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Adolescent impulsivity as a sex- and subtype-dependent predictor of impulsivity, alcohol drinking, and dopamine D₂ receptor expression in adult rats

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Abstract

Impulsivity is a personality trait associated with a heightened risk for drug use and other psychiatric conditions. Because impulsivity-related disorders typically emerge during adolescence, there has been interest in exploring methods for identifying adolescents that will be at risk to develop substance use disorders in adulthood. Here, we used a rodent model to assess inhibitory control (impulsive action) and impulsive decision making (impulsive choice) during adolescence (43–50 days old) or adulthood (93–100 days old) and then examined the impact of development on these impulsivity traits by retesting rats 50 days later. Impulsive action was not stable from adolescence to adulthood in males and was lowest in adult males, relative to adolescents and females. Impulsive choice was stable across development and unaffected by age or sex. Next, we examined the connection between our model of impulsivity and two measures relevant to substance abuse research: the initiation of voluntary alcohol drinking and dopamine D₂ receptor (D₂R) expression in the prelimbic prefrontal cortex. Consumption of saccharin-sweetened ethanol during 30 min sessions in adulthood was associated with adolescent, but not adult, impulsive action, particularly in males. Prelimbic D₂R expression was reduced in individuals with high levels of impulsive choice and this relationship appeared to be strongest among females. The results of this study demonstrate that impulsive choice, along with its connection to D₂R expression, is relatively unchanged by the process of development. For impulsive action however, individual levels of impulsivity during adolescence predict drinking in adulthood despite changes in the measure during development.

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Authors' contribution

LRH, JMG, OAAA, AGK, and BWR were responsible for the design of the behavioral portion of the experiment. LRH, OAAA, AGK and QG collected behavioral data. LRH, APB, QG and RG were responsible for the western blot portion of the experiment. LRH and JMG drafted the manuscript. All authors critically reviewed content and approved the final version for publication.

Introduction

Individuals who are highly impulsive are at greater risk for substance abuse, legal trouble, and some psychiatric disorders (Kreek *et al.* 2005; Perry & Carroll 2008; Fox *et al.* 2010; Dalley *et al.* 2011; Jupp & Dalley 2014). With our growing understanding that adolescence is a time of life when impulsivity-related disorders typically emerge (Casey & Jones 2010), there has been increased focus on identifying adolescents that will go on to experience problems later in life. For example, the Adolescent Brain Cognitive Development (ABCD) Study is a recently-launched multisite initiative examining whether the risk for mental disorders, such as substance abuse, is linked to brain development and longitudinal changes in behavior during adolescence. Rodent models of adolescence have also been used to investigate impulsivity and other risk factors (Andrzejewski *et al.* 2011; Burton & Fletcher 2012; Doremus-Fitzwater *et al.* 2012; McClure *et al.* 2014). These have a distinct advantage of allowing for thorough evaluation of neurobiological mechanisms, but to date few rodent studies have taken a longitudinal approach like that used in the ABCD Study.

There is considerable development within the brain during adolescence, particularly within the prefrontal cortex (PFC) and the striatum (Lenroot & Giedd 2006; Raznahan *et al.* 2014), regions involved in controlling and driving motivated behavior, respectively (Somerville & Casey 2010; Dalley *et al.* 2011; Dalley & Robbins 2017). Notably, behavior becomes more controlled and less impulsive from adolescence to adulthood (Steinberg *et al.* 2008; Shulman *et al.* 2014; Charles *et al.* 2016), perhaps as a result of maturation within the PFC and striatum (Ernst *et al.* 2009), and this occurs in a sex-specific fashion. For example, girls have better impulse control by the end of adolescence (Shulman *et al.* 2014). In rodents, females lose more neurons within the PFC from adolescence to adulthood (Willing & Juraska 2015), while males undergo more overproduction and pruning of striatal dopamine D₂ receptors (D₂R) (Andersen *et al.* 1997). Elimination of D₂Rs also occurs in the PFC during adolescence (Andersen *et al.* 2000), though no study has examined whether this pruning is sex dependent. Because PFC D₂Rs are involved in regulating impulse control (Simon *et al.* 2013), sex differences in this age-dependent pruning could contribute to sex differences in behavior during both adolescence and adulthood.

Identifying adolescents that will go on to become impulsive adults could improve the identification of at-risk populations during a critical window of vulnerability (Newton *et al.* 2011; Conrod *et al.* 2013). In this study we developed a rodent model to test for longitudinal changes in two subtypes of impulsivity, impulsive action and choice. Impulsive choice is the preference for immediate reward over long term gain, while impulsive action is related to poor inhibitory control (Dalley *et al.* 2011). These subtypes are dissociable and not correlated in humans or rats (Broos *et al.* 2012), although both may independently be associated with the expression of D₂R mRNA in the PFC (Simon *et al.* 2013). To test both subtypes during the short window of rat adolescence, we developed a novel action-choice task that allows for the rapid testing of both behaviors. We then assessed the extent to which these measures change, both at the individual and group levels, across adolescent development or during adulthood. Lastly, we measured ethanol drinking and D₂R expression in the PFC. This approach allowed us to determine whether a brief measurement of

adolescent impulsivity could be used to predict behavior and dopamine function during adulthood.

Methods

Subjects

A total of 178 male and female outbred Sprague-Dawley rats completed the study. These rats were born in our animal facility from non-sibling breeders obtained from Harlan (Indianapolis, IN, USA) and were weaned on postnatal day (P) 22. They were housed in groups of 2–3 same-sex littermates for the duration of the experiment. Rats were kept on a 12-h reversed light/dark cycle (lights off at 0900h), in a temperature controlled room and with water available *ad libitum* throughout the study. Rats were weighed daily around 1200h and all food was removed from home cages 2–4 h prior to the start of each operant session. Food was returned within 1 h of session completion and was available *ad libitum* at all other times. We have found this procedure leads to sufficient motivation during operant sessions while maintaining body weight at an average of $102 \pm 0.65\%$ of the free-fed weight, estimated from a group of free-fed controls. Experimental procedures were approved by the Institutional Animal Care and Use Committee at the University of Illinois, Urbana-Champaign, and were consistent with the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 2011).

Action-choice task

The timeline for training and testing is shown in Fig. 1a. Rats were assigned to complete initial training and the first impulsivity test during adolescence ($n = 51$ females and 38 males) or adulthood ($n = 47$ females and 42 males). From P27–P40 or P77–P90, rats completed the initial pre-training period (see supplemental methods). Briefly, they were handled and habituated to the food restriction process for 4 days, then given pre-exposure to the reinforcer (45mg food pellets; Bioserv, #F0021, Frenchtown, NJ). Next, they completed two magazine training sessions, 6 days of autoshaping and lever press training, and two forced choice sessions. Starting with the forced choice sessions, responses on the “large reward lever” yielded 3 pellets, while the “small reward lever” yielded 1 pellet. Next, rats begin training on the action-choice task (Fig. 1a) starting at P41 or P91. During two sessions, rats chose between the small or large reward levers, with reward delivery occurring 1s after a lever press. Choice trials were separated by a 20 s intertrial interval (ITI), where the cue lights above the lever were extinguished and responses on the levers had no consequence. Each session consisted of 3 blocks of 8 trials, with 2 exemplar trials (one lever available) at the start of each block. For exemplar trials, omissions (60s without a lever press) led to an ITI followed by repeating the exemplar trial. Repeated bias against the same lever (a difference of 10 exemplar omissions across two sessions) led to removal from the study ($n = 12$ adolescent females, 1 adolescent male, 2 adult females, 6 adult males).

Impulsive action was tested over three sessions (Fig 1a) wherein lever press responses during the ITI resulted in a signaled 5-s timeout (TO). During this TO, the ITI was interrupted and the houselight was illuminated; subsequent lever presses during the TO would reset the 5 s timer and thus extend the TO length. Responses during the ITI or TO

were scored as “premature responses” and could extend the length of each ITI to a maximum of 60 s. During the next five sessions (Fig 1a), impulsive choice was measured by introducing a delay discounting component to the task (adapted from Foscue *et al*, 2012). This was accomplished by delaying delivery of the large reward while the delivery of the small reward remained fixed at 1 s. The delay to delivery of the large reward increased progressively in each session, from 5 s in session 1 to 75 s in session 5. Responses during the 20 s ITI had no consequence. Retesting was conducted 50 days after the start of the initial test (Fig. 1a) using the same methods as described above, except rats were run in different operant chambers and the reward value assigned to each lever was reversed.

Ethanol Self Administration

After completing retesting, rats remained undisturbed in their home cages for 20 days before the start of ethanol self-administration (SA; Fig. 1a). During the 15 sessions of ethanol SA, rats were taken to a separate testing room and placed in individual cages containing a bottle of water and three sipper tubes that each contained 5 mL of a sweetened ethanol solution (10% v/v ethanol and 0.2% saccharin). These sessions lasted 30 min and rats were returned to their home cages immediately afterwards.

Tissue collection for Western blot analysis of D₂R expression

At least 30 days after the completion of ethanol SA, we collected tissue from the final cohort of rats used in the study ($n = 30$ adolescent test, 31 adult test). Bilateral punches (1.0 mm) were obtained from the prelimbic region (AP +2.5–4.7, ML $\pm$ 1.0, DV –3.0–4.0) and we examined expression of D₂Rs (Abcam, AB21218) and the housekeeping protein vinculin (Abcam, AB18058) via standard Western blot analysis (supplemental methods). A control sample containing tissue combined from 6 experimentally naïve rats was included on each gel and the relative intensity of D₂Rs was determined for each sample by the following equation: $[(D_2R/Vinculin)/(Control\ D_2R/Control\ Vinculin)]$.

Data Analysis

We analyzed test and retest performance with three-way repeated measures ANOVA for both premature responding (impulsive action) and delay discounting (impulsive choice), with session and test as within-subjects factors and sex as the between-subjects factor. We also examined drinking and performance during the initial test using three-way ANOVA with session or block as the within subjects factor and sex and age-of-first-test as the between-subjects factors.

For all further analyses, impulsive action was defined as the average number of premature responses across the three action test sessions. Impulsive choice was defined as the slope of the discounting curve across the five sessions it was measured. Three-way ANCOVA was used to examine the relationship between impulsivity during the first set of tests and later impulsivity, drinking, and D₂R expression; in each of these, initial impulsivity was the continuous variable and sex and age were the between-subjects factors. For each ANCOVA, significant interactions with the continuous variable were investigated with pre-planned tests of linear regression within each group. For drinking and D₂R expression, we interrogated significant ANCOVA results by specifically comparing rats with high ($\geq 66^{\text{th}}$ percentile) or

low (33rd percentile) levels of impulsive action or choice, relative to other rats of the same age and sex, in order to model the “extremes” of the personality trait. These data were analyzed with three-way ANOVA. All ANOVA interactions were further investigated with Tukey HSD post-hoc tests. All statistical tests were performed in SAS (version 9.4) using PROC GLIMMIX and PROC REG. Alpha level was set to $p < 0.05$ and correlations were conducted with two-tailed distributions.

Results

Impulsive action and choice task

During the two training sessions immediately before the tests of impulsivity, we found significant effects of age and sex on responding during the ITI (Fig. 1b). Specifically, adolescent rats made fewer ITI lever presses during training sessions, which was when these responses had no consequence (Fig. 1b left). There were main effects of age ($F_{1,174} = 42.3$, $p < 0.001$) and session ($F_{1,174} = 40.6$, $p < 0.001$) as well as an age $\times$ session interaction ($F_{1,174} = 24.8$, $p < 0.001$). Adults, but not adolescents, decreased their ITI responding by the second training session. There was also an interaction between age and sex ($F_{1,174} = 7.78$, $p = 0.006$), with females having a higher level of ITI responding than males during adulthood. During the impulsive action test sessions the number of premature responses decreased across sessions (Fig. 1b, right; $F_{2,347} = 75.4$, $p < 0.001$) and were sex-dependent ($F_{1,174} = 4.34$, $p = 0.039$). Premature responding was also affected by interactions between sex and age ($F_{1,174} = 12.3$, $p < 0.001$), sex and session ($F_{2,347} = 4.40$, $p = 0.013$), and a three-way interaction between sex, age and session ($F_{2,347} = 3.14$, $p = 0.044$). Specifically, premature responding was highest in female adults relative to male adults and female adolescents. However, when premature responses were normalized to account for the baseline differences in ITI responding (Fig. 1c), adolescents and females displayed higher levels of impulsive action relative to adults and males [main effects of sex ($F_{1,174} = 4.82$, $p = 0.029$) and age ($F_{1,174} = 25.6$, $p < 0.001$)]. In addition, normalized impulsive action decreased across sessions ($F_{2,347} = 79.1$, $p < 0.001$) and there was an interaction between session and sex ($F_{2,347} = 3.69$, $p = 0.026$), with females having a steeper decrease across sessions. Males appeared to have larger reductions in impulsivity by adulthood, but the sex by age interaction did not reach significance ($F_{1,174} = 3.25$, $p = 0.073$). There were no effects of age or sex on impulsive choice (supplemental figure S1).

We next examined the stability of impulsive action across the 50 days between the initial test and the retest. In rats first tested as adolescents (Fig. 2a), impulsive action decreased from the adolescent test to the adult retest ($F_{1,87} = 43.3$, $p < 0.001$). It also decreased across sessions during both testing phases ($F_{2,174} = 67.6$, $p < 0.001$), though there was a test by session interaction ($F_{2,173} = 6.71$, $p = 0.002$) driven by the steeper reduction in premature responses across the initial test sessions. The results were similar in rats first tested as adults (Fig. 2b), with lower levels of impulsive action during retest ($F_{1,87} = 107.9$, $p < 0.001$), a decrease in premature responding across sessions ($F_{2,174} = 61.0$, $p < 0.001$), and a session by test interaction ($F_{2,174} = 7.93$, $p < 0.001$). However, among rats first tested as adults, females were more impulsive than their male counterparts ($F_{1,87} = 13.1$, $p < 0.001$). For these rats, sex interacted with test ($F_{1,87} = 6.68$, $p = 0.011$) and session ($F_{2,174} = 7.43$, $p < 0.001$) and

the effect of sex was most pronounced during the first session of the initial test. Analyzing the stability of impulsive action with ANCOVA revealed that impulsive action at retest was influenced by a rat's level of impulsive action during the initial test ($F_{1,170} = 31.1$, $p < 0.001$). The relationship between test and retest behavior was affected by interactions with sex ($F_{1,170} = 4.76$, $p = 0.031$) and age-of-first-test ($F_{1,170} = 6.87$, $p = 0.010$). Further investigation of these interactions using pre-planned comparisons of stability within each group revealed that impulsive action was stable from adolescent test to adult retest in females (Fig. 2c; $R^2 = 0.197$, $p = 0.001$) but not in males (Fig. 2d; $R^2 = 0.037$, $p = 0.249$). In rats first tested as adults, however, impulsive action was stable from test to retest in both females (Fig. 2e; $R^2 = 0.400$, $p < 0.001$) and males (Fig. 2f; $R^2 = 0.247$, $p = 0.001$).

In the same manner, we next analyzed the stability of impulsive choice from an initial test to a retest 50 days later. During delay discounting sessions, rats first tested as adolescents (Fig. 3a) increased their preference for the small, immediate reward as delay increased ($F_{4,348} = 255.72$, $p < 0.001$), but this was unaffected by retest or sex. Rats first tested as adults (Fig. 3b) also shifted their preference as delay increased (Fig. 3b; $F_{4,348} = 271$, $p < 0.001$), but these rats also had lower levels of impulsive choice (i.e., less preference for the small reward) during the retest sessions ($F_{1,87} = 20.74$, $p < 0.001$). Analyzing the stability of impulsive choice with ANCOVA revealed that impulsive choice at retest was influenced by an individual's level of impulsive choice during the initial test (Fig. 3c–f; $F_{1,170} = 28.7$, $p < 0.001$), but there were no interactions to suggest that this stability was related to either sex or the age-of-first-test.

Ethanol drinking

Ethanol consumption (Fig. 4a) was highest in females ($F_{1,173} = 173$, $p < 0.001$). Drinking increased across blocks of sessions ($F_{2,346} = 13.5$, $p < 0.001$) and there was an interaction between session block and sex ($F_{2,346} = 4.97$, $p = 0.007$), such that drinking increased across blocks in males only. Using ANCOVA to examine the relationship between overall average drinking and the average level of impulsive action, we found that drinking was influenced by sex ($F_{1,169} = 23.8$, $p < 0.001$), age of first test ($F_{1,169} = 10.8$, $p = 0.001$), and by an interaction between age of first test and the average level of impulsive action ($F_{1,169} = 7.48$, $p = 0.007$). We explored this interaction by first examining drinking in rats with high or low levels of impulsive action (Fig. 4b) and found that drinking depended on both sex ($F_{1,107} = 74.0$, $p < 0.001$) and on the interaction between age-of-first-test and impulsive action level ($F_{1,107} = 5.55$, $p = 0.020$), with a trend toward higher drinking among rats that were especially impulsive during adolescence ($p = 0.058$). Further investigation of this interaction, using pre-planned comparisons to measure the relationship between drinking and the entire continuum of impulsivity, revealed that adolescent impulsive action was significantly correlated with drinking for males (Fig. 4d; $R^2 = 0.157$, $p = 0.014$) and there was a trend toward a modest correlation for females (Fig. 4c; $R^2 = 0.070$, $p = 0.061$). There was no positive correlation between drinking and adult impulsive action for females (Fig. 4e; $R^2 = 0.002$, $p = 0.756$) or males (Fig. 4f; $R^2 = 0.075$, $p = 0.083$). There was no relationship between an individual's level of impulsive choice and drinking (data not shown; $ps > 0.15$).

D₂R expression

Finally, we examined the relationship between prelimbic PFC dopamine D₂R expression (Fig. 5a, b) and impulsivity using ANCOVA. There was no effect of sex or age of first test on D₂R expression (supplemental figure S2; all p s > 0.5). However, we found that D₂R expression was negatively associated with impulsive choice ($F_{1,53} = 6.61$, $p = 0.013$) and this relationship was mediated by an interaction with sex ($F_{1,53} = 6.03$, $p = 0.017$). We explored this interaction by first examining D₂R expression in rats with extremely high or low levels of impulsive choice (Fig. 5b) and found that D₂R expression was lower in rats with high levels of impulsive choice ($F_{1,30} = 8.52$, $p = 0.007$), but here there was no main effect or interactions with sex or age-of-first-test (p s > 0.25). We further investigated the ANCOVA interaction between sex and impulsivity level using pre-planned comparisons to measure the relationship between D₂R and the entire continuum of impulsivity within each group. Here we found that impulsive choice was negatively correlated with D₂R expression in females, whether first tested as adolescents (Fig. 5c; $R^2 = 0.474$, $p = 0.013$) or as adults (Fig. 5e; $R^2 = 0.415$, $p = 0.007$). However, there was no relationship between impulsive choice and D₂R expression in males first tested as adolescents (Fig. 5d; $R^2 = 0.055$, $p = 0.349$) or adults (Fig. 5f; $R^2 = 0.017$, $p = 0.649$). There was also no relationship between impulsive action and D₂R expression (data not shown; all p s > 0.15).

Discussion

In this longitudinal study, we sought to examine developmental changes in impulsivity to determine if adolescent behavior could be used to predict an individual's impulsivity, alcohol drinking and PFC dopamine receptor expression during adulthood. Impulsivity is thought to be a key risk factor for mental health issues that emerge during adolescence (Kreek *et al.* 2005; Perry & Carroll 2008; Fox *et al.* 2010; Dalley *et al.* 2011), but it is unclear if individual differences persist as impulsiveness decreases during development. Improving identification of vulnerable individuals during adolescence, before the onset of mental health issues or substance abuse, would greatly improve preventative efforts. The ABCD Study has recently begun a longitudinal analysis of the interrelationships between adolescent behavior, brain development and substance use in humans, but to our knowledge there exists only one longitudinal study of impulsivity in rodents (McClure *et al.* 2014). Moreover, current rodent models of impulsivity typically focus on only one aspect and thereby ignore the multifaceted nature of this personality trait. Therefore, we developed the action-choice task in order to rapidly and reliably model two aspects of impulsivity during adolescence and adulthood.

It is hypothesized that the PFC is less able to exert top-down control over behavior during adolescence, with behavior maturing and becoming more controlled as the PFC develops (Ernst *et al.* 2009). In line with this notion, we found that adolescent rats were less able to inhibit inappropriate responses that led to a timeout rather than a food reward compared to their adult counterparts. This suggests adolescents have higher levels of impulsive action relative to adults, which is consistent with another study in rats that used similar methods (Burton & Fletcher 2012) and with human studies showing a relative deficit in inhibitory control during adolescence (Steinberg *et al.* 2008; Shulman *et al.* 2014; Charles *et al.* 2016).

However, we did not find any evidence for an age difference in impulsive choice, which is in contrast to human studies (Steinberg *et al.* 2009; Achterberg *et al.* 2016). While human adolescents seem to have higher levels of both subtypes of impulsivity, it would seem that for our novel rodent task only impulsive action is elevated during adolescence.

Our results may have been influenced by some aspects of our task design. For example, in the current study we motivated responding by removing food for approximately 4–6 hours per day, which is an approach that does not lead to weight loss. Our finding that adult males exhibit less impulsive action than adolescent males or adult females is consistent with another study using similar food restriction methods (Burton & Fletcher 2012). In contrast, studies using more traditional food restriction methods that cause bodyweight loss of ~15% have reported that males engage in more premature responding (Bayless *et al.* 2012; Hammerslag *et al.* 2014). Studies using weight-loss inducing food restriction also reveal large age differences in impulsive choice (Doremus-Fitzwater *et al.* 2012; Lukkes *et al.* 2015). Another methodological factor potentially contributing to our finding of no age difference in impulsive choice is that this behavior was always tested after impulsive action. Testing in the late stages of adolescence may obscure an effect of age on impulsive choice. Lastly, the current action-choice task introduced a signaled timeout only during impulsive action tests. This was done to insure our impulsive action task was compatible, but did not interfere, with a previously developed rapid assessments of delay discounting (Adriani & Laviola 2006; Foscue *et al.* 2012; Doremus-Fitzwater *et al.* 2012). Studies that used the 5-choice serial reaction time or similar tasks include a signaled timeout in every session (Lovic *et al.* 2011; Burton & Fletcher 2012; Feja & Koch 2014; Anastasio *et al.* 2014). Thus, the design and relative novelty of our task, especially the impulsive action assessment, should be considered when interpreting and comparing the current results to others.

The process by which inhibitory control increases during adolescent development may be influenced by mesocorticolimbic development (for review: Ernst *et al.* 2009). In this study, we found that impulsive action changed unpredictably in males: highly impulsive adolescents did not necessarily exhibit high levels of impulsivity relative to their peers during adulthood. In contrast, individual differences in impulsive action were relatively unchanged across development for females. Males and females differ in mesocorticolimbic development, with females having a greater reduction in PFC neurons (Willing & Juraska 2015) and males exhibiting more overproduction and pruning of dopamine D₁ receptors and D₂Rs in the dorsal striatum and nucleus accumbens (NAc; Andersen *et al.* 1997). Thus, it may be that males have less stability of impulsive action because of the overproduction and pruning of dopamine receptors in the striatum and NAc. It is also possible that the stability of impulsive action was disrupted because males were undergoing puberty. Impulsive action was tested from P42 to 45. The onset of puberty varies across laboratory animal colonies, but tends to occur around P45 for male rats and much earlier (~P35) in their female counterparts (Drzewiecki *et al.* 2016; Kang *et al.* 2016). Impulsive choice was tested from P46–50 and was stable from adolescence to adulthood for both males and females, consistent with another study in both sexes (McClure *et al.* 2014). Interestingly, dopamine receptor mRNA expression in the NAc of adults is associated with impulsive action but not with impulsive choice (Simon *et al.* 2013) and males experience larger changes in dopamine receptor density within the NAc during adolescence (Andersen *et al.* 1997). Thus, it may be

that sex differences in the maturation of dopamine receptors within the NAc, rather than sex differences in PFC development, are driving the subtype- and sex-specific effects of development on impulsivity.

Although PFC dopamine receptor mRNA is thought to be associated with both impulsive action and impulsive choice (Simon *et al.* 2013), we found that PFC D₂R expression was related only to impulsive choice. Another study looking at evoked dopamine release found that high levels of impulsive choice were associated with less responsive dopamine terminals in the medial PFC, while there was no such relationship between impulsive action and dopamine release (Diergaarde *et al.* 2008). Therefore, it may be that impulsive choice is associated with reduced functionality of the PFC dopamine system, as measured by evoked release and receptor protein expression, while impulsive action is simply associated with reduced mRNA. Interestingly, the relationship between impulsive choice and D₂R expression was present whether rats were first tested during adolescence or adulthood, suggesting that this relationship persisted through the period of D₂R pruning in the PFC (Andersen *et al.* 2000). Moreover, the relationship between delay discounting and D₂R expression appears to be stronger in females. The reason for this sex difference is unclear and highlights the need for more research on sex differences in PFC dopamine receptors. If this is replicated in humans, it will reinforce the need to consider the role of sex differences when examining D₂R function and impulsivity as factors in drug abuse vulnerability (Volkow *et al.* 2006, 2009).

In this study, we demonstrated a relationship between an adolescent measurement of impulsivity and the initiation of ethanol drinking in adulthood. We have previously hypothesized that drinking is more strongly related to impulsivity in males (Hammerslag & Gulley 2016), while anxiety may be a better predictor of drinking for females (Varlinskaya *et al.* 2015; Hammerslag & Gulley 2016). In this study the relationship between impulsive action and drinking was significant only in males, however there was a trend toward the same relationship in females and thus the importance of this sex difference is unclear. A recent study in humans found that impulsivity predicted AUDIT score for males, through an interaction with positive alcohol expectancy, but that there was no effect of impulsivity on this index of problem drinking in females (Ide *et al.* 2017). When we first assessed impulsivity during adulthood, instead of adolescence, we were unable to demonstrate any relationship between impulsive action and drinking. To our knowledge this study is the first to investigate this relationship, though others have found that impulsive action predicts cocaine and nicotine self-administration (Dalley *et al.* 2007; Diergaarde *et al.* 2008). Previous studies have examined the connection between impulsive choice and ethanol drinking, however, with mixed results. For example, higher levels of impulsive choice in a non-traditional maze-based task predicted greater drinking (Poulos *et al.* 1995). A more recent operant study found no connection between impulsive choice and drinking (Diergaarde *et al.* 2012), consistent with the results of the current study. A limitation of our voluntary drinking model is that rats only had access to saccharin-sweetened ethanol, therefore it is unclear if they would have still engaged in drinking if alternatives had been available. Nevertheless, the approximate levels of intake (0.5–1.0 g/kg in 30 min) are in line with what has been demonstrated in operant studies using saccharin-sweetened (Roberts *et al.* 1999) and unsweetened (Diergaarde *et al.* 2012) solutions. Though adolescent impulsivity

may be a useful predictor of future alcohol consumption, it is impulsive action, and not impulsive choice, that seems to be the important factor in rats.

In summary, we examined developmentally regulated changes in two facets of inhibitory control, impulsive action and choice, and how this was influenced by sex. We found that in Sprague-Dawley rats, impulsive action changes unpredictably across development for males, but not for females, and that adolescents have greater levels of impulsive action relative to adults. Our results support to the notion that development within the mesocorticolimbic system mediates the increases in impulse control that accompany the transition to adulthood. Future studies should investigate whether it is the development of NAc dopamine receptors that is key to sex and age differences in impulsive action, as we hypothesize here. Meanwhile, impulsive choice was relatively unaffected by sex and did not seem to change as rats matured from adolescence to adulthood. Indeed, dopamine D₂R expression in the PFC seems to have long-lasting ties to impulsive choice. This suggests that the relationship between D₂R and delay discounting, as well as each individual's relative level of impulsive choice, is unchanged by the process of adolescent development. Thus, an adolescent measurement of impulsive choice can be used to predict both behavior and PFC D₂R expression during adulthood. Although impulsive choice seems to be a relatively constant measure of impulsivity across development, it should be noted that impulsive action was the most strongly correlated with drinking later in life. Thus, it may be that adolescent impulsive action is a useful measure for predicting future drug use vulnerability but is less useful for predicting future levels of impulsivity. Overall, our results demonstrate the extent to which the development of impulsivity is influenced by sex, subtype, and even study design factors like food restriction. Future studies of age and sex differences should take these considerations into account to improve our models of impulsivity and related disorders, such as ADHD and addiction.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Experimental timeline and lever press behavior in the impulsive action test

(A) The overall timeline of the experiment (top), including the training and testing sequence (middle) and an overview of the task rules (bottom). One aspect of the basic rules was modified for each set of impulsivity tests. During the impulsive action tests, the ITI was changed so that each lever press led to a signaled 5-s timeout. During the impulsive choice tests, the delay to large reward delivery was extended (up to 75 s) while the delay to delivery of the small reward (1 s) was unchanged. (B) Adults of both sexes responded more throughout the ITI period during the first two training sessions, whereas adult males and adult females had the least and most premature responding, respectively, during the impulsive action test sessions. *** $p < 0.001$ adults vs adolescents (collapsed across sex); + $p < 0.05$, +++ $p < 0.001$ adult females vs adult males; ### $p < 0.001$ female adult vs female adolescent within session (C) When premature responding was normalized to account for group differences in ITI responding during training, age and sex differences were apparent: adolescents and females were the more impulsive groups. *** $p < 0.001$ adults vs adolescents (collapsed across sex); +++ $p < 0.001$ females vs males within session (collapsed across age)

Figure 2. Test and retest measures of impulsive action and their correlation within sex
 Impulsive action in rats first tested as (A) adolescents (P43-45) or as (B) adults (P93-95) was higher during the initial test, relative to the retest. * $p < 0.05$ and *** $p < 0.001$ test vs retest within session, collapsed across sex; +++ $p < 0.001$ females vs males, collapsed across testing period (C-F) Mean premature responses action across the three test sessions during the initial test (x-axis) and the retest 50 days later (y-axis) in rats first tested as adolescents (C: female, $n = 51$; D: male, $n = 38$) or adults (E: female, $n = 47$; F: male, $n = 42$). Performance is not stable in males first tested as adolescents, but is stable in all other groups.

Each symbol denotes the value for an individual rat. The line of best fit, along with the 95% confidence interval of that best fit (dotted line), is included when there is a statistically significant relationship between the variables ($p < 0.05$).

Figure 3. Test and retest measures of impulsive choice and their correlation within sex
 Impulsive choice in rats first tested as (A) adolescents (P46-50) or (B) adults (P96-100). Rats first tested as adults were less impulsive upon retest. *** $p < 0.001$ vs retest, collapsed across delay (C-F) The slope of the discounting curve across the five delay discounting sessions during the initial test (x-axis) and the retest 50 days later (y-axis) in rats first tested as adolescents (C: female, $n = 51$; D: male, $n = 38$) or adults (E: female, $n = 47$; F: male, $n = 42$). Impulsive choice was stable from test to retest in all groups. Each symbol denotes the value for an individual rat. The line of best fit, along with the 95% confidence interval of

that best fit (dotted line), is included when there is a statistically significant relationship between the variables ($p < 0.05$).

Figure 4. Ethanol drinking and its relationship to impulsive action

(A) Drinking in males and females first tested as adolescents or adults. Females drank more overall, while males increased their drinking across blocks of sessions. +++p < 0.001 females vs males, collapsed across block; ***p < 0.001 vs block 1 in males. (B) Consumption for rats classified as having high (66th percentile) or low (33rd percentile) levels of impulsive action based on behavior during adolescence (left side) or adulthood (right). There was a significant interaction between impulsivity level and age, with a non-significant trend toward higher drinking in highly impulsive rats first tested as adolescents (p

= 0.058). +++ $p < 0.001$ females vs males, collapsed across level of impulsivity and age of first test (C-F) The relationship between drinking and premature responding is shown for rats first tested as adolescents (C: females, $n = 51$; D: males, $n = 38$) or adults (E: females, $n = 47$; F: males, $n = 42$). In males first tested as adolescents, individual drinking was positively correlated with premature responding; this relationship did not reach significance for females ($p = 0.061$). Each symbol denotes the value for an individual rat. The line of best fit, along with the 95% confidence interval of that best fit (dotted line), is included when there is a statistically significant relationship between the variables ($p < 0.05$).

Figure 5. D2R expression in the prelimbic cortex and its relationship with impulsive choice. (A) Location of the bilateral, 1 mm punches taken from the prelimbic medial PFC and representative samples from one gel (Ctl – control; adolF – adolescent test females; adolM – adolescent test males; adulF – adult test females; adulM – adult test males). (B) Relative D2R expression [(D2R/Vinculin)/(Control D2R/Control Vinculin)] for rats classified as having high (66th percentile) or low (33rd percentile) levels of impulsive choice based on their first test in adolescence or adulthood. High impulsive choice was associated with reduced D2R expression in males and females. ** $p < 0.01$, vs low impulsive choice,

collapsed across age of first test and sex. (C-F) Relationship between D2R expression and impulsive choice (slope of discounting curve) in rats first tested as adolescents (C: female, n = 12; D: male, n = 18) or adults (E: female, n = 16; F: male, n = 15). Impulsive choice, whether first tested during adolescence or adulthood, was negatively correlated with D2R expression in females but not in males. Each symbol denotes the value for an individual rat. The line of best fit, along with the 95% confidence interval of that best fit (dotted line), is included when there is a statistically significant relationship between the variables ($p < 0.05$).