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Reinforcing Efficacy of Amphetamine in Adolescent and Adult Male Rats

Lauren Chantel Payne

REINFORCING EFFICACY OF AMPHETAMINE

IN ADOLESCENT AND ADULT MALE RATS

by

LAUREN CHANTEL PAYNE

Under the Direction of Kyle J. Frantz

ABSTRACT

Rationale: Amphetamine abuse by adolescents predicts long-term drug dependence. Heightened vulnerability to drug abuse could be due to higher sensitivity to drug's reinforcing effects. Rodents are used to study age-related sensitivities to drugs. **Objective:** We compared intravenous amphetamine self-administration between adolescent and adult male rats on an operant schedule of reinforcement measuring the reinforcing efficacy of a drug. **Methods:** After surgery, adolescent and adult rats acquired lever-pressing behavior reinforced by amphetamine infusions. **Results:** Both age groups exhibited more infusions per session as dose increased. However, neither the number of infusions per session nor total amphetamine intake differed across age groups. **Conclusion:** Although rapid transition is reliable to test reinforcing properties of stimulants, results suggest that amphetamine is an equally efficacious reinforcer among both age groups. In regards to humans, these results suggest that other factors, like social influences, explain higher rates of drug intake by adolescent compared with adult humans.

INDEX WORDS: amphetamine, age, adolescent, rat

REINFORCING EFFICACY OF AMPHETAMINE IN ADOLESCENT AND ADULT MALE RATS

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LIST OF ABBREVIATIONS

FR= fixed ratio schedule of reinforcement

FR1 = fixed ratio 1 schedule of reinforcement

FR2= fixed ratio 2 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

h = hour

s = second

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Introduction

Drug abuse is a problem in the United States. In 2006, 112 million Americans 12 years and older reported using an illicit drug at least once in their lifetime (SAMHSA 2005). Specifically, the stimulant amphetamine has remained a popular drug of abuse, with 99% of stimulant treatment admissions relating to methamphetamine abuse (SAMHSA 2005). Adolescent initiation of drug use predicts long-term drug abuse and dependence (Laviola et al. 1999-this reference needs to be a human study, not animal). Although not yet reaching the rate of adult illicit drug use, adolescent initiation of amphetamine abuse has increased significantly since 1994 (Johnston et al. 2005). These statistics require research into factors underlying adolescent vulnerability to illicit drug use, abuse, and dependence.

Adolescence is a period in development characterized by behavior such as increased interest in novelty-seeking, social interactions among peers, and overall "risky or reckless" behavior, that spans across species, from humans to nonhuman primates and rodents (Adriani et al. 1998; Arnett 1992; Smith 2003; Spear 2000). In specifically researching human adolescent drug abuse, perhaps the ideal subjects would be humans; however, administering drugs of abuse to humans is unethical. Another experimental model of choice would be nonhuman primates, as they are closely related to humans with regard to development and behavior; however, they are extremely expensive. Rodents, while more removed from humans in terms of species similarities, have been validated as good models in behavioral assays related to drugs of abuse (e.g. Ator and Griffiths 2003; Laviola et al. 1999; Spear 2000).

Rodent adolescence can be defined as the timeframe from 7-10 days before the start of puberty through the first few days afterward [Post Natal Days (PND) 35-55]. Behavioral characteristics often displayed by adolescent rodents include increased novelty-seeking and increased time spent in social interactions compared with younger and older rodents (Adriani et al. 1998; Izenwasser 2005; Spear 2000; Smith 2003). Despite these similarities to adolescent humans, adolescent rodents have not been used extensively for research on vulnerability to drugs of abuse.

Drug-related behavioral reinforcement paradigms are often used to investigate the reinforcing potency and efficacy of a stimulus. Reinforcing stimuli can include food, alcohol, and intravenous (i.v) drug infusions. More specifically, i.v. drug self-administration in rats models some aspects of drug abuse in humans (Ator and Griffiths 2003; Caine et al. 1993; Richardson and Roberts 1996). In this model, operant behavior (lever-pressing) is reinforced by i.v. drug infusions. The test subjects control their drug intake during the test sessions. Two well-validated schedules of reinforcement are Fixed Ratio (FR) and Progressive Ratio (PR). FR is a simple schedule in which a rat must make a set number of lever presses to receive each drug infusion. FR is a straightforward method for animals to acquire self-administration, and a dose-effect function which contains both an ascending and descending limb usually results from FR studies (Fig 1; Green et al. 2002).

An alternative to FR is the PR schedule, in which the number of presses required to obtain each drug infusion increases gradually during a single session; this forces the subject to meet increasing behavioral demands throughout the session to obtain the drug.

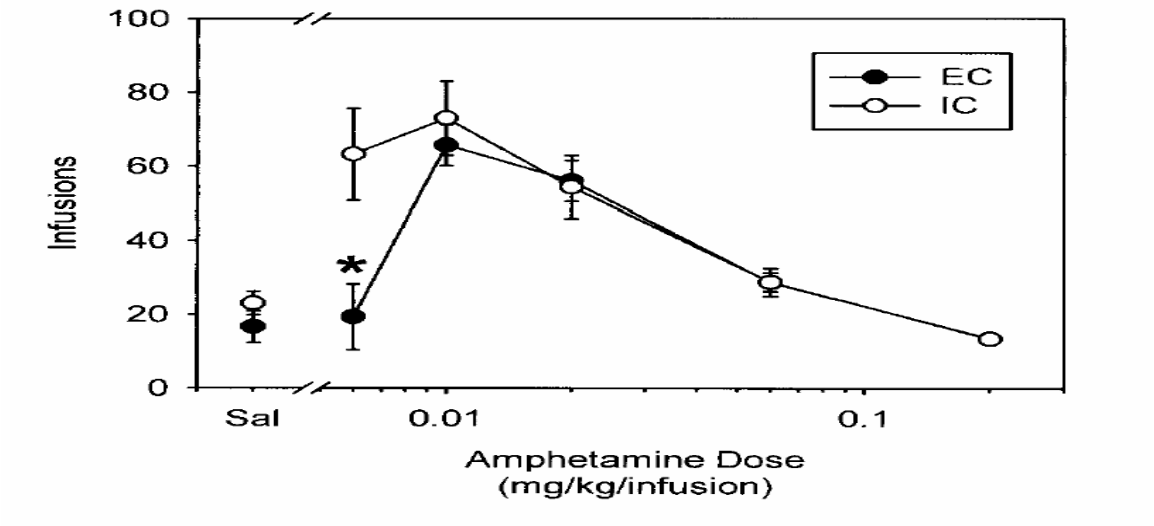
This is a more reliable approach than FR to measure reinforcing efficacy of a stimulant, because it allows for easier interpretation of results identified in the PR monotonic dose-effect function (Fig. 1; Green et al. 2002). In addition, PR uses breakpoint to interpret drug reinforcing efficacy. By definition, breakpoint is the highest ratio of lever presses to drug infusion a subject completes before quitting. Thus, rats generally control their individual PR session durations, as sessions stop when rats quit responding for a certain length of time (e.g. 60 min; Suto et al. 2002, 2003). Breakpoint cannot be analyzed statistically, however, due to its establishment from an exponential function. Ratios at the high end of the scale will have larger variance than values at the low end of the scale, possibly masking group differences (Richardson and Roberts 1996). Therefore, the number of infusions per session is analyzed, as in an FR session.

A previous project from our group compared age and sex differences in amphetamine self- administration by adolescent and adult Sprague-Dawley rats (Shabhazi et al. 2007). Acquisition was tested on an FR schedule for 14 days, and adolescent males received more drug infusions than adult males on a 0.05 mg/kg/infusion amphetamine dose. Higher rates of lever pressing in adolescents vs. adults suggest adolescent hypersensitivity to amphetamine's reinforcing effects, i.e. they might represent an upward shift in the dose-effect function (Fig. 2; Piazza et al. 2000). An alternative interpretation is that adolescents are less sensitive to the reinforcing effects of amphetamine; higher rates of pressing maintained by lower amphetamine doses could be part of a rightward shift in the typical dose-effect function. These conflicting interpretations of FR results have led to the use of alternative schedules, such as the PR

schedule of reinforcement outlined above. Its monotonic dose-effect function is likely to produce clearer results than FR, therefore, the present study used a PR schedule to compare amphetamine's reinforcing efficacy in adolescent vs. adult male rats. On the present PR schedule, higher infusions per session would support the first interpretation of the Shabhazi et al. (2007) study, whereas lower infusions per session would support the second interpretation (Fig. 3).

Based on previous studies that have reported hyposensitivity to amphetamine among adolescent rodents (Adriani and Laviola 2003; Bolanos et al. 1998), we predicted adolescent rats would be less sensitive to amphetamine's reinforcing effects than adults, therefore taking fewer drug infusions than adults on the PR schedule of reinforcement. In order to test adolescent rats on PR during adolescence, we used a rapid transition FR to PR protocol, as per the Suto et al. 2002, 2003 studies. FR was first used to facilitate the association between behavior (lever press) and consequence (drug infusion) in this operant conditioning model. After FR criteria were met, subjects transitioned to a PR schedule for the remainder of the study. Three amphetamine doses (0.00, 0.05, and 0.2 mg/kg/infusion) were tested on PR; thus, subjects that acquired self-administration within the initial 5 days of testing on FR1 and FR2 schedules during adolescence (PND 35) or adulthood (PND 85-88) at start of testing were tested on a PR schedule during late adolescence (PND 42 and beyond) or older adulthood (PND 92 and beyond). Results from this assay should reveal age differences in amphetamine's reinforcing efficacy in male rats.

Fixed Ratio (FR) Schedule of Reinforcement



Progressive Ratio (PR) Schedule of Reinforcement

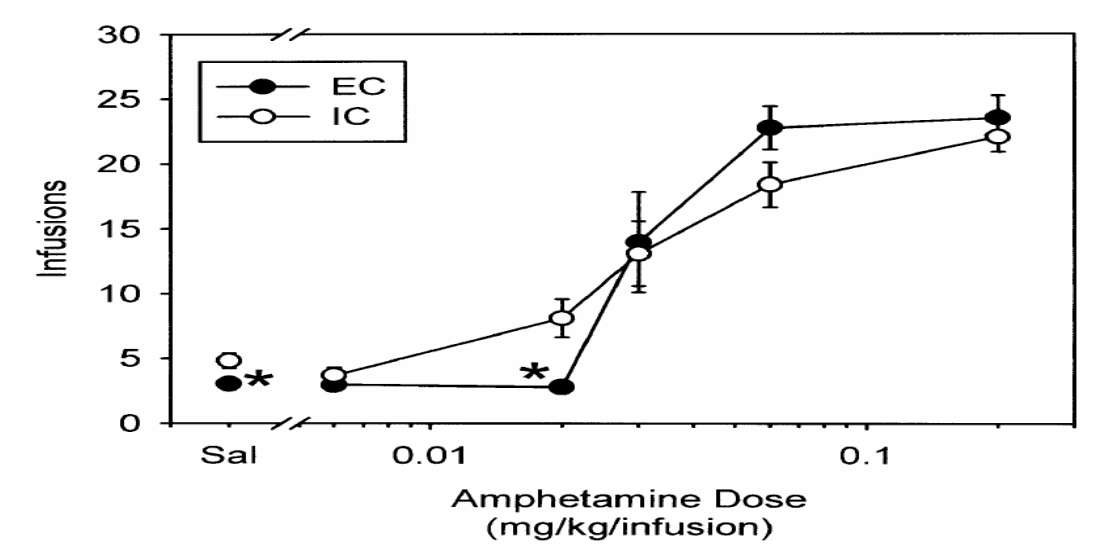


Figure 1. Sample dose-effect functions from fixed ratio and progressive ratio schedules of reinforcement in amphetamine self-administration by adult rats (Green et al. 2002).

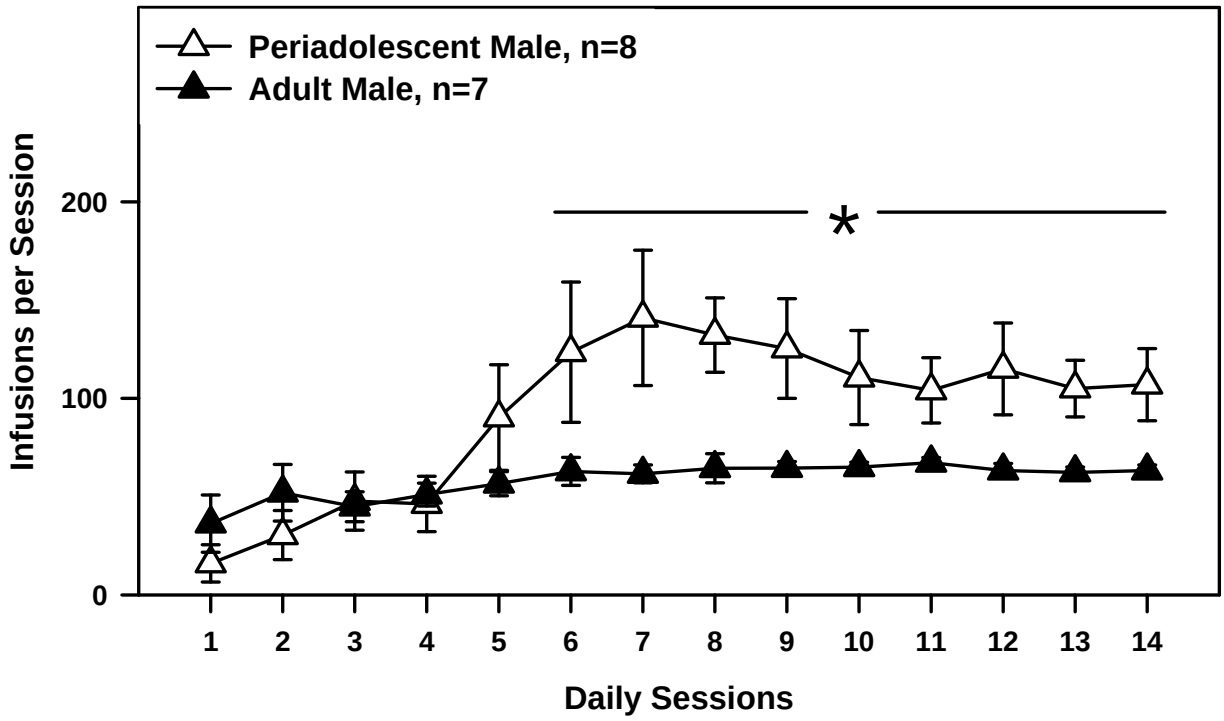
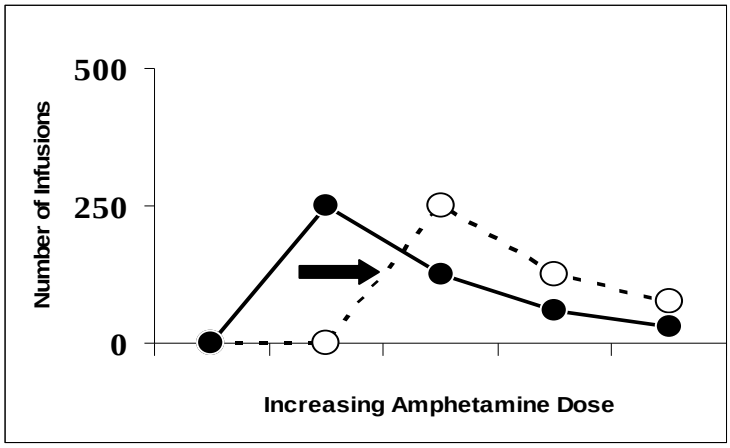
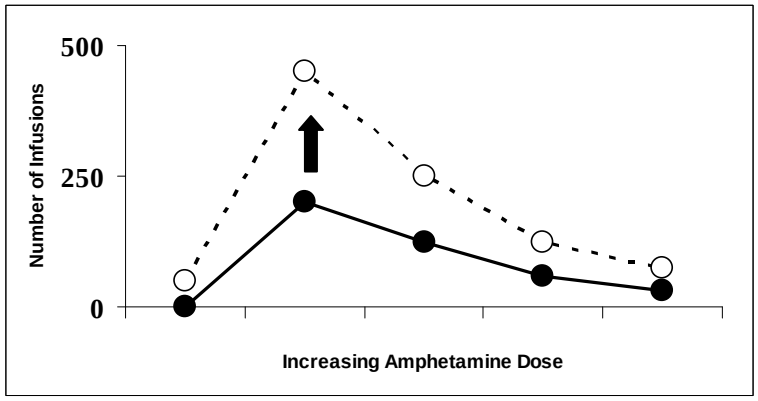


Figure 2. Number of infusions per session during acquisition of lever-pressing maintained by 0.05 mg/kg/infusion amphetamine on a fixed ratio 1 schedule of reinforcement (Shahbazi et al. 2007).

Fixed Ratio Dose-Effect Function: Rightward shift shows hyposensitivity.



Fixed Ratio Dose-Effect Function: Upward shift shows hypersensitivity.



Progressive Ratio Dose-Effect Function: <u>Upward</u> and d<u>ownward</u> shifts show hyper- or hyposensitivity, respectively.

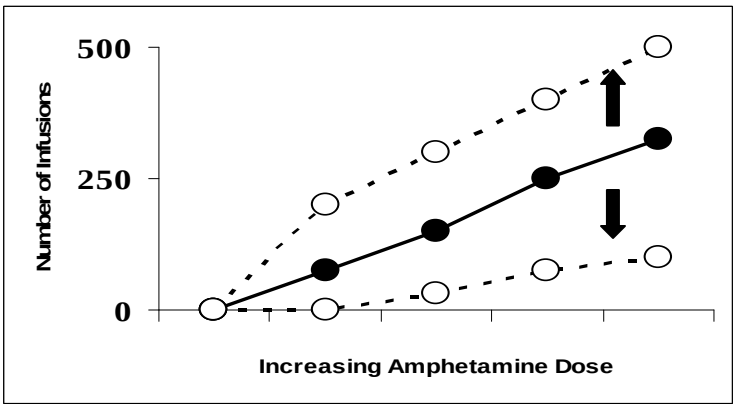


Figure 3. Possible interpretations of FR results from Shahbazi (2007) on fixed ratio schedule of reinforcement, and potential resolution by testing on progressive ratio schedule of reinforcement

Materials and Methods

Subjects

Sixteen male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) arrived in the laboratory at either PND 22 (adolescents) or PND 72 (adults). Rats were housed in groups of 2-3 in a humidity and temperature-controlled vivarium (20-22˚C), on a 12/12 hr light/dark cycle (lights turned off at 0500 or 0600 h). Subjects acclimated one week before experimentation and had access to food and water *ad libitum* throughout the study except during behavioral test sessions. Beginning two days after arrival, rats were weighed and handled daily by the experimenters. Body weights for all rats gradually increased throughout the experiment, suggesting no adverse drug effects on physical health. All procedures followed the Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research (NRC 2003).

Drugs

d-Amphetamine sulfate salt was purchased from Sigma Chemicals (St. Louis, MO). Amphetamine stock solutions were diluted with saline for drug dose titration based on body weight. The drug was titrated each time a rat gained 10 g or more (usually every 1-2 days). Methohexital sodium (1% Brevital Sodium) was purchased from King Pharmaceuticals, Inc. (Bristol, TN). Both amphetamine and Brevital solutions were filtered with 25μm syringe filters (Fisher Scientific, Hampton, NH).

Equipment

Intravenous (i.v) catheters for self-administration were made in the lab, as previously described (Caine et al. 1993; Shabhazi et al. 2007). Briefly, catheters were assembled from metal guide cannulae bent at a right angle and fitted with 14-cm lengths of silastic tubing stretched over the cannulae. Polypro mesh was cut into 3-cm diameter circles and secured to the guide cannula assembly with methylmethacrylate. Tissue growth around the mesh secured the catheter in place after implantation.

The self-administration equipment included operant boxes enclosed in sound-attenuating, ventilated cubicles (Med Associates, Inc., St. Albans, VT). Presses on an active lever triggered a syringe pump with a 6 rpm motor to deliver 0.1 ml of drug solution (Med Associates, Inc., St. Albans, VT) over 2 sec via a stainless steel swivel and a polyethylene tube attached to the catheter portal on the rat's back. Each reinforced response lit a cue light above the active lever, which remained lit throughout the infusion. The cue light, house light, and white noise were not present during a 10 s time-out (TO) after infusion delivery. Progressive ratio (PR) testing included two levers. Lever pressing during TO and presses on the inactive lever were recorded but did not result in any scheduled consequences. Drug delivery and data collection were controlled by Med Associates, Inc. software (Med PC IV).

Surgical Procedures

Catheters were implanted into the rat as previously described (Caine et al. 1993, Shabhazi et al. 2007). Briefly, rats were anesthetized with an isofluorane/oxygen vapor

mixture (4-5% for initial anesthetization and 1.5-3% during surgery) while catheter tubing was passed subcutaneously from the subject's back to the right jugular vein. The tubing was inserted into the vein previously punctured with a 25 gauge needle, and tied carefully with suture thread. During post-surgical recovery, rats received 1.5-2.0 ml Timentin antibiotic (Ticarcillin Disodium and Clavulanate Potassium; 100 mg/ml, i.v.) twice daily on the first two days post-surgery, then once daily after each session throughout the experiment. Catheters were also flushed daily with 1.5-2.0 ml heparinized saline (100 USP units/1ml) pre- and post- session. Catheters were flushed with 0.1-0.4 ml of Brevital sodium (1% methohexital sodium) weekly during testing to ensure i.v. catheter patency. If muscle tone was not lost within 5s, the catheter was concluded to be defective and the subject's data were not included in the analyses. Four rats failed this Brevital test, leaving twelve subjects in most of the data analysis to follow.

Acquisition of Self-Administration on a Fixed Ratio (FR) Schedule of Reinforcement

After four to seven-day post-surgical recovery, rats were tested for spontaneous acquisition of amphetamine self-administration (PND 35 or PND 85-88 at start) on a protocol modeled after the methods of Lorrain and Vezina (1999). A rapid transition from FR to PR schedules of reinforcement was performed in order to ensure adolescent rats began PR testing during the adolescent period, which is generally defined as PND 35-55. (See Fig. 4 for a timeline of experimentation.) During FR1, rats received a single i.v. priming infusion of 0.2 mg/kg amphetamine at the start of each session, and sessions ceased immediately after a subject satisfied the criterion of obtaining at least an

additional 10 infusions, or 2 h elapsed, whichever came first. After FR1, rats proceeded to an FR2 schedule, in which 2 lever presses were required to receive one i.v. drug infusion. Both FR1 and FR2 sessions began with 1 lever extended into the operant chamber and a house light and white noise turned on. Lever-pressing was reinforced by an i.v. infusion of 0.2 mg/kg/0.1 ml amphetamine over 2 s. A cue light above the lever remained on during the infusion, followed by a 10 s timeout (TO) during which the cue light, white noise, and house light remained off.

The FR2 criterion of at least 10 additional infusions (after the priming infusion) during the 2 h session was required for a subject to move to PR testing. Rats that satisfied both the FR1 and FR2 criteria within the initial 5 days of testing were then moved to a PR schedule for the remainder of the study. FR1 and FR2 were used to provide a simple method for subjects to acquire the self-administration behavior prior to challenge with the more complex PR schedule. An amphetamine dose of 0.2 mg/kg was chosen for the FR phase after extensive pilot work with various doses and because previous studies have reported this dose as sensitizing and reinforcing in rats (Vezina et al. 2002; Lorrain and Vezina 2000).

PR Testing

On the PR schedule, the number of responses required to obtain each drug infusion increased gradually within the session, according to an exponential ratio: 1, 3, 6, 9, 12, 17, 24, 32, 42, 56, 73, 95, 124, 161, 208 (Roberts et al. 1989). The daily PR sessions lasted for a maximum of 6 h, or until 1 h elapsed without a drug infusion. The 6

h session length was used to prevent early termination of the session prior to the rat ceasing its drug-seeking behavior. During PR (but not FR) conditions, a second, inactive lever was extended into the chamber but no priming infusions were given; other parameters (cues, TO) in PR remained the same as FR.

PR testing was initiated using either 0.05 or 0.2 mg/kg/infusion amphetamine doses for 5 consecutive days. Rats were divided into two dose groups counterbalanced for infusions obtained during the first few days of PR testing. In the following week, saline substitution was tested (see below). In the third and final week of testing, the alternate amphetamine dose was tested. Each test phase was separated by a two-day recess, including a Brevital test for catheter patency on the second day.

Saline Substitution

Saline substitution was inserted between weeks of amphetamine testing, in part to determine whether lever-pressing behavior was maintained by amphetamine in the weeks prior and subsequent to saline substitution. Saline was tested on the same PR schedule as amphetamine.

Data Analysis and Statistics

Changes in body weight were analyzed in a two-way ANOVA, with cohort x days as factors. The two cohorts analyzed were rats that acquired self-administration in the present study and rats that did not acquire self-administration from Shabhazi et al. (2007) for comparison. Days to reach criterion on FR1 and FR2 were analyzed in a two-way

analysis of variance (ANOVA), with age x schedule as factors. The number of drug infusions, session duration, and lever discrimination on PR testing were averaged over the last four days of testing at each dose for each rat, and total amphetamine intake was summed over the last four days of testing at each dose. The number of drug infusions per session as well as session durations were analyzed using two-way ANOVAs with dose x age as factors. A three-way ANOVA was performed on lever presses (active vs. inactive lever presses), with dose x lever x age as factors. Breakpoints are indicated with infusions per session on graphs, but are not statisticall analyzed (see Introduction). Active lever presses were presses on the active lever that led to drug infusions, whereas inactive presses were on the inactive lever that did not advance the rat toward obtaining another infusion. Follow-up one-way ANOVAs and post-hoc tests (pairwise t-tests) were conducted as appropriate for each dependent variable.

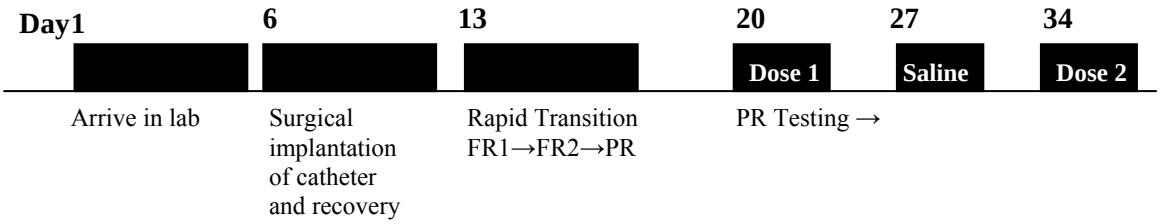


Figure 4. Timeline of experimentation.

Results

Changes in Body Weight in Acquired vs. Non-Acquired Rats

Body weight increased at a similar rate among rats that acquired self-administration in this study and those that did not acquire self-administration (from Shabhazi et al. 2007; Fig. 5). However, body weight differed throughout acquisition testing between adult acquired vs. non-acquired rats. A two-way ANOVA on body weight among adult acquired vs. non-acquired rats, with cohort x days as factors, revealed a main effect of cohort [$F_{(1,12)}$= 13.16, p=0.003] and days [$F_{(9,108)}$= 17.46, p< 0.01]; however, the cohort x days interaction [$F_{(9,108)}$= 1.97, p=0.05] was not significant. The difference in acquired and non-acquired adults is likely due to the different initial weights between groups. Among adolescent rats, a main effect of days [$F_{(9,72)}$=146.46, p<0.01] and cohort x days interaction [$F_{(9,72)}$= 4.07, p<0.01] suggested gradual weight gain, but it did not differ by cohort [$F_{(1,8)}$<0.001, p=0.95].

Rates of Acquisition on FR1 and FR2

Adolescent and adult rats reached criterion levels of pressing on FR1 and FR2 at approximately the same rate (Fig. 6). A two-way ANOVA on the number of days required to reach criterion levels of pressing on each successive schedule revealed no significant main effects of schedule [$F_{(1,15)}$=1.18, p=0.30] or age [$F_{(1,15)}$=0.39, p=0.54], nor was the schedule x age interaction significant [$F_{(1,15)}$=0.35, p=0.57].

Infusions Per Session on PR Schedule

Infusions per session on the PR schedule increased with dose per infusion, but did not vary by age (Fig. 7 and 8). A two-way ANOVA with dose x age as factors, number of infusions being the dependent measure, revealed a significant main effect of dose [$F_{(2,20)}$=8.38, p=0.002], but neither the main effect of age [$F_{(1,10)}$ = 0.018, p=0.90], nor a significant dose x age interaction was significant [$F_{(2,20)}$=0.52, p=0.60]. These statistics were calculated using only rats that were tested on all doses (0.00, 0.05, and 0.2 mg/kg/infusion; n= 5 adolescents and n= 7 adults). A follow-up one-way ANOVA and paired t-tests on the dose factor revealed that rats took more infusions at the 0.05 or 0.2 mg/kg/infusion doses compared with saline ($p<0.05$), and more infusions at 0.2 compared with 0.05 mg/kg/infusion as well.

Total Amphetamine Intake During PR Testing

Total amphetamine intake increased with dose per infusion, but did not vary by age (Fig. 9). A 2-way ANOVA with dose x age as factors, total intake being the dependent measure, revealed a significant main effect of dose [$F_{(1,10)}$=23.64, p=0.01], but no main effect of age [$F_{(1,10)=}$0.66, p=0.43], nor a significant interaction between dose and age [$F_{(1,10)}$=0.73, p=0.40].

Active vs. Inactive Lever Presses

Presses on the active lever outnumbered inactive lever presses on all doses, and active lever presses increased as dose increased. However, no age difference in active or inactive presses was identified (Fig. 10). Thus, a three-way ANOVA with dose x age x lever type as factors, lever presses being the dependent measure, revealed a significant main effect of dose [$F_{(2,20)}$ =4.48, p=0.025] and response type [$F_{(1,10)}$ =5.50, p=0.041], as well as a significant dose x response interaction [$F_{(2,20)}$=4.32, p=0.028], but no main effect of age [$F_{(1,10)}$=0.35, p=0.57], nor interactions between dose x age [$F_{(2,20)}$= 0.16, p=0.85] or response x age [$F_{(1,10)}$=0.41, p=0.54]. Follow-up tests revealed that active lever presses increased as the dose increased, whereas presses on the inactive lever did not differ by dose. Moreover, significant discrimination between active and inactive responding occurred at all amphetamine doses.

Session Duration

PR session duration did not differ by dose or age (Fig. 11). A two-way ANOVA with age x dose as factors, session duration being the dependent measure, revealed no significant main effect of dose [$F_{(2,20)}$=1.92, p=0.17], age [$F_{(1,10)}$= 2.21, p=0.17], nor a significant dose x age interaction [$F_{(2,20)}$=0.61, p=0.55].

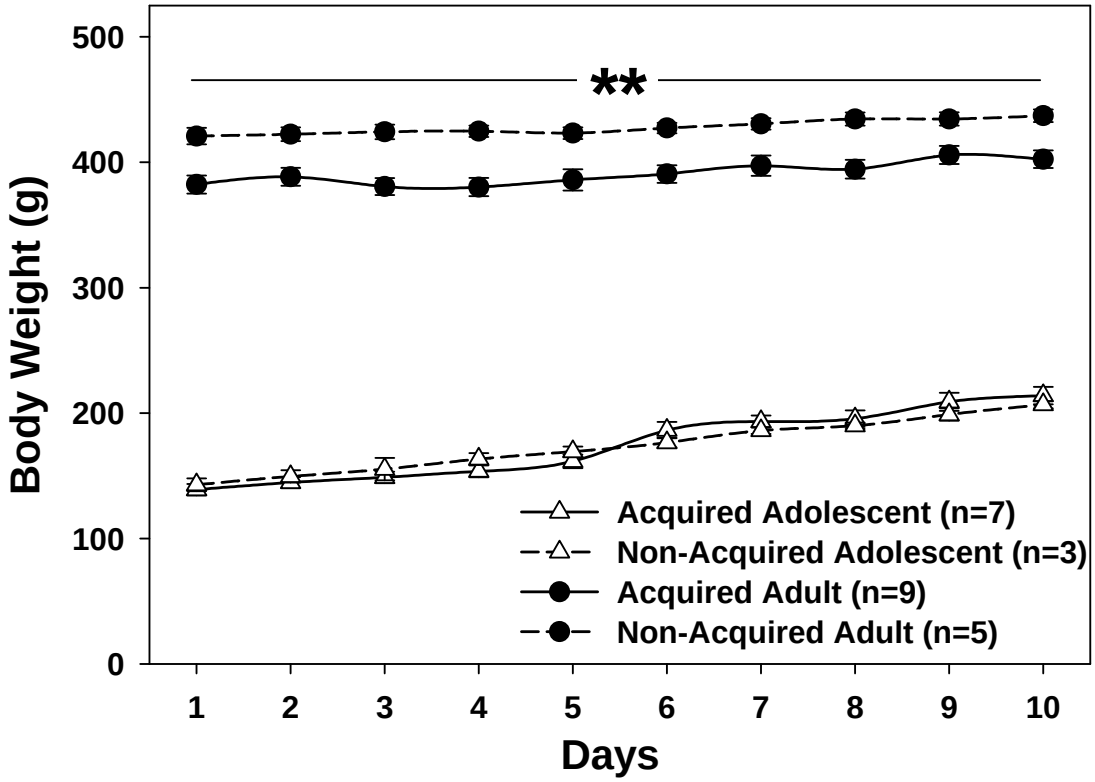


Figure 5. Body weight increased over days in experimentation for adolescent and adult male rats, similarly in rats that did vs. did not reach acquisition criteria by day 10. [All rats that did not reach acquisition criteria derived from a separate experiment (Shahbazi et al. 2007)]. Values are mean ± SEM. A significant main effect of adult cohort is indicated (**$p<0.01$).

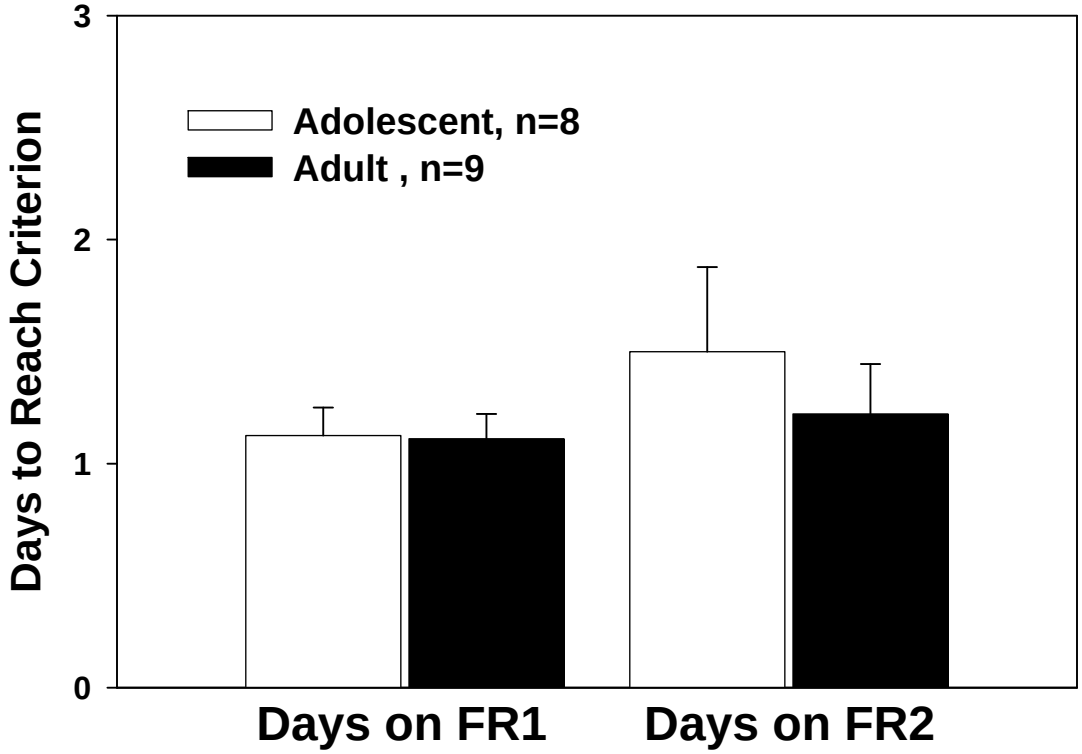


Figure 6. Adolescent and adult rats reached the criterion number of presses on FR1 and FR2 schedules at approximately the same rate. Bars represent mean ± SEM days on each schedule before moving to the next schedule (see Methods).

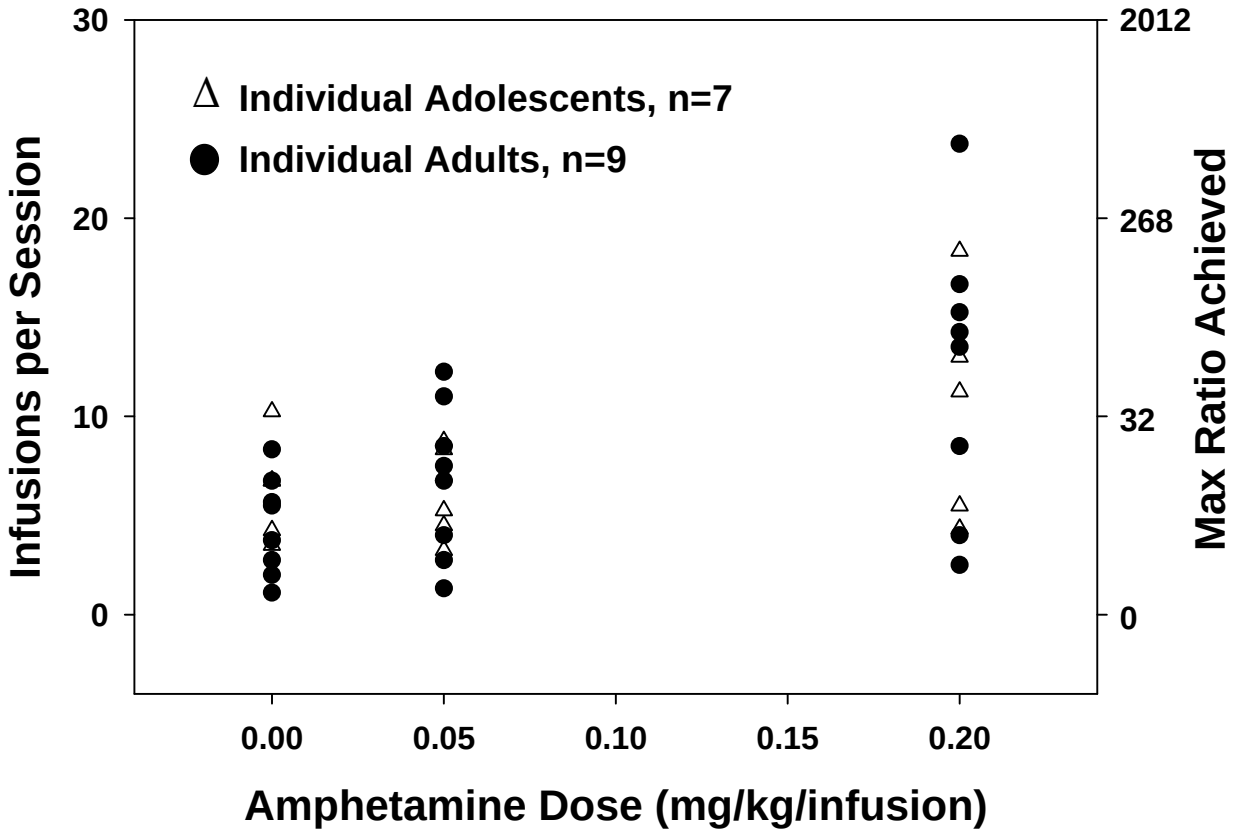


Figure 7. Average number of infusions for individual adolescent and adult rats, over the last four self-administration sessions at each amphetamine dose.

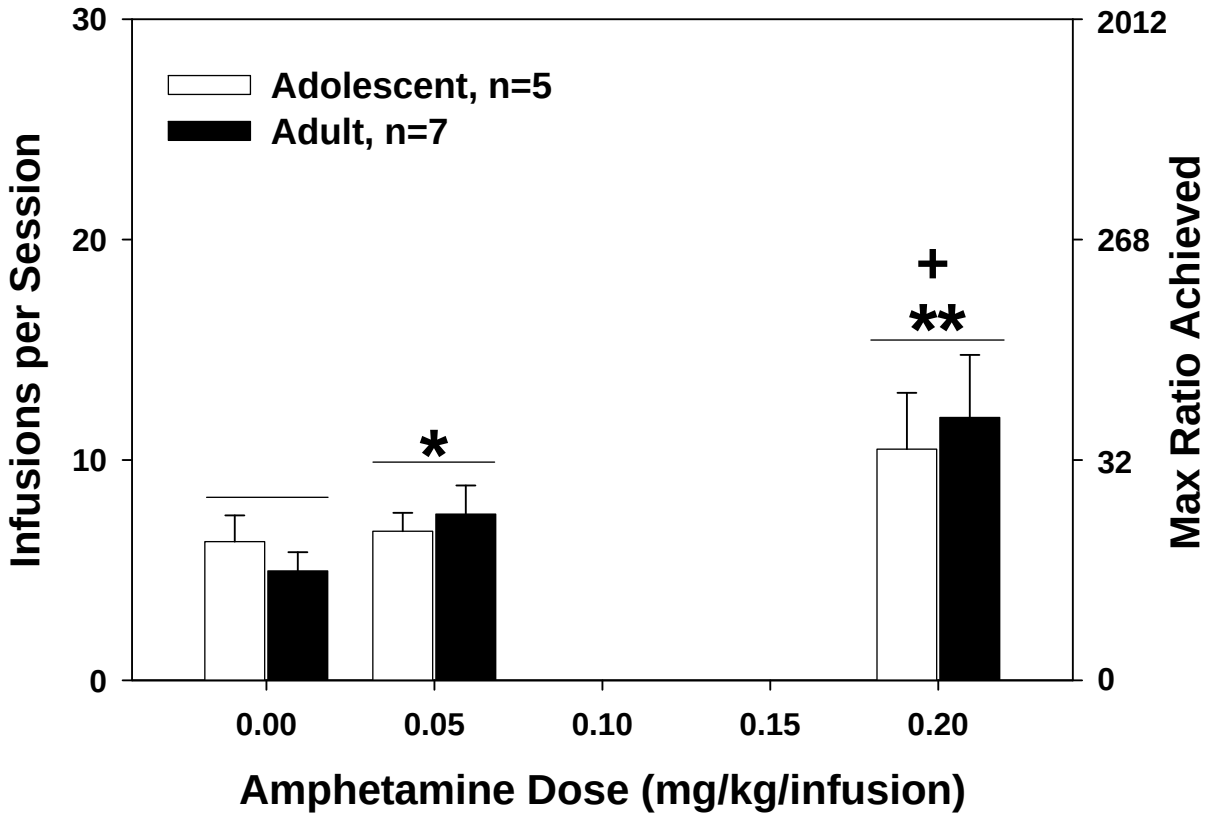


Figure 8. Average number of infusions for adolescent and adult rats, over the last four self-administration sessions at each amphetamine dose. Bars represent mean ± SEM. Only those rats that self-administered all doses are included. Significant differences from saline conditions (0 mg/kg/infusion; * $p<0.05$, ** $p<0.01$) and the low amphetamine dose (0.05 mg/kg/infusion; + $p<0.05$) are indicated.

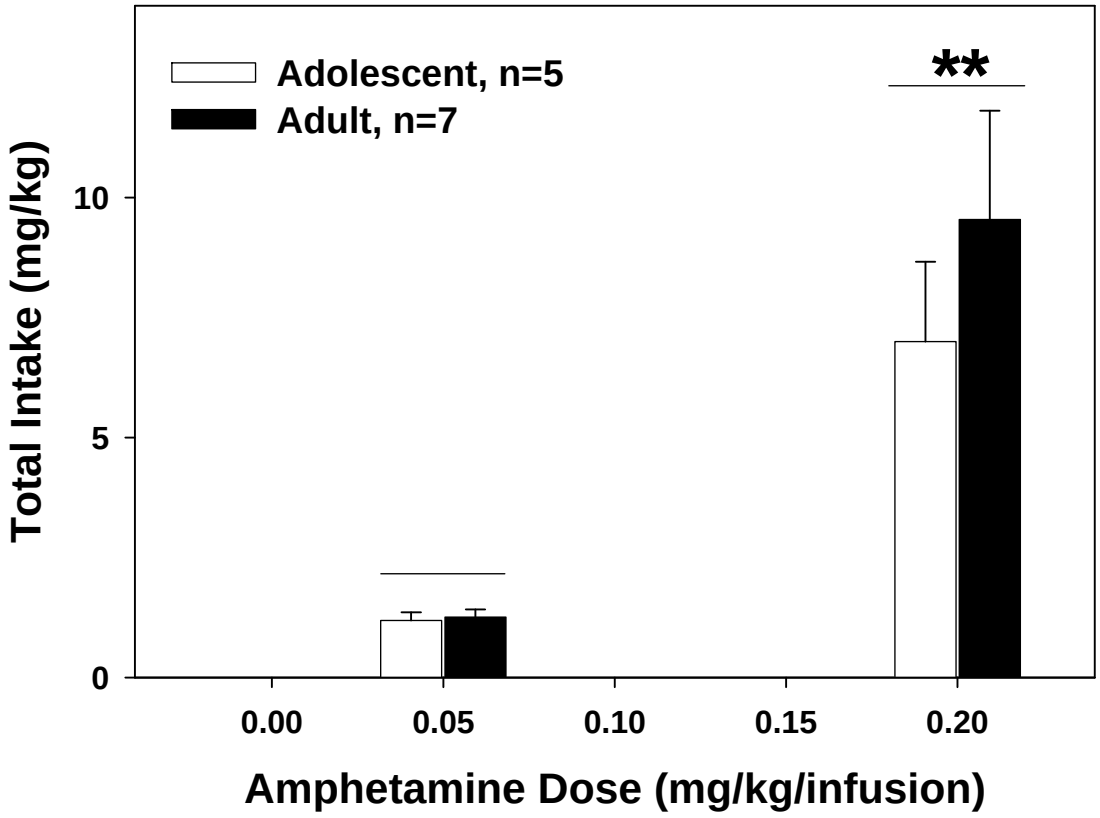


Figure 9. Total amphetamine intake (mg/kg) summed over the last four sessions at each amphetamine dose. Bars represent mean ± SEM. Only those rats that self-administered all doses are included. Significant main effects of dose are indicated (**$p<0.01$).

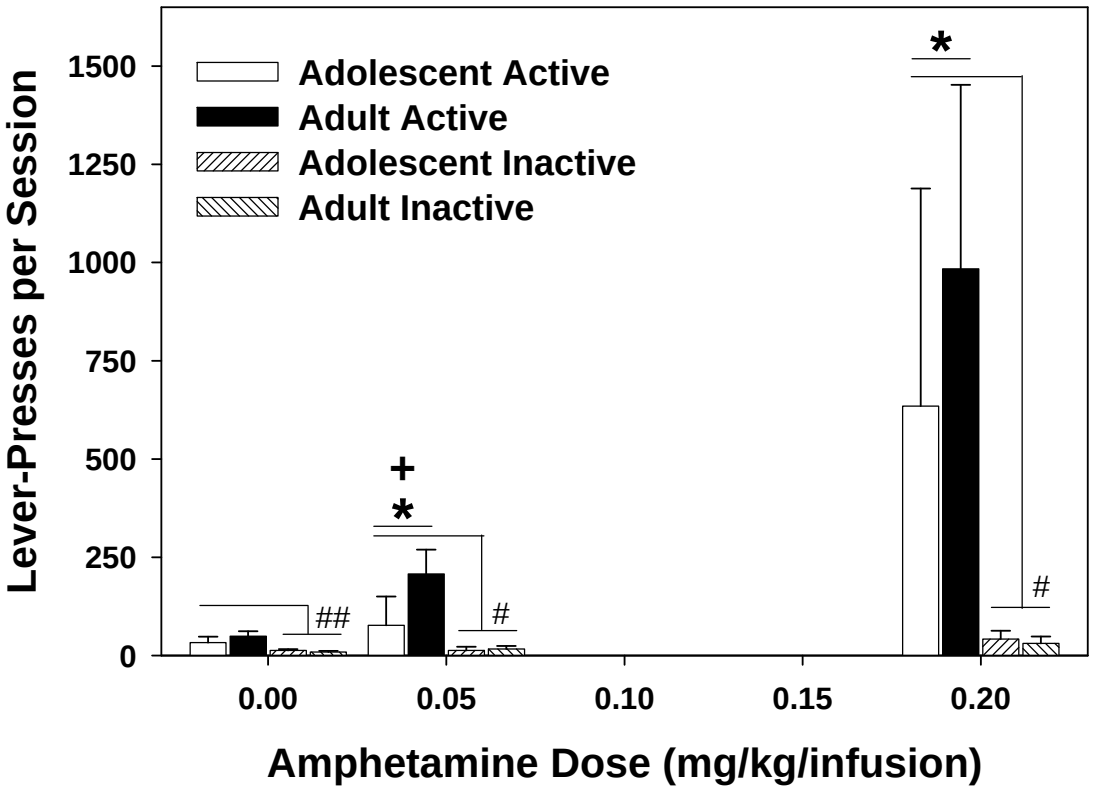


Figure 10. Active vs. inactive lever-presses per session averaged over all sessions at each amphetamine dose. Bars represent mean ± SEM. Significant differences between levers are indicated at each dose (# $p<0.05$, ## $p<0.01$), along with differences between active lever-presses across doses (*$p<0.05$ different from saline; +$p<0.05$ different from 0.2 mg/kg/inf). Presses did not vary by age. Only those rats that self-administered all doses are included

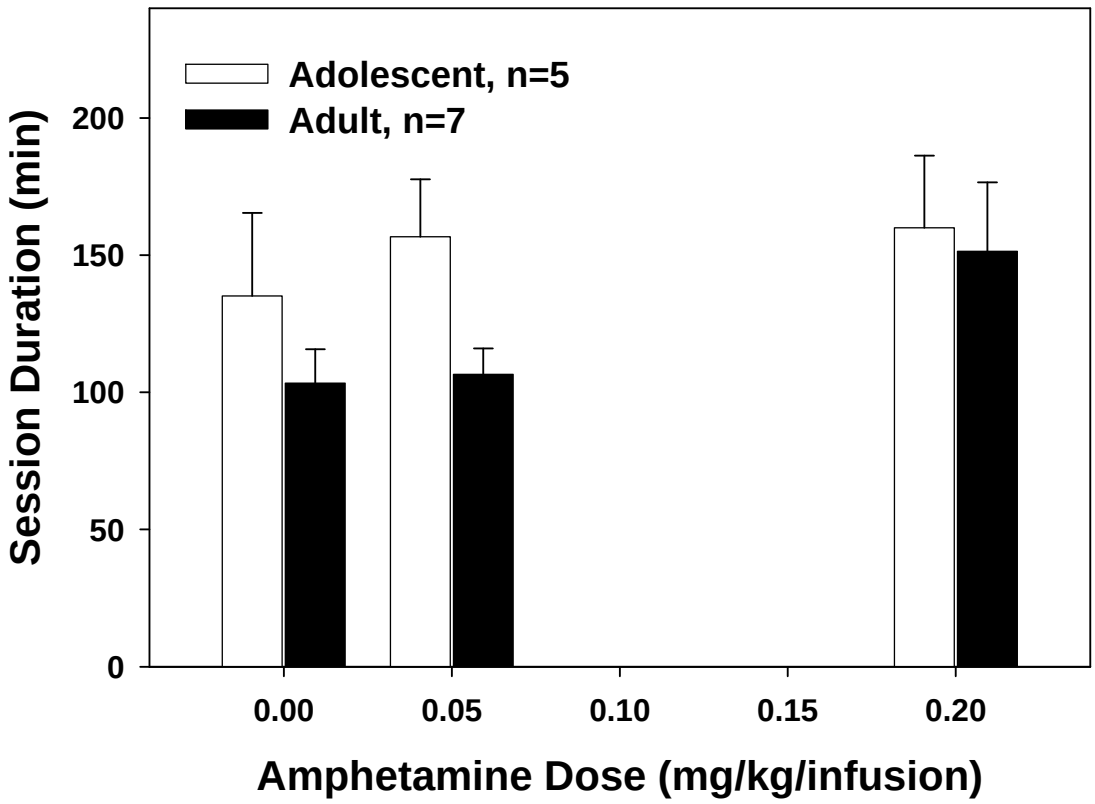


Figure 11. Duration of self-administration sessions averaged over the last four sessions at each amphetamine dose. Bars represent mean ± SEM. Only those rats that self-administered all doses are included.

Discussion

The present results suggest that amphetamine's reinforcing effects are similar in adolescent and adult male rats in the i.v drug self-administration model. On a PR schedule of reinforcement, both age groups demonstrated the expected dose-effect function, with maximal number of infusions increasing as amphetamine dose increased (Green et al. 2002; Vezina et al. 2002). Similarly, total amphetamine intake increased with amphetamine dose. However, neither measure differed by age group. These data do not support the hypothesis of adolescent hyposensitivity to amphetamine reinforcement. The data could suggest that previously reported age differences in response to amphetamine do not extend to reinforcing efficacy.

Even though few studies have reported i.v. drug self-administration in adolescent rats (Belluzi et al. 2005, Frantz et al. 2006; Shabhazi et al. 2007), several aspects of the current study assist in validating this rapid transition FR-PR method as effective in identifying the reinforcing efficacy of a drug during adolescence. First, this protocol supported the expected dose-dependent responding in three different cohorts (n=16 rats). The dose-effect function exhibited on the PR schedule corresponds to other reports of a monotonic dose-effect function on PR, with number of infusions and total drug intake received increasing as dose increases (Green et al. 2002; Vezina et al. 2002). Second, the maximal infusions received in this study were similar to maximal infusions obtained in the Green et al. 2002 study. Third, the lower infusions taken during saline substitution in comparison to infusions during the two amphetamine doses help to validate the assertion that responding on the PR schedule was maintained by amphetamine (Arnold and Roberts,

1997; Shabhazi et al. 2007). Fourth, clear active vs. inactive lever discrimination was observed in both adolescents and adults on all three test doses, suggesting that subjects made efficient associations between levers and drug availability. Finally, gradual increases in body weight in both age groups in the present study indicated that the health of the rats was maintained throughout the experiment. Therefore, the present results are generally in agreement with previous amphetamine and cocaine studies reporting that the rapid transition FR-PR is an effective method of reinforcing self-administration in rats (Lorrain and Vezina 2000; Suto et al. 2002, 2003).

The present results contrast outcomes from Shabhazi et al. (2007). In the prior study, adolescent and adult male rats acquired self-administration on FR over 14 consecutive days, and adolescents took more infusions than adults on the 0.05 mg/kg/infusion amphetamine dose. Two possible interpretations emerged. First, adolescents could be *more* sensitive to the reinforcing effects of amphetamine, suggested if the increased amphetamine intake by adolescents is part of an upward shift in the dose-effect function (Piazza et al. 2000). Second, adolescents could be *less* sensitive to the reinforcing effects of amphetamine, suggested if the increased intake is part of a rightward shift in the dose-effect function. On the present PR schedule, higher infusions per session would support the first interpretation, whereas lower infusions per session would support the second. In fact, the complete lack of age differences in the present PR study failed to support either interpretation. If validated in future replications of this experiment (see below), these results imply that adolescents will take more amphetamine than adults if response requirements are “easy”, but that they demonstrate adult levels of

intake if behavioral demands are higher (e.g. on a PR schedule). Alternatively, these results could indicate that higher rates of amphetamine intake by adolescents on the FR schedule could be mediated by general motor activation rather than increased goal-directed behavior (see next; Adriani et al. 1998; Collins et al. 2004).

Motor activation comes into question when interpreting the Shabhazi results, as the observed age differences could be due to generalized motor activation among adolescents when compared to adults after exposure to amphetamine, which has been reported in several previous studies (Adriani et al. 1998; Collins et al. 2004). However, other studies have indicated lower locomotor activation and neurochemical sensitivity in adolescents after exposure to amphetamine or its derivatives (Laviola et al. 1999; Bolanos et al. 1998; Kokoshka et al. 2000). Moreover, qualitative visual analysis of video recordings of a representative rat from each age group in the present study suggests no age difference in locomotor activity after exposure to amphetamine. Overall, further investigation into the motor and neurochemical effects of amphetamine could help to interpret the Shabhazi study as well as current results.

The present results are consistent with other elements of the Shabhazi report and several experiments showing similar amphetamine-induced behavior in adolescent and adult subjects. The Shabhazi study reported no age difference between male rats in the number of infusions on the FR schedule at a lower amphetamine dose (0.025 mg/kg/infusion). Likewise, other studies have identified no age differences in response to amphetamine or other psychomotor stimulants, such as cocaine (Adriani et al 1998; Andersen et al. 2001; Frantz et al. 2006; Shram et al. 2007). Altogether the literature on

adolescent sensitivity to amphetamine is mixed, suggesting that adolescent vulnerability to stimulants is a complex issue.

Several limitations of the present method should be considered. First, although the rapid transition FR-PR method did produce the expected dose-dependent responding on PR, some rats never acquired stable lever-pressing behavior on this schedule. Out of 61 rats tested on the rapid transition protocol, eight adults and five adolescents did not acquire lever-pressing behavior and did not advance from FR to PR. In addition, out of six cohorts tested on variations of the rapid transition schedule (varying amphetamine dose, time-out and session durations, priming infusions, etc.), only the three cohorts reported at present (n=12 rats) demonstrated the expected dose-dependent responding. These results suggest that the rapid transition schedule may produce only tenuous associations between lever-pressing and i.v amphetamine infusions. Moreover, active lever presses during infusions and timeouts were not recorded. Shabhazi et al. (2007) reported higher timeout presses in adolescents, which could indicate high impulsivity and lower behavioral control by environmental stimuli (Laviola et al. 1999; Adriani and Laviola 2003). However, visual analysis of a representative adolescent and adult rat in the present study showed little pressing in either rat during infusions and timeouts**,** suggesting that timeout presses were not common throughout the experiment.

Another methodological concern is that infusion volume remained constant for all rats throughout the study. Amphetamine doses were titrated for each animal based on body weight by changing the concentration of drug solution, while infusion volume (0.1 ml) remained constant. Therefore, the higher volume to body weight ratio in adolescents

compared with adults could exert unknown aversive effects on adolescents. Nevertheless, current studies in our lab involving cocaine self-administration (Li et al. 2007) indicate no effect of titration method on either age group self-administering cocaine. Finally, five adolescent and seven adult rats successfully completed testing on all amphetamine doses for the present study, which is a low sample size and leads to very low statistical power of analysis. Coupled with high variability in lever-pressing among individual animals, the data collected with such low subject numbers could be inconclusive, as a small sample size could fail to show real age differences in self-administration on the PR schedule.

The present lack of age differences in amphetamine reinforcement is surprising in light of the major developmental changes in brain reinforcement circuitry that occur during adolescence in rats (as well as primates). Some of the structural and neurochemical changes in the mesocorticolimbic system during adolescence include a several-fold increase in dopamine receptor density (that undergoes pruning prior to adulthood) and an increase in dopamine transporter density in the striatum (that subsequently decreases through old age; Izenwasser 2005). Previous studies reporting amphetamine and other psychomotor stimulant effects on this circuit have shown that these drugs stimulate dopamine levels in the nucleus accumbens, which should affect general motor behavior as well as responding reinforced by the drug (e.g. Frantz et al. 2006). However, motor and reinforcing properties of amphetamine can be dissociated from each other (Cirulli and Laviola 2000). Therefore, although there are structural differences between adolescents and adults in the brain regions thought to underlie both

motor activity and behavioral reinforcement (e.g. nucleus accumbens), these differences could change motor activity but not reinforcing efficacy of amphetamine.

Future research may clarify the current findings. First, the present test on the rapid-transition FR-PR schedule should be replicated to increase the sample size and confirm the lack of age differences between adolescents and adults. In addition, other group comparisons (e.g. young adults, senescent adults, strains, and sex) could be tested on this schedule to determine whether the present results generalize to broader rodent models, as other studies have reported sex (Shabhazi et al. 2007) and rat strain differences in drug self-administration (Shram et al. 2007). Finally, if the rapid-transition schedule continues to produce tenuous associations between behavior and reinforcers, then the self-administration protocol may need to be modified to allow rats to train longer on FR prior to moving to PR. This could include increasing the number of daily FR sessions for each rat, or PR session duration (to ensure stable self-administration training in the operant paradigm on FR, or avoid early termination of the rat's motivation to obtain an infusion during PR).

Overall, rodents appear to be good behavioral models for some aspects of human drug-related behavior, and the progressive ratio schedule of reinforcement appears to define the reinforcing efficacy of a drug. To the extent that results from studies using animal models can be applied to the human condition, the current study suggests that adolescents are just as vulnerable as adults to the reinforcing effects of amphetamine. Coupled with poor decision-making, extended amounts of leisure time, new access to money, and an increase in the influence of peers on behavior that can occur during human

adolescence, effective behavioral reinforcement by amphetamine may make teenagers more likely than adults to initiate drug intake and progress toward drug dependence. As is the case for all measures of animal behavior, individual variability related to genes and environment is likely to influence decisions regarding drug use or abuse during both adolescence and adulthood.

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