of cocaine are observed, despite lower motor activation by cocaine in periadolescents (Frantz, 2000).

A second possible explanation for differences in vulnerability to psychostimulant drugs between adolescents and adults may be hormonal changes. Testosterone, estrogen, and corticosterone all increase around adolescence. Acute testosterone attenuates amphetamine-induced activity (Forgie and Stewart, 1993). Conversely, high level estrogen is associated with increased response to amphetamine (Becker et al., 1982). Hormones may affect the mesocorticolimbic dopamine regions involved in drug processes. For example, testosterone or estradiol (its aromatized metabolite) influences the mesolimbic dopamine system (Mitchell and Stewart, 1989), although the specific relationship between testosterone and dopamine is equivocal (Mitchell and Stewart, 1989; Becker, 1999) With regard to estrogen, it may increase dopamine release as well as associated GABAergic neuron excitability (Becker, 1999).

Moreover, corticosterone levels determine individual vulnerability to amphetamine self-administration (Piazza et al., 1991). High circulating levels of corticosterone may sensitisize an animal's response to amphetamine by an action on the dopamine system; dopamine transmission is necessary for psychostimulant self-administration, dopamine cell bodies posses corticosterone receptors, and corticosterone stimulate dopamine neurons.

A third possible explanation for differences in vulnerability to psychostimulant drugs between adolescents and adults is that the brain is being reorganized neuroanatomically and neurochemically during adolescence. As discussed earlier, mesocorticolimbic dopamine circuitry is implicated in the motor activating and reinforcing effects of psychostimulants (Koob, 1992).

This system and related inputs continue to mature throughout periadolescence. Such reorganization may underlie developmental changes in responsiveness to psychostimulants drugs.

Psychomotor stimulant drugs: focus on amphetamine

In the present study, age and sex differences in vulnerability to the psychostimulant drug, amphetamine, are investigated. Psychostimulant drugs such as the amphetamines and cocaine significantly influence mental functioning and behavior (Julien, 1998). All psychostimulants increase dopamine and norepinephrine (to a lesser extent) in the nucleus accumbens, which is a structure associated with drug-related behavior. Psychostimulants have limited therapeutic use, and all have significant side effects, toxicities, and patterns of abuse. Interest in amphetamines involves two different areas: (1) therapeutic use in the treatment of narcolepsy (irresistible sleepiness), attention deficit disorder, and obesity, and (2) compulsive misuse and dependency.

The psychostimulant drug used for this study is d-amphetamine. At low doses (2.5-20 mg/kg body weight), amphetamine causes increased alertness, euphoria, excitement, wakefulness, reduced fatigue, loss of appetite, mood elevation, increased motor and speech activity, and a feeling of power. At moderate doses (20 to 50 mg/kg body weight), added effects of amphetamine include slight tremors, restlessness, increased motor activity, insomnia, and agitation. At high doses of amphetamine (and when it is used chronically), side effects include stereotyped behaviors (purposeless, repetitive acts), as well as unexpected outbursts of aggression and violence, paranoid

delusions, and severe anorexia. These effects may be due to an indirect action involving the presynaptic release of dopamine and norepinephrine and to a lesser degree, direct stimulation of postsynaptic catecholamine receptors. In addition, amphetamines affect the autonomic nervous system to cause vasoconstriction, hypertension, tachycardia, and other "alerting responses".

In terms of pharmacological actions, amphetamine inhibits reuptake of dopamine and causes release of dopamine from presynaptic terminals (Jones et al., 1998). Amphetamine can act directly at the dopamine transporter and on vesicular storage of dopamine. The time course of amphetamine-induced reverse transport of dopamine (transporter-mediated release of dopamine) is much faster than that of vesicle depletion, so dopamine release in response to amphetamine occurs mainly by reverse transport (Sulzer et al., 1995).

Overall, d-amphetamine is commonly abused, particularly among adolescents, but it has not been tested in a rat model of adolescent drug vulnerability. Therefore, we are investigating the possibility that periadolescent rats are more vulnerable to amphetamine self-administration than adults by using the i.v. amphetamine self-administration paradigm in periadolescent and adult rats.

Sex differences in drug-related behavior

In spite of general reports that male and female animals vary in behavioral responses to drugs, few studies have investigated sex differences in drug reinforced behavior, particularly during adolescent development. Such basic research is critical to understanding trends in human male and female drug use. Among persons aged 12 or older, males (13%) are twice as likely as females (6%) to be classified with substance dependence or abuse (Administration, 2003). Men are also more likely to report current illicit drug use than women (10% vs. 6%). However, specific rates of non-medical psychotherapeutic drug use are the same for males and females (3%; Administration, 2003). Among youths, the rate of substance dependence or abuse among females (9%) is not different from the rate among males (Administration, 2003), suggesting that fewer boys but more girls abuse drugs than their adult counterparts. Girls are more likely than boys to smoke as well (14% vs. 12%; Administration, 2003). Therefore, it is clear that female drug intake is an important issue of study, especially among younger females.

Cocaine use has been studied extensively. In the last decade, cocaine abuse by women has increased quickly, and sex differences exist in the patterns of cocaine use and addiction (Griffin et al., 1989; Hu et al., 2004). Women start using cocaine at a younger age than men, become addicted faster, and enter treatment at a younger age (Griffin et al., 1989). In addition, cocaine cues induce more drug craving in female than male addicts (Robbins et al., 1999). These trends make it particularly important to study female psychostimulant intake, including amphetamines.

Female rodents are generally more responsive to psychostimulant drugs than males. For example, adult females produce more amphetamine-induced net rotations than males (Becker et al., 1982). Similarly, adult females show more cocaine-induced horizontal activity than males and exhibit greater sensitization of behaviors in response to repeated administration of cocaine (Laviola et al., 1995; Becker et al., 2001).

In addition, there are sex differences in reinforcing effects of psychostimulant drugs. With regard to the acquisition of methamphetamine self-administration (Roth and Carroll, 2004), female rats acquire faster than males. Similarly, female rats acquire cocaine self-administration more rapidly and at a higher percentage than males, and then self-administer more cocaine than males (Lynch and Carroll, 1999; Hu et al., 2004). Similarly (Lynch and Carroll, 1999), heroin self-administration is sexually dimorphic. In terms of "motivation" to self-administer psychostimulants, female rats work harder than males to obtain methamphetamine or cocaine on a PR schedule of reinforcement (Roberts et al., 1989; Roth and Carroll, 2004).

However, some studies fail to show sex differences in reinforcing effects of drugs. For example, acquisition of cocaine or heroin self-administration on a FR schedule is not sexually dimorphic (Roberts et al., 1989; Stewart, 1996). These findings indicate that schedules of reinforcement and specific paradigms are important factors in studying sex differences in the reinforcing effects of psychostimulant drugs.

Fewer studies investigate adolescent sex differences in vulnerability to psychostimulant drugs. Periadolescent rats of both sexes show sensitization to the locomotor activating effects of cocaine (Laviola et al., 1995)**.** However, periadolescent

females sensitize to the locomotor-stimulant effects of nicotine over a 7-day treatment period while male periadolescent rats do not (Collins and Izenwasser, 2004). There are also adolescent sex differences in cross-sensitization between nicotine and amphetamine or cocaine (Collins and Izenwasser, 2004; Collins et al., 2004). However, these results show increased sensitivity among males not females. These data suggest that male adolescent smokers may be particularly vulnerable to the risk of stimulant abuse (Collins et al., 2004). Overall, these data underscore the need to understand the drugs and conditions that differently affect male vs. female adolescent subjects.

Sex differences in vulnerability to psychostimulant drugs may be explained by several factors including: 1) different pharmacokinetics of psychostimulant drugs; 2) interaction between drugs and gonadal hormones; and 3) direct effects of sex chromosomes on brain structure and function. These factors are not mutually exclusive.

Several studies indicate sex differences in pharmacokinetics of psychostimulant drugs (Becker et al., 1982; Festa et al., 2004). For example, when whole brain or striatal levels of amphetamine produced by systemic administration of the drug are measured, a significantly higher brain level of amphetamine in females than males is observed (Becker et al., 1982). In general, females often metabolize drugs slower than males, and therefore drug effects are greater and/or longer (Festa et al., 2004).

A second possible explanation for sex differences in vulnerability to psychostimulant drugs is gonadal hormones. For example**,** intact female rats in the estrus phase of the estrous cycle show more net motor rotations than male rats, even when the amphetamine dose is adjusted to produce similar brain levels of amphetamine in males

and females (Becker et al., 1982). Female rats in proestrus and estrus phases also show higher horizontal activity following cocaine injections compared to females in diestrus II (Sell et al., 2000). Ovariectomized rats treated with estrogen or with estrogen and progesterone show more horizontal activity following cocaine injection than ovariectomized or ovariectomized rats treated only with progesterone (Sell et al., 2000). With respect to repeated cocaine, ovariectomized female rats treated with estrogen show greater sensitization of rotational behavior (in both magnitude and rate of sensitization) compared to ovariectomized females, castrated males, and intact males (Hu and Becker, 2003). Therefore, gonadal hormones influence responsivity to motor effects of psychostimulants, at least in females.

Gonadal hormones also affect the reinforcing effects of psychostimulants. For example, on a PR schedule of reinforcement, female rats in the estrus phase reach higher break points than females in other stages (Roberts et al., 1989). Moreover, treatment of ovariectomized females with estradiol facilitates the acquisition of cocaine self-administration behavior but has no effect on cocaine self-administration behavior in male rats (Jackson et al., 2005). Thus, the effects of some gonadal hormones on the acquisition of cocaine self-administration are sexually dimorphic.

However, some studies contradict these results regarding effects of ovarian hormones on drug-taking behavior. No differences across estrous cycle are observed in cocaine self-administration on a FR schedule of reinforcement (Roberts et al., 1989). Also, no differences between ovariectomized and intact females are observed in rate of acquisition of heroin self-administration (Stewart, 1996). Nevertheless, most studies

suggest that interactions between gonadal hormones and drugs explain at least some of the difference between female and male responsivity to drugs. Becker et al. (Becker et al., 1982) suggests these effects are mediated by dopamine in the striatum.

Some evidence indicates that pharmacokinetics and gonadal hormones cannot explain all the sex differences in drug sensivity. For example, ovariectomized and castrated rats show different rates of acquisition of cocaine self-administration (Hu et al., 2004), suggesting that even without circulating gonadal hormones, sex differences remain. Thus, an additional possible explanation for sex differences is the direct effect of genes on cell morphology and function (Carruth et al., 2002). An interesting line of research with mice indicates that the genetic sex of the brain can be made independent of gonadal phenotype. In this case, cells in the neonatal striatum maintain a different phenotype consistent with genetic sex not gonadal sex, suggesting that sex chromosomes contribute directly to sex differentiation. Given that the striatum influences responsivity to psychostimulant drugs, direct gene effects may join differential pharmacokinetics and gonadal hormones to determine overall sex differences in drug responsivity.

Operant paradigms and schedules

In the present study, an operant conditioning paradigm in which lever-pressing behavior is maintained by i.v. drug delivery is used to create an animal model of human drug intake. As reviewed by Mazure (Mazur, 1998), operant conditioning (also known as instrumental conditioning) is a procedure in which a specific behavior is enhanced through the process of reinforcement. The delivery of a reinforcer is contingent on the

subject's behavior; no reinforcer is delivered until the subject produces the specific response targeted for conditioning. The assumption is that a behavior will be repeated if its consequences are pleasurable, rewarding, or at least reinforcing to the subject. Operant conditioning has two parts: the behavior (something the learner does), and the consequence (something that happens as a result of that behavior). In the i.v. drug self-administration paradigm, lever-pressing is the behavior and drug infusion is the consequence. If lever-pressing increases when followed by drug infusion, then drug infusion is interpreted as reinforcing. The amount of lever-pressing behavior is thought to be a measure of the reinforcement value of the reinforcer. For example, if subjects acquire lever-pressing behavior quickly, then the reinforcement value is high. Different schedules of reinforcement determine how much lever-pressing behavior is required to receive a reinforcer, and under what timetable. Fixed ratio and progressive ratio are two common reinforcement schedules.

A reinforcement schedule is a rule that states under what conditions a reinforcer will be delivered. When every occurrence of the operant response is followed by a reinforcer, this schedule is called continuous reinforcement (or Fixed Ratio 1 schedule; FR1). The rule for reinforcement in a FR schedule is that a reinforcer is delivered after every **n** response, where **n** is the size of the ratio. For example, in a FR 20 schedule, every 20 responses will be followed by a reinforcer.

In a variable-ratio schedule (VR), the number of required responses is different from reinforcer to reinforcer. A special example of a variable-ratio schedule is progressive ratio schedule of reinforcement (PR). The rule for reinforcement on a PR

schedule is that **n,** the size of the response ratio is escalated through a series such as 1, 2, 4, 6, 9, 12, 16, 20, 36, 48, etc. within a single operant conditioning session (Roberts et al., 1989). PR schedules can be used to estimate the reinforcing effectiveness of a self-administered drug by determining the "break point" at which the subject stops responding (Caine, 1993). Higher break points are associated with higher reinforcing effectiveness of the drug or drug dose. Other ways to analyze PR data are number of infusions and break points on active lever. Any of these measures on the PR schedule of reinforcement appears to reflect difference in motivation to obtain a drug (Roberts et al., 1989).

In many PR self-administration studies, a FR schedule is imposed first and the number of infusions for each animal is recorded. If the subjects meet certain stability criteria (for example, rats whose daily total infusions fall within ± 10% of the mean number of infusions in 3 consecutive days will be considered stable), then they will be run on PR schedule on the following day (Roberts et al., 1989).

Operant behavior that is maintained by i.v. drug delivery is the most direct and perhaps the most relevant animal model of human drug self-administration (Caine, 1993). In the present study, we employ both FR and PR schedules of reinforcement in an i.v. amphetamine self-administration paradigm. On the PR schedule, we also test two doses of amphetamine for comparison. Together, these schedules and doses provide a profile of the reinforcing effects of amphetamine in our subject populations: periadolescent and adult, male and female Sprague-Dawley rats.

RESEARCH QUESTIONS

We aim to test the hypothesis that the reinforcing effects of amphetamine will be higher during periadolescence than adulthood, and that this effect is exacerbated in females compared with males. We plan to test this hypothesis by using the i.v. amphetamine self-administration paradigm in periadolescent and adult, male and female rats. Four experimental questions form the basis of our studies:

1. Do rates of acquisition of amphetamine self-administration differ between periadolescent and adult rats?

2. Do rates of acquisition of amphetamine self-administration differ between male and female rats?

3. Does amount of amphetamine intake differ between age and sex- groups, among those rats that acquired self-administration?

4. Are rats that acquired amphetamine self-administration as periadolescents more motivated than adults to take amphetamine, as measured by break points in the PR schedule of reinforcement? Is this motivation sexually dimorphic?

MATERIALS AND METHODS

Subjects

Male and female Sprague-Dawley rats (Charles River Laboratories, Wilmington MA) arrived at the laboratory at either PND 22 (periadolescent) or 77 (adult). They were housed in groups of 2-3 in a humidity and temperature controlled (20-22°C) vivarium, on a 12/12 hr light/dark cycle. Animals had 1 week to acclimate to these conditions, and they had ad libitum access to food and water throughout experimentation. The total number of rats used in these experiments was 76. All procedures in this study were conducted in strict adherence to the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Drugs

d-Amphetamine Sulfate salt was purchased from Sigma Chemicals (St. Louis, MO). Methohexital sodium (1%, Brevital Sodium) was purchased from King Pharmaceuticals, Inc. (Bristol, TN).

Equipment

The i.v. catheters for the self-administration experiment were made as previously described (Caine, 1993) with minor modifications. The self-administration chambers consisted of operant boxes enclosed in sound-attenuating, ventilated environmental cubicles (Med Associates, Inc., St. Albans, VT). Two levers extended into the chamber at the start of each session. Pressing on the active lever activated a syringe pump with a 5

rpm motor (Med Associates, Inc., St. Albans, VT) for 2 sec to deliver 0.1 ml of drug solution via a stainless steel swivel and a polyethylene tube attached to the catheter portal on the animal's back. Each reinforced response lit a cue light above the lever which stayed on throughout the duration of the infusion. The cue light, house light, and white noise were not present during a 20 sec time-out (TO), and lever presses on the other lever were recorded but not reinforced. Drug delivery and data collection were controlled by a Med Associates, Inc. software system.

Surgical procedures

Catheters were implanted in general accordance with the procedure of Caine et al. (Caine, 1993). Rats were anesthetized with an isoflurane/oxygen vapor mixture (4-5% for initial anesthetization and 1.5-3% during surgery) and catheter tubing was passed subcutaneously from the animal's back to the right jugular vein, inserted into the vein previously punctured with a 25 gauge needle, and tied gently with suture thread. During recovery, rats received 0.2 ml Timentin (Ticarcillin Disodium and Clavulanate Potassium; 100mg/ml, i.v.) twice daily on the first two days after surgery, then once daily throughout the experiment. Catheters were flushed daily with heparinized saline (100 USP units/1 ml) throughout the experiment.

Acquisition of intravenous amphetamine self-administration

Following a 4-7-day post-surgical recovery, testing of spontaneous acquisition of amphetamine self-administration began (PND 35-38 or 90-92). Sessions were 2 hr in duration and performed daily for 14 days during the light phase of the light/dark cycle. Sessions began when 2 levers extended into the operant chamber. Non-contingent drug injections were not administered. Lever-pressing was reinforced by i.v. injection of 0.05 mg/kg/0.1ml infusion amphetamine under a FR1, time-out 20 (TO20) schedule. The concentration of the self-administered amphetamine solution for both periadolescent and adult rats was titrated daily to adjust for weight change. Responding on the inactive lever was also recorded but had no scheduled consequences. Rats were defined as having acquired self-administration when their lever-pressing behavior met the following criteria for at least 3 successive sessions and throughout the remainder of the acquisition period: a) the number of responses on the active lever exceeded 2 times the number of presses on the inactive lever to demonstrate lever-discrimination and b) the number of responses on the active lever was greater than 12. (Twelve is the average number of non-reinforced lever-presses made by amphetamine-naïve periadolescent and adult rats in the absence of amphetamine). A separate group of 30 rats acquired amphetamine self-administration at 0.025 mg/kg/0.1ml infusion, but only their subsequent maintenance phase data are discussed at present.

Patency of the i.v. catheters was tested one day before the first and immediately after the last test session by administering the ultra short-acting barbiturate anesthetic (Brevital Sodium, 1% methohexital sodium) through the catheter. If muscle tone was not

lost within 3 sec, then the catheter was assumed to be faulty and the subject was not included in analyses. This test was also conducted on an as-needed basis at the end of maintenance phase testing (see below) to confirm catheter patency.

Maintenance of intravenous amphetamine self-administration

After the acquisition phase, rats were tested under a FR1 schedule for three consecutive days, and those rats whose daily number of infusions fell within ±10% of the mean number of infusions over those three days were considered stable. On the following day, stable rats were tested under a PR schedule (in which the number of presses for a single infusion increases gradually within a single session under the following progression 1, 2, 4, 6, 9, 12, 16, 20, 36, 48, etc; Roberts et al., 1989). Sessions were 4 hr in duration during the light phase of the light-dark cycle. Sessions began when 2 levers extended into the operant chamber. Non-contingent injections were not administered. Lever-pressing was reinforced by i.v. injection of amphetamine under a PR, TO20 schedule of reinforcement in 2 different dose groups. Each subject was assigned randomly to receive either 0.05 mg/kg/0.1 ml infusion or 0.0125 mg/kg/0.1 ml infusion. The concentration of the self-administered amphetamine solution for both periadolescent and adult rats was titrated daily to adjust for weight change. Responding on the inactive lever was also recorded but had no scheduled consequences.

Statistical analyses

Fisher's Exact Test of data in a 2x2 Table was used to compare the proportion of subjects that acquired self-administration over 14 days acquisition testing (see sample table below). Both age- and sex-groups were compared. A Mann-Whitney U test was used to compare the number of days to acquisition of amphetamine self-administration between age- and sex-groups.

The number of drug infusions over daily sessions among those rats that acquired self-administration by day 14 was analyzed using two-way analysis of variance (ANOVA) with age and day or sex and day as factors. A Huynh-Feldt Epsilon adjustment on degrees of freedom and F values in ANOVA was conducted due to consistent violation of sphericity. Follow up one-way ANOVAs and post-hoc tests were conducted as appropriate. Total drug intake during week 1 and week 2 were compared between age and sex groups using separate t-tests. Lever discrimination was analyzed by two-way repeated measures ANOVA within age and sex groups, with days and levers as factors.

For the maintenance phase data, dose-response comparisons between 0.05 mg/kg/0.1ml infusion and 0.0125 mg/kg/0.1 ml infusion, as well as age and sex differences on the PR schedule of reinforcement were analyzed using t-tests. Both number of infusions and break points were analyzed.

RESULTS

Rate of acquisition

Significant sex- but not age-differences were observed in rate of acquisition. Individual Fisher's Exact Tests conducted on the difference in proportions of rats acquiring self-administration on each day of testing revealed no significant differences between age groups in the rate of acquisition of amphetamine self-administration (% rats acquired; Table 1). However, sex comparisons within the periadolescents age groups showed that significantly higher percentage of periadolescent females acquired amphetamine self-administration compared with periadolescent males (Table 2; $p = 0.05$ on day 4 to 5 and day 12 to 14). Conversely, sex comparison within the adult age groups failed to show significant differences in percent acquisition of amphetamine self-administration.

The Mann-Whitney U Test conducted on the number of days to acquisition revealed no significant differences between periadolescent (PND 35-51) vs. adult (90-105) *male* rats in the rate of acquisition of amphetamine (p=0.29; Figure 1 and Table 3). Similarly, no significant differences were seen between periadolescent (PND 35-51) vs. adult (90-105) *female* rats in the rate of acquisition of amphetamine (p=0.11; Figure 2 and Table 3). However, sex comparisons within the periadolescent age groups showed that periadolescent female rats acquired amphetamine self-administration significantly faster compared to periadolescent male rats (p=0.05; Figure 3 and Table 3). Although no significant differences were observed between adult male vs. female rats in the rate of acquisition of amphetamine (p=0.13; Figure 4 and Table 3), when adults and periadolescents were combined, again the significant difference between sexes was apparent, with females acquiring faster than males (p= 0.01, Table 3).

Number of infusions over daily sessions and overall amount of drug intake

Significant age differences were observed in males on drug intake, but no significant sex differences were observed. Periadolescent males took more infusions than adult males during the second week of acquisition testing. A two-way repeated measures ANOVA conducted on number of infusions over daily sessions among only those rats that acquired self-administration by day 14 revealed significant differences between periadolescent vs. adult male rats during the second week of acquisition (significant age effect over days 6-14; $F_{(1,13)}$=5.70, p= 0.03; Figure 5). However, the days effect and days x age interaction were not significant ($F_{(4, 52)}$ =0.76, p=0.56 and $F_{(4, 52)}$ =0.94, p=0.45,

respectively). An independent sample t-test was conducted on total drug intake during week 1 and week 2. Periadolescent males had a higher drug intake during the second week of acquisition compared to adult males ($t_{(13)}$ =2.57, p=0.02; Figure 6).

Among female rats that acquired amphetamine self-administration, no significant age-differences were observed in number of infusions (age effect; $F_{(13, 10)}$ =1.08, p=0.46; Figure 7) but the days effect was significant ($F_{(4, 13)}$ =13.79, p<0.01; Figure 7). Nor were significant age-differences observed in amount of drug intake ($t_{(22)}$ =0.40, p=0.70 for the first week and $t_{(22)}$ =1.46, p=0.16 for the second week; Figure 8).

Similarly, no significant sex-differences between periadolescent male vs. female rats were observed in number of infusions (sex effect; $F_{(18, 1)}$ =0.991, p=0.33; Figure 9), but the days effect was significant ($F_{(5, 96)}$ =14.81, p<0.001). The days x sex effect was significant (**F**$_{(5, 96)}$ = 2.49, p=0.03). No significant sex-differences were observed in amount of drug intake either ($t_{(18)}$ =0.95, p=0.35 for the first week and $t_{(18)}$ =0.97, p=0.34 for the second week; Figure 10). Finally, no significant sex-differences were observed in number of infusions among adults (sex effect; $F_{(13, 5)}$ =0.75, p=0.69; Figure 11) although the days effect was significant ($F_{(5, 85)}$ =5.73, p<0.001). Nor were significant sex-differences observed in amount of drug intake ($t_{(17)}$ =-0.35, p=0.73, for the first week and $t_{(17)}$ =1.30, p=0.21 for the second week; Figure 12).

Active vs. inactive lever presses during acquisition

Lever discrimination was robust in all age- and sex-groups. Two-way repeated measures ANOVAs were conducted on the active vs. inactive lever presses within age and sex groups, with days and levers as factors. Among periadolescent males that acquired amphetamine self-administration, a significant lever effect was observed ($F_{(1, 14)}$ =7.26, p=0.02, Figure 13). However, the effect of days (within levers; $F_{(2, 27)}$ =2.48, p=0.10) and days x levers interaction ($F_{(2, 27)}$ =2.7, p=0.086) were not significant.

Among adult males that acquired amphetamine self-administration, a significant lever effect was also observed ($F_{(1, 14)}$ =35.09, p<0.001; Figure 14). However, neither the days effect nor days x lever interaction was significant ($F_{(2, 19)}$ = 0.54, p=0.55 and $F_{(2, 19)}$ = 0.14, p=0.82, respectively).

Among periadolescent female rats that acquired amphetamine self-administration, a significant lever effect was observed ($F_{(1, 22)}$ =34.94, p<0.001; Figure 15). A significant days effect was observed ($F_{(5, 106)}$ =4.61, p=0.001). However, a significant days x lever interaction was observed ($F_{(5, 106)}$ =6.44, p<0.001). Post hoc analysis revealed significant differences from day 3 to 14 (p<0.003).

Among adult female rats that acquired amphetamine self-administration, a significant lever effect was observed ($F_{(1, 22)}$ =72.07, p<0.001; Figure 16). However, neither a days effect ($F_{(2, 51)}$ =1.33, p=0.28), nor a days x lever effect ($F_{(2, 51)}$ =2.25, p=0.11) was significant.

Maintenance

A separate group of rats that were trained on 0.025 mg/kg/infusion for acquisition phase was combined with rats that were trained on 0.05 mg/kg/infusion and used for PR schedule of reinforcement. No significant effect of training dose on PR schedule was observed (analyses for acquisition among these rats were not shown).

Male rats that acquired amphetamine self-administration as periadolescents (termed "young adults") did not "work as hard" as male rats that acquired self-administration as adults (still termed "adults"). At the low dose of amphetamine (0.0125 mg/kg/infusion), young adult (PND 48-63) males earned a significantly lower number of infusions on the PR schedule compared to adult males (PND 103-117; $t_{(12)}=2.28$, $p=0.04$; Figure 17), although no significant difference occurred at the high dose of amphetamine (0.05 mg/kg/infusion; $t_{(12)}=1.93$, $p=0.08$; Figure 17). With regard to break points on the active lever, at the low dose, no significant differences were observed between young adult vs. adult males ($t_{(12)}=2.05$, $p=0.06$; Figure 18), whereas, at the high dose, young adult males achieved considerably lower break points compared to adult males ($t_{(12)}=2.19$, $p=0.05$; Figure 18).

Contrary to male rats, female young adults worked significantly harder than adult females. At the low dose, young adult females had significantly greater infusions compared to adult female rats ($t_{(18)} = -2.78$, $p=0.01$; Figure 19). At the high dose, no significant differences were observed in number of infusions ($t_{(18)} = -1.56$, $p=0.13$; Figure 19). In the same way, at the low dose, young adult females worked to significantly higher break points compared to adult females ($t_{(18)} = -2.05$, $p=0.05$; Figure 20), although at the high dose, no significant differences were observed in break points between age-groups ($t_{(18)} = -0.92$, $p=0.37$; Figure 20).

With regard to direct sex-comparisons, young adult females but not adult females achieved a greater number of infusions and higher break points compared with age-matched males. At both doses of amphetamine, young adult females had a significantly greater number of infusions compared to young adult male rats ($t_{(14)} = -3.81$, $p<0.01$ for low dose and $t_{(14)} = -3.79$, $p<0.01$ for high dose; Figure 21). Similarly, at both doses of amphetamine, young adult female rats worked to significantly higher break points compared to young adult male rats ($t_{(14)} = -2.11$, $p=0.05$ for low dose and $t_{(14)} = -2.70$, $p=0.02$ for high dose; Figure 22).

Conversely, no significant sex differences were observed in number of infusions on the PR schedule between adult male vs. females ($t_{(16)} = 1.19$, $p=0.25$ for low dose and $t_{(16)} = 0.30$, $p=0.77$ for high dose; Figure 23). In the same way, no significant differences were observed in break points between adult male vs. female rats ($t_{(16)} = 0.99$, $p=0.34$ for low dose and $t_{(16)} = 0.11$, $p=0.91$ for high dose; Figure 24).

For most age and sex groups, the higher dose of amphetamine (0.05 mg/kg/infusion) produced a greater number of infusions and higher break points than the low dose (0.0125 mg/kg/infusion). For young adult males, the number of infusions were similar for both doses, although the break points on the high dose was greater than the low dose, ($t_{(10)} = 1.91$, $p = 0.08$ and $t_{(10)} = 2.42$, $p = 0.04$, respectively). For adult males, the number of infusions and the break points were greater on the high dose than the low dose ($t_{(12)} = 6.23$, $p < 0.001$ and $t_{(12)} = 3.74$, $p < 0.01$, respectively). Similarly, for young adult female rats, the number of infusions and the break points were greater on the high dose than the low dose ($t_{(18)} = 4.52$, $p < 0.001$ and $t_{(18)} = 3.34$, $p < 0.01$, respectively). For adult female rats as well, the number of infusions and the break points were greater on the high dose than the low dose ($t_{(18)} = 3.78$, $p < 0.01$, $t_{(18)} = 2.36$, $p = 0.03$, respectively), as well.

Table 1-**Results of Fisher's Exact Test for a 2 x 2 Contingency table on the differences in proportions of rats acquiring self-administration over 14 days acquisition testing (age differences)**

Day	Male Periadolescent vs. Adult	Female Periadolescent vs. Adult
1	1.00	1.00
2	1.00	1.00
3	0.97	0.96
4	0.98	0.98
5	0.95	0.95
6	0.96	0.95
7	0.92	0.95
8	0.84	0.95
9	0.73	1.00
10	0.73	1.00
11	0.73	1.00
12	0.73	1.00
13	0.73	1.00
14	0.73	1.00

Table 2**- Results of Fisher's Exact Test for a 2 x 2 Contingency table on the differences in proportions of rats acquiring self-administration over 14 days acquisition testing (sex differences)**

Day	Periadolescent Male vs. Female	Adult Male vs. Female
1	1.00	1.00
2	1.00	1.00
3	0.30	0.30
4	*0.05	0.10
5	*0.05	0.10
6	0.11	0.23
7	0.20	0.23
8	0.33	0.23
9	0.32	0.08
10	0.16	0.08
11	0.16	0.08
12	*0.05	0.08
13	*0.05	0.08
14	*0.05	0.08

Table 3- **Average numbers of days to acquisition**
(Mean ± SEM, n= 10-12 per group)
All rats are included.

	Male	Female	**p=0.01
Periadolescent	8.83±1.40	5.42±0.89	*p=0.05
Adult	7.20±1.72	3.92±0.48	p=0.13
	p=0.29	p=0.11	

Table 4**- PR schedule results: age differences**

		Male Young Adult vs. Adult	Female Young Adult vs. Adult
0.0125 mg/kg/inf	Infusions	$t_{(12)} = 2.28$ *p = 0.04	$t_{(18)} = -2.78$ **p = 0.01
	Break points	$t_{(12)} = 2.05$ P = 0.06	$t_{(18)} = -2.05$ *p = 0.05
0.05 mg/kg/inf	Infusions	$t_{(12)} = 1.93$ p = 0.08	$t_{(18)} = -1.56$ p=0.13
	Break points	$t_{(12)} = 2.19$ *p = 0.05	$t_{(18)} = -0.92$ p = 0.37

Table 5**- PR schedule results: sex differences**

		Periadolescent Male vs. Female	Adult Male vs. Female
0.0125 mg/kg/inf	Infusions	$t_{(14)} = -3.81$ **p < 0.01	$t_{(16)} = 1.19$ p = 0.25
	Break points	$t_{(14)} = -2.11$ *p = 0.05	$t_{(16)} = 0.99$ p = 0.34
0.05 mg/kg/inf	Infusions	$t_{(14)} = -3.79$ **p < 0.01	$t_{(16)} = 0.30$ P = 0.77
	Break points	$t_{(14)} = -2.70$ *p = 0.02	$t_{(16)} = 0.11$ P = 0.91

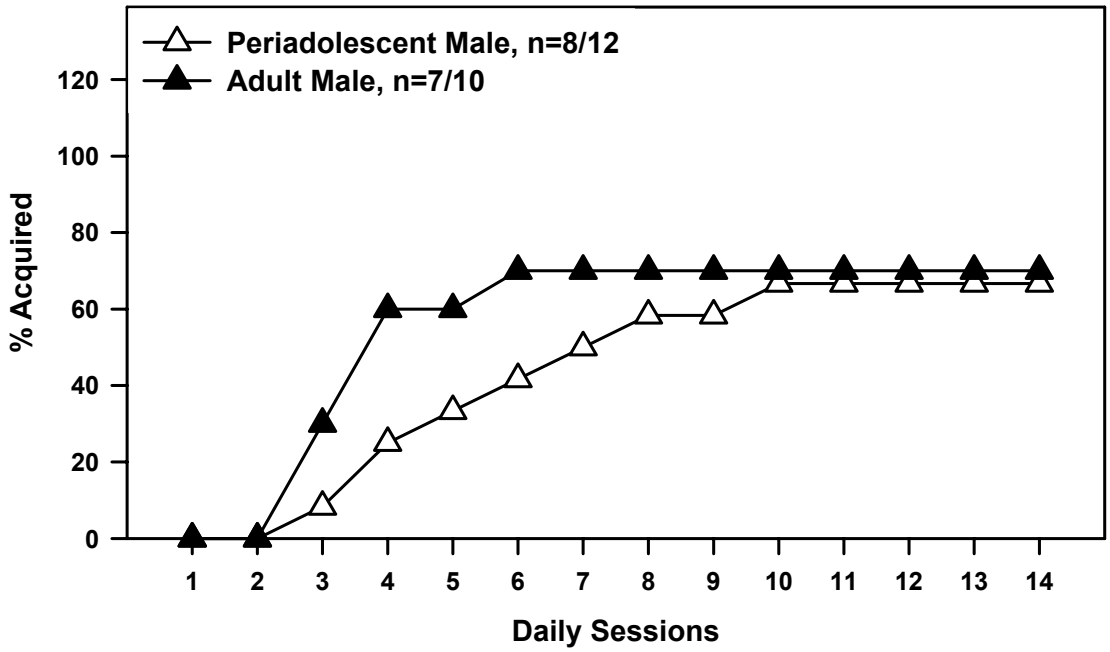

Figure 1 **Percent acquisition in periadolescent (PND 35-51) vs. adult (PND 90-105) males.** Percent rats acquiring stable self-administration of 0.05 mg/kg/infusion amphetamine across daily test sessions in periadolescent (open triangles) or adult (closed triangles) male rats. (See Methods for definition of acquisition.) Final proportion of rats exhibiting stable behavior on day 14 indicated in the legend.

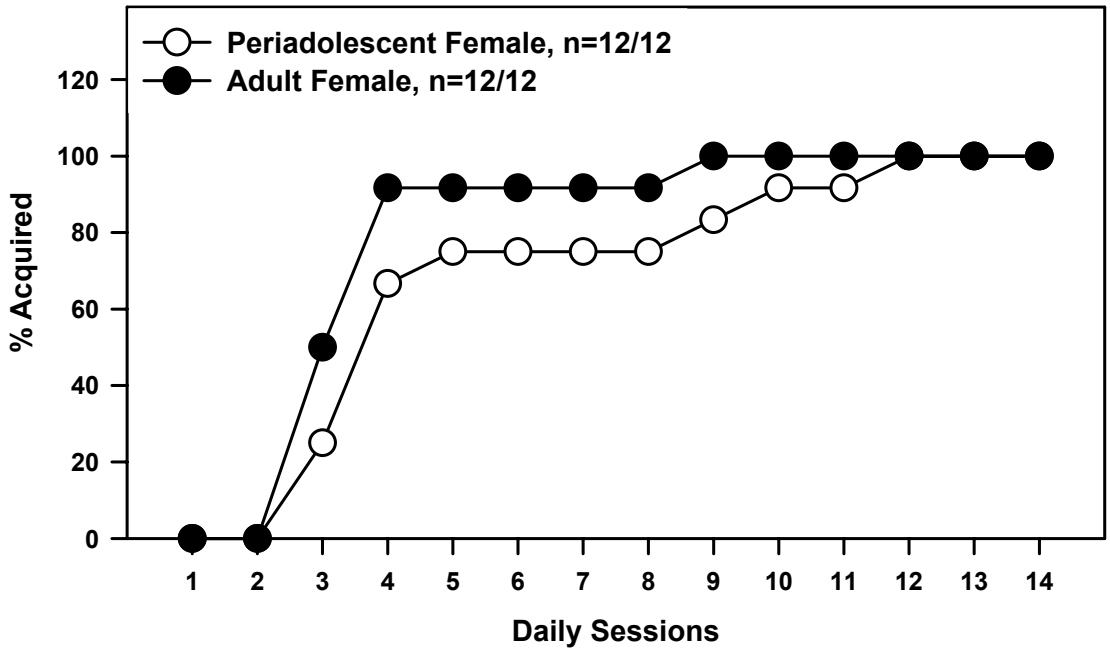

Figure 2 **Percent acquisition in periadolescent (PND 35-51) vs. adult (PND 90-105) females.** Percent of rats acquiring stable self-administration of 0.05 mg/kg/infusion amphetamine across daily test sessions in periadolescent (open circles) or adult (closed circles) female rats. (See Methods for definition of acquisition.) Final proportions of rats exhibiting stable behavior on day 14 are indicated in the legends.

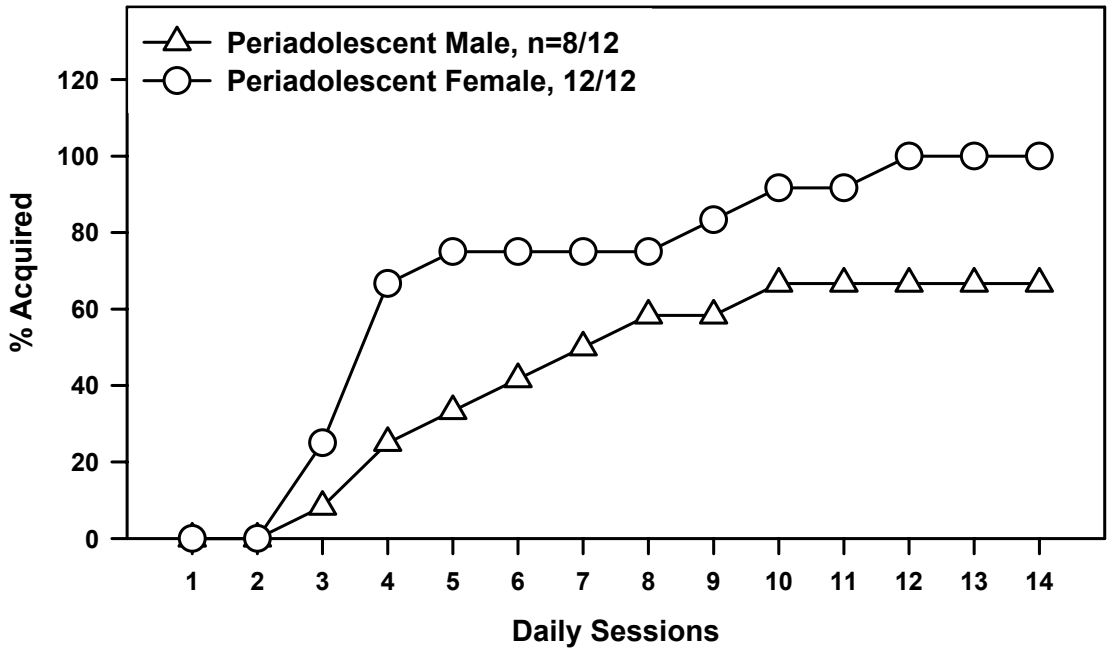

Figure 3 **Percent acquisition in periadolescent (PND 35-51) male vs. females.** Percent of rats acquiring stable self-administration of 0.05 mg/kg/infusion amphetamine across daily test sessions in periadolescent male (open triangles) or female (open circles) rats. (See Methods for definition of acquisition.) Final proportions of rats exhibiting stable behavior on day 14 are indicated in the legends. These data are the same as Figures 1+2, replotted for direct sex comparisons.

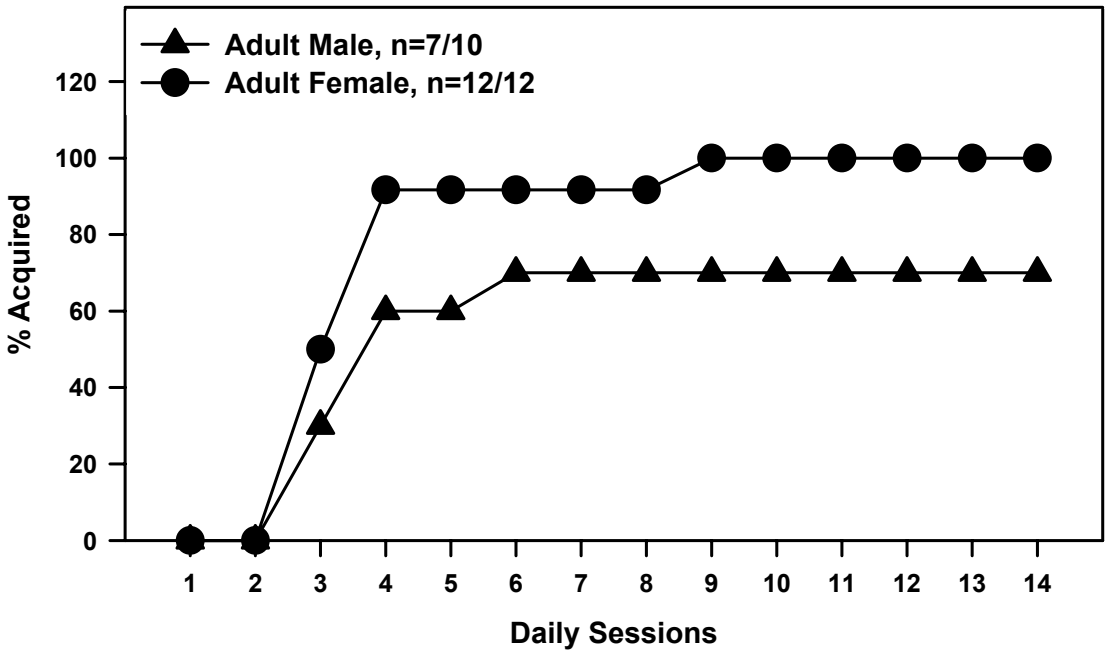

Figure 4 **Percent acquisition in adult (PND 90-105) male vs. females.** Percent of rats acquiring stable self-administration of 0.05 mg/kg/infusion amphetamine across daily test sessions in adult male (closed triangles) or female (closed circles) rats. (See Methods for definition of acquisition.) Final proportions of rats exhibiting stable behavior on day 14 are indicated in the legends. These data are the same as Figures 1 +2, replotted for direct sex comparisons.

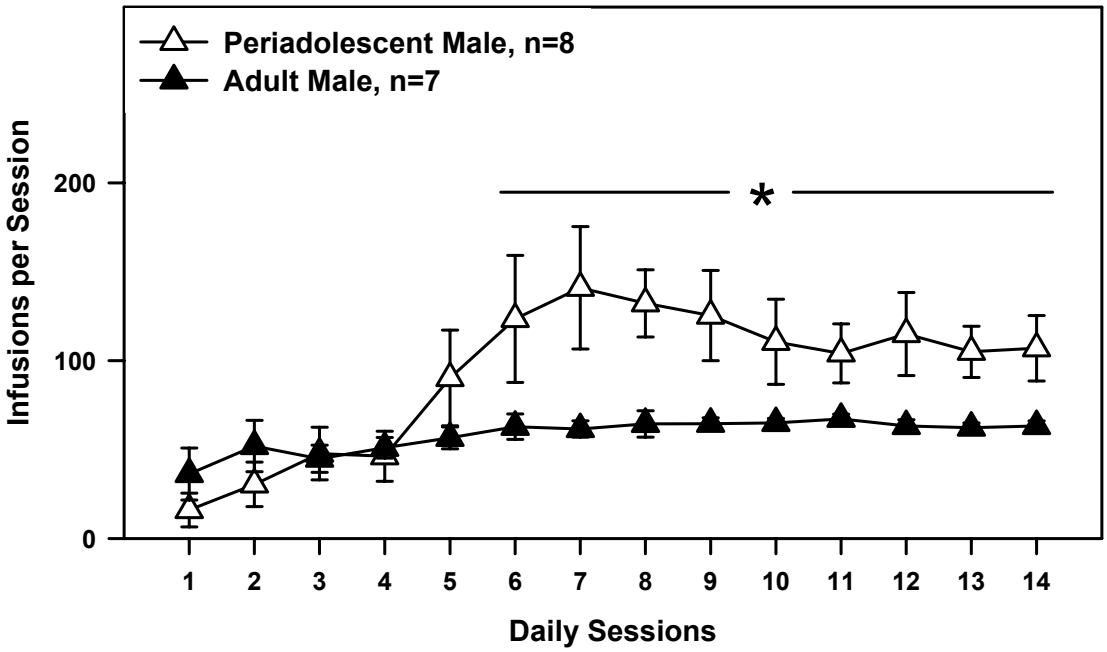

Figure 5 **Number of infusions over daily sessions by periadolescent (PND 35-51) vs. adult (PND 90-105) males.** Among male rats that acquired amphetamine self-administration, periadolescent males (open triangles) took more infusions than adult males (closed triangles) during the second week of acquisition (significant age effect over days 6-14, *p=0.03). Numbers per group indicated in legend.

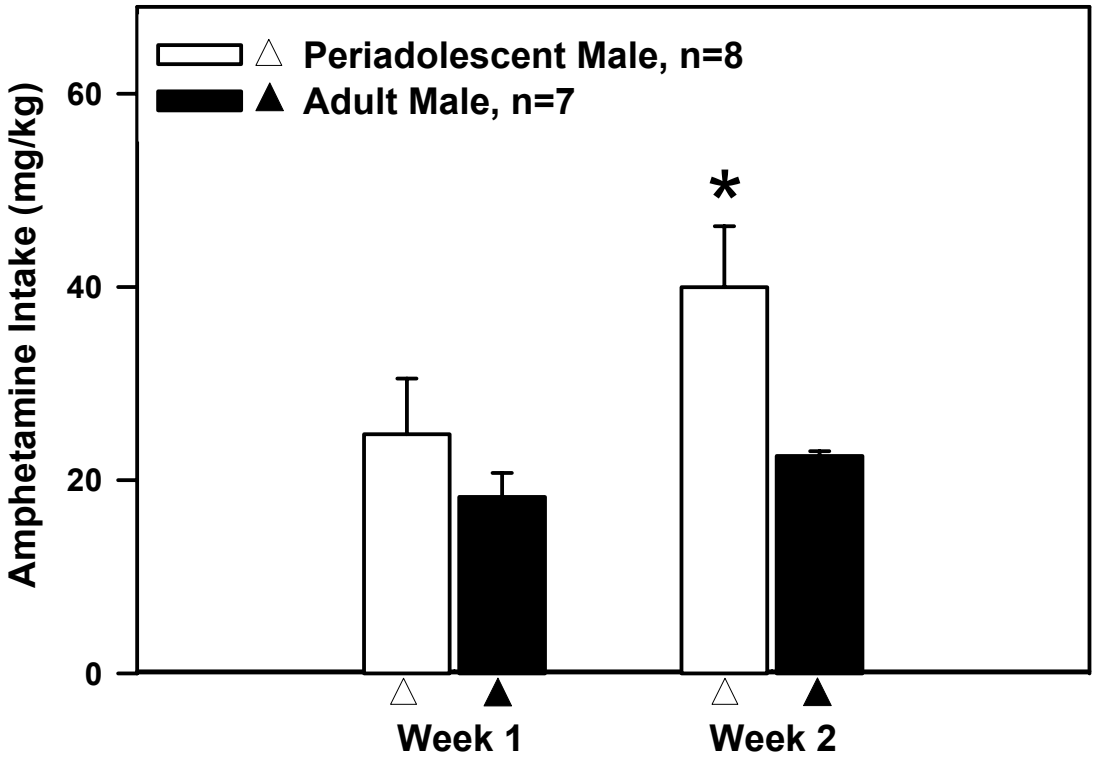

Figure 6 **Total drug intake by periadolescent (PND 35-51) vs. adult (PND 90-105) males.** Among male rats that acquired amphetamine self-administration, periadolescent (open triangles) had a significantly higher drug intake during the second week of acquisition compared to adult males (closed triangles; *P=0.02). Numbers per group indicated in legend.

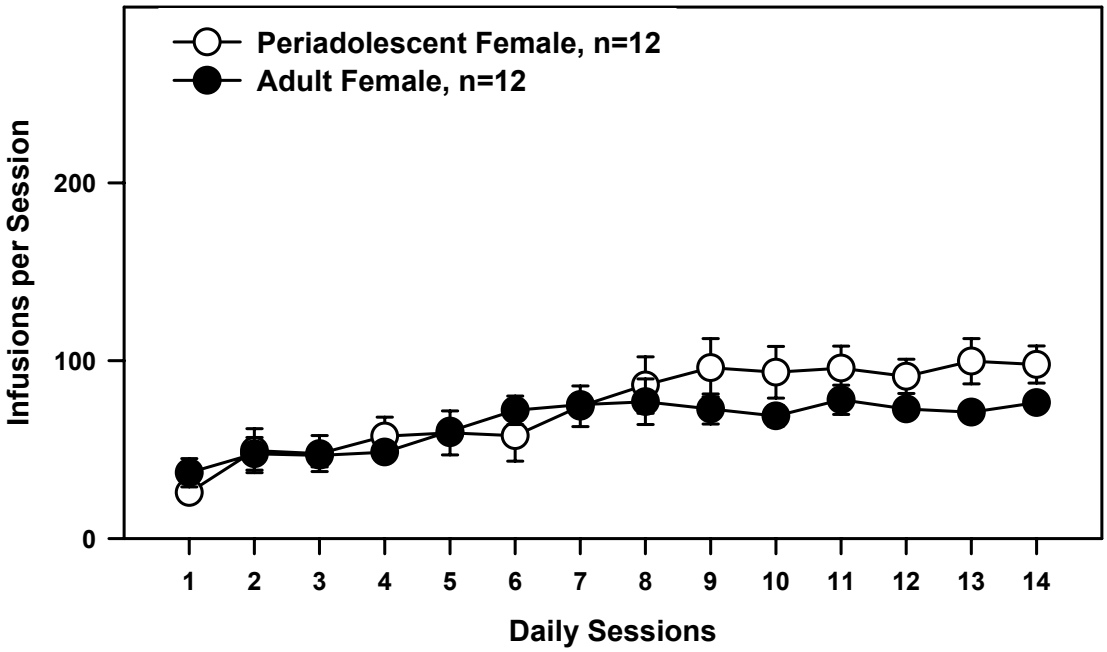

Figure 7 **Number of infusions over daily sessions by periadolescent (PND 35-51) vs. adult (PND 90-105) females.** Among female rats that acquired amphetamine self-administration, no significant differences were seen in number of infusions between periadolescent (open circles) vs. adult (closed circles) female rats. Numbers per group indicated in legend.

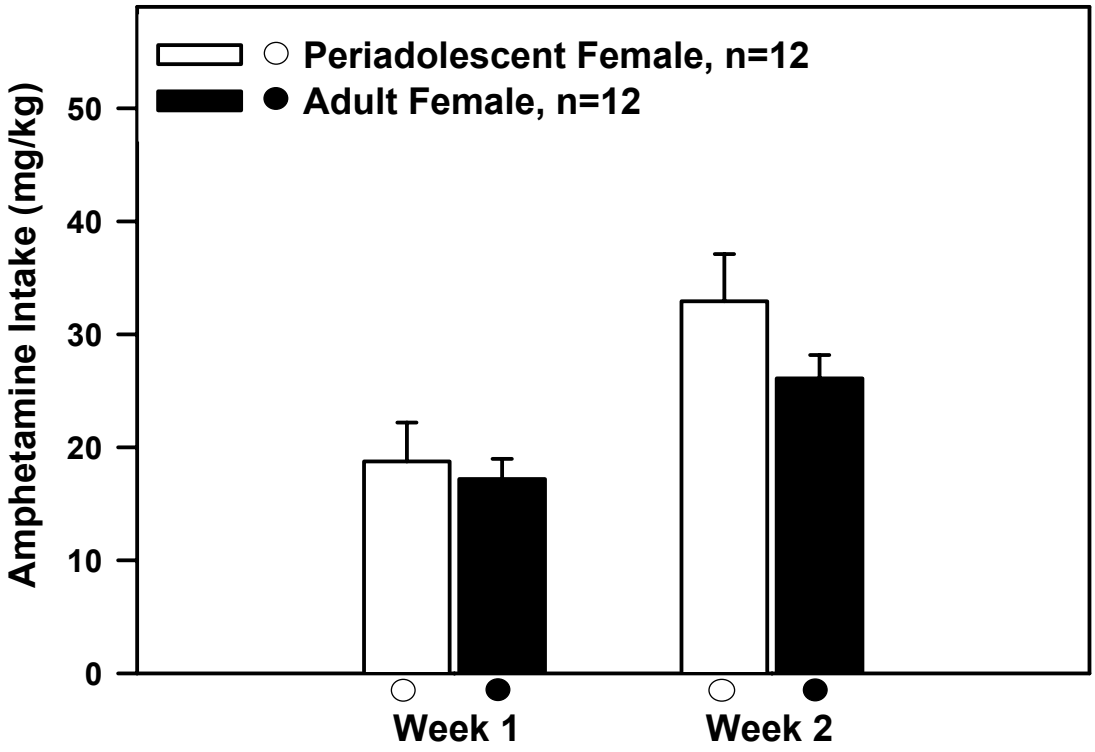

Figure 8 **Total drug intake by periadolescent (PND 35-51) vs. adult (PND 90-105) females.** Among female rats that acquired amphetamine self-administration, no significant differences were seen between periadolescent (open circles) vs. adult (closed circles) female rats. Numbers per group indicated in legend.

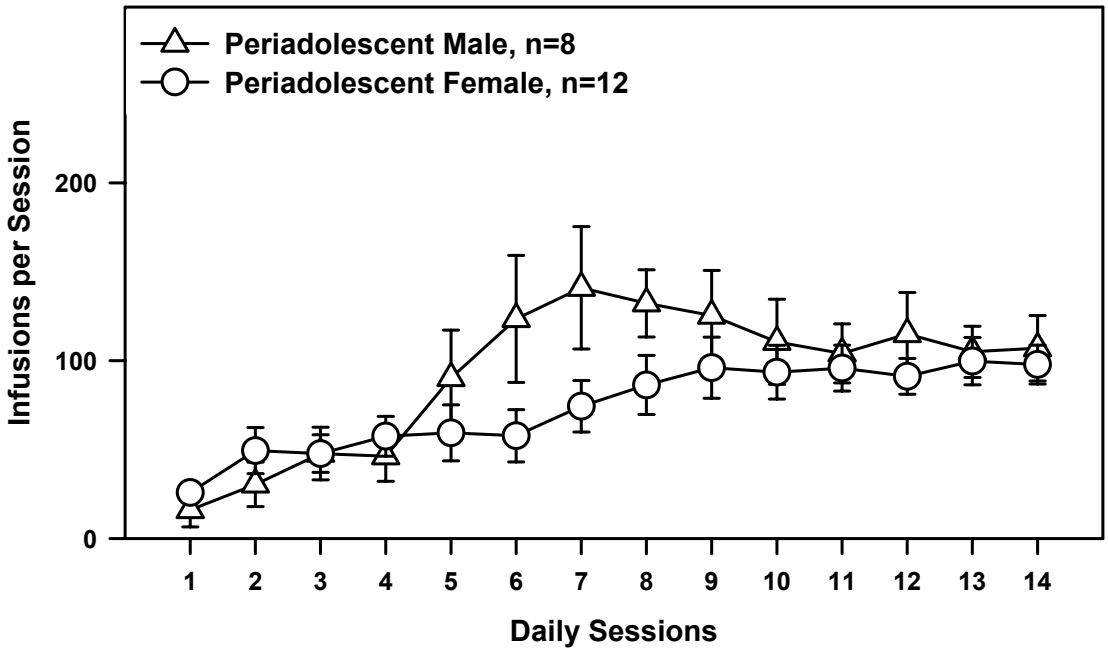

Figure 9 **Number of infusions over daily sessions by periadolescent (PND 35-51) male vs. females.** Among periadolescent rats that acquired amphetamine self-administration, no significant differences were seen in number of infusions between periadolescent male (open triangles) vs. female (open circles) rats. Numbers per group indicated in legend. These data are the same as in Figures 5+7, replotted for direct sex comparisons.

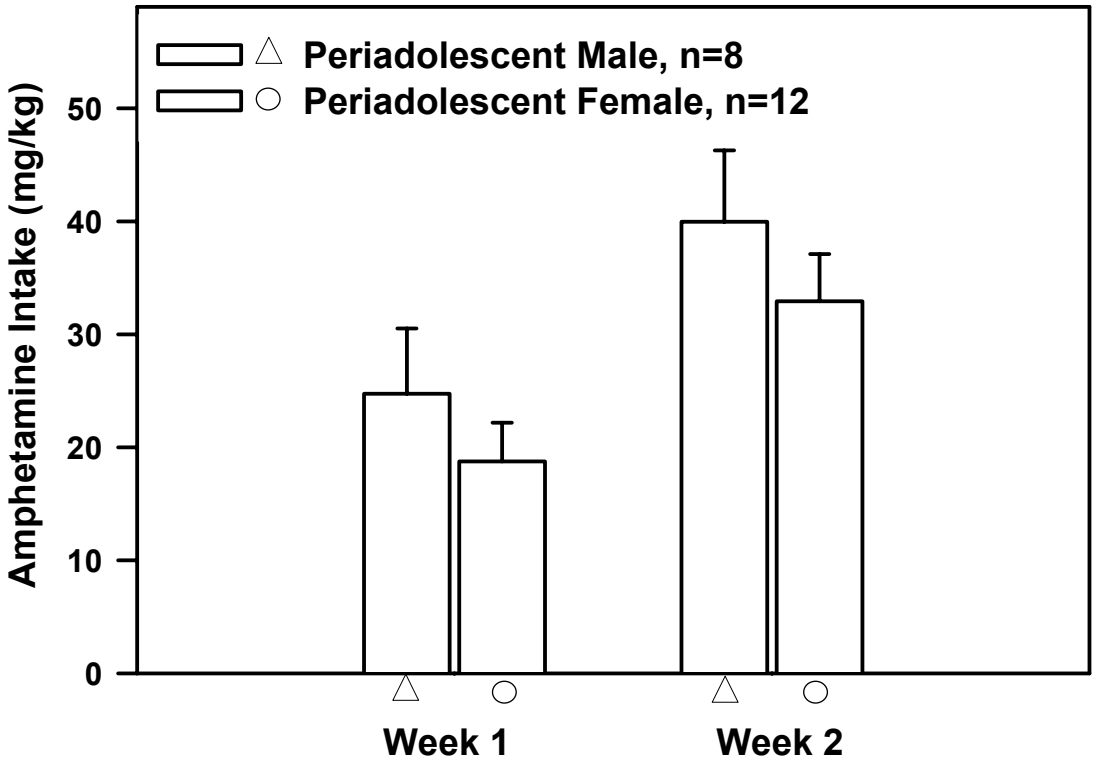

Figure 10 **Total drug intake by periadolescent (PND 35-51) male vs. females.** Among periadolescent rats that acquired amphetamine self-administration, no significant differences were seen between periadolescent males (open triangles) vs. females (open circles). Numbers per group indicated in legend. These data are the same as in Figures 6+8, replotted for direct sex comparisons.

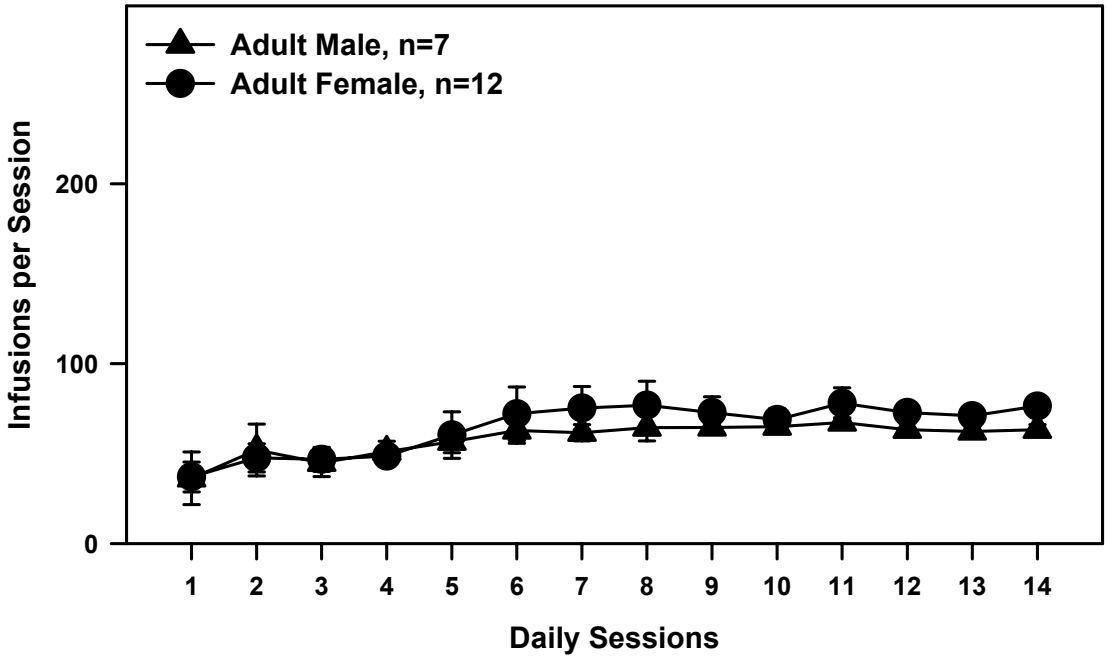

Figure 11 **Number of infusions over daily sessions by adult (PND 90-105) male vs. females.** Among adult male and female rats that acquired amphetamine self-administration, no significant differences were seen between adult male (closed triangles) vs. female (closed circles) rats. Numbers per group indicated in legend. These data are the same as in Figures 5+7, replotted for direct sex comparisons.

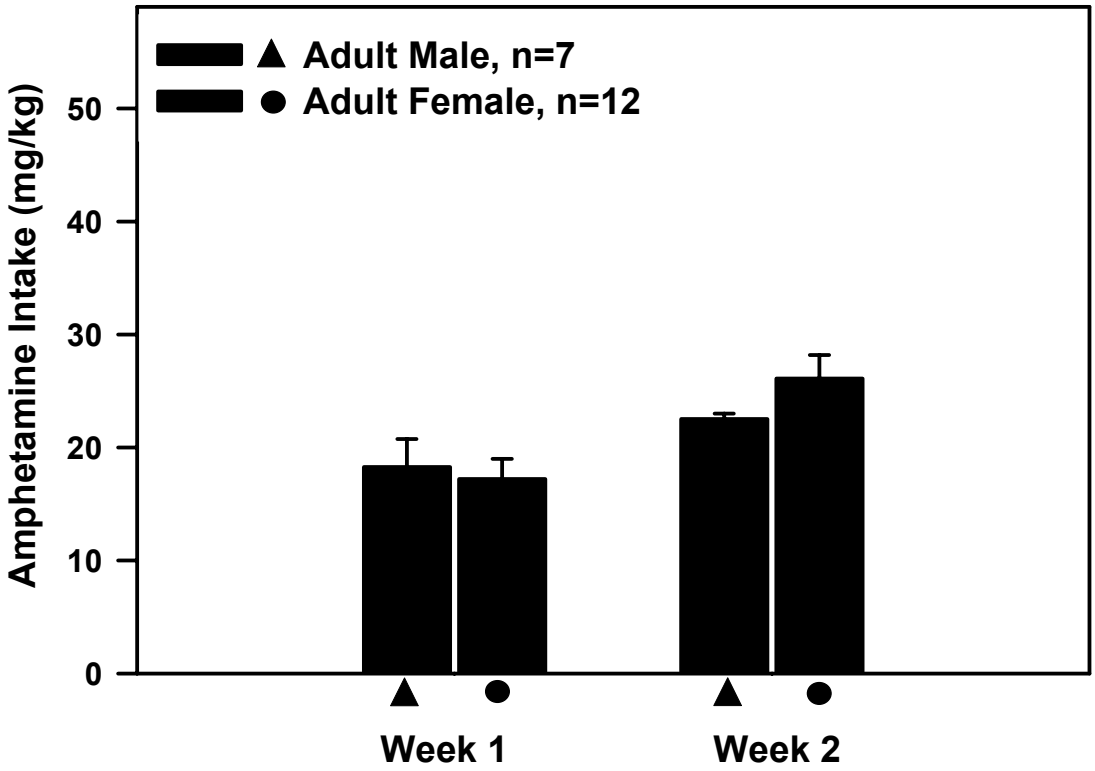

Figure 12 **Total drug intake by adult male vs. females.** Among adult (PND 90-105) male and female rats that acquired amphetamine self-administration, no significant differences were seen between adult male (closed triangles) vs. female (closed circles) rats. Numbers per group indicated in legend. These data are the same as in Figures 6+8, replotted for direct sex comparisons.

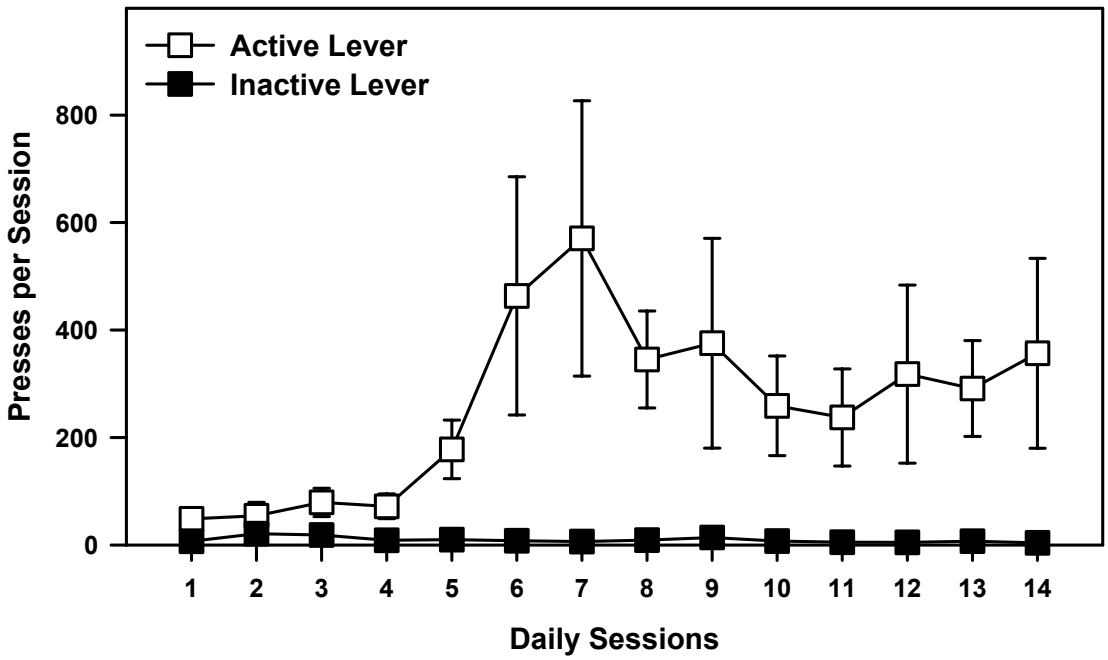

Figure 13 **Active vs. inactive lever presses by periadolescent males.** Only periadolescent male rats that acquired amphetamine self-administration (n=8) are included; they showed significant lever discrimination between active (closed squares) vs. inactive (open squares) lever responses.

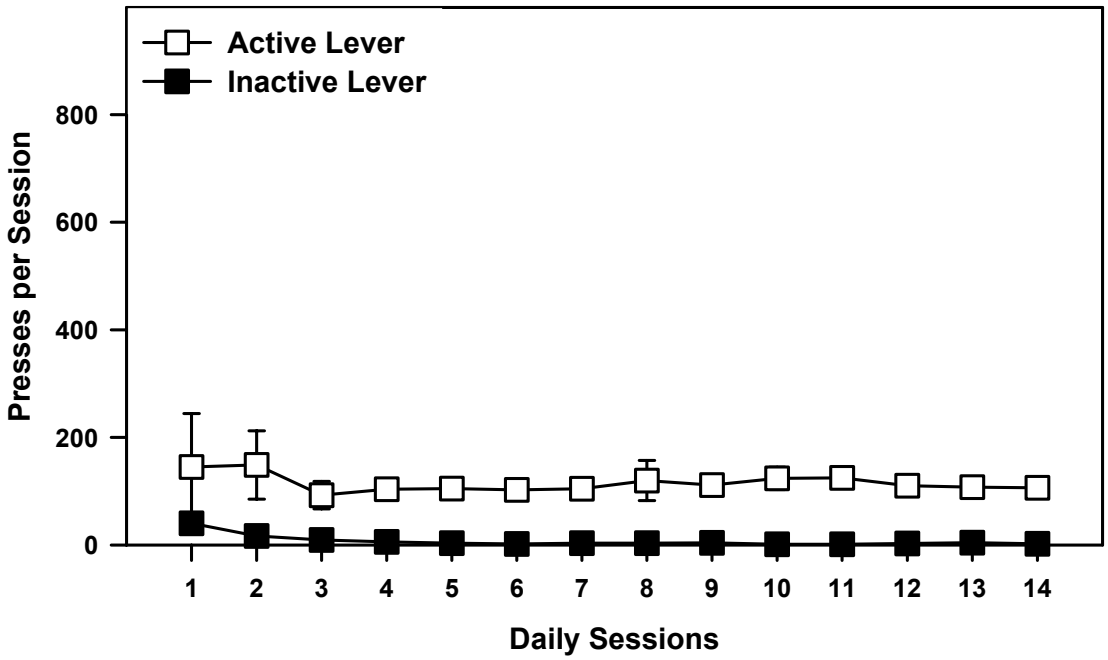

Figure 14 **Active vs. inactive lever presses by adult males.** Only adult male rats that acquired amphetamine self-administration (n=7) are included; they showed significant lever discrimination between active (closed squares) vs. inactive (opened squares) lever pressing.

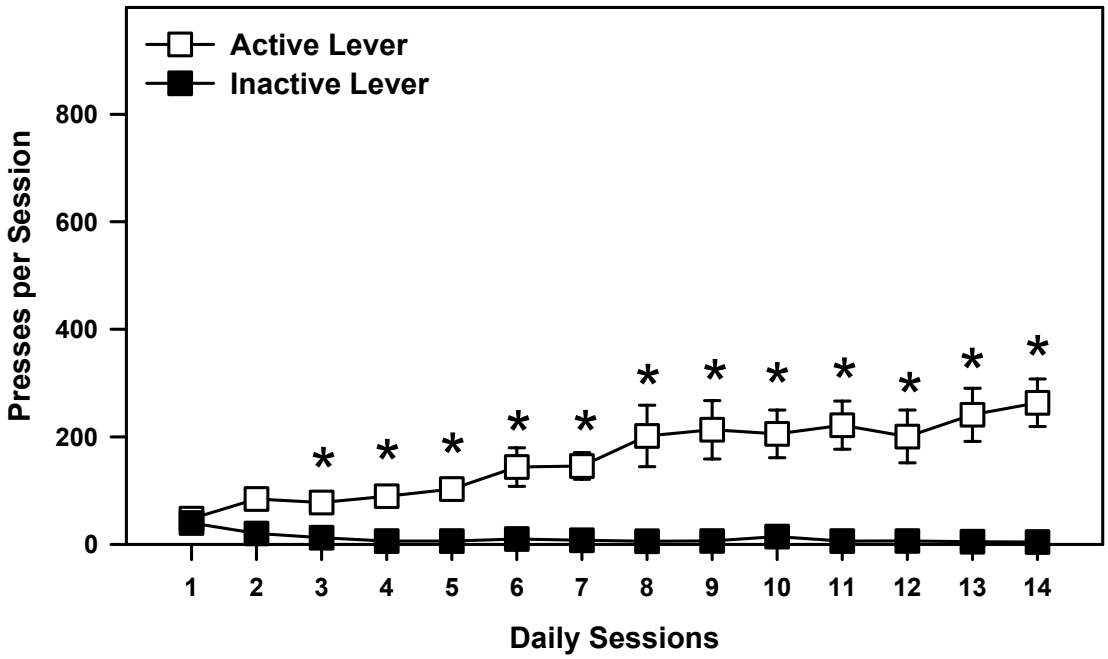

Figure 15 **Active vs. inactive lever presses by periadolescent females.** Only periadolescent female rats that acquired amphetamine self-administration (n=12) are included; they showed significant discrimination between active (closed squares) vs. inactive (open squares) lever pressing (*$p<0.003$).

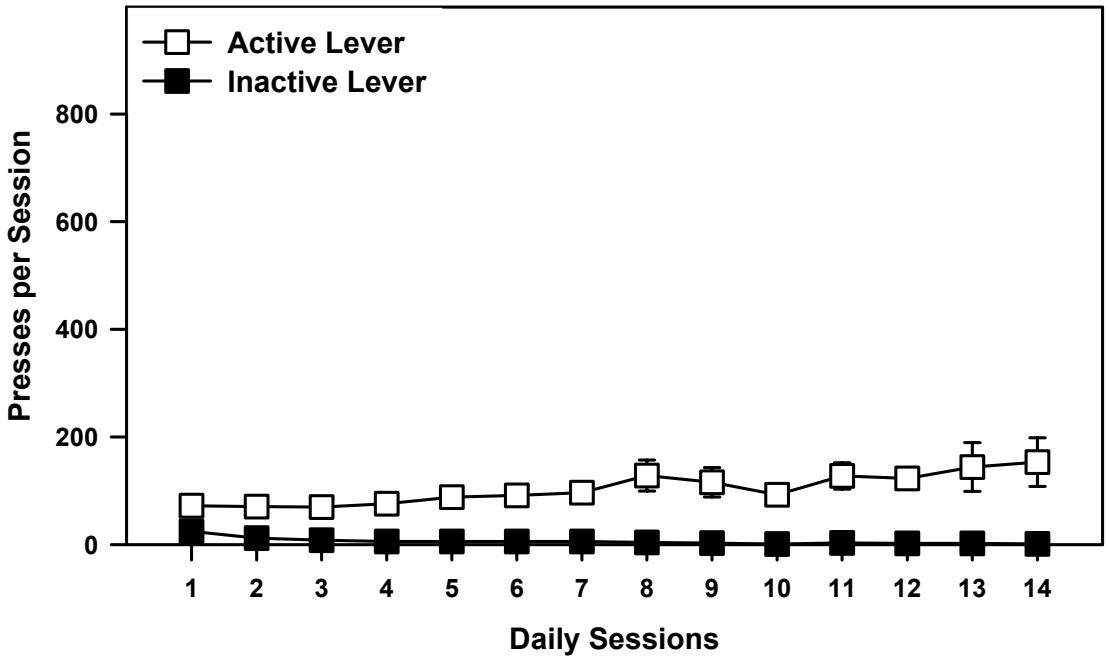

Figure 16 **Active vs. inactive lever presses by adult females.** Only adult female rats that acquired amphetamine self-administration (n=12) are included; they showed significant discrimination between active vs. inactive lever presses.

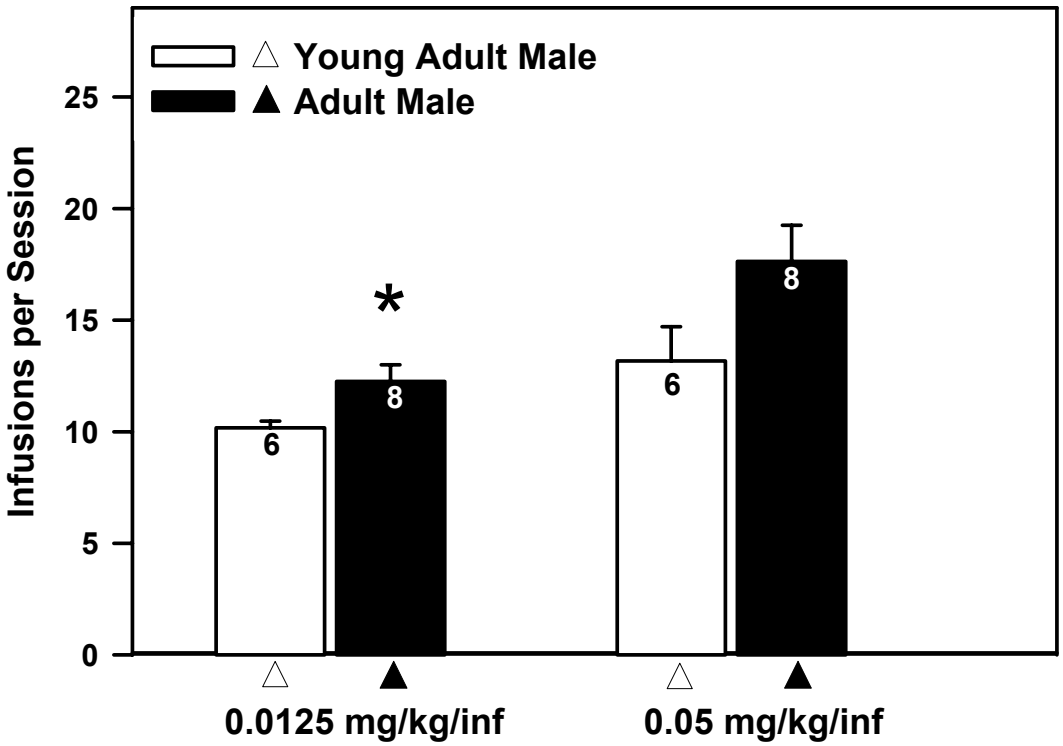

Figure 17 **Number of infusions earned on PR schedule by young adult (PND 48-63) vs. adult (PND 103-117) males.** At the low dose of amphetamine (0.0125 mg/kg/infusion), adult males (closed triangles) had significantly greater number of infusions compared to young adult male (open triangles) rats (*p= 0.04). Numbers on bars indicate n per group.

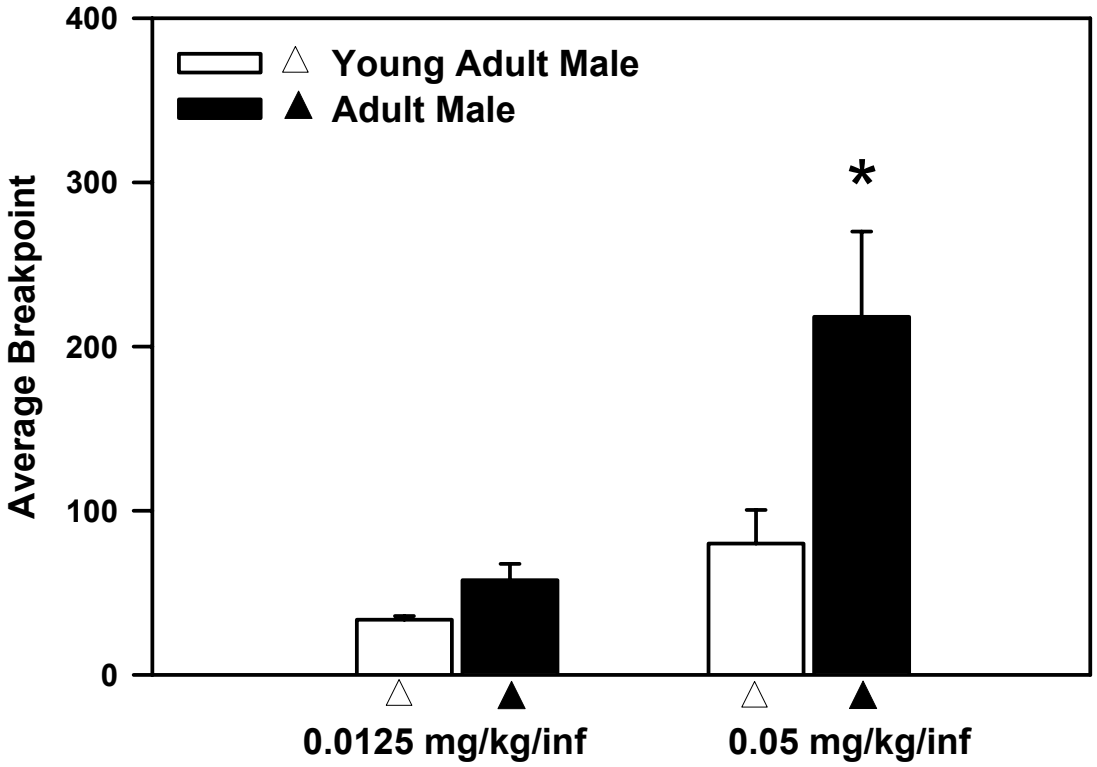

Figure18 **Break points in responding on the PR schedule by young adult (PND 48-63) vs. adult males (PND 103-117).** At the high dose of amphetamine (0.05 mg/kg/infusion), adult male (closed triangles) rats worked to significantly higher break points compared to young adult male (open triangles) rats (*p= 0.05). Numbers on bars indicate n per group.

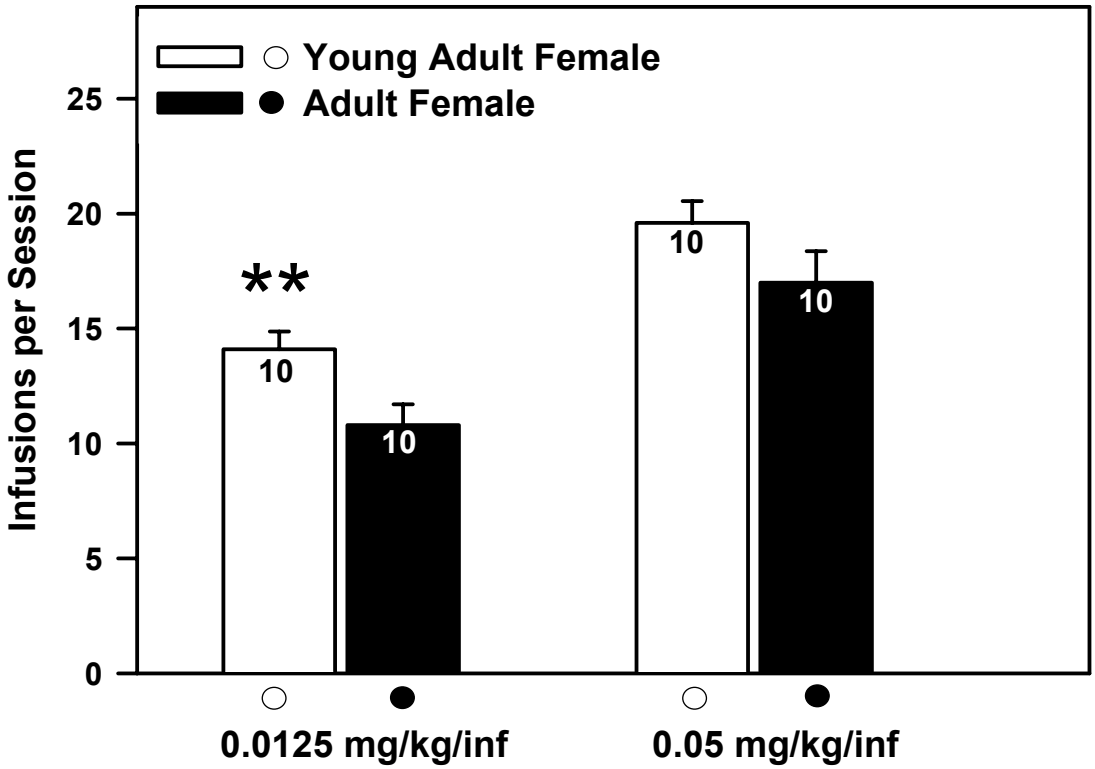

Figure 19 **Number of infusions earned on PR schedule by young adult (PND 48-63) vs. adult (103-117) females.** At the low dose of amphetamine (0.0125 mg/kg/infusion), young adult females (open circles) had significantly greater infusions compared to adult female (closed circles) rats (**p= 0.01). Numbers on bars indicate n per group.

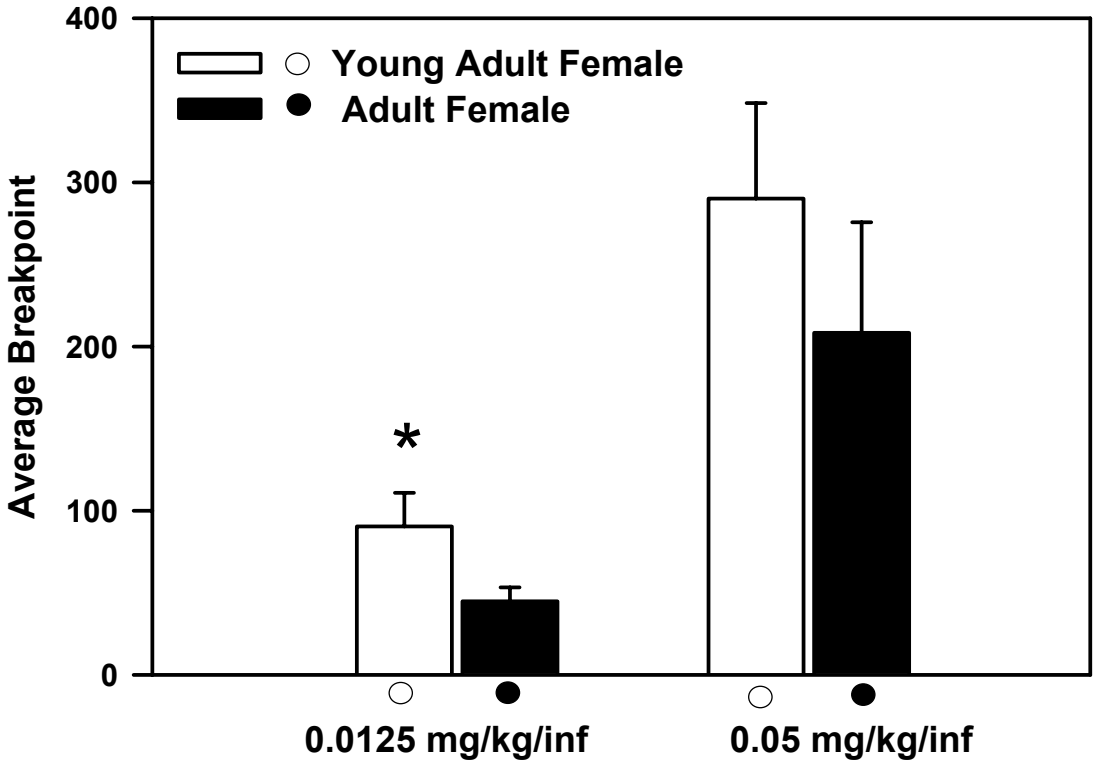

Figure 20 **Break points in the responding on the PR schedule by young adult (PND 48-63) vs. adult (PND 103-117) females.** At the low dose of amphetamine (0.0125 mg/kg/infusion), young adult (open circles) females worked to significantly higher break points compared to adult (closed circles) female rats (*p= 0.05). Numbers on bars indicate n per group.

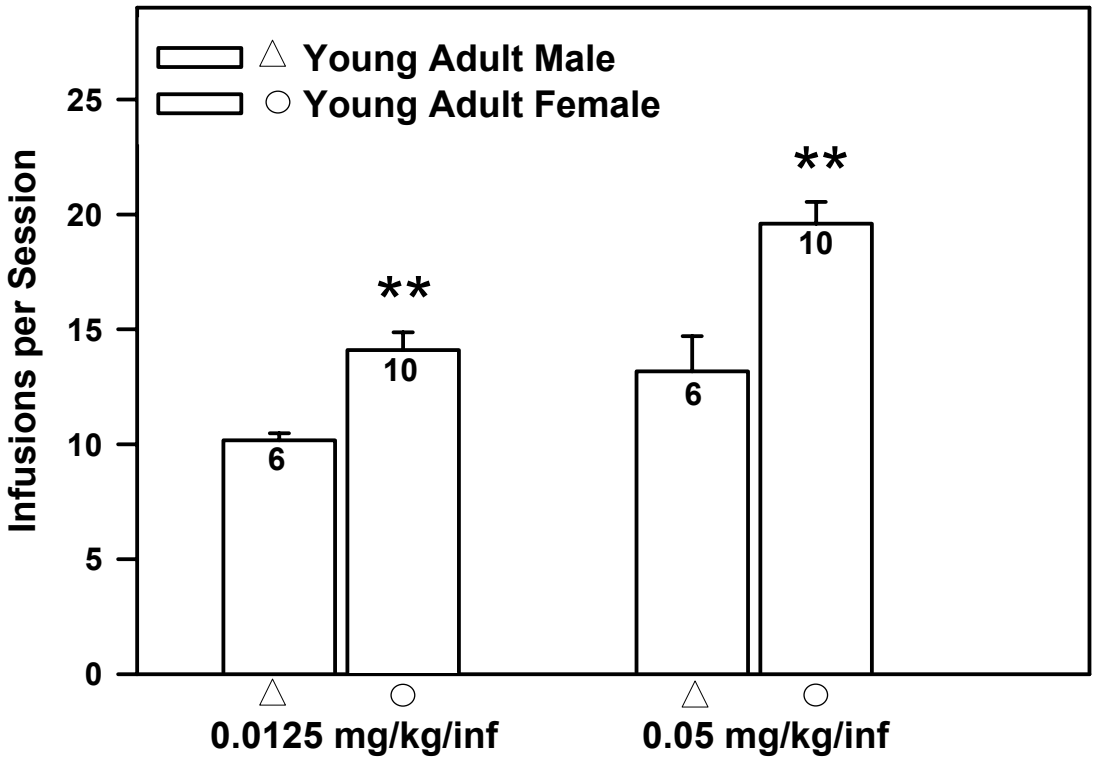

Figure 21 **Number of infusions earned on PR schedule by young adult (PND 48-63) male vs. females.** At both doses of amphetamine (0.0125 and 0.05 mg/kg/infusion), young adult females (open circles) had significantly greater number of infusions compared to young adult male (open triangles) rats (**p<0.01 for low dose and **p< 0.01 at high dose). Numbers on bars indicate n per group. These data are the same as in Figures 17+19, replotted for direct sex comparisons.

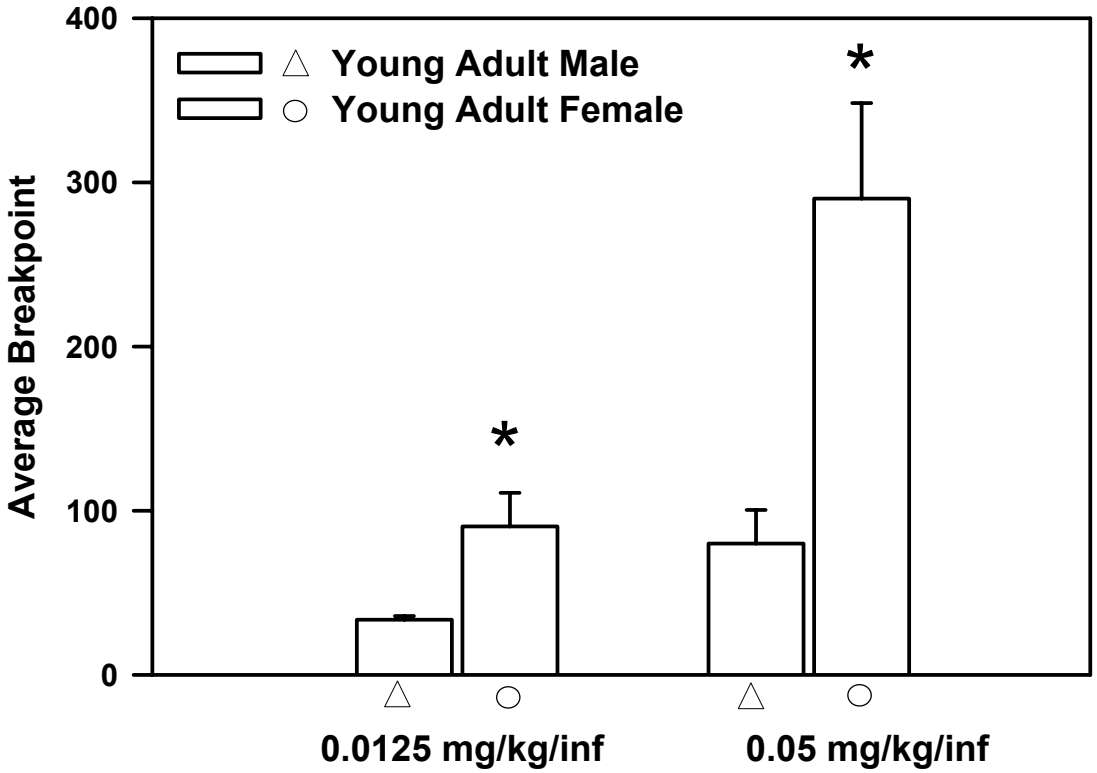

Figure 22 **Break points in responding on the PR schedule by young adult (PND 48-63) male vs. females.** At both doses of amphetamine (0.0125 and 0.05 mg/kg/infusion), young adult female (open circles) worked to significantly higher break points compared to young adult male (open triangles) rats (*p= 0.05 for low dose and *p=0.02 for high dose). Numbers on bars indicate n per group. These data are the same as in Figures 18+20, replotted for direct sex comparisons.

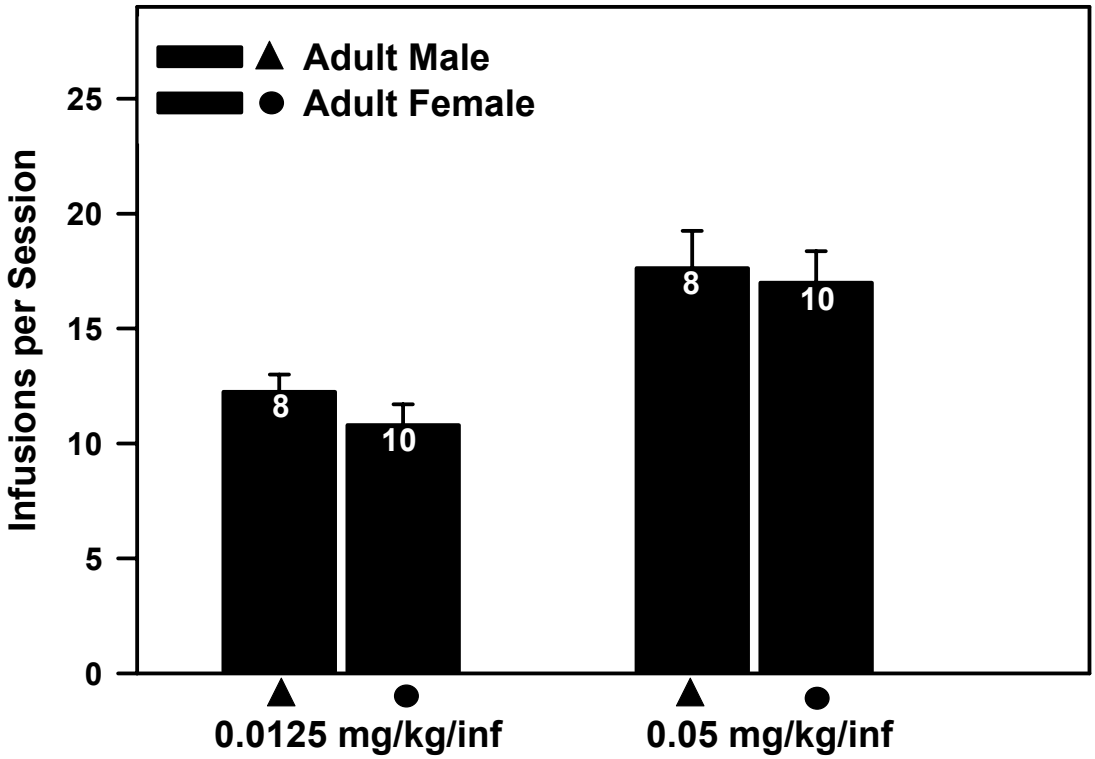

Figure 23 **Number of infusions on PR schedule by adult (PND 48-63) male vs. females.** At both doses of amphetamine (0.0125 and 0.05 mg/kg/infusion), no significant differences were seen between adult male (closed triangles) vs. female (closed circles) rats. Numbers on bars indicate n per group. These data are the same as in Figures 17+19, replotted for direct sex comparisons.

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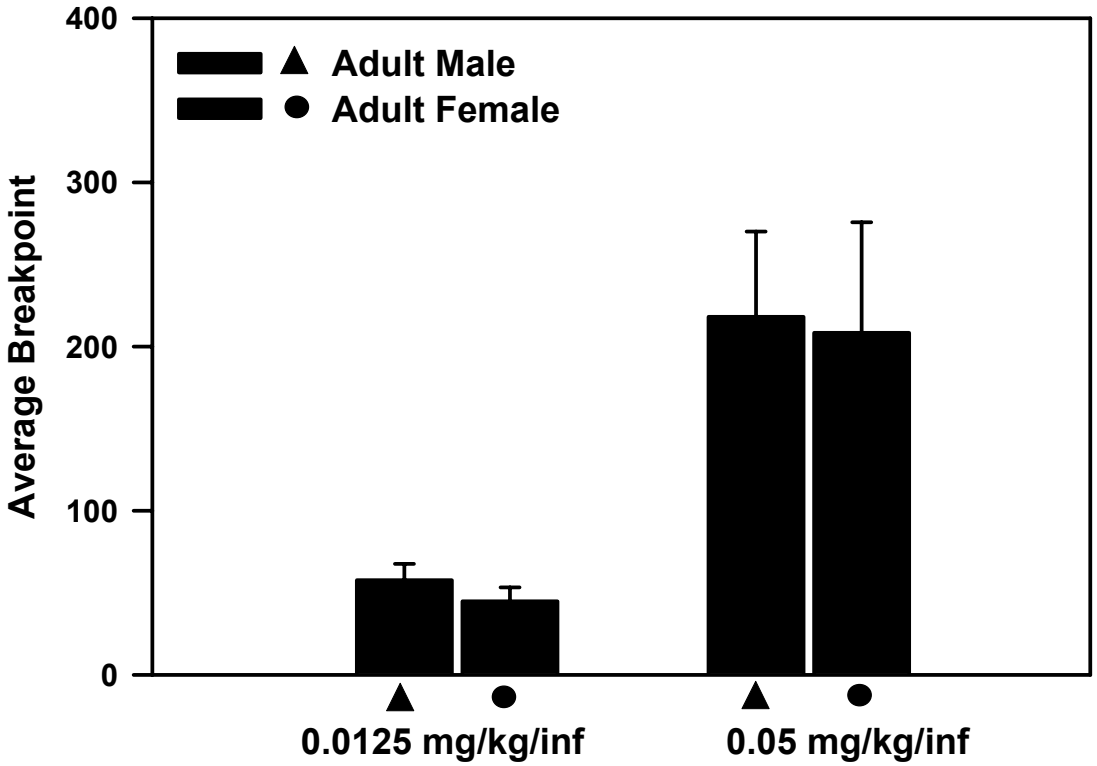

Figure 24 **Break points in responding on the PR schedule by adult (PND 103-117) male vs. females.** At both doses of amphetamine (0.0125 and 0.05 mg/kg/infusion), no significant differences were seen between adult male (closed triangles) and female (closed circles) rats. Numbers on bars indicate n per group. These data are the same as in Figures 18+20, replotted for direct sex comparisons.

DISCUSSION AND CONCLUSION

Age, sex, and schedule of reinforcement are important contributing factors to the present findings. Our age-related hypothesis that amount of amphetamine intake differs between age groups was supported, but only in male rats in the acquisition phase. Also, our age-related hypothesis that periadolescents are more motivated than adults to take amphetamine was supported, but only in female rats. Our sex-related hypothesis was supported by several measures, such as the rate of acquisition among periadolescents and number infusions and break points among young adults in the maintenance phase. The present study points out the complexity of behavioral analysis in the drug self-administration model.

Our age-related hypothesis that rates of acquisition of amphetamine self-administration differ between periadolescent and adult rats was not supported by the rate of acquisition data. We did not observe age differences in rate of acquisition as measured by percent rats acquired or days to acquisition. Our findings are consistent with reports of no difference between age groups in the rate of acquisition of i.v. cocaine self-administration (Frantz, 2000), but they are not consistent with nicotine studies (Belluzzi et al., 2005). In this regard, Belluzzi and colleagues suggest that nicotine and acetaldehyde mixtures are highly reinforcing during early adolescence with a substantial decline in reward value occurring during later adolescence and in adulthood. Reasons for discrepancies between this and our study may include different psychostimulants and a different paradigm such as nose-poke holes instead of levers, only 5 daily 3 hr sessions,

and slightly different age groups. Also, it is possible that if we had used a very low dose of amphetamine, we may have observed differences in rate of acquisition (0.05 mg/kg/infusion is a mid-range dose).

Our age-related hypothesis was supported by amount of drug intake, but only in males. Among those male rats that acquired amphetamine self-administration, periadolescent males took more amphetamine during the second week of acquisition testing compared to adult males. This finding is consistent with reports that periadolescents have higher nicotine intake compared to adults (Belluzzi et al., 2005). This may be explained by higher basal level of corticosterone in periadolescent than adult males (Laviola et al., 2002) and the interaction of corticosterone with dopamine systems (Piazza et al., 1991). High circulating levels of corticosterone may sensitisize an animal's response to amphetamine by an action on the dopamine system; dopamine cell bodies posses corticosterone receptors, and corticosterone stimulate dopamine neurons (Piazza et al., 1991). Also, this may be explained by lower aversive effects of amphetamine on periadolescent males than adult males, or higher hedonic effects of amphetamine on periadolescent males than adult males (Laviola et al., 1999; Levin et al., 2003).

However, we did not observe age differences in drug intake among female rats. This may be explained by the observation that in female rats, the first ovulation occurs between PND 35-45 (Ojeda et al., 1986; Ojeda et al., 2003). If vaginal opening and ovulation take place during our acquisition testing, then periadolescent females may be more like adult females with respect to gonadal hormones than periadolescent males are like adult males. Therefore, effects of ovarian hormones on drug-related brain regions

will be similar in periadolescent and adult females. For example, estradiol increases dopamine release in the striatum and decreases dopamine reuptake in the nucleus accumbens (Hu et al., 2004; Jackson et al., 2005).

Our sex-related hypothesis that females are more sensitive to the reinforcing effects of amphetamine was supported by the rate of acquisition in periadolescent rats. Periadolescent females acquired amphetamine self-administration significantly faster than periadolescent males, as measured by percent rats acquired and number of days to acquisition. When periadolescent and adult female rats were combined, females acquired amphetamine self-administration faster compared to males. Also, more females tended to acquire amphetamine self-administration than males in each age group, further suggesting increased sensivity to amphetamine in females compared with males. These findings are consistent with other reports that female rats are more vulnerable to the acquisition of psychostimulant self-administration (e.g. Lynch and Carroll, 1999; Hu et al., 2004; Roth and Carroll, 2004).

However, our sex-related hypothesis was not supported by amount of drug intake. We did not observe sex differences in amount of drug intake among male and female rats. The lack of sex differences in rate of acquisition and drug intake in our *adult* rats parallels other reports that cocaine self-administration on a FR1 schedule of reinforcement is not different in adult male vs. female rats (Roberts et al., 1989), suggesting that different paradigms produce different results depending on factors such as specific psychostimulant, duration of daily sessions, priming injections, schedule of reinforcement, etc.

All groups showed significant lever discrimination between active vs. inactive levers. Interestingly, only periadolescent male rats showed higher rates of "inappropriate" presses during drug infusion and time out compared to other groups. A high rate of "inappropriate" presses may indicate impulsive behavior or intense stereotypy. This behavior may be explained by a high corticosterone level in periadolescent males and its interaction with dopamine receptors and amphetamine (Piazza et al., 1991; Laviola et al., 1995; Laviola et al., 2002)

Our age-related hypothesis that periadolescents are more motivated to take amphetamine than adults was only partially supported. Young adult males failed to work as hard as adults to obtain amphetamine. This is contrary to our hypothesis and may be explained by higher levels of testosterone in young adult males compared to adult males. The highest peak of testosterone occurs at PND 50-60 (Ojeda, 1994) and testosterone level in plasma declines by more than 50% in Sprague-Dawley rats between 3-24 months of age (Kaler and Neaves, 1981). Indeed, Forgie and Stewart (Forgie, 1994) reported that testosterone has a suppressive effect on amphetamine induced-motor activity (but see (Martinez-Sanchis et al., 2002), and thus it may also decrease amphetamine's reinforcing effects in young adult males in the present paradigm.

Contrary to males, on the PR schedule, young adult female rats worked harder than adult females. These findings support our hypothesis and are consistent with reports on nicotine that young adult females (that acquired self-administration as periadolescents) self-administer more nicotine than adult rats (that acquired self-administration as adults; Levin et al., 2003). This may be explained by a lessened perception of aversive effects of amphetamine or a different hedonic set point in rats that acquired self-administration during adolescence (Levin et al., 2003).

Our sex-related hypothesis that female rats are more motivated than males to take amphetamine was partially supported in the maintenance phase. Young adult females worked harder than young adult males. Young adult females achieved higher break points and therefore higher number of infusions than age-matched males. These findings support our hypothesis and are consistent with previous behavioral studies on sex differences in adult rats (Roberts et al., 1989; Hu et al., 2004; Roth and Carroll, 2004; Roth et al., 2004). For example, female rats are more motivated to self-administer methamphetamine or cocaine than adult male rats under a PR schedule of reinforcement (Roth and Carroll, 2004; Roberts et al., 1989).

However, no sex differences were observed in adult rats on PR schedule of reinforcement. These findings may be explained by differences in the paradigms, such as lower dose of methamphetamine (0.02mg/kg/infusion), automated priming, and 6-hr daily sessions in the Roth and Carroll study (Roth and Carroll, 2004). In Roberts et al. (Roberts et al., 1989) study, cocaine was tested, and vaginal lavages were conducted daily on female rats. Some studies suggest that vaginal lavage affects the psychological effects of psychostimulants (Walker et al., 2002).

There are several limitations to the present study. First, we did not assess estrous cycle in female rats. We did not attempt to monitor this possible variant because vaginal lavage, which is used to assess cycle stage in female rats, not only serves as a rewarding stimulus, but also decreases stimulant-induced activity (Walker et al., 2002). Therefore, it might result in inaccurate behavioral comparisons of female to male rats. Also, it is unclear if estrous cycle affects acquisition of stimulant self-administration, although Roberts et al. (Roberts et al., 1989) suggest that it does not. Preliminary studies are ongoing to address this concern in our paradigm.

Second, we did not assess circulating levels of adrenal and gonadal hormones in our subjects. We did not attempt to monitor this possible variant because the present study was an initial exploration of behavior. Future studies will include analysis of neurohormonal mechanisms.

Third, we do not have a specific age-related control group that would be informative. If a separate group of males and females was trained to self-administer during periadolescence and tested on the maintenance phase during later adulthood (PND 103-117), they would provide information comparing effects of age at acquisition with age at testing. Currently, we cannot tell whether differences in maintenance phase results are due to long-term effects of adolescent vs. adult acquisition or acute effects of young adult vs. adult testing.

In conclusion, the present study shows age differences in total drug intake among male rats, such that periadolescent males take more amphetamine compared to adult males. Also, the present study demonstrates sex differences in rate of acquisition among periadolescent rats, such that periadolescent females acquire amphetamine self-administration faster compared to periadolescent males. Furthermore, this study demonstrates age differences among young adult and adult rats in motivation to self-administer amphetamine, such that young adult males are less motivated to self-administer amphetamine compared to adult males but young adult females are more motivated to self-administer amphetamine than their adult counterparts. This study also indicates significant sex differences in young adult rats, such that young adult females are more motivated to self-administer amphetamine than age-matched males.

Translating data from rodents to humans may be risky, due to obvious physiological and social differences between the species. Nevertheless, robust lever pressing, impulsive behavior, higher drug intake, and lower motivation to work for amphetamine in periadolescent male rats compared with all other age- or sex-groups may

resemble the increased sensitivity of periadolescent human males reported in national surveys (Administration, 2003; Johnston, 2004). Moreover, faster rates of acquisition during adolescence for females and higher motivation to work for amphetamine of young adult female rats that acquired self-administration as periadolescents compared to other age- and sex-groups resemble recent clinical studies in humans that show females start cocaine use at younger ages and get addicted faster compared to males (Griffin et al., 1989). Therefore, understanding the neural mechanisms of these effects in rats might aid human drug-related concerns.

Future research is necessary to determine the effects of hormones (gonadal and adrenal) on amphetamine self-administration in periadolescent and adult, male and female rats. Also neurochemical and molecular approaches, as well as pharmacokinetic studies, will help to identify the central nervous system mechanisms involved in drug vulnerability in periadolescent and adult, male and female rats. The long-term impact of these studies includes helping to form effective treatment and prevention programs for drug dependent individuals of all ages and both sexes.

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