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Age and sex differences in behavioral flexibility, sensitivity to reward value, and risky decision-making

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Abstract

Compared to adults, adolescent behavior is often characterized by reduced behavioral flexibility, increased sensitivity to reward, and increased likelihood to take risks. These traits, which have been hypothesized to confer heightened vulnerability to psychopathologies such as substance use disorders (SUDs), have been the focus of studies in laboratory animal models that seek to understand their neural underpinnings. However, rodent studies to date have typically used only males and have adopted standard methodological practices (e.g., weight loss inducing food restriction) that are likely to have a disparate impact on adolescents compared to adults. Here, we used adolescent and adult Sprague-Dawley rats of both sexes to study instrumental behavior tasks that assess behavioral flexibility (strategy shifting and reversal learning; Exp. 1), sensitivity to reward value (outcome devaluation; Exp. 2), and risky decision making (probability discounting; Exp. 3). In Exp. 1, we found that adolescents were faster to acquire reversal learning than adults but there were no differences in strategy shifting. In Exps. 2 and 3, adolescents and adults were equally sensitive to changes in reward value and exhibited similar reductions in preference for a large reward when reinforcement probability was decreased. However, adolescents responded more efficiently and earned reinforcers at a higher rate than their same-sex, adult counterparts. Together, these findings provide only limited support for the existence of an “adolescent-typical” phenotype in Sprague-Dawley rats and instead suggest that age differences in the expression of these behaviors may depend on conditions such as pubertal status and motivational state.

Keywords

adolescence; risk-taking; behavioral flexibility; reward sensitivity

Introduction

Adolescence, which is the transitional period between childhood and adulthood that is characterized by physical, socioemotional, hormonal, and behavioral changes, is a time when psychopathologies such as SUD often emerge (Chen, Storr, & Anthony, 2009). Prominent theories of adolescent behavior propose that delayed neurobehavioral

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development leads to a relative imbalance between the early-maturing reward system and the late-maturing cognitive control system, which underlies an individual's self-regulation abilities (Casey, Jones, & Somerville, 2011; Shulman, Harden, Chein, & Steinberg, 2015; Steinberg, 2010; Steinberg, 2008). This imbalance is hypothesized to contribute to the adolescent-typical behaviors, such as increased risk taking, that are associated with risk for developing SUDs (Jupp & Dalley, 2014; Mitchell et al., 2013). Behavioral flexibility, one component of cognitive control, is the ability to inhibit inappropriate behaviors to promote more adaptive ones in response to environmental changes (Brown & Tait, 2014). In humans, behavioral flexibility tends to increase from late childhood to young adulthood with girls performing better than boys at younger adolescent ages and boys increasing performance across adolescence (Kalkut, Han, Lansing, Hodnack, & Delis, 2009). Age is negatively associated with risk taking both in laboratory tasks of risky choice (Cauffman et al., 2010; Eshel, Nelson, Blair, Pine, & Ernst, 2007; Gardner & Steinberg, 2005) and in self-report measures (Gardner & Steinberg, 2005). In addition, males tend to make more risky choices than females (van Leijenhorst, Westenberg, & Crone, 2008). Sensation- or reward-seeking peaks in adolescence and males generally exhibit higher levels than females (Shulman et al., 2015). Moreover, pubertal status and age have been shown to be dissociable in humans, as pubertal status positively correlates with adolescent sensation-seeking even when controlling for age (Martin et al., 2002). Collectively, these results are in line with a hypersensitive reward system and protracted maturation of the cognitive control system in adolescents and highlight the influence of sex in these behaviors.

Studies that utilize rodent models of adolescence, which have potential advantages for investigating underlying neural mechanisms of age differences in behavior, have typically focused on responses to psychoactive drugs (Kantak, Goodrich, & Uribe, 2007; Shahbazi, Moffett, Williams, & Frantz, 2008; Shram, Funk, Li, & Le, 2008; Anker & Carroll, 2010; Anker, Baron, Zlebnik, & Carroll, 2012; Hankosky, Westbrook, Haake, Marinelli, & Gulley, 2017). These studies using drug reinforcers can be challenging to interpret, however, because it is difficult to separate the contribution of adolescent developmental changes in general reward and cognitive control processes from those induced by the drugs themselves. Notably, adult rats that self-administer psychostimulants have been shown to have deficits in reward processing (Green, Dykstra, & Carelli, 2015), impaired behavioral flexibility (Cox et al., 2016), and increased risk taking (Mitchell et al., 2013). An alternative approach that avoids these potentially confounding drug effects on behavior is to substitute non-drug reinforcers in the analysis of potential age effects.

Rodent studies of age differences in behavioral flexibility that use positive (food) or negative (escape from water) reinforcement have reported mixed results (Simon, Gregory, Wood, & Moghaddam, 2013; Newman & McGaughy, 2011; Willing, Drzewiecki, Cuenod, Cortes, & Juraska, 2016), with factors such as pubertal onset potentially contributing to disparate findings (Willing et al., 2016). Studies of age differences in reward processing also highlight the importance of the peripubertal period in males, as there is a peripubertal peak in motivation for (Friemel, Spanagel, & Schneider, 2010) and consumption of palatable reward (Friemel et al., 2010; Marshall, Liu, Murphy, Maidment, & Ostlund, 2017). In the only study to concurrently investigate age and sex differences in a Pavlovian approach task, our lab found that females and adolescents were less sensitive to reward devaluation (Hammerslag

& Gulley, 2014). In adults, sex differences have been reported where females were insensitive to devaluation of a food reinforcer (Quinn, Hitchcott, Umeda, Arnold, & Taylor, 2007). One explanation for reduced response suppression following reward devaluation is that adolescents and females maintain a greater value representation of the outcome due to their relative hypersensitivity to reward. However, our previous study only examined age and sex differences when the reward was devalued. To date, a single rodent study in males reported age differences in risky choice, with adolescents exhibiting less sensitivity to reward probability compared to adults (Zoratto, Laviola, & Adriani, 2013). Sex differences in risky choice have only been examined in adults, with males generally displaying more risky behavior than females (for review see Orsini & Setlow, 2017). Thus, although there appears to be emerging evidence of age and sex differences in reward sensitivity, behavioral flexibility, and risk taking, the current literature is mostly limited to either male or adult subjects, respectively, which precludes analysis of interacting effects of age and sex in these behavioral domains.

The goal of the present study was to address these gaps by using adolescent and adult Sprague-Dawley rats of both sexes to concurrently examine age and sex differences in behavioral flexibility using an operant strategy-shifting task (Exp. 1), reward value sensitivity in an instrumental outcome devaluation task (Exp. 2), and risk-based decision making in a probability discounting task (Exp. 3). In line with leading theories of adolescent vulnerability that propose adolescents are hypersensitive to reward and exhibit poor cognitive control (Casey et al., 2011; Shulman et al., 2015; Steinberg, 2010), we predicted that adolescents would display less behavioral flexibility, enhanced sensitivity to reward value, and increased risky choices relative to adults. In addition, consistent with previous rodent studies using non-drug reinforcers (Hammerslag & Gulley, 2014; Orsini & Setlow, 2017; Quinn et al., 2007), we predicted that these differences would be more pronounced in males relative to females.

Methods

Subjects

Subjects were 147 Sprague-Dawley rats (69 male, 78 female) born in-house from breeders originally obtained from Envigo (Indianapolis, IN, USA). Rats were weaned on postnatal day (P) 22 and housed 2-3 per cage in a temperature-controlled room on a 12-h reversed light/dark cycle (lights off at 0900). Rats were weighed daily beginning on P25 (Exp. 1 & 3) or P33 (Exp. 2). A subset of the rats (38 male, 43 female) were checked daily for markers of pubertal onset, which are vaginal opening in females and preputial separation in males, (Castellano et al., 2011; Korenbrot, Huhtaniemi, & Weiner, 1977). These checks began on P30 and continued until all rats reached puberty. In this sample, average pubertal onset was estimated to be at 34.9 ± 0.2 days for females (range: 32-39 days) and 43.6 ± 0.2 days for males (range: 41–47 days).

Water was available in the homecage *ad libitum* throughout the study. Food was available *ad libitum* until the start of the behavioral training when it was limited to 20 h/day. Specifically, food was removed from the homecage 2 h prior to behavioral sessions and was returned 0.5-2 h after their conclusion. Rats maintained on this regimen exhibit sufficient motivation

during instrumental behavior sessions, but maintain body weights that are not significantly different from those in age-matched, free-fed controls during the peri-adolescent period of rapid growth (Fig. 1A). All experimental procedures were approved by the Institutional Animal Care and Use Committee at the University of Illinois, Urbana-Champaign, and followed the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011).

Apparatus

Rats were trained and tested in standard operant chambers (Coulbourn Instruments; Whitehall, PA, USA) that were housed inside sound-attenuating cubicles. The cubicles were equipped with fans that provided ventilation and masked extraneous noise. One wall of each operant chamber was equipped with a centrally located food trough outfitted on either side with a response module (retractable levers in Exp. 1, Exp. 2; recessed nosepoke ports in Exp. 3) that were equidistant (87 mm) from the trough. White cue lights were located above each response module and a white houselight was located near the chamber ceiling on the wall opposite the food trough. Sessions were recorded and analyzed using Graphic State software (Coulbourn Instruments; Allentown, PA).

Exp. 1: Strategy Shifting

The timeline for training and testing for the 23 male and 25 female rats used in Exp. 1 is shown in Fig. 1B. Training began with one session of “magazine training”, during which 40 pellets (45 mg; Bio-Serv F0021 Dustless Precision Pellets) were delivered individually into the food trough on a random time 100-sec schedule. Subsequently, rats were trained during four 30-min sessions (1 session/day) to respond on both levers on a fixed ratio 1 (FR1) schedule of reinforcement. The order in which the levers were presented alternated each day. Rats that did not acquire lever pressing were manually shaped using successive approximations. Following lever press training, rats were trained during seven sessions to respond within 10-sec of lever extension into the chamber. Each rat’s side bias was then established in a single session as described by Floresco, Block, and Tse (2008).

Daily strategy-shifting sessions consisted of 120 trials separated by a 20-sec intertrial interval (ITI). During each trial, one of the two cue lights located above the levers was illuminated and both levers were extended into the chamber. Individual cue lights were presented pseudorandomly across trials, such that no cue light could be presented on more than two consecutive trials. Rats were first trained to use a visual strategy by reinforcing them for pressing the lever that had a cue light illuminated above it, regardless of the lever’s spatial location (i.e., left or right side of pellet delivery trough). An incorrect choice resulted in retraction of the levers, 10-sec illumination of the houselight, and a return to the ITI. Trials continued until rats achieved eight consecutive correct choices and had completed at least 30 trials.

On the day following acquisition of this performance criterion, or after a maximum of five visual strategy training sessions, rats began sessions where they could complete a within-session shift to an egocentric response strategy. This within-session shift, which occurred following eight consecutive correct choices using the visual strategy, required rats to respond

according to lever position (e.g., the left lever), regardless of cue light location, for reinforcement to occur. The location of the reinforced lever for the response strategy was determined individually for each rat such that it was the lever opposite of the rat's side bias observed during initial training. Trials continued until rats achieved a criterion of eight consecutive correct choices or a maximum of 120 trials. Rats received up to a maximum of three sessions to complete the within-session shift. Rats were shifted from the visual strategy to the response strategy as shifting in this direction has been shown to be medial PFC-dependent, while the opposite shifting direction was not (Floresco et al., 2008).

Following acquisition of the response strategy or the maximum of three sessions, rats received two daily response strategy sessions consisting of 75 trials each. One day following these sessions, the response strategy was reversed using a within-session design. The session started with the original response strategy reinforced until rats had eight consecutive correct lever choices. Subsequently, the rule was reversed such that they were reinforced for pressing on the previously non-reinforced lever (e.g. the right lever). As before, trials continued until rats achieved a criterion of eight consecutive correct choices. For all stages of the task, trials where rats failed to respond on a lever within 10-sec of trial onset were terminated, scored as an omission, and were followed by a 10-sec illumination of the houselight before the ITI commenced. Omitted trials were repeated until rats made a response within the required time.

Exp. 2: Outcome Devaluation

The timeline for training and testing for the 28 male and 32 female rats used in Exp. 2 is shown in Fig. 1B. Rats began training with two daily 60-min sessions of pre-exposure to two food reinforcers: 45-mg food pellets (Bio-Serv F0021 Dustless Precision Pellets) and 66.7% sweetened condensed milk (SCM; Meadow Gold). In these sessions, which were intended to reduce neophobia, rats were individually placed in cages and given free access to the food reinforcers for 30 min each. The following day, rats began training in the operant chambers with a single session of "magazine training" that lasted for 30 trials. For these sessions, the houselight was off, levers were retracted, and the food trough was illuminated for 5 s when a reinforcer was delivered on a random time 60 s (RT-60). Reinforcer type (food pellet or SCM) was counterbalanced across rats and a single food pellet or 0.04 ml dipper cup of SCM was delivered for each trial. Training continued with two sessions of autoshaping that each involved up to 25 reinforcer deliveries as a result of lever pressing (30 min duration). For each trial, the houselight and a cue light above a single extended lever was illuminated and the position of the lever (left or right of the food trough) was counterbalanced across rats. When a response occurred (FR1) or a time interval elapsed (RT-60), the lever was retracted, the houselight was extinguished, and the assigned reinforcer was delivered along with illumination of the food trough for 5 s. During two additional training sessions on subsequent days, reinforcers were only delivered following a lever press (FR1). Rats that made fewer than 10 responses during these sessions were manually shaped for 30 min.

Following this initial training, rats received sessions with reinforcement on a random/variable interval 30 s (RI-30) schedule that ended after 50 outcomes were earned, 20-min elapsed without a lever press, or 90 total min elapsed. Three RI-30 sessions preceded

extinction test set 1 and three additional RI-30 sessions occurred prior to extinction test set 2. A single test set consisted of two extinction test days (one of each value condition) separated by one re-training RI-30 session. On extinction test days, rats received a 60-min pre-feeding session in which they were given *ad libitum* access to either the instrumental outcome (devalued condition) or the alternative reinforcer (valued condition). Immediately following pre-feeding, rats were placed in the operant chambers for a 10-min test under extinction conditions in which lever presses were recorded but had no consequence. Following the extinction test, rats were returned to their pre-feeding cages for a 15-min post-test consumption where they had simultaneous access to both reinforcers. The order of outcome value condition for the test days was counterbalanced across rats.

Exp. 3: Probability Discounting

The timeline for training and testing for the 18 male and 21 female rats used in Exp. 3 is shown in Fig. 1B. During the two days prior to the first training session, rats were given ~25 food pellets (45 mg; Bio-serv F0021 Dustless Precision Pellets) in their homecage on two successive days. Training began with one session of magazine training during which the nosepoke ports were covered and the houselight extinguished. A food pellet was delivered and the magazine light illuminated for 5 sec on an RT-30 reinforcement schedule for a total of 30 deliveries. On the following day, the nosepoke ports were uncovered and assignment of the ports to a small (1 food pellet) or large (3 food pellets) reward was counterbalanced across rats. Illumination of a green light inside one port signaled that the port was active and rats were trained to nosepoke on an FR1 schedule for a total of 25 reinforcements/session or 30 min had elapsed. Only one port was illuminated per session and the active port was alternated for four daily sessions. If the rat failed to nosepoke within 10 s following illumination of the active port, the trial was scored as an omission, and the houselight was illuminated to begin the 20-s ITI to repeat the trial.

Test sessions consisted of 12 forced choice trials followed by 12 free choice trials. During the forced choice trials, one active port was illuminated randomly each trial for a total of 6 presentations of each port. During the free choice trials, both ports were active and illuminated. Omitted trials were repeated following the ITI. Responses in the small, certain reward port were reinforced with delivery of 1 pellet at 100% probability. Responses in the large, potentially risky reward port were reinforced with delivery of 3 pellets at 100, 66.7, 33.3, or 16.7% probability that descended across sessions. In 23 rats (10 male, 13 female), four sessions at 100% were followed by three sessions each at 66.7, 33.3, and 16.7% probabilities. In the remaining 16 rats (8 male, 8 female), the procedure was modified slightly so that they received 100% sessions with only 6 forced choice trials until each rat in the group showed a preference (higher than 50% choice) for the large, risky port before they were allowed to proceed to the three sessions at each of the other probabilities. This change, which resulted in the first cohort of rats having 4 sessions at 100% probability and the second having 7-8 sessions, was implemented due to concerns about satiation from the large number of pellets rats received during the 100% probability forced choice trials. However, subsequent analysis of the test session data with age, sex, probability, and cohort as factors reveal there was no main effect of cohort ($F_{1,31} = 3.54$; $p = 0.0695$) and no interactions with

cohort (all F 's < 2.16 ; all p 's > 0.15). Thus, the data from these two cohorts were grouped for all subsequent analyses.

Data Analysis

Dependent measures used to assess cognitive flexibility (Exp. 1) included the number of trials to criterion, errors to criterion, and omissions for each strategy (visual, response, and response reversal). Dependent measures for each strategy were analyzed with two-way ANOVA (age $\times$ sex). The relationship between trials to criterion performance on each strategy and the subsequent strategies was assessed using linear regression analyses.

For outcome devaluation (Exp. 2), dependent measures were rate of lever pressing and reinforcement rate during acquisition and the RI-30 sessions, consumption during the feeding sessions before and after extinction tests, and lever presses during extinction tests. Paired t -tests were conducted on lever presses during the extinction test sets to reveal any statistically significant differences in lever presses between the value conditions. Significance of the obtained p -values was evaluated after first adjusting for multiple comparisons using false discovery rate (less than 5%; Benjamini & Hochberg, 1995). A mixed factorial ANOVA was conducted on lever presses during the test sets (age $\times$ sex $\times$ test set $\times$ value condition). Mixed factorial ANOVAs were conducted on lever pressing rate (lever presses/min) and rate of reinforcer earned (g/kg/min) during the acquisition period (age $\times$ sex $\times$ session) and RI-30 sessions (age $\times$ sex $\times$ session). Pre-feeding consumption (g/kg) on extinction test days was analyzed using mixed factorial ANOVA (age and sex as between subject factors; test set and reinforcer as within subject factors). For the post-test consumption (g/kg) data, separate factorial ANOVAs for pellets and SCM were conducted (age $\times$ sex $\times$ test set $\times$ value condition as factors).

The dependent measure used to assess risk-based decision making (Exp. 3) was the percent choice of the large reward nosepoke port during the free choice trials on the last session at each probability of large reinforcement. A three-way mixed ANOVA was conducted with age, sex, and probability (within subjects) as factors. Reinforcement rate adjusted for body weight (g/kg/min) and omissions during the free choice trials were also analyzed for the final session at each probability using a three-way mixed ANOVA. To assess reward discrimination, two-way ANOVA (age $\times$ sex) was conducted on the percent large reward choice and reinforcement rate (g/kg/min) in free choice trials on the final session of 100% probability of large reinforcement. For all analyses, Tukey post-hoc comparisons were conducted when appropriate.

Results

Exp. 1: Strategy Shifting

A total of 23 male and 25 female rats began Exp. 1, but one rat was excluded from the final analyses due to a technical problem ($n = 1$ adult female). Thus, final group sizes were 12 each for adolescent females and adolescent males, 12 adult females, and 11 adult males. Shown in Fig. 2 are the total number of trials required to meet the performance criterion for each strategy. For the initial strategy on which rats were trained (visual), a total of 12 rats

failed to achieve the acquisition criterion of 8 consecutive correct ($n = 2$ adolescent males, $n = 2$ adolescent females, $n = 5$ adult males, $n = 3$ adult females), resulting in a broad performance range of as few as 111 trials to the maximum of 960 trials (which was the maximal number of visual strategy trials a rat could receive). Despite this range, two-way ANOVA of the group data for both trials and errors to criterion for the visual strategy revealed no significant main effects or interactions (Fig. 2A; errors to criterion not shown). Linear regression analyses demonstrated that trials to criterion performance on the visual strategy did not significantly predict subsequent trials to criterion performance for the egocentric response strategy ($\text{Adj } R^2 = 0.0077$, $p = 0.25$) or the response reversal ($\text{Adj } R^2 = 0.0191$, $p = 0.17$). Thus, we present performance on these strategies including the data from all rats, whether or not they met acquisition criterion for the visual strategy.

Following the visual strategy, rats were required to shift to an egocentric response strategy. Compared to the initial strategy, the response strategy was learned by all rats in considerably fewer trials that ranged from 8 to 114 trials (Fig. 2C). Still, there were no group differences in response strategy performance, as two-way ANOVA of trials and errors to criterion revealed no significant main effects or interactions. Subsequently, when the contingency for the response strategy was reversed such that rats had to make an alternate response (e.g., press the left lever instead of the right), there were age differences in performance (Fig. 2E). Two-way ANOVA of trials to criterion revealed a significant main effect of age ($F_{1,43} = 4.21$; $p = 0.046$), with adolescents requiring significantly fewer trials to reach criterion than adults. Linear regression analysis showed that the number of trials required to reach criterion for the response strategy did not significantly predict the trials to criterion for the subsequent response reversal ($\text{Adj } R^2 = 0.0451$, $p > 0.08$). In addition, all rats met the acquisition criterion for the reversal. Analysis of total errors to criterion for the response reversal revealed no significant main effects or interactions.

Total omissions during each strategy also are presented in Fig. 2. There were no significant differences in omissions during the visual (Fig. 2B) and response reversal strategies (Fig. 2F). However, adolescents had more omissions during the egocentric response strategy compared to adults (Fig. 2D; age: $F_{1,6} = 6.64$, $p = 0.042$). Importantly, omissions did not explain differences in performance as there were no differences in trials to criterion for the response strategy.

Exp. 2: Outcome Devaluation

A total of 28 male and 32 female rats began Exp. 2. However, ten rats were excluded from the final analyses due to their failure to learn to lever press ($n = 3$ adolescent females, $n = 2$ adolescent males), failure to meet the criterion of at least one lever press during the test sessions ($n = 1$ adolescent female, $n = 3$ adult male), or a technical problem ($n = 1$ adult female). Thus, the final group sizes were 13 adolescent females, 12 adolescent males, 14 adult females, and 11 adult males.

Lever pressing rate was assessed across two autoshaping and two lever press training sessions during the initial lever press acquisition period (Fig. 3A). Reinforcer type (food pellet or SCM) was not used as a factor because mixed ANOVA revealed there was no significant main effect of reinforcer type and it did not interact with age, sex, or session. We

observed significant main effects of age ($F_{1,46} = 23.65$, $p < 0.0001$) and session ($F_{3,46} = 100.48$, $p < 0.0001$), as well as a significant age $\times$ session interaction ($F_{3,46} = 11.87$, $p < 0.0001$). Post-hoc analysis revealed no age difference in lever pressing rate during autoshaping (sessions 1 and 2), but adults had a higher lever pressing rate than adolescents during both lever press training sessions, 3 ($p = 0.0387$) and 4 ($p < 0.0001$). For both adolescents and adults, response rate increased across these initial training sessions (adolescents: from sessions 2-4, all p 's < 0.03 ; adults: each session, all p 's < 0.01). Because body size differs considerably between adolescents and adults, and this may influence both capacity and motivation to make lever press responses that lead to reinforcement, we also analyzed responding during these RI-30 sessions with a body weight-adjusted rate of reinforcement measure (g of reinforcer/kg/min; Fig. 3C). This analysis indicated a main effect of session ($F_{3,46} = 36.10$, $p < 0.0001$), such that rats increase their reinforcement rate across all sessions (all p 's < 0.01). In addition, adult females had higher reinforcement rates than adult males ($p = 0.027$), while adolescent reinforcement rates were similar in both sexes (age $\times$ sex: $F_{1,46} = 4.23$, $p = 0.045$).

Lever pressing rate during RI-30 sessions is shown in Fig. 3B. Across these sessions, all rats (regardless of age and sex) increased their rate of lever pressing (session: $F_{7,46} = 13.70$, $p < 0.0001$) and adolescents pressed the lever at a slower rate than adults (age: $F_{1,46} = 6.60$, $p = 0.014$). Mixed factorial ANOVA also revealed a significant age $\times$ session interaction ($F_{7,46} = 2.64$, $p = 0.022$). Specifically, adolescents pressed the lever at a slower rate than adults during the sessions prior to the first test set (sessions 1-3) and session 5. However, this age effect diminished or no longer existed in the RI-30 sessions that followed the first devaluation test set (sessions 6-8). In these sessions, adolescents responded at higher rates similar to those in adults. Analysis of reinforcement rate during RI-30 sessions revealed that adolescents (age: $F_{1,46} = 110.64$, $p < 0.0001$) and females (sex: $F_{1,46} = 63.42$, $p < 0.0001$) had a higher rate of reinforcement compared to adults and males, respectively. Mixed factorial ANOVA also revealed a significant main effect of session ($F_{7,46} = 4.74$, $p < 0.001$; Fig. 3D, significance not shown). Specifically, rats earned the reinforcers at the lowest rate in session 1 ($p = 0.011$ vs. session 2) but this rate remained from session 2 onward.

During the pre-feeding phase of the devaluation tests, rats were given unlimited access to consume one of the reinforcers for 60 min. A mixed factorial ANOVA of the consumption data during the pre-feeding sessions across test sets 1 and 2 revealed there was no main effect of test set so these data were collapsed. As shown in Fig. 4, all rats consumed more SCM than pellets during the pre-feeding sessions (main effect of reinforcer: $F_{1,46} = 489$, $p < 0.0001$). In addition, females consumed more reinforcer than males (main effect of sex: $F_{1,46} = 18.9$, $p < 0.0001$), but the significant sex $\times$ age interaction ($F_{1,46} = 10.6$, $p = 0.0022$) and the subsequent post-hoc tests revealed this effect was driven primarily by the consumption differences in adult females compared to adult males.

Immediately following the pre-feeding phase, rats were given a brief extinction test and lever presses during these sessions are shown in Fig. 5. Rats displayed a significant reduction in lever presses when the reinforcer was devalued compared to valued (value condition: $F_{1,184} = 62.4$, $p < 0.0001$). Paired t-tests revealed that all groups exhibited a significant reduction in lever presses when the reinforcer was devalued compared to valued

(p 's < 0.05), with the exception of adolescent females in the second test set. In addition, mixed factorial ANOVA revealed several significant interactions (age $\times$ sex: $F_{1,184} = 6.09$, $p = 0.015$; age $\times$ test set: $F_{1,184} = 4.04$, $p = 0.049$; age $\times$ sex $\times$ test set: $F_{1,184} = 3.91$, $p = 0.049$). These interactions resulted because adult males had fewer lever presses than adolescent males during the second test set collapsed across value condition, but this effect was not present in females (Fig. 5B).

Following the extinction tests, rats were given simultaneous access to both reinforcers for 15 min in order to evaluate the efficacy of the devaluation procedure (Fig. 6). As we observed for the pre-feeding phase, there was no significant main effect of test set so these data were collapsed for subsequent analyses. Importantly, each group consumed fewer pellets when pellets were devalued through pre-feeding than when the value of pellets remained intact through pre-feeding of the alternate reinforcer (Fig. 6A; value condition: $F_{1,46} = 375$, $p < 0.0001$). This pattern, which was also evident in SCM consumption (Fig. 6B; value condition: $F_{1,46} = 284$, $p < 0.0001$), suggests that pre-feeding was a successful manipulation to change the value of the reinforcers. Despite all groups being sensitive to a change in value of both rewards, adult females consumed more pellets than adult males when the value of pellets was intact (age $\times$ sex $\times$ value condition: $F_{1,46} = 5.29$, $p = 0.026$; sex $\times$ value condition: $F_{1,46} = 4.33$, $p = 0.043$), while adolescents consumed more SCM than adults when SCM was devalued (age $\times$ value condition: $F_{1,46} = 6.11$, $p = 0.0172$; age: $F_{1,46} = 36.54$, $p < 0.0001$). Females also consumed more SCM than males, regardless of value condition (sex: $F_{1,46} = 9.45$, $p < 0.0035$).

Exp. 3: Risk-Based Decision Making

A total of 18 male and 21 female rats began and subsequently completed Exp. 3, yielding group sizes of 11 adolescent females, 8 adolescent males, 10 adult females, and 10 adult males. Percent choice of the large reward port and reinforcement rate adjusted for body weight are shown in Fig. 7. Rats reduced their choice of the large reward as the probability of reinforcement decreased (Fig. 7A; probability: $F_{3,105} = 8.46$, $p < 0.0001$). On the last day when the probability of large reinforcement was 100%, two-way ANOVA of percent large reward choice revealed that adults tended to make the large reward choice more than adolescents (age: $F_{1,35} = 4.17$, $p = 0.0487$), but this group difference was modest and appeared to be largely driven by adult males (Fig. 7B).

Reinforcement rate adjusted for body weight was examined for the free choice trials in the final session at each probability (Fig. 7C). Adolescents had higher reinforcement rates than adults at all probabilities (age: $F_{1,35} = 36.41$, $p < 0.0001$). Reinforcement rates of rats was significantly reduced when the probability of large reinforcement decreased to 16.7% (probability: $F_{3,105} = 7.67$, $p < 0.0001$). In addition, females earned more reinforcer than males (sex: $F_{1,35} = 68.68$, $p < 0.0001$). On the final day of the 100% probability of large reinforcement, two-way ANOVA of reinforcement rate revealed that adolescents (Fig. 7D; age: $F_{1,35} = 14.26$, $p < 0.001$) and females (sex: $F_{1,35} = 20.08$, $p < 0.0001$) had higher reinforcement rates than adults and males, respectively.

Analysis of omissions during free choice trials on the final day of each probability revealed no significant main effects of interactions (data not shown). Further analyses were

performed to address concerns of potential group differences in satiation within a session. Mixed factorial ANOVAs (age $\times$ sex $\times$ trial $\times$ port) were conducted to examine omissions and latency to nosepoke upon illumination of the ports during the free choice trials of the final session where the large reward had a 100% probability of reinforcement. These analyses revealed no significant main effects or interactions, with the exception of a significant main effect of port ($F_{1,28} = 4.58$, $p = 0.0411$) on the latency to nosepoke upon port illumination, reflecting that rats took longer to nosepoke into the large reward port compared to the small reward port.

Discussion

In the present study, we tested rats under minimal, weight maintaining food restriction conditions in multiple instrumental behavior tasks that allowed investigation of potential age- and sex-dependent differences in behavioral flexibility, reward value sensitivity, and risk-based decision making. In the strategy-shifting task, we found that adolescent rats, regardless of sex, exhibited faster acquisition of reversal learning, suggesting adolescents have greater behavioral flexibility than adults. In both the outcome devaluation and risk-based decision making tasks, adolescents of both sexes responded in a more efficient manner than their same-sex adult counterparts, earning reinforcers at a significantly higher rate. However, adolescent and adult rats of both sexes were equally sensitive to devaluation, suggesting no age difference in updating outcome value representations. The only sex difference in this task was in adults, with adult females consuming more reward than adult males during the pre-feeding session. In addition, we found that adolescents and adults, regardless of sex, made similarly risky choices in the probability discounting task. Overall, our results are only partially consistent with our *a priori* hypotheses and the predictions of leading theories of adolescent vulnerability.

In the strategy-shifting task, we found that adolescent rats displayed enhanced behavioral flexibility compared to adult rats, regardless of sex. This effect was specific to reversal learning, as adolescents acquired the extradimensional shift from visual to response strategies just as rapidly as adults. It is important to note, however, that rats in the present study required considerably more trials to acquire the visual strategy than those in previous studies (Floresco et al., 2008; Cox et al., 2016), which could be due to strain differences in visual acuity (Kumar, Talpos, & Steckler, 2015). Like Kumar and colleagues' study, some of our Sprague-Dawley rats failed to acquire the initial visual discrimination. Notably, we found that performance on the visual strategy did not significantly predict subsequent performance in the shift to response strategy. The direction of shift from the visual strategy to the egocentric response strategy has been shown to be medial PFC-dependent due to the cost of switching from an easier to a more difficult strategy (Floresco et al., 2008). Since the visual strategy in the present study seemed to be rather difficult for our rats to acquire, the strategy shift to response in our task may not have been sensitive to medial PFC function and this may explain the similar performance between adolescents and adults.

The lack of age differences in the extradimensional shift is consistent with a previous study (Newman & McGaughy, 2011) when adolescents were tested at similar ages (P50 and P53) as our experiment (P44-50). In addition, our finding that adolescents exhibit greater

behavioral flexibility in reversal learning compared to adults is consistent with some previous findings (Simon et al., 2013), but not others (Newman & McGaughy, 2011). Differences in study methodology, such as stimulus complexity, rat strain, food restriction, and adolescent pubertal status may explain the disparity. Newman and McGaughy (2011) employed the intradimensional/extradimensional attentional set-shifting task in which rats were required to dig in pots for food reinforcers. Stimulus complexity in this task may have influenced their finding of adolescents needing more trials to acquire a compound discrimination (Newman & McGaughy, 2011); adolescents may be more susceptible to distraction by irrelevant stimuli. The pots in this study varied in multiple dimensions making them a much more complex stimulus set compared to a tone (Simon et al., 2013) or light cue (the present study). This complexity may have contributed to the earlier study's finding that adolescent rats displayed less cognitive flexibility compared to adults. Differences in stimulus complexity may also lead to differences in switch cost due to task difficulty. Regarding rat strain, Newman and McGaughy (2011) used Long Evans rats to show adolescents were less flexible than adults, whereas we and Simon et al. (2013) found adolescent Sprague-Dawley rats were more flexible than adults. Adolescents and adults are differentially sensitive to motivational factors, such as food restriction, as adolescents exhibited more perseverative responding during extinction compared to adults when food deprived (Sturman, Mandell, & Moghaddam, 2010). Newman and McGaughy (2011) food restricted rats to 90% free-feeding weight of aged-matched controls, whereas those in the present study were fed *ad libitum* ~20 h/day and experienced no weight loss compared to rats fed *ad libitum* 24 h/day. The greater food restriction employed by Newman and McGaughy (2011) relative to that in the present study may have contributed to their finding of adolescent behavioral inflexibility by increasing perseverative responding. Lastly, regarding the influence of pubertal stage, it was recently reported that male and female Long Evans rats who reached puberty within days of training in a Morris water maze task had significantly better performance on the reversal learning stage of the task compared to pre-pubertal rats (Willing et al., 2016). Thus, despite similar testing ages for the adult groups in ours and the previous studies (Simon et al., 2013, Newman & McGaughy, 2011), the age and presumed pubertal status of the adolescent groups may contribute to inconsistent findings. Future studies are required to pinpoint the precise role that these various methodological factors play in laboratory rodent assessments of age and sex differences in behavioral flexibility.

Many adolescent changes in neural regions associated with behavioral flexibility seem to be most prominent during the peri-pubertal period. Previous work has shown that behavioral flexibility is sensitive to changes in prefrontal cortex (PFC) function (Dalton, Wang, Phillips, & Floresco, 2016; Ghods-Sharifi, Haluk, & Floresco, 2008; Floresco et al., 2008), with different subregions controlling distinct components of flexible behavior. Specifically, inactivation of the medial PFC impaired extradimensional set-shifts, while sparing (Floresco et al., 2008) or even enhancing reversal learning (Dalton et al., 2016). In contrast, inactivation of the orbitofrontal cortex (OFC) impaired reversal learning, while sparing set-shifts (Ghods-Sharifi et al., 2008). Substantial neuroanatomical changes, including pruning of neurons (Markham, Morris, & Juraska, 2007; Willing & Juraska, 2015) and synapses (Drzewiecki, Willing & Juraska, 2015), have been reported to occur at pubertal onset in the

medial PFC in rats of both sexes. These neuroanatomical changes may underlie adult-like performance in set-shifts in the postpubertal adolescent rats in the present study and the previous literature discussed above (Newman & McGaughy, 2011). However, it remains unclear whether there are peri-pubertal neuroanatomical changes in the OFC, and if they play a role in age-dependent differences in reversal learning.

The current study and other recent findings (Marshall et al., 2017; Hankosky et al., 2017) suggest that reward sensitivity in adolescent rodents is likely dependent on multiple factors, including motivational state and pubertal status. During the acquisition period for lever pressing in Exp. 2, adolescents had a lower response rate compared to adults. This effect persisted during the RI-30 sessions leading up to the first test set. Although the response rate was lower in adolescents, an age difference in rate of reinforcement emerged during the RI-30 sessions. Adolescents did not differ from adults in their rate of reinforcement during the acquisition period, but displayed a higher rate of reinforcement compared to adults throughout the RI-30 sessions. These results may suggest that adolescent rats are more behaviorally efficient under the RI-30 schedule of reinforcement as they respond less frequently during this schedule but still earned reinforcers at a higher rate than their adult counterparts. Adolescents' reduced response rate during random interval schedules is consistent with a previous study (Andrzejewski et al., 2011), although those authors did not report analysis of reinforcement rate normalized to body weight. The lower response rate in adolescents may be due to more physical effort required to lever press compared to adults. However, our findings from Exp. 3 where the required response was a nosepoke rather than a lever press argue against this as we found a similar age difference in rate of reinforcement. Thus, even when the effort cost of the response is reduced (a nosepoke vs. a lever press), age differences in reinforcement rate persist. Together, our results highlight the importance of analyzing age differences in reinforcement in a manner that takes age differences in body size into account.

Despite evidence for a peri/post-pubertal peak in reward consumption (Friemel et al., 2010; Marshall et al., 2017), motivation (Friemel et al., 2010), and cocaine self-administration in males (Wong, Ford, Pagels, McCutcheon, & Marinelli, 2013), several studies measuring instrumental responding for non-drug reinforcers report either comparable responding (Sturman & Moghaddam, 2011; Kim, Simon, Wood, & Moghaddam, 2016; Naneix, Marchand, Di Scala, Pape, & Coutureau, 2012) or decreased responding in adolescents compared to adults (present study; Andrzejewski et al., 2011; Sturman et al., 2010; Hankosky et al., 2017). One factor that could contribute to the apparent discrepancies between reward consumption and instrumental responding is whether the measure accounts for differences in body weight. Studies of reward consumption and drug self-administration adjust for differences in body weight, while measures of instrumental responding for non-drug reinforcers typically do not. As such, studies of sex differences in non-drug reward processing have found that females consume more reward per body weight when given *ad libitum* access (present study; Marshall et al., 2017; Hammerslag & Gulley, 2014), but seem to respond for reinforcer similarly or more than males (present study; Hankosky et al., 2017) depending on whether the measure is normalized to body weight. In the present study, as the adolescents increase in body size across RI-30 sessions, they increase their responding in order to maintain their elevated reinforcement rate (adjusted for body weight) relative to

adults. Studies examining reward consumption normalized to body weight have consistently found that adolescent males consume more reward than their adult counterparts (Friemel et al., 2010) regardless of caloric content (Marshall et al., 2017). While these data suggest that adolescent reward hypersensitivity does not depend on age differences in caloric need, other studies that account for body weight but use non-caloric reinforcers do not clearly support this interpretation (Hankosky et al., 2017, Shram et al., 2008). Future studies are needed to understand how age differences in caloric need due to body size may influence instrumental responding.

Despite age differences in response and reinforcement rate during RI-30 sessions leading up to the first test set, we found no group differences in sensitivity to reward devaluation during the first test set (when adolescent rats were P42-P44), which coincides with mean pubertal onset in our sample of adolescent males. We did not find evidence for adolescent hypersensitivity to reward value when the reward value was intact in either test set, as adolescents did not respond significantly more than adults during the valued condition. Furthermore, all rats reduced their lever pressing behavior when the outcome was devalued compared to valued, suggesting that adolescents and adults are equally sensitive to a change in relative reward value. As reward value sensitivity is only one aspect that may contribute to reward sensitivity, future research is needed to investigate age and sex differences in other aspects of reward sensitivity, such as Pavlovian-to-instrumental transfer and progressive ratio responding to measure motivation.

In risk-based decision making, adolescents chose the large option less than adults when the probability of large reinforcement was 100%. However, the choices of adolescents and females during the free choice trials led to higher reinforcement rate per body weight in these groups compared to their adult and male counterparts. Despite this initial difference, adolescents and adults showed similar declining percent choices of the large, risky option during subsequent sessions with decreasing probabilities of large reinforcer delivery, suggesting that rats of both ages and sexes were sensitive to the change in reinforcement probability. However, all rats, regardless of age or sex, continued to choose the large, risky option more than 50% of the time even at the lowest reinforcement probability tested (16.7%), despite the fact that the more advantageous choice was the small, certain option with this low probability of large reinforcement. We did not find evidence for adolescent rats making more risky choices compared to adults, which conflicts with a previous study showing adolescent males exhibit increased risky choices (Zoratto et al., 2013).

As suggested above, procedural variables may explain these discrepant findings. Zoratto and colleagues (2013) investigated risky choice using a home-cage probabilistic delivery paradigm. Rats in their study were pair-housed in a cage partitioned with a sliding door that was only closed during testing sessions. Since this task occurred in the home-cage where rats were socially housed, there may have been a social influence on decision making (Zoratto et al., 2013). Notably, a study in humans showed that the presence of peers increased risk taking in adolescents but not adults (Gardner & Steinberg, 2005). In addition, the food restriction method employed by Zoratto and colleagues (2013) may have affected risky choice in a different manner than the minimal food restriction used in the present study. Previous studies in adult males showed that the probability discounting curve is less

steep in free-fed rats compared to those adults restricted to ~85% of their free-feeding weight (St. Onge & Floresco, 2009). Given these points, future studies of risk taking behavior in rodent models of adolescence should examine these methodological factors as potential determinants of age differences in risky behavior.

Influential theories of adolescent vulnerability (Casey et al., 2011; Shulman et al., 2015; Steinberg, 2010) propose that some of the behavioral traits adolescents often express, including poor cognitive flexibility, hypersensitivity to reward, and the tendency to behave in a risky manner, contribute to the adolescent's heightened susceptibility to psychopathologies. The present findings in a rodent model of adolescence are inconsistent with a straightforward interpretation of these theories and instead highlight the importance of factors such as pubertal status and motivational state. The onset of puberty is a notable adolescent event that occurs earlier in females compared to males. The experimental design of the present study did not allow for investigation of males and females at comparable pubertal stages, which may have impacted our ability to detect sex differences in the adolescent group. Future investigations are needed, as only recently have rodent studies of adolescent behavior begun to accommodate for the differential pubertal timing between sexes (Willing et al., 2016). In addition, there is a growing interest in human studies to examine pubertal development as a factor in relation to adolescent neurobehavioral development, especially in domains of reward processing and cognitive control.

A recent longitudinal study (Braams, van Duijvenvoorde, Peper, & Crone, 2015) found an adolescent peak in nucleus accumbens activity in response to reward similar to that reported previously (Galvan et al., 2006). Braams et al. (2015) extended previous findings by showing that pubertal development was linearly associated with the nucleus accumbens activity, such that the greatest nucleus accumbens response occurred during mid-puberty. Testosterone levels were also positively correlated with nucleus accumbens activity during adolescence, suggesting that pubertal hormones may play a role in the heightened reward response (Braams et al., 2015). In addition, sensation seeking peaks during adolescence in males but not females (Steinberg et al., 2008) and is positively correlated with pubertal development even when controlling for age (Steinberg et al., 2008; Martin et al., 2002), suggesting that pubertal status and age can have dissociable contributions to adolescent reward behavior.

In contrast to heightened reward system activation, the extent of orbital frontal cortex activation in adolescents was also more comparable to children than adults (Galvan et al., 2006). These results are consistent with a relatively immature cognitive control system. An overactive reward system and reduced cognitive control system is proposed to result in increased adolescent risk taking, which seems to be dependent on sex. Consistent with the peak in sensation seeking in adolescent males (Steinberg et al., 2008), self-report of risk taking behavior was associated with males, and positively correlated with pubertal development (Collado-Rodriguez, MacPherson, Kurdziel, Rosenberg, & Lejuez, 2014). Other studies of cognitive control have shown that cognitive flexibility increases across adolescence in males with females displaying more flexible behavior than males earlier in adolescence (Kalkut et al., 2009). Overall, the human literature suggests that adolescent

hypersensitivity to reward may be tied to pubertal development, with important sex differences in the development of reward processing and cognitive control behaviors.

In conclusion, the present study found that adolescent rats earn higher reinforcement rates than adults under minimal food restriction. Although this result is consistent with our *a priori* predictions that adolescents would be hypersensitive to reward, our remaining findings were contrary to expectations. Namely, adolescents exhibited greater behavioral flexibility and had similar risk preference and sensitivity to reward value compared to adults. Thus, most of our findings were not in line with theories of adolescent vulnerability that have been largely supported by studies in humans. Importantly, the present study suggests that adolescent behaviors that may confer vulnerability to psychopathology are not evident under all circumstances in rodent models. Rather our findings and that of the broader rodent literature in adolescent behavioral development speak to the influence of factors, such as pubertal status and motivational state in these discrepancies. Future work is needed to investigate the methodological, and ultimately neural, determinants of adolescent-typical behaviors in rodent models.

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Figure 1.

Summary of body weight gain during the periadolescent period and the experimental timeline for the study. (A) Shown are data from rats in the adolescent groups that were allowed access to food for ~20h/day during training and testing and those from the adult groups that had not yet begun the restricted access procedure and thus had food available 24h/day. The data points for rats in the 24h access groups are plotted with a nudge of 0.3 x-axis units so they are not obscured by the data points from the restricted groups. Separate two-way ANOVAs revealed significant main effects of postnatal day (males: $F_{30,1615} = 466$, $p < 0.0001$; females: $F_{30,1871} = 305$, $p < 0.0001$). In males, there was a significant age by

group interaction ($F_{30,1615} = 1.88$, $p = 0.0028$), reflecting that weights were only significantly different between groups on P49 ($*p < 0.05$). **(B)** Experimental timeline showing the training and testing ages for each experiment. Mean age of pubertal onset for each sex in the subset of rats that underwent puberty checks is indicated by their respective symbols.

Figure 2.

Trials to criterion (TTC) and omissions during acquisition of the (A,B) visual strategy, (C,D) response strategy, and (E,F) response reversal in the strategy-shifting task. Rats ($n = 11-12/\text{group}$) met the acquisition criterion when they achieved 8 consecutive correct responses.

Those that failed to meet the criterion for the visual strategy were assigned the total number of trials they received, which was 960. $*p < 0.05$ vs. adults

Figure 3.

Responding during the instrumental outcome devaluation task ($n = 11-14/\text{group}$). Rates of lever pressing (lever presses/min) are shown during the acquisition period (A) and the RI-30 sessions (B) with breaks in the graph corresponding to extinction testing days. $*p < 0.05$ and $***p < 0.001$ vs. adolescents, collapsed across sex. (C) Rates of reinforcement adjusted for body weight (g/kg/min) are shown for the acquisition period. $\#p < 0.05$ adult females vs. adult males. During the RI-30 sessions (D), there were main effects of age ($p < 0.0001$) and sex ($p < 0.0001$) for rates of reinforcement.

Figure 4. Reward consumption during the pre-feeding phase of the outcome devaluation experiment. Consumption data for pellets and sweetened condensed milk (SCM) are adjusted for body weight and averaged across the two test sets. *** $p < 0.0001$ vs. adult females, within reinforcer type.

Figure 5.

Total number of lever presses during the 10 min extinction sessions are shown for test set 1 (A) and test set 2 (B). Each test set consisted of two extinction sessions immediately following the pre-feeding phase. The extinction test days were separate by one RI-30 session. * $p < 0.05$, ** $p < 0.01$ vs. devalued within group in paired t-test; @ $p < 0.05$ vs. adolescent males collapsed across value condition

Figure 6.

Post extinction test consumption (g/kg) of pellets (**A**) and SCM (**B**) by value condition.

During the pre-feeding phase, rats were given the reinforcer they expected in the test session (devalued condition, which was pellets for A and SCM for B) or the alternate reinforcer (valued condition, which was SCM for A and pellets for B). *** $p < 0.0001$ vs. devalued within group; @ $p < 0.05$ vs. adult females with pellets valued; ### $p < 0.0001$ vs. adults with SCM devalued

Figure 7.

Responses in the large reward nosepoke port during free-choice trials of the risky decision-making task. Data ($n = 8-11/\text{group}$) are presented as the percent of large reward choices on (A) the last session when each probability of reinforcement was tested and (B) during the last session when the probability of reinforcement was fixed at 100%. Rate of reinforcement adjusted for body weight (g/kg/min) is shown for (C) the last session at each probability of reinforcement for the large reward choice with main effects of age ($p < 0.0001$) and sex ($p < 0.0001$), and (D) during the final session at 100% probability. * $p < 0.05$, *** $p < 0.001$ vs. 100.0%, collapsed across age and sex; + $p < 0.05$, +++ $p < 0.05$ vs. 66.7% collapsed across age and sex; ### $p < 0.001$ vs. females collapsed across age; @ $p < 0.05$ vs. adolescents