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The Developmental Inter-Relationships Between Activity, Novelty Preferences, and Delay Discounting in Male and Female Rats

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Abstract

Increased locomotion, novelty-seeking, and impulsivity are risk factors associated with substance use. In this study, the inter-relationships between activity, novelty preferences, and delay discounting, a measure of impulsivity, were examined across three stages: juvenile/early adolescence (postnatal Day [P] 15, 19, and 42 for activity, novelty, and impulsivity, respectively), adolescent/late adolescent (P28, 32, 73), and adult (P90, 94, 137) in male and female rats. Our estimates of impulsive choice, where animals were trained to criterion, revealed an age $\times$ sex interaction where early adolescent females had the lowest levels of impulsivity. The relationships of activity and novelty to impulsivity significantly changed across age within each sex. Early adolescent males with high activity, but low novelty preferences, were more impulsive; however, low activity and high novelty preferences were related to high impulsivity in adult males. Female activity gradually increased across age, but did not show a strong relationship with impulsivity. Novelty preferences are moderately related to impulsivity into adulthood in females. These data show that males and females have different developmental trajectories for these behaviors. Males show greater sensation-seeking (e.g., activity) and risky behavior (e.g., novelty preferences) earlier in life, whereas these behaviors emerge during adolescence in females.

Keywords

impulsivity; delay discounting; sex differences; development; novelty; rat

INTRODUCTION

The transition to adolescence is marked by the normal appearance of both sensation-seeking and risky behaviors (Khurana et al., 2015). In a prospective study, human data from the Philadelphia Trajectory Study of teenagers 10–19 years of age show that risky behavior (e.g., impulsivity) and sensation-seeking tendencies are associated with progression to elevated drug use or experimentation, respectively (Khurana et al., 2015). Findings in adult

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animals show that the risky behaviors of novelty preferences and impulsivity are associated with greater (Mitchell et al., 2014) and more compulsive self-administration of cocaine (Belin, Berson, Balado, Piazza, & Deroche-Gamonet, 2011) or other drugs (Bird & Schenk, 2013). In animals, these risky behaviors occur more frequently during adolescence than adulthood (Laviola, Macri, Morley-Fletcher, & Adriani, 2003; Sonntag et al., 2014) and much of preclinical work has focused on adolescence to examine risky behaviors, with few studies examining drug-taking in adolescence (Wong, Ford, Pagels, McCutcheon, & Marinelli, 2013). In humans, impulsive behavior generally declines across childhood, adolescence, and adulthood (Green, Myerson, & Ostaszewski, 1999). The prevention of drug use may be improved by earlier identification of those vulnerable earlier in life than adolescence (Stanis & Andersen, 2014).

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Establishing unique relationships between activity (e.g., locomotor activity in response to a novel environment) and novelty-seeking may help predict more complex risky behaviors that can lead to early identification of vulnerable individuals (Schramm-Sapota et al., 2011; Stanis & Andersen, 2014). Activity and novelty preferences are relatively simple tasks that take days to assess and provide the advantage that the animal does not mature too much during the assessment. In adult rats, locomotor activity is associated with quicker acquisition of drug-taking behavior (Piazza, Deminiere, Le Moal, & Simon, 1989, 1990), but not compulsive use (Hooks, Jones, Smith, Neill, & Justice, 1991). Locomotor activity in young adolescent rats (aged postnatal Day [P] 31) positively correlated with the amount of cocaine self-administered later in life; this relationship was the same at P68 (Schramm-Sapota et al., 2011). Although activity as a predictive factor has been examined, the simpler, developmental relationships between activity (which correlates with cocaine self-administration), novelty preference, and impulsivity remain to be investigated for unique maturational changes.

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In humans, delay discounting, a measure of impulsive choice where the reinforcement is devalued with increasing delays in receipt (Mazur, 1997), and its relationship to executive function, is associated with the progression from early teen experimental drug use to more serious substance use issues (Khurana et al., 2015). Delay discounting is found in many species: humans (Olson et al., 2007; Steinberg et al., 2009), primates (Woolverton, Myerson, & Green, 2007), rodents (Adriani & Laviola, 2003; Bizot, Le Bihan, Puech, Hamon, & Thiebot, 1999; Bizot et al., 2007), and pigeons (Mazur, 1997). In male rodents, delay discounting is elevated in adolescents relative to adults (Adriani & Laviola, 2003; Doremus-Fitzwater, Barreto, & Spear, 2012). Sex differences in this maturational process of impulsivity are notably sparse from the literature (Perry, Nelson, Anderson, Morgan, & Carroll, 2007) or have not been found in operant tasks (Doremus-Fitzwater et al., 2012; Eubig, Noe, Floresco, Sable, & Schantz, 2014) or in a t-maze (Freund et al., 2014).

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As an initial step toward determining how activity and novelty preference are related to developmental changes in impulsive choice, all three of these behaviors were assessed in males and females. Most studies perform group analyses to examine age differences and miss the individual variation that may yield more insight (Schramm-Sapota et al., 2011). In the current study, individual correlations within age and sex may provide new information about their developmental relationship to impulsivity. Our goal is to identify whether the

nature of the predictive relationships between these variables changes across age and sex and how “outliers” may appear. As only 8–15% of individuals who experiment with substances of abuse become addicted (Everitt & Robbins, 2013), these three behaviors (or their combination) may reveal a unique *early* vulnerability that is related to impulsivity that is assessed weeks later.

Activity, novelty preferences, and impulsive choice, as measured by delay discounting, were determined within each subject beginning in juvenility, adolescence, and adulthood in male and female rats in the current study. We used a within-subject, within-session, operant delay discounting task with delays of 0, 10, 20, 40, and 60 s that was partly based on the methods of Evenden and Ryan (1996). Five major hypotheses were tested that build upon the literature. First, typical activity will change across development: subjects will become less active with maturation (Laviola et al., 2003). Sex differences will be observed where females will demonstrate greater activity than males (Torres-Reveron & Dow-Edwards, 2005). Second, novelty preferences are predicted to peak in adolescence and decrease in adulthood (Adriani, Chiarotti, & Laviola, 1998), and will be higher in adolescent males than females (Palanza, Morley-Fletcher, & Laviola, 2001) with no enduring sex difference in adulthood. Third, impulsivity is predicted to decrease with age (taking into account that rats are young adolescents at the time of testing), but will not show sex differences (Doremus-Fitzwater et al., 2012; Eubig et al., 2014). Fourth, the inter-relationship between activity and impulsivity will transition with age, noting the drawback of subjects having matured during impulsivity training and testing. Others (Perry et al., 2007) have shown no relationship in adults between activity and delay discounting for food. We predict that this relationship will be more complex developmentally, with males showing the highest levels of activity and impulsivity earlier in life (Casey, Jones, & Somerville, 2011; Laviola et al., 2003) that declines with maturation. Older females with the highest levels of activity will also show more impulsivity, in keeping with observations of Perry et al. (2007) who demonstrated that low saccharin-preferring females had higher activity and greater impulsivity. Fifth, elevated novelty preferences will correlate with greater impulsivity by adulthood, consistent with greater likelihood for drug use in animals with these characteristics (Belin, Mar, Dalley, Robbins, & Everitt, 2008; Belin et al., 2011). In contrast, younger rats will not show this relationship and will be more impulsive, but not novelty-preferring, until adolescence or later. Female adolescents will show a high degree of both novelty preference and impulsivity (Doremus-Fitzwater et al., 2012).

METHODS

Subjects

Male and female Sprague-Dawley rats ($n = 55$ in total) were obtained from Charles River Laboratories (Boston, MA) and group-housed with three subjects/cage after weaning at P21. Males and females were in separate cages within the same vivarium. They were maintained on a 12:12 hr light/dark cycle (lights on at 0600) with experimental sessions conducted between 0700 and 1200 hr. Food and water were available ad libitum, with the exception of food being restricted (described below) when rats were undergoing behavioral training and testing in the delay discounting task. All experimental procedures were approved by the

IACUC at McLean Hospital and were consistent with the Principles of Laboratory Animal Care (NIH Publication no. 85-23). Three age groups were used starting at P15 (juvenile), P28 (adolescent), and P90 (adult) based on previous achievements of key developmental milestones (Andersen, 2003). The juvenile rats were obtained in litters from Charles River Laboratories on P9 and weaned on P21 after testing; the remaining rats matured in our vivarium. The schedule of training and testing of activity, novelty preferences, and delay discounting is shown in Figure 1.

Locomotor Activity

Locomotor activity was recorded for 20 min on Day 1 as part of the first day of habituation of novelty preferences by examining the number of beam breaks within the apparatus (Macri, Laviola, Leussis, & Andersen, 2010). Briefly, a two-chamber apparatus that differed by wall patterns was used (7 × 8.5 inches each side; Med Associates, St. Albans, VT). Subjects were confined to one side and activity was recorded on Day 1. Subjects were assessed at P15, 28, and 90.

Novelty Preferences

The same subjects ($n = 8-11$ /group) from Day 1 activity were repeatedly placed into the initial chamber described above for 20 min for 3 consecutive days, based on previous methods (Macri et al., 2010). The initial side of the habituation chamber was counterbalanced across subjects. On Day 4, subjects remained in the home cage for 24 hr. On Day 5, when subjects were P19, 32, and 94, the door to the second chamber was opened and the total time spent on the novel side during a 20-min session was recorded and used as the dependent variable of novelty preferences

Delay Discounting

Apparatus—Trapezoidal-shaped operant chambers (Lafayette Instruments, Lafayette, IN) with a Bussey–Saksida Touch Screen (Indianapolis, IN) for responding displayed two different stimuli. Touchscreens required minimal force for a response, making them well suited for animals of all ages. Each operant chamber was enclosed in a sound-attenuating box with a fan for ventilation and white noise. A pellet dispenser delivered grain pellets into the magazine. Responses into the magazine during the training phases were detected by a photobeam located across its entry. Training and experimental responses were detected by nosepoke contact within the presented symbol on the touch screen. The entire setup was controlled by MatLab (Mathworks; Natick, MA) using custom programs and the Biopsychology Toolbox (Rose, Otto, & Dittrich, 2008).

Delay Discounting Task—Subjects were food deprived (to a target goal of 90% of free-feeding weight within a few days; and fed an amount of chow that was based on age-matched control levels) starting at P19, 32, and 94. Subjects ($n = 7-11$ /group) began training at P21, 34, and 96 to measure increases in impulsive choice with a delay discounting task shown in the schematic in Figure 2. Subjects were first trained to initiate each session by poking their nose in the food hopper. This poke produced a square symbol on the right or left of the touch screen (whose position was counterbalanced across subjects) and the presentation of one food pellet (Grain-Based Dustless Precision Pellets; 45 mg, Bio-Serv,

Frenchtown, NJ). Once the animal reached a criterion of 60 rewards in 90 trials, then two symbols appeared: one signaling immediate reward and the other signaling the delayed reward. The side of presentation of the immediate or delay symbol was counterbalanced across subjects, but stayed constant for each individual subject. To assure sufficient learning of what the symbols represented (immediate vs. delayed reinforcer presentation), the criterion was set to responding 45 times out of 50 for the delay square for 2 consecutive days. The final phase of testing was initiated when the rat poked his nose in the central food well and then both symbols were presented. Selection of the delay square resulted in the delivery of four pellets after a delay of 0, 10, 20, 40, or 60 s presented in ascending order. A total of five blocks of 12 trials were conducted within each daily session that lasted 100 min. At the beginning of each block, the time of the delay was introduced in two forced choice trials (with only the delay square presented at the beginning of each block of 12 trials) to signal a change in delay condition. After the subject poked the screen and received the pellet, the magazine lights went off, the symbol was removed from the screen, and there was a 100 s delay before the symbols reappeared on the screen to initiate the next trial. Subjects were tested for 3 consecutive days or until behavior was stable (standard error of less than .15 across the 3 days of testing). The average variability across the 3 days (and within each delay) was $.125 \pm .006$. The average number of large reinforcers from the 10 trials at each delay (data were not used from the two forced trials) was averaged across the 3 days and was used for data analysis. We have found that this multi-phased training session resulted in stable learning and performance as measured by the average number of large reinforcements received at each across the 3 days.

Statistical Analyses

Overall ANOVAs (SPSS, v.20) were used to analyze the influence of Age and Sex on activity levels (beam breaks) and novelty preferences (time spent in novel environment). Pearson bivariate correlational analyses were conducted to determine the inter-relationships between activity, novelty preference, and the halfway point between the number of large reinforcements received out of the maximum achieved. This indifference point was calculated based on the best fit of the relationship between delay and the number of large reinforcements received for each individual subject where R^2 was greater than .8. The point of 50% of the large reinforcements (referred to as DDT₅₀ for delay discounting at 50% of the large rewards received) was then determined from this fitted equation. As the relationship between delay and the number of large reinforcements is often non-linear, the calculated fit of the line to estimate the DDT₅₀ is more appropriate than estimating the delay at which 50% of large reward responding occurred (which implies a linear relationship; Doremus-Fitzwater et al., 2012). Statistical significance was determined with a Pearson's two-tailed test (SPSS, v. 20). After the individual correlational analyses, comparisons of correlations across ages were compared by recoding each group of subjects via their correlational coefficients (see Introduction to SAS. UCLA: Statistical Consulting Group, http://www.ats.ucla.edu/stat/mult_pkg/faq/general/citingats.html (accessed 8/31/15). Significance was set at $p < .05$. All subsequent comparisons were corrected with Bonferroni correction.

A standard statistical mixed ANCOVA was used to determine differences in delay discounting as a function of Age (3) $\times$ Sex (2) $\times$ [delay], with delay as a within-subject factor with five levels; activity was used as a covariate to account for activity-related differences in responding. The criterion for sensitivity to delay was considered significant when delay as a factor was significant or significantly interacted with Age or Sex (Mar & Robbins, 2007). All subjects displayed such a sensitivity to delay. The average of the number of large reinforcements received across 3 days of testing was used as the dependent variable. Following the significant three-way interaction, subsequent analyses were analyzed for each Sex. Both the number of large reinforcements and estimates of DDT₅₀ were analyzed in this fashion.

RESULTS

Activity and Novelty Differences As a Function of Age and Sex

Activity was assessed at P15, 28, and 90 in male and female rats, with novelty preferences assessed in the same animals four days later. ANOVA shows a significant main effect of Age ($F_{2,43} = 7.51, p = .002$) that interacted with sex at a trend level (Age $\times$ Sex interaction: $p = .06$) for activity levels. Sex alone did not have a significant influence (Fig. 3). Post hoc comparisons with Bonferroni correction shows that the P28 subjects significantly differed from P15 rats ($p = .003$) and P90 ($p = .02$). Novelty preferences only demonstrated a trend for an Age main effect: $p = .08$, with no interactions (Fig. 3).

Correlational Analyses: Activity and Novelty Preferences

Correlations were used to investigate whether activity predicts novelty preferences. When the overall study sample was placed into the correlational matrix, a significant inverse relationship was found between activity and novelty preference, but not for any of the other variables ($r = -.36; n = 50; p = .01$). Figure 4 shows that the more active the subjects were, the less time they spent exploring the novel chamber. This relationship was strengthened when males only were considered ($r = -.56; n = 29; p = .002$). Activity and novelty preferences did not significantly correlate in females ($p = .58$).

The average number of days each subject took until the final data collection point for delay discounting is shown in Figure 1. While testing commenced during P19, 32, and 94, subjects spent a significant number of days testing that differed as a function of both Age and Sex (which did not interact; Age: $F_{2,41} = 13.32, p < .001$; Sex: $F_{1,41} = 10.28, p = .003$). Subjects that began as juveniles took the least amount of time to reach criterion relative to the other ages, reaching early adolescence at completion; females took longer than males to reach criterion. As a result of a few weeks of testing, the early behaviors (activity and novelty) are correlated with later stages shown for each age (Fig. 1). Data for the delay discounting correspond to early adolescent, late adolescent, and adult time points.

Correlation was used to determine the relationships between individual activity and novelty preferences and the indifference point of DDT₅₀ and differences between these inter-relationships across age. A larger value for DDT₅₀ indicates less impulsivity, as the subjects wait for the larger reinforcer. As these relationships are expected to differ across age and

sex, individual correlations within these conditions were performed to better elucidate their patterns. Figure 5 shows the relationship between DDT₅₀ and activity. Overall, activity for all subjects did not predict DDT₅₀: $R = .12$; $n = 55$; $p = .4$. Within individual correlations of activity and impulsivity, only the P90/128 males demonstrated trend-level significance of $p = .09$, suggesting that this relationship needs to mature. Indeed, we found that P15 males differ from P28 and 90 ($p < .05$), and P28 differs from P90 ($p < .001$). Only in female P28 rats was the activity/DDT₅₀ relationship different from other ages: between P15 and P28 ($p < .05$) and between P28 and P90 ($p < .05$).

Figure 6 illustrates the unique relationships between DDT₅₀ and novelty preferences at each age and sex. This relationship was significant overall, with an $R = .31$; $n = 55$; $p = .02$, although none of the individual analyses were significant. Pearson's R are presented. Cross-age comparisons of the different correlation coefficients within each sex revealed that P19 males differ from P32 and 90 ($p < .05$), and P32 differs from P90 ($p < .001$). Female P32 rats only differed from P19 subjects ($p < .05$).

Delay Discounting

The number of large reinforcers received was analyzed using ANCOVA, with activity as a covariate to account for the potential influence that activity may have on discounting. Inclusion of activity as a covariate strengthened the overall analysis ($\text{Age} \times \text{Sex} \times [\text{delay}] = F_{2,43} = 5.51$, $p = .007$), and the p -value declined to $p = .02$ when this covariate was removed from the analysis. Activity itself was not significant as a covariate ($p = .37$). An $\text{Age} \times \text{Sex}$ interaction was also observed: ($F_{2,43} = 3.51$, $p = .04$). Finally, a main effect of [delay] was significant ($p = .0001$).

To simplify these analyses, we divided the data by Sex and continued to use activity as a covariate when it strengthened the analysis. In males, activity had a significant influence on the discounting data ($F_{1,21} = 9.47$, $p = .006$). Age overall was significant ($F_{2,21} = 5.59$, $p = .01$), but did not interact with [delay] ($p = .07$; Fig. 7). In contrast, female discounting was not affected by activity ($p = .12$). Without this covariate, an $\text{Age} \times [\text{delay}]$ interaction was observed in females: ($F_{8,88} = 2.30$, $p = .028$; Fig. 7). Post hoc tests with Bonferroni post hoc test indicates that significant differences occurred at the 40 s delay, where the early adolescent females were less impulsive than adults ($p < .01$).

The indifference point, the delay point when 50% point of large reward was received (DDT₅₀), was also analyzed (Tab. 1). An $\text{Age} \times \text{Sex}$ ANOVA was significant ($F_{2,46} = 4.96$, $p = .01$). When analyzed separately for Sex, DDT₅₀ was not significant across Age in males ($F_{2,25} = 2.53$, $p = .1$), but was significantly different across Age in females: $F_{2,21} = 6.81$, $p = .005$. An Age main effect ($F_{2,46} = 4.29$, $p = .02$) in females followed by a Bonferroni post hoc test shows that the DDT₅₀ in early adolescent females was significantly higher than in adolescent ($p = .005$) and adult females ($p = .06$), suggesting lower impulsivity at this age. Late adolescents had trend-level differences from adults ($p = .09$).

DISCUSSION

Our results show that the relationships between activity, novelty preferences, and impulsivity change in an age- and sex-dependent way. The ages that were examined in this study are significantly younger than most studies, as we began our assessments at P15 for activity and P19 for novelty preferences (e.g., P15/19). This latter age corresponds to childhood (Andersen, 2003), when most preventative intervention strategies are implemented (Stanis & Andersen, 2014). As predicted, activity levels decreased with age, but reached trend-level significance ($p = .06$) for sex differences at our selected ages. Novelty preferences also failed to significantly differ across maturation. We also found hypothesized age differences in discounting for males, with early adolescent males showing high discounting. Surprisingly, females at an early adolescent assessment point (P44) demonstrated the least impulsivity compared with all other groups when no sex differences were predicted (Doremus-Fitzwater et al., 2012; Eubig et al., 2014). Analysis of DDT₅₀ supports this conclusion. DDT₅₀ remained low (more impulsivity) in the males at all ages, but early adolescent females had the highest value (least impulsivity) compared with all other conditions.

The relationship between activity and DDT₅₀ changes across age and sex (Fig. 5). The slope suggests that greater activity is related to lower DDT₅₀ (more impulsivity) in early adolescent males. This slope significantly differs from the late adolescents and adults, where it flattens with maturation and significantly transitions to show that lower activity levels are observed within a greater distribution of impulsivity scores. In contrast, early adolescent females start with a similarly flat relationship and moderate levels of activity are related to a range of impulsivity scores. This relationship contracts in late adolescent females, as most subjects increased their amount of impulsivity and significantly differ from the early adolescent and adult females; adult females show a negative correlation where high activity is found at the lowest DDT₅₀ levels (more impulsivity). These data are consistent with our hypothesis and data by Perry et al. (2007) in adult females. Adult females may not only acquire self-administration quicker (which has been related to activity levels; Becker & Hu, 2008), but may also be more vulnerable to compulsive use. However, as compulsive use has not been tested in females this relationship between activity, impulsivity, and compulsive drug use is speculation. We hypothesized that late adolescent females would show a higher risk profile, as adolescent females have higher levels of activity relative to adult females (Palanza et al., 2001). One reason that this observation did not occur may be that our animals were too young. Our “late” adolescent animals were in the very earliest stages of puberty at P28 during the activity assessment, unlike most studies that typically use subjects that are between P35 and 45.

The importance of novelty preference behavior lies in its consistent relationship to later drug-seeking and drug-taking behavior (Bardo, Rowlett, & Harris, 1995; Bardo, Donohew, & Harrington, 1996; Exner & Clark, 1993). In the current study, novelty preferences did not significantly differ across age. Again, adolescent males and females typically demonstrate high levels of both impulsivity and novelty preferences and our P32 age may have been too young to capture these age differences in males (Adriani & Laviola, 2003; Pinkston & Lamb, 2011), although novelty preferences (Laviola et al., 2003; Sonntag et al., 2014) and impulsivity demonstrated the strongest correlation in P32/69 males (Fig. 6). This

is surprising given that our “adolescent” group was still quite young (P32) and shows approximately the same degree of novelty preferences than the adult group. However, these data also show that impulsivity in the majority of P32 males is higher than P94 males. By adulthood, the DDT₅₀ increases and few animals show the phenotype of high novelty preferences and high impulsivity. Curiously, a single juvenile/early adolescent male had both high preferences and high impulsivity, which raises the possibility of early identification of a “high risk subject.” Future studies will test this hypothesis by following animals throughout development. Juvenile females demonstrate the lowest risk profile, with low impulsivity and low novelty preferences. This relationship significantly and strongly changes by P32, and at this stage, both males and females significantly differed from the youngest age. Sex differences in novelty preferences have been shown in adolescent mice, where males are more novelty-preferring than females (Palanza et al., 2001). We observed no sex differences in novelty preferences at our younger adolescent age. By adulthood, the significant transition to the observed negative slope between higher novelty preferences and lower DDT₅₀ (e.g., more impulsivity) in adults is the result of more adults showing less impulsive behavior. The transition of the activity/novelty relationship with DDT₅₀ may also predict individuals at risk for addiction or a decline in vulnerability to abuse drugs of abuse, which typically occurs during the mid 20s in humans (Collado, Felton, MacPherson, & Lejuez, 2014; O’Brien & Anthony, 2005). This window of vulnerability may occur later in rats, as young (P31) or late (P68) adolescent rats demonstrated a positive relationship between activity and the amount of cocaine self-administration (Schramm-Sapota et al., 2011).

The assessment of age differences of impulsive choice at younger ages in animals has used two approaches: either a t-maze (Bizot et al., 2007; Freund et al., 2014) or an operant paradigm (Adriani & Laviola, 2003; Doremus-Fitzwater et al., 2012; McClure, Podos, & Richardson, 2014). The t-maze uses shorter delays (delay: 0–15 vs. 0–60 s in an operant task) (Bizot et al., 2007; Freund et al., 2014). Data from a t-maze indicate that juvenile male rats are more impulsive than older ages; age differences are evident at the short delay and are not further modulated (e.g., fewer selections for the larger reinforcers) when faced with increasing delays (Andersen et al., unpublished). However, the operant paradigm uses longer delays (0–60 s), which may selectively accentuate age differences in the perception of time that are apparent in young animals (Wittmann & Paulus, 2008). Age differences that adolescents (P30–49) are more impulsive than adults (>P60) are typically (Adriani & Laviola, 2003), but not always observed (P60ish vs. P35ish; McClure et al., 2014). Of course, subtle differences in methodology could also explain these age differences.

Our estimates of the DDT₅₀ point for each age were based on non-linear curve fitting using the equation of the line to estimate the delay at which the 50% of responding for the large reinforcer occurred. This approach may be more precise than that used by others (Doremus-Fitzwater et al., 2012; Harty, Whaley, Halperin, & Ranaldi, 2011), where a ratio of the (number of rewarded pokes at the long delay hole/[total rewarded pokes])* 100 has been used to quantify impulsive choice. The ratio metric has also been used as an index of conditioning, although we trained our animals to criteria at each phase. Differences in the length of time it took each group to reach Phase 4 of testing suggest that conditioning may depend on age and sex (Fig. 1). By limiting the amount of training and testing to pre-set days (Adriani & Laviola, 2003; Adriani, Seta, Dessi-Fulgheri, Farabollini, & Laviola, 2003;

Doremus-Fitzwater et al., 2012), the possibility remains that previous estimates of age differences in impulsivity also reflect differences in training; to this end, this procedure has been referred to as a modified delay discounting paradigm. Similar to our assessment here, the majority of adult delay discounting studies trains their subjects to criterion (Winstanley, Theobald, Dalley, & Robbins, 2005).

Sex differences have been described in delay discounting in rats and mice (Koot, van den Bos, Adriani, & Laviola, 2009; Perry et al., 2007). For example, low saccharin-preferring females showed more discounting than their male counterparts (Perry et al., 2007) and we have shown that juvenile females have a flatter discounting curve than juvenile males, but in a t-maze test that utilized shorter delays than those the current study (Freund et al., 2014). The observation in the early adolescents (this study) is consistent with a greater exploratory tendency described in female mice (Koot et al., 2009), where these subjects may be more or just as likely to explore the small reinforcer side rather than focusing primarily on the large side. However, previous research has failed to find evidence for sex differences in discounting in adolescent and adult rats, respectively (Doremus-Fitzwater et al., 2012; Eubig et al., 2014). Our estimates of the indifference point for our early adolescent females at P44 are elevated (e.g., 34.1 s, less impulsivity) compared to 18.5 s at P37 (Doremus-Fitzwater et al., 2012). In contrast, our later adolescent females yielded indifference measures that are similar to the findings of Doremus-Fitzwater when both studies started training at the same age (16.1 vs. 18.5 s at P34 and P31, respectively). This observation raises the question of whether the age of initiation of training is the critical variable and whether the need to be trained to criteria or limiting training and testing days is sufficient. The other factor that may have an influence is the level of gonadal hormones. Ovarian hormones influence the perception of timing where estrogen increases impulsivity (Pleil, Cordes, Meck, & Williams, 2011; Smith, Sierra, Oppler, & Boettiger, 2014). Consistent with that observation, the DDT₅₀ in our late adolescent females is the lowest and stays low into adulthood in the females. The younger animals (P19/44) had steadily rising levels of estrogen, but estrogen levels were relatively low during the training phase when they were young. Hormonal variations were not controlled for in our study, in part because our data were averaged across three days of testing and thus three different phases of the cycle. Also, food restriction may also contribute to the appearance of sex differences (Koot et al., 2009). Females are more sensitive to mild food deprivation than males and will discount more under mild food deprivation (Koot et al., 2009). Our subjects were ~90% of free feeding weight and most studies deprive their rats to 80–85%, which increases impulsivity in males more than females (Adriani et al., 2003). The use of sweetened reinforcers (chocolate food pellets vs. grain pellets used here) may also bias adolescents to consume more reinforcements, as adolescents consume more sweet solutions than adults (Wilmouth & Spear, 2009). This observation could explain lower discounting rates in the adolescent group of Doremus-Fitzwater et al. (2012).

In the operant task, however, the potential limitation of presenting the delay in ascending order could have differentially affected discounting at the longer delays. Although others have shown that varying the delay presentation can reduce this concern (Slezak & Anderson, 2009), we did not vary our schedule. The within-subject, within-day paradigm offers the advantage of reproducible delayed responding across days (which did not differ by more

than 15%) and removes the possibility that subjects may be responding less as a function of experience (prior day's delays as in other studies (Adriani & Laviola, 2003; Doremus-Fitzwater et al., 2012) rather than waiting longer within each session. With this in mind, the only difference that we observed in males was at the 60 s delay, where early adolescents differed from the other ages. The number of trials was lowered from 12 to 8 for the juveniles to address concerns about satiation, but no differences in discounting were observed.

From an evolutionary viewpoint, sensation-seeking is associated with individuation from family and increasing the chances of finding a mate. Male teenagers (13–20 year olds) show more sensation-seeking than females (Wilson & Daly, 2006). Earlier development of risk-taking (novelty preferences and impulsivity) will further increase the likelihood of individuation in males. In females, early individuation may be maladaptive although greater impulsivity could facilitate mate selection. The shift in the relationship between novelty preferences and DDT₅₀ (where the DDT₅₀ is gradually increasing [less impulsivity]) illustrates that fewer adult females are risk-takers, especially when compared to adolescent females. The slope of the line in Figure 6 shows how this relationship is changing, although adolescents and adults did not significantly differ.

Overall, our study highlights important age- and sex-dependent developmental trajectories in impulsive choice behavior. We have also identified how two identified risk factors (novelty preferences and impulsivity) that have been associated with increased substance use later in life (Khurana et al., 2015; Scheres et al., 2006) change across age and sex. Identification of risk factors early in life allows for investigation of intervention measures (Stanger et al., 2012). Furthermore, these behavioral data offer insight into the maturation of their underlying neural circuitry and will be the subject of future studies.

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FIGURE 1.

Timeline showing the ages of testing of subjects. Activity (Act) and novelty preferences (Nov) were single day tests, whereas the Mean $\pm$ SE is presented for the final age of testing for impulsivity. The solid arrow represents males and the open arrow represents females. Age and Sex differences were present (statistics in the text).

FIGURE 2.

The training and testing paradigm for delay discounting. Three different training phases were conducted, with different symbol presentation and pellet reinforcers dispensed as shown. The fourth phase tested subjects for 3 consecutive days or until responding stabilized (<15% variability across all delays).

FIGURE 3.

Age differences between activity levels and novelty preferences for males and females.

Mean $\pm$ SE presented. *P28 subjects significantly differed from P15 rats ($p = .003$) and P90 ($p = .02$)

FIGURE 4.

Correlations between activity, a measure of sensation-seeking behavior, and novelty preferences, a measure of risk-taking behavior, with all subjects included on the left; males only on the right. A significant correlation was observed for the male only analysis.

FIGURE 5.

The amount of locomotor activity in response to a novel environment versus the delay indifference point when 50% of the large reinforcers are received (DDT₅₀). Each age group and both sexes are presented with their individual Pearson R -values showing the strength of the relationship and significance level. Cross-age comparisons of the different relationships within each sex revealed that P15 males differ from P28 to 90 ($p < .05$), and P28 differs from P90 ($p < .001$). The female activity/DDT₅₀ relationship differed between P15 and P28 ($p < .05$) and between P28 and P90 ($p < .05$).

FIGURE 6.

Time spent on the novel side versus DDT₅₀. Each age group and both sexes are presented with their individual Pearson R -values showing the strength of the relationship and significance level. Cross-age comparisons of the different relationships within each sex revealed that P19 males differ from P32 to 90 ($p < .05$), and P32 differs from P90 ($p < .001$). Female P32 rats only differed from P19 subjects ($p < .05$).

FIGURE 7.

Delay discounting curves as a function of age. Following a significant interaction of Age $\times$ Sex $\times$ [delay], males and females were analyzed separated. Data presented here are covaried for activity levels for males, but not for females as activity was not a significant covariate. Means $\pm$ SE presented. *Shows that early adolescents (both sexes) were significantly different from late adolescents and adults at all delays in males, but only at the 40 s delay in females; $p < .05$, Bonferroni corrected.

Table 1Estimated Indifference Point for DDT₅₀ (in Seconds)

Stage of Completion (m/f)	Males (<i>n</i>)	Females* (<i>n</i>)
Early adolescent (P30/44)	19.8 3 2.1 (8)	34.1 3 5.8 (7) **, ***
Late adolescent (P69/79)	19.3 3 2.6 (11)	16.1 3 1.9 (9)
Adult (P127/148)	28.7 3 4.1 (9)	22.5 3 2.9 (8)

Means ± SE (number of subjects).

* Age main effect in females: $F_{2,34} = 6.8$, $p = .005$.** Different from late adolescent females; $p < .05$.*** Trend-levels difference from adult females; $p = .06$.