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Extinction and drug-induced reinstatement of cocaine-seeking following self-administration or conditioned place preference in adolescent and adult rats

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ABSTRACT

Adolescence marks a particularly vulnerable period to developing substance use disorders, and people who start using drugs in adolescence are more likely to relapse. A limited number of studies have investigated age difference in relapse following re-exposure to the drug after a period of abstinence. Using a cocaine self-administration paradigm, we showed no age difference in acquisition or extinction of self-administration. Interestingly, adolescent rats displayed impaired cocaine-primed reinstatement of cocaine-seeking. Using the same dose as that self-administered in the first experiment, we then investigated age differences in acquisition and extinction of conditioned place preference, as well as locomotor sensitization. While there were no differences in locomotor activity or acquisition of preference, adolescents failed to extinguish their preference, even when the number of extinction sessions was doubled from what adults received. Taken together, these results suggest that while cocaine has similar rewarding and reinforcing effects regardless of age, adolescent may attribute stronger salience to the drug-associated context. In addition, re-exposure to cocaine itself may not be a strong relapse trigger in adolescence. Overall, these findings suggest that we should focus more on

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alleviating drug-context salience compared to re-exposure to substance in order to reduce relapse of drug-seeking in adolescents.

Keywords: adolescence, cocaine, context, extinction, self-administration, conditioned place preference

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1. Introduction

A major hurdle in the treatment of substance use disorders is the vulnerability to relapse even after extended periods of abstinence (Simpson, Joe, Fletcher, Hubbard, & Anglin, 1999). Evidence suggests that an early age of onset may predict the severity of addiction later in life (S. A. Brown et al., 2009; Chen, Storr, & Anthony, 2009; Dawson, Goldstein, Patricia Chou, June Ruan, & Grant, 2008; Grant & Dawson, 1998; King et al., 2007; O'Brien & Anthony, 2005; Robins & Przybeck, 1985). In addition, adolescent-onset drug users are more resistant to treatment interventions and more vulnerable to relapse, a result apparent even after controlling for the duration of substance use (Anthony & Petronis, 1995; Catalano, Hawkins, Wells, Miller, & Brewer, 1991; Perepletchikova, Krystal, & Kaufman, 2008).

Common relapse triggers include exposure to cues related to the drug-taking experience, stress, or the drug itself (Carter & Tiffany, 1999; de Wit & Stewart, 1981; Drummond, 2000; Perry, Zbukvic, Kim, & Lawrence, 2014; Wemm & Sinha, 2019). Consistent with observations in humans, we have shown that adolescent rats display greater cue-induced reinstatement of cocaine-seeking following cue extinction (Zbukvic et al., 2016). Extinction of a drug-associated cue is the reduction in responding elicited by drug cues following repeated cue presentations without the drug (O'Brien et al. 1990). Cue extinction forms the basis of cue exposure therapy, one of the most common behavioral treatments of substance use disorders (Conklin and Tiffany 2002). While resistance to cue extinction in adolescence provides insights into age-related relapse propensity to cues, other relapse triggers need to be

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studied to further understand adolescent vulnerability to substance use. Therefore, the first aim of the present study was to examine drug-induced reinstatement following cocaine self-administration in adolescent and adult rats. Notably, rats were trained to lever press for cocaine in the absence of any discrete drug-associated cue to minimize any contribution of discrete cue-induced responding in our assessments (Kim et al., 2015). Rats then underwent progressive ratio schedule of self-administration, lever extinction, then cocaine-primed reinstatement. Previous studies showed no age effects in acquisition of cocaine self-administration with a range of doses (0.3-1.0 mg/kg) (Frantz, O'Dell, & Parsons, 2007; Kerstetter & Kantak, 2007; Li & Frantz, 2009, 2017; Li et al., 2018; Madsen, Zbukvic, Luikinga, Lawrence, & Kim, 2017; Wong et al., 2013; Zbukvic et al., 2016) and motivation to self-administer cocaine as measured by progressive ratio (Zbukvic et al., 2016). The age of adolescent rats at the start of experimentation in those studies ranged between postnatal days (P) 35 and 37, considered early adolescence based on the updated hormonal and cognitive rodent developmental milestones (Bell, 2018; Madsen & Kim, 2016; Perry et al., 2020). It should be noted that one study showed that when P42 rats start cocaine self-administration, they pressed more than adults at 0.3 mg/kg, although P35 rats did not differ from adults (Wong et al., 2013). Notably, all those studies paired a discrete cue (e.g., light) with each cocaine infusion. Drug-associated discrete cues can facilitate the development and persistence of drug-taking (Perry, Zbukvic, Kim, & Lawrence, 2014), and without such a facilitator, there may be age differences in the development of cocaine self-administration.

As for instrumental extinction, studies have reported that adolescent rats respond significantly more than adults (Anker & Carroll, 2010), less than adults (Li & Frantz, 2009, 2017), or the same (Li et al., 2018; Zbukvic et al., 2016). When challenged with a 10 mg/kg cocaine-prime, Li and Frantz (2009) observed no age difference in drug-induced reinstatement, although adolescent rats were tested as adults (P79-80). In a subsequent study, Li and Frantz (2017), found that adolescent onset of cocaine self-administration led to reduced reinstatement compared to adult-onset following cocaine-priming test in adulthood (i.e., after a 60-day period of abstinence). In contrast, Anker and Carroll (2010) reported *more* responding in the adolescent-onset group tested in late adolescence (~P56). In their study, adolescents received significantly more cocaine infusions during the last 5 days of

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self-administration, so it is unclear whether the age effects on reinstatement were due to self-administration differences initial cocaine intake.

The second aim of this study was to assess cocaine-induced reinstatement following acquisition and extinction of cocaine conditioned place preference (CPP). Critically, the cocaine dose used for CPP was based on the self-administered dose in the first experiment, to maximize the ethological validity of our CPP assessment. This way, we could test potential age differences in 1) CPP at a self-administered dose rather than the experimenter-chosen dose; and 2) locomotion during acquisition of CPP and following cocaine-prime. Locomotor sensitization is thought to be an indicator of drug-induced neuroadaptations manifesting as robust behavioral change (Robinson & Berridge, 1993, 2000), which leads to heightened activity in response to acute drug priming. Literature suggests that adolescent rats (age range P28-52) display impaired locomotor sensitization compared to adults (Collins & Izenwasser, 2002; Frantz et al., 2007; Laviola, Wood, Kuhn, Francis, & Spear, 1995). However, it is yet unknown how locomotion may be associated to cocaine-primed reinstatement in adolescent and adult rats.

Previous studies have shown that adolescents show stronger CPP (age P38-44 at the start of conditioning), suggesting that drug-associated contexts might be more salient in adolescence (Brenhouse & Andersen, 2008; Brenhouse, Sonntag, & Andersen, 2008). We thus hypothesized that adolescents will show heightened cocaine self-administration and CPP, both of which involve drug-context learning (Perry et al., 2014). Extinction may also be delayed. If locomotion is related to relapse, cocaine-induced reinstatement may be reduced in adolescents, based on studies showing that adolescent rats display impaired locomotor sensitization to cocaine (Collins & Izenwasser, 2002; Frantz et al., 2007; Laviola et al., 1995).

2. **Material and Methods**

2.1. Animals

A total of 64 male Sprague-Dawley rats were bred in-house at The Florey Institute of Neuroscience and Mental Health, Melbourne, Australia. Adolescent rats were postnatal day (P) 35 (±1) and adult rats were P70 (±1) at the start of behavioral training. In experiment 1 rats were pair-housed until surgery, then individually

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housed following surgery. In experiment 2, rats were pair-housed throughout experimentation. All rats were housed under a 12:12 light/dark cycle (lights off 7 a.m.) with food and water available *ad libitum*. All testing was conducted during the dark phase. All rats were handled 3 times prior to surgery (experiment 1) or behavioral training (experiment 2). All procedures were carried out in accordance with The Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (NHMRC, 2013) and approved by the Animal Care and Ethics Committee at the Florey Institute of Neuroscience and Mental Health.

2.2. Surgeries

In experiment 1, three days prior to first self-administration day, a catheter was implanted into the right jugular vein of all rats in experiment 1, as described previously (Zbukvic et al., 2016). In brief, rats were anesthetized with isoflurane vaporized with oxygen and injected with meloxicam (3 mg/kg, i.p.). Catheters were constructed in-house as described previously (Madsen et al., 2017) and consisted of guide cannulas (22 gauge, PlasticsOne, VA, USA) and three layers of Silastic tubing (adult length 14 cm; adolescent length 12 cm; Dow Corning, USA). Catheters were flushed daily for 2 days following surgery with 0.05 mL of heparinized saline (90 IU/mL; Pfizer, NY, USA) containing 40% Neomycin antibiotic (amoxicillin sodium; Aspen Australia, NSW, Australia).

For the duration of the experiment catheters were flushed daily with 0.05 mL of 10 and 50 IU/mL antibiotic-heparin solution before and after cocaine self-administration, respectively. Patency was tested once at the end of self-administration using 0.04 mL of ketamine (100 mg/mL) for adult and 0.02 mL for adolescent rats immediately followed by 0.05 mL of 10 IU/mL antibiotic/heparin solution. Any rat that failed to show loss of muscle tone within 5 s was removed from the study.

2.3. Cocaine self-administration, lever extinction, and cocaine-primed reinstatement

In experiment 1, rats were trained to self-administer cocaine (cocaine hydrochloride; Johnson Matthey Macfarlan Smith, Edinburgh, UK) dissolved in saline in standard operant conditioning chambers, as described previously (Zbukvic et al., 2016), except no discrete cue associated with cocaine delivery (e.g., light) was ever

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present. Daily 2-hour sessions were conducted. During these sessions, active lever presses resulted in the intravenous delivery of cocaine (0.3 mg/kg/infusion) over 2.7 s. A discriminative vanilla scent was present underneath the active lever whenever the active lever was present (i.e., even during lever extinction) to indicate the spatial location of the active lever. Vanilla was never explicitly paired with cocaine delivery. Inactive lever presses had no consequences. For the first 5 days rats received cocaine on a fixed ratio (FR) 1 requirement. For the final 5 days of self-administration, responding occurred on a FR3 requirement. Following the last day of self-administration, rats received a single 4-hour progressive ratio session, in which the number of active lever response required to receive a cocaine reward increased incrementally. The progressive ratio schedule was adapted from Richardson & Roberts (1996).

Twenty-four hours following the final self-administration session, rats received daily 30-min lever extinction sessions for 7 days in which pressing on either active or inactive lever had no consequences. Twenty-four hours following the last lever extinction day, rats received a single intraperitoneal injection of either a cocaine prime (10 mg/kg) or saline then were immediately tested for drug-induced reinstatement for 60-min under lever extinction condition. Prime assignment was random. An additional two days of lever extinction was provided before rats underwent a second reinstatement session, in which they received whichever prime they did not receive in the first reinstatement session.

2.4. Acquisition, extinction, and cocaine-primed reinstatement of cocaine conditioned place preference

In experiment 2, the CPP apparatus (Med Associates Inc, VT, USA) consisted of two main chambers with differences in visual (wall patterns) and tactile (floor texture) cues. The time spent in each chamber and locomotor activity was recorded via horizontal optic sensor beams and specific software for the apparatus (Activity Monitor, Med Associates Inc). Locomotor activity was measured as the distance moved in meters. All locomotor analyses were performed on the total distance travelled during each session.

Before each session rats were habituated to the experimental room for at least 15 min. On day 1 (baseline), rats were allowed access to both chambers for

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20 min then placed back in their home cage. A combination of unbiased and biased allocation was used. More specifically, rats with a neutral preference during baseline (40–60% for either side) were randomly allocated their cocaine-paired side (unbiased allocation). For the remainder of the animals, the drug was paired with the side that was least preferred (biased allocation). On days 2–11 (conditioning) rats received alternating intraperitoneal injections of cocaine or saline then were immediately confined into one of the two conditioning chambers for 20 min. Cocaine dose received on each conditioning day (**Table 1**) was matched to the sum of average dose received every two days during cocaine self-administration in experiment 1 (e.g. average dose received in self-administration day 1 + 2 = dose received on day 1 of CPP).

On days 12-18 (extinction), rats were allowed to freely explore both chambers for 15 min with no injection, to first test for CPP then to subsequently extinguish their preference. Preference score was calculated as the percentage of time spent in the cocaine-paired chamber compared to the entire test. These sessions were 15 mins because pilot experimentation showed that from extinction day 4, rats started to fall asleep from ~18 min in the apparatus, presumably due to habituation.

All adult rats received 7 days of extinction. Adolescents rats either received 7 days or 14 days of extinction, based on pilot experiment that showed resistance to CPP extinction in adolescents. Following the last day of extinction, rats underwent a 20-min reinstatement session, intraperitoneally receiving either a cocaine prime (10 mg/kg) or saline. Prime assignment was random. The first reinstatement session was followed by another 2 days of extinction, and a second reinstatement session in which they received whichever prime they did not receive in the first reinstatement session.

2.5. Data Analysis

Statistical analyses were performed using IBM SPSS Statistics 26 (IBM Corp., NY, USA) and GraphPad Prism 8 (GraphPad Software, CA, USA). Self-administration, lever extinction and reinstatement (experiment 1), and locomotor sensitization during CPP acquisition, CPP score during extinction and reinstatement, and locomotion during reinstatement (experiment 2) were analyzed using repeated measures analysis of variance (ANOVA). Significant interactions were followed up with post

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hoc ANOVAs, Tukey multiple comparisons (if comparing more than 2 groups) or t-tests with Bonferroni correction (if comparing 2 groups) as appropriate. In experiment 1, lever discrimination and breakpoint data were analyzed using independent t-tests. In experiment 2, correlation analyses were carried out using Pearson tests. Data are available upon request.

3. Results

3.1. No Age Differences in Cocaine Consumption and Motivation to Self-Administer

Experiment 1 investigated cocaine self-administration in adolescent and adult rats (**Figure 1A**). We first examined the first five days of self-administration (acquisition phase). Three-way repeated-measures ANOVA [within-subjects factors: Lever Type (active vs inactive) and Day (IVSA days 1-5); between-subjects factor: Age (adults vs adolescents)] revealed a significant main effect of Lever Type [$F(1,21) = 9.275$, $p < 0.01$], Day [$F(4,84) = 5.830$, $p < 0.001$], and a significant Lever Type x Day interaction [$F(4,84) = 3.755$, $p < 0.01$]. There were no effects or interactions involving Age (smallest $p = 0.35$). To understand the Lever Type x Day interaction, we assessed the effect of Lever Type per Day. Bonferroni-adjusted post hoc tests indicated more active lever presses than inactive lever presses for Day 5 ($p < 0.01$; **Figure 2A**), suggesting that rats successfully acquired self-administration as evidenced by lever discrimination, regardless of age. We then analyzed the last 5 days of self-administration (maintenance phase). Three-way repeated measures ANOVA [within-subjects factors: Lever Type and Day (IVSA days 6-10); between-subjects factor: Age] revealed a significant main effect of Lever Type [$F(1,21) = 25.119$, $p < 0.001$], but no effect of Day or Age (smallest $p = 0.18$), and no interactions (smallest $p = 0.17$), indicating stable active lever pressing regardless of age. Consistent with active lever presses, analyses of infusions received (**Figure 2B**) revealed a main effect of Day [$F(9,189) = 7.075$, $p < 0.001$], but no effect of Age or Age x Day interaction (smallest $p = 0.22$).

Consistent with the repeated measures ANOVA above, % lever discrimination over the last five days of stable responding was comparable in adults and adolescents [$t(21) = 1.735$, $p = 0.10$; **Figure 2C**). There was also no effect of Age on

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breakpoint during the progressive ratio session [t(21) = 0.253; p = 0.803], suggesting that rats displayed similar maximum number of consecutive active lever presses to obtain a cocaine infusion regardless of age (**Figure 2D**). Similarly, a two-way ANOVA of progressive ratio data revealed a main effect Lever Type [F(1,21) = 13.093; p = 0.002], but no effect or interaction involving Age (smallest p = 0.63), suggesting that rats pressed more on the active lever regardless of age (**Figure 2E**).

3.2. No Age Difference in Lever Extinction

Three-way repeated measures ANOVA revealed a significant main effect of Lever Type [F(1,21) = 18.643, p < 0.001], Extinction Day [F(6,126) = 9.093, p < 0.001], and a significant Lever Type x Day interaction [F(6,126) = 3.601, p < 0.005]. There were no effects or interactions involving Age (smallest p = 0.10). To understand the Lever Type x Day interaction, we assessed the effect of Lever Type per Extinction Day. Bonferroni-adjusted post hoc tests indicated more active lever presses than inactive lever presses for days 1,2, and 4-6 (ps < 0.05). These results show that extinction led to a reduction in active lever presses more than inactive lever presses, so that by the last day the rats were no longer pressing active lever more than the inactive lever **(Figure 3A).**

3.3. Adolescents Show Impaired Cocaine-Primed Reinstatement Following Extinction of Self-Administration

A three-way repeated measures ANOVA [within-subjects factors Prime (cocaine vs saline) and Lever Type (active vs inactive); between-subjects factor Age (adults vs adolescents)] revealed a significant main effect of Prime [F(1,21) = 25.910; p < 0.001], Lever Type [F(1,21) = 12.836; p < 0.005] and Age [F(1,21) = 5.605; p < 0.05], and a Prime x Lever Type x Age interaction [F(1,21) = 6.714; p < 0.017]. To understand the three-way interaction, we analyzed each Lever Type separately. In active lever presses (**Figure 3B**), there was a Prime x Age interaction [F(1,21) = 6.067; p < 0.05]. Follow-up Bonferroni-adjusted post hoc tests indicated that adult rats reinstated their cocaine-seeking upon cocaine-prime (p < 0.001) whereas adolescents did not (p = 0.30), and the difference between adolescents and adults was significant for the cocaine-primed active lever responding (p < 0.005) but not for the saline-primed active lever responding (p > 0.99). There were no effects in inactive lever presses (smallest p = 0.32; **Figure 3C**). When only the first 20 min of

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the reinstatement session were analyzed, the results were the same, with adult rats reinstating their preference ($p < 0.01$) and adolescent failing to reinstate ($p = 0.99$; data not shown). Adolescent rats appear impaired in cocaine-primed reinstatement of lever pressing compared to adult rats.

3.4. No age effects in locomotor sensitization to cocaine injections

Experiment 2 investigated conditioned place preference (CPP) in adolescent and adult rats (**Figure 1B**). Cocaine dose received on each conditioning day (**Table 1**) was matched to the sum of average dose received every two days during cocaine self-administration in experiment 1 (e.g. average dose received in self-administration day 1 + 2 = dose received on day 1 of CPP). This way, total dose of cocaine received were comparable across experiments 1 and 2. Two-way repeated measures ANOVA [within-subjects factors Day (conditioning days 1-10), between-subjects factor Group (adults 7 days extinction vs adolescents 7 days extinction vs adolescents 14 days of extinction)] revealed a significant main effect of Day [$F(9,342) = 21.319$, $p < 0.001$] **(Figure 4A)**. There were no effects or interactions involving Group (smallest $p = 0.35$), indicating that locomotion was similar across adolescent and adult rats. The significant effect of Day was followed up with Tukey post hoc multiple comparisons, which showed that rats moved more on days 5, 7, and 9 (i.e., last 3 days of cocaine injection) compared to all the other days (ps < 0.05). Those days were not different from each other (smallest $p > 0.99$). In addition, rats moved more on days 1 and 3 compared to day 2 (ps < 0.05). There were no other significant differences between the days (smallest $p = 0.14$). Taken together, all rats developed sensitization of cocaine-induced locomotor activity across conditioning sessions.

3.5. No age effects in cocaine conditioned place preference

A two-way repeated-measures ANOVA [within-subjects factor Day (baseline vs test); between-subjects factor Group (adults 7 days extinction vs adolescents 7 days extinction vs adolescents 14 days of extinction)] revealed a significant main effect of Day [$F(1,38) = 39.496$, $p < 0.001$], but no effect of Group or Day x Group interaction (smallest $p = 0.56$; **Figure 4B)**. This suggests that all three groups similarly acquired a preference to the cocaine-paired side. To investigate potential age differences in the relationship between preference and locomotion at test, we conducted

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correlation analyses per age. Pearson tests revealed no correlation between locomotion and preference to the cocaine-paired side at test for adolescents and adults (r^2s = 0.02-0.03; ps > 0.05; **Figure 4C**)

3.6. Adolescent rats display impaired extinction of conditioned place preference

We first examined preference for the cocaine-paired side during the first seven days of extinction that every subject received. A two-way repeated-measures ANOVA [within-subjects factor Day (extinction days 1-7), between-subjects factor Group (adults 7 days extinction vs adolescents 7 days extinction vs adolescents 14 days of extinction)] revealed a trend towards a significant main effect of Day [$F(6,228) = 1.959$; $p = 0.072$] and a significant Group x Day interaction [$F(12,228) = 2.357$ $p < 0.007$], indicating that the change in preference over extinction days was different across the groups. This interaction was further investigated by conducting a one-way repeated measures ANOVA of Day per Group, which allowed assessment of whether CPP decreased over the days in each group, regardless of the number of days of extinction. Preference significantly decreased across extinction days in the adult group [$F(6,90) = 3.129$; $p = 0.008$] but not in either of the adolescent groups (smallest $p = 0.11$) **(Figure 5A)**. This suggests that adolescent rats display impaired extinction of CPP, even after 14 days of extinction.

3.7. Cocaine prime test

A two-way repeated measures ANOVA [within-subjects factor Prime (cocaine vs saline); between-subjects factor Group (adults 7 days extinction vs adolescents 7 days extinction vs adolescents 14 days of extinction)] was performed to analyze conditioned place preference following priming with cocaine or saline **(Figure 5B).** It revealed a main effect of Prime [$F(1,38) = 14.866$; $p < 0.001$] and a trend towards a significant Prime x Group interaction [$F(2,38) = 2.900$; $p = 0.067$]. Because adolescents did not extinguish their preference, one-way ANOVA per drug condition showed that in saline condition adult rats showed lower preference to the cocaine-paired chamber to adolescents ($p < 0.05$) whereas no age differences were observed in cocaine prime ($p > 0.05$), a result consistent with Bonferroni-adjusted t-tests. This is not surprising considering that adolescents failed to significantly extinguish their cocaine place preference.

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Two-way repeated measures ANOVA [within-subjects factor Prime; between-subjects factor Group] was performed to analyze locomotor activity following priming injections **(Figure 5C),** revealing a main effect of Prime [$F(1,38) = 35.269$; $p < 0.001$], but no Group effect or Prime x Group interaction (smallest $p = 0.51$). This suggests that cocaine prime increased locomotor activity regardless of age or number of extinction sessions. To investigate the potential relationship between locomotor activity and preference at cocaine-prime test, we conducted correlation analyses per age. Pearson tests revealed no correlation between locomotor activity and preference to the cocaine-paired side upon cocaine priming (r^2s = 0.01-0.06; ps > 0.05; **Figure 5D**)

4. Discussion

In the present study we showed that adult and adolescent rats similarly acquire cocaine self-administration and CPP. While no age differences were observed in lever extinction, adolescent rats failed to extinguish their CPP. Cocaine priming following instrumental extinction resulted in impaired reinstatement of lever responding in adolescent rats compared to adult rats. While cocaine-primed reinstatement in adolescents could not be assessed following CPP extinction, locomotor activity was similar across age groups at all stages of experiment 2, suggesting that impairment in drug-primed reinstatement following self-administration is unlikely caused by age differences in cocaine-induced locomotion.

4.1 Acquisition of self-administration and CPP

While previous studies showed no differences in acquisition of cocaine self-administration when infusions were paired with a cue (Frantz et al., 2007; Kerstetter & Kantak, 2007; Li & Frantz, 2009, 2017; Li et al., 2018; Madsen et al., 2017; Zbukvic et al., 2016), it is the first time no age difference in absence of any discrete cues has been shown. We also found no age differences in lever discrimination and motivation to self-administer cocaine, consistent with our previous study that involved a light cue paired with cocaine (Zbukvic et al., 2016). In addition, a recent study showed no age differences in dopamine release in the nucleus accumbens (NAc) following a systemic injection of cocaine (Corongiu et al., 2020), a key region involved in instrumental behaviors. Overall, these results suggest that cocaine has similar rewarding and reinforcing effects regardless of age.

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In contrast, Anker and Carroll (2010) found that adolescent rats self-administered more cocaine than adults. In their study, acquisition of self-administration for 6 hours daily (~10 days) did not differ between adolescents and adults, but age differences emerged during the 2 hours daily (2 sessions a day) maintenance phase over 5 days (Anker & Carroll, 2010). We have shown previously that 6 hours daily cocaine self-administration over 10 days did not differ between adolescent and adult rats (Madsen et al., 2017). It is possible that adolescents may begin to self-administer more cocaine than adults when the paradigm becomes more chronic than 10 days.

4.2 Lever and CPP extinction

We did not observe any age differences in lever extinction between adolescents and adults when rats received daily 30-min extinction sessions. This is in contrast with Anker and Carroll (2010), who showed increased cocaine-seeking during daily 2 x two-hour extinction sessions in adolescent rats. However, these findings may be the result of higher adolescent cocaine consumption during self-administration (Anker and Carroll, 2010). On the other hand, Li and Frantz (2009) observed lower levels of lever responding across six hour-long extinction sessions in adolescent rats. A potential explanation is that in long extinction sessions, adolescents are less perseverative with lever pressing. In addition, they later showed that adolescent rats respond less than adults in the first two one-hour extinction sessions (Li and Frantz, 2017). Notably, rats were extinguished after a 60-day abstinence period, and lower responding could indicate decreased recall of the operant memory acquired during self-administration.

Similar to lever extinction, we found that adult rats extinguished their CPP for the cocaine-paired chamber after 7 days of extinction. This supports a previous report showing that context-only extinction is as effective as daily lever extinction in adult rats to reduce cocaine-seeking (Kim et al., 2015). In contrast, adolescent rats failed to extinguish their CPP, even after 14 sessions. This result is more dramatic than the previous report that adolescents need ~75% more extinction sessions than adult rats to extinguish their cocaine CPP (Brenhouse & Andersen, 2008). Considering that rats were conditioned with the same dose of cocaine that was self-administered, differences in extinction behavior between our two experiments

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suggest that instrumental action-outcome behavior may be easier to inhibit compared to context-drug memories in adolescents. Both paradigms involve contextual learning (Perry et al. 2014). Self-administration additionally involves instrumental learning, with rats associating lever presses with drug reward. Notably, lever pressing is an effortful activity in rodents (Salamone, Yohn, López-Cruz, San Miguel, & Correa, 2016). It may be easier to 'give up' when it is no longer reinforced. Context-cocaine association may be enough to maintain the place preference but not lever pressing in adolescents. Such a finding also suggests that discrete cue-cocaine associations may be stronger than context-cocaine associations, with the former potentiating high levels of lever pressing in adolescents even after successful extinction (Zbukvic & Kim, 2018). It would be informative to investigate potential age differences in instrumental extinction of cocaine self-administration via nose-pokes, a less effortful action for rodents.

Failure to extinguish drug-context memories in adolescents in the present study may be due to prefrontal cortex (PFC) immaturity. The PFC undergoes dramatic changes in structure and neurochemistry during adolescence (Cullity, Madsen, Perry, & Kim, 2019; Crews et al., 2007; Dwyer and Leslie, 2016; Ernst and Luciana 2015; Kim, Perry, Ganella, & Madsen, 2017; Willing & Juraska, 2015; Zbukvic, Park, Ganella, Lawrence, & Kim, 2017). PFC function is necessary for context and cue extinction (Laurent & Westbrook, 2009), and there is evidence in humans and rodents that resistance to cue extinction is associated with reduced PFC plasticity in adolescence (Ganella et al., 2018; Kim, Li, & Richardson, 2011). While the PFC is also involved in instrumental extinction (Gass & Chandler, 2013; Peters, Kalivas, & Quirk, 2009), other brain regions such as NAc play a more critical role (Millan et al., 2011; Gibson et al., 2019). There is evidence that the NAc matures faster than the PFC in rodents and humans (Mengler et al., 2014; Mills, Goddings, Clasen, Giedd, & Blakemore, 2014), explaining why context extinction but not instrumental extinction was impaired in adolescent rats.

4.3 Cocaine-induced reinstatement of lever pressing and CPP

Surprisingly, adolescent rats displayed impaired cocaine-induced reinstatement of lever responding compared to adults. This is in line with a previous report showing less responding in adolescent-onset cocaine self-administration group upon cocaine-

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priming in adulthood (Li and Frantz, 2017). In contrast, Anker and Carroll (2010) and Li and Frantz (2009) reported either enhanced cocaine-induced reinstatement in adolescent rats or no age differences. In Anker and Carroll (2010), adolescent rats self-administered more cocaine and displayed impaired lever extinction behavior, which likely influenced drug-induced reinstatement results. In Li and Frantz (2009), adolescent rats were tested for drug-induced reinstatement 30 days following self-administration at P79-80, which is well into adulthood compared to P53-56 (late adolescence) in the present study. In addition, both studies included a discrete cue paired with each cocaine infusion during self-administration training, but not during drug-primed reinstatement (Anker and Carroll, 2010; Li and Frantz, 2009). There is evidence that cue-pairing may cause specific neural adaptations in the mesocorticolimbic circuit critically involved in reinstatement (Kalivas & Volkow, 2005), which could explain the discrepancies between studies.

Age effects in drug-induced reinstatement observed in the present study are unlikely to be caused by age differences in cocaine metabolism, because there are no age differences in brain or plasma cocaine levels following injection of a similar dose to our study (Caster, Walker, & Kuhn, 2005). In addition, a recent study showed no age differences in NAc dopamine concentration upon injection of 10 mg/kg of cocaine (Corongiu et al., 2020). Cocaine-induced locomotion likely did not differ during reinstatement test, based on no age differences in inactive lever pressing (experiment 1) and locomotion (experiment 2).

A potential explanation is that the negative impact of cocaine withdrawal after self-administration is less intense in adolescents than adults, given the positive correlation between withdrawal severity and drug-primed reinstatement of cocaine-seeking (Buffalari, Baldwin, & See, 2012). Consistent with this idea, literature indicates that adolescent rats display reduced alcohol and nicotine withdrawal severity (Doremus, Brunell, Varlinskaya, & Spear, 2003; O'Dell, Bruijnzeel, Ghozland, Markou, & Koob, 2004). Potential age differences in cocaine withdrawal severity need to be assessed in the future.

It has also been proposed that cocaine-taking relies on a "satiety threshold" whereby the high from the previous drug injection must reach below a threshold to enable subsequent drug-taking (Tsibulsky & Norman, 1999). This process is

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modulated by dopamine action in the NAc (Suto & Wise, 2011). Injection of cocaine induces a greater dopamine increase in the NAc of adolescent rats compared to adults (Walker & Kuhn, 2008), and lower levels of dopamine transporters have been observed in the NAc of adolescent rats (Matthews, Bondi, Torres, & Moghaddam, 2013). Those findings suggest that adolescents may have experienced more prolonged concentration of synaptic dopamine following a 10 mg/kg cocaine prime compared to adults, staying too high above the satiety threshold to engage in cocaine-seeking. However, a recent microdialysis study showed that in NAc shell, dopamine levels return to baseline more quickly in adolescent rats compared to adult rats following a systemic injection of 10 mg/kg cocaine (Corongiu et al. 2020). Such age difference appeared well after the injection (~60 minutes post-injection), whereas in our study, the entire reinstatement session lasted 60 minutes, with most lever pressing occurring earlier rather than later in the session. Future studies using more temporally accurate tests (e.g., voltammetry) is required to test whether reduced cocaine-induced reinstatement may be related to NAc dopamine levels in adolescents.

4.4 Hyperlocomotion and sensitization to cocaine

Locomotion increased over time in response to cocaine injections at the self-administered dose in adolescents and adults (Table 1). This is consistent with a study reporting an age-independent increase in locomotion in response to an increasing dose of cocaine within the range used in the present study (5-25 mg/kg) (Caster et al., 2005). In contrast, Frantz and colleagues (2007) found delayed sensitization in adolescent rats when injected with similar doses. In their study, cocaine was injected every five days, whereas cocaine injections occurred every second day in our study, which may explain the contrasting findings. Another study found that cocaine-induced locomotion in adolescent rats remained constant across daily injections while it increased in adults (Collins and Izenwasser, 2002). Discrepancies could be due to several methodological differences between our studies. In that study, rats were injected twice daily, receiving 20 mg/kg before testing and 30 mg/kg in their homecage four hours later while all our animals receiving cocaine only prior to testing. In addition, we may have observed similar age differences with more injection days, considering that adolescents appeared to show a sudden increase in locomotion compared to adults (Figure 4A). Although there

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were no statistically significant age-related interactions on locomotion during CPP acquisition, there appears to be a difference in day 5 locomotion between age groups (Figure 4A). Notably, rats received the highest dose of cocaine on day 5 (23.2 mg/kg), doubling that of the previous cocaine day. Lower locomotor activity in adults on day 5 may suggest transition into stereotypy due to the abrupt increase in cocaine dose, because locomotion and stereotypy are mutually exclusive (Fog, 1969).

In the present study, all groups displayed hyperlocomotion to the 10 mg/kg cocaine prime compared to saline, regardless of the number of extinction sessions. The lack of difference in locomotion between the 7 and 14 days of extinction groups suggests a long-lasting effect of sensitization to cocaine. It has been hypothesized that repeated drug exposure leads to neuroadaptations resulting in excessive attribution of incentive salience to the drug and drug-related stimuli, which can in turn lead to compulsive drug-seeking, -taking and increased vulnerability to relapse (Robinson & Berridge, 1993, 2000). Our data suggest this is not the case, as there were no differences in locomotion between groups, regardless of whether rats extinguished or reinstated their preference. This dissociation between the motor effect of cocaine and drug-induced reinstatement has previously been reported following extinction of CPP and self-administration (Ahmed & Cador, 2006; R. M. Brown, Short, & Lawrence, 2010), and suggests that sensitization measured by locomotion do not play a role in drug-induce relapse propensity.

4.5 Limitations and conclusions

It should be noted that in the present study the housing conditions were different between the two experiments. In experiment 1, rats were single-housed post-surgery as is common practice in cocaine self-administration literature (Madsen et al., 2017; Zbukvic et al., 2016; Kerstetter and Kantak, 2007; Anker and Carroll, 2010), whereas they were pair-housed in the second experiment, which is in line with CPP and locomotor sensitization literature (Zakharova et al., 2009; Badanich et al., 2006; Frantz et al., 2007; Collins and Izenwasser, 2002). There is evidence that individual housing from P21 can affect drug-induced locomotion, modestly enhance self-administration acquisition, and anxiety levels (Hall, Huang, Fong, Pert, & Linnoila, 1998; Lu, Shepard, Hall, & Shaham, 2003). In our study, total dose of cocaine

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received per rat were kept the same between the self-administration and CPP experiments. However, while the total dose of cocaine received across experiments was the same, we cannot assume the rats' cocaine experience was similar between the experiments because the potency between the two routes (intravenous vs intraperitoneal) may vary (Ma et al., 1999). Different housing treatments can lead to different stress levels in adolescents compared to adults (Burke et al., 2017). However, Li and Frantz (2017) showed that isolated (referred to as 'impoverished') housing did not differentially impact adult vs adolescent-onset of cocaine self-administration when tested for cocaine-induced reinstatement in adulthood. There is one study simultaneously assessing adult vs adolescent-onset of drug exposure showing that intravenous vs intraperitoneal route of administration does lead to significant differences in age-associated effects on conditioned fear, although they used methamphetamine and not cocaine (Luikinga et al., 2019), which highlights that direct comparisons between experiments 1 and 2 in the present study is limited.

In the present study we only investigated sensitization by measuring distance travelled in response to cocaine injections. It would be informative to look at stereotyped behaviors such as repeated head movement, rearing and sniffing considering the critical role they play in behavioral sensitization (Robinson and Berridge, 1993). Importantly, similar stereotyped behaviors have been obesvered in people with cocaine use disorder (Fasano et al., 2008), and preclinical evidence suggest an age effect on such behaviors (Caster et al., 2005; Laviola et al., 1995).

Our data showed that adolescent rats are impaired in cocaine-induced reinstatement after acquisition and extinction of instrumental action-outcome behavior in absence of a discrete cue. This difference is unlikely to be caused by age differences in locomotion but could be caused by differences in withdrawal severity and satiety threshold. We also reported that adolescent rats fail to extinguish their drug-context memories. Our results, along with previous studies, suggest that adolescent vulnerability to substance use is critically related to environmental cues, and future studies should focus on ways to reduce the salience of drug-associated cues and contexts rather than the drug itself to treat young people with cocaine use disorder.

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Table 1. CPP cocaine injection schedule and doses calculations

Self-administration days	Group mean (±SEM) cocaine (mg/kg)	CPP conditioning day	Cocaine dose administered

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	consumed in 2 days		during CPP conditioning(mg/kg)
1,2	7.6 ± 2.1	1	7.6
3,4	12.5 ± 0.2	3	12.5
5,6	23.2 ± 2.7	5	23.2
7,8	16.8 ± 2.3	7	16.8
9,10	16.5 ± 1.1	9	16.5

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Figure legends

Figure 1: Experimental groups and timeline. (A) Adult and adolescent rats underwent cocaine self-administration (0.3 mg/kg/infusion). All rats underwent a single progressive ratio session the after the final day of self-administration. Rats then received lever extinction. Following extinction, rats received either a saline prime or a cocaine prime (10 mg/kg i.p.) and were tested for reinstatement of active lever pressing. **(B)** Adult and adolescent rats were conditioned using alternating daily cocaine or saline injections (i.p.). Adult rats then received 7 days of extinction. Adolescent rats received either 7 or 14 days of extinction. Following extinction, rats received either a saline prime or a cocaine prime (10 mg/kg i.p.) and were tested for preference reinstatement.

Figure 2: Cocaine self-administration, consumption and motivation to self-administer. (A) Mean (±SEM) daily lever responses. Examination of the first five days (acquisition) showed that responding on the active lever was higher than inactive lever only for day 5 in both groups ($p < 0.05$). Examination of the last five days (maintenance) showed that there was a main effect of lever type ($p < 0.001$) but no other significant effects or interactions (ps > 0.05), suggesting stable cocaine-taking. **(B)** Mean (±SEM) daily cocaine infusions (0.3 mg/kg/infusion). Number of infusions received increased over self-administration days for both groups ($p < 0.05$). **(C)** % active lever discrimination (+SEM) was similar across age. **(D)** Mean breakpoint (+SEM) and **(E)** active vs inactive lever pressing during the progressive

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ratio session. There were no age differences. Adult n = 9; adolescent n = 14. FR = fixed ratio.

Figure 3: Lever extinction and drug-primed reinstatement. (A) Mean (±SEM) daily lever responses. Active lever responses decreased over lever extinction days in both groups, with no difference between active and inactive lever responses by final extinction day. **(B)** Mean (+SEM) active lever presses following injection of either a saline or cocaine prime (10 mg/kg, i.p.). Adult rats reinstated their cocaine-seeking upon cocaine prime ($p < 0.001$) whereas adolescents did not ($p = 0.30$). Active lever responses were higher upon cocaine prime (*$p < 0.005$) in adults but not in adolescents. Active lever responses were similar in both groups upon saline prime. **(C)** Mean (+SEM) inactive lever presses following injection of either a saline or cocaine prime (10 mg/kg, i.p.). There were no age effects or interactions (ps < 0.05). Adult n = 9; adolescent n = 14.

Figure 4: Locomotor sensitization to cocaine injections and conditioned place preference. (A) Mean (±SEM) daily total distance travelled. Rats received cocaine i.p. on days 1, 3, 5, 7 and 9 (dose schedule in **Table 1**). They received saline on days 2, 4, 6, 8 and 10. There was no difference in locomotion between groups (ps > 0.05). Rats moved more on days 5, 7, and 9 compared to all other days (*ps < 0.05). Rats moved more on days 1 and 3 compared to day 2 (# $p < 0.05$). **(B)** % time spent in cocaine-paired chamber (+SEM). All rats acquired a preference to the cocaine-paired chamber following conditioning (*$p < 0.05$). **(C)** Correlation between total distance travelled and % time spent in cocaine-paired chamber at cocaine-primed test. Locomotor activity did not correlate with preference at any age (ps > 0.05). Adult (7 days of extinction) n = 16; adolescent (7 days of extinction) n = 12; adolescent (14 days of extinction) n = 13.

Figure 5: Conditioned place preference extinction and preference following drug prime (A) Daily % time spent (±SEM) in cocaine-paired chamber. Preference significantly decreased across extinction days in adult rats ($p < 0.05$), but not in either adolescent groups (ps > 0.05). **(B)** % time spent in cocaine-paired chamber (+SEM) following injection of either saline or cocaine prime (10 mg/kg). Adult rats reinstated their preference for the cocaine-paired chamber upon cocaine prime (*p <

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0.001). Adolescent groups showed cocaine-side preference regardless of saline or cocaine prime (ps > 0.05). **(C)** Mean (+SEM) total distance traveled during cocaine- or saline-primed reinstatement session. All groups displayed similarly increased locomotor activity in response to cocaine compared to saline (*p < 0.05). **(D)** Correlation between total distance travelled and % time spent in cocaine-paired chamber at cocaine-primed test. Locomotor activity did not correlate with preference at any age (ps > 0.05). Adult n = 16; adolescent (7 days of extinction) n = 12; adolescent (14 days of extinction) n = 13.

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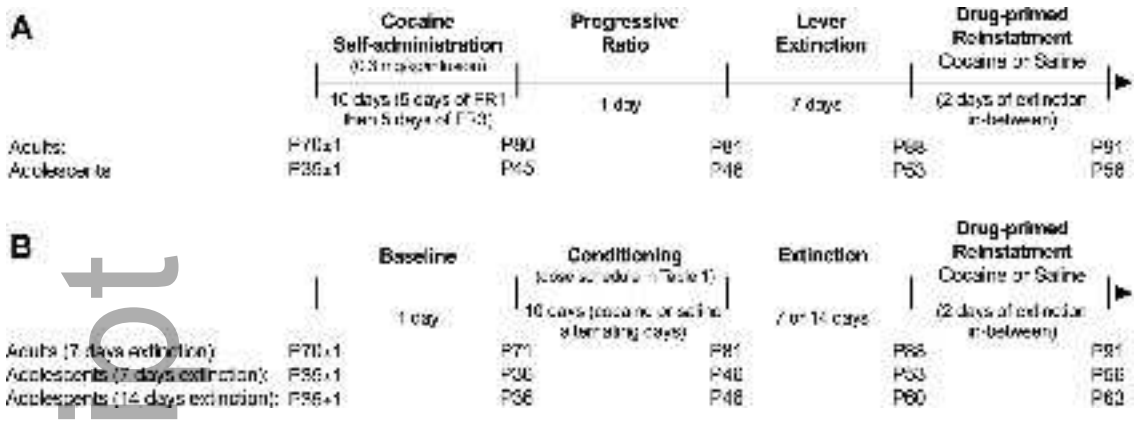

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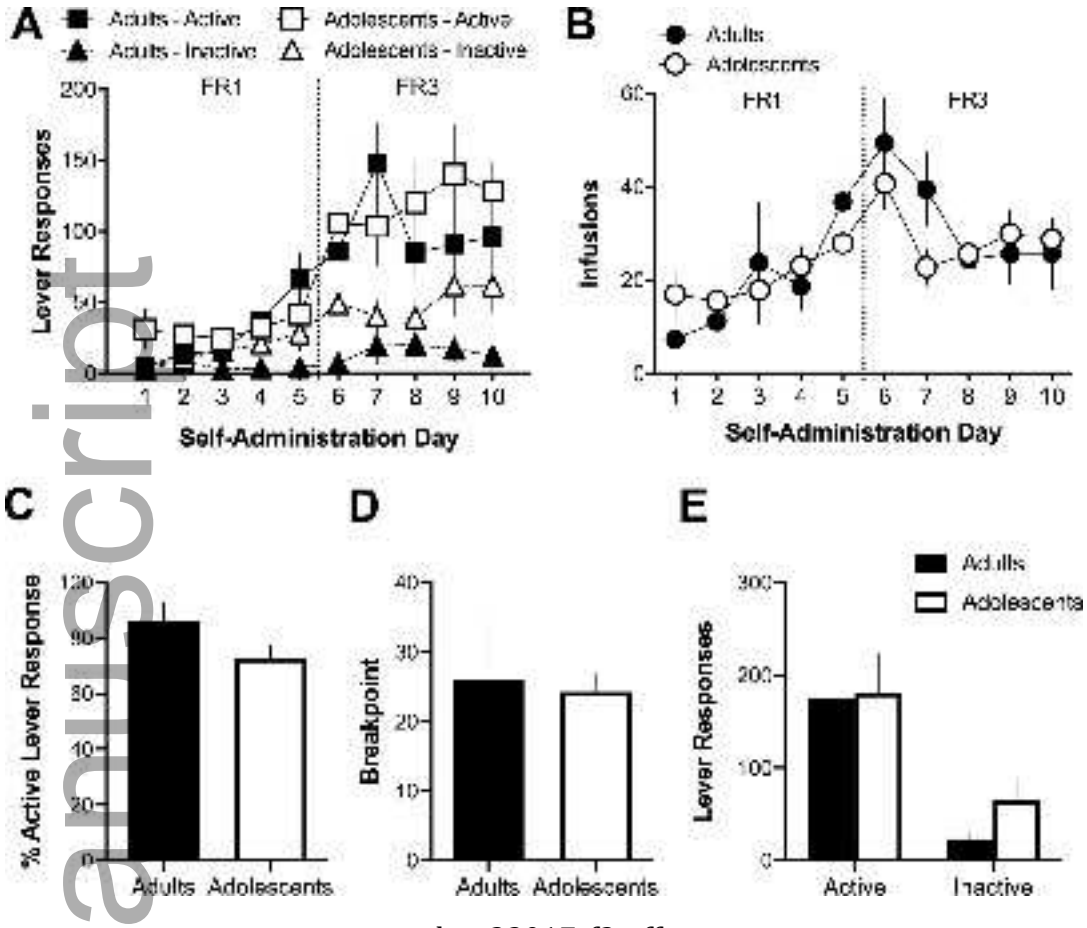

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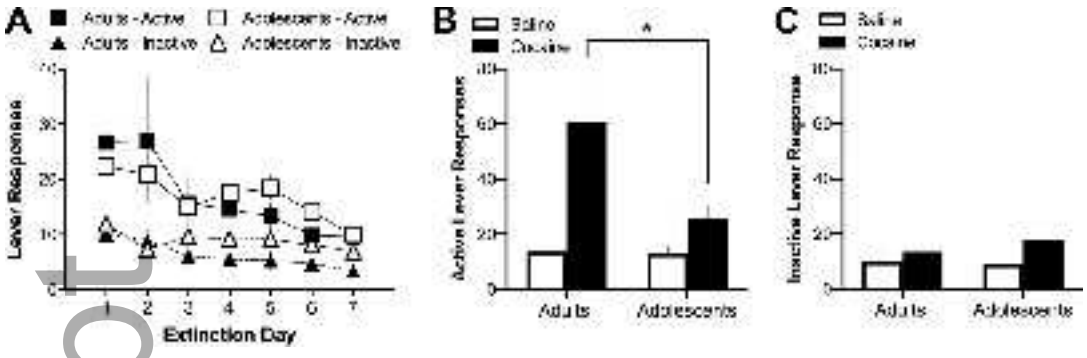

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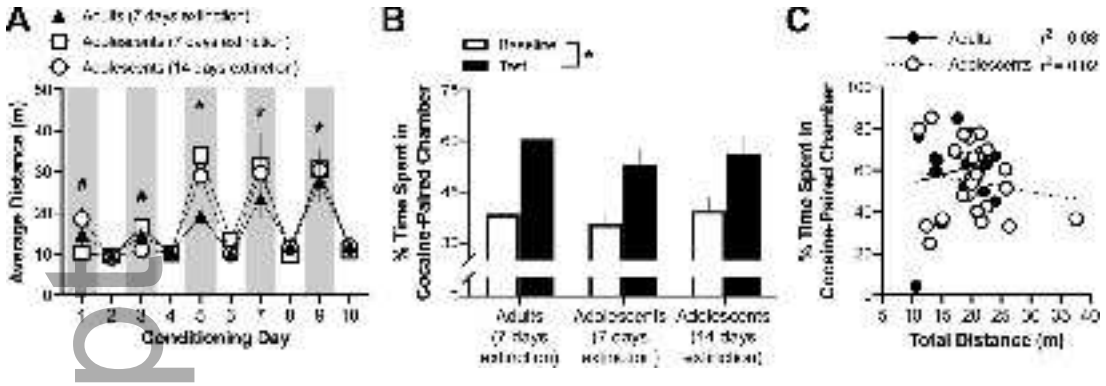

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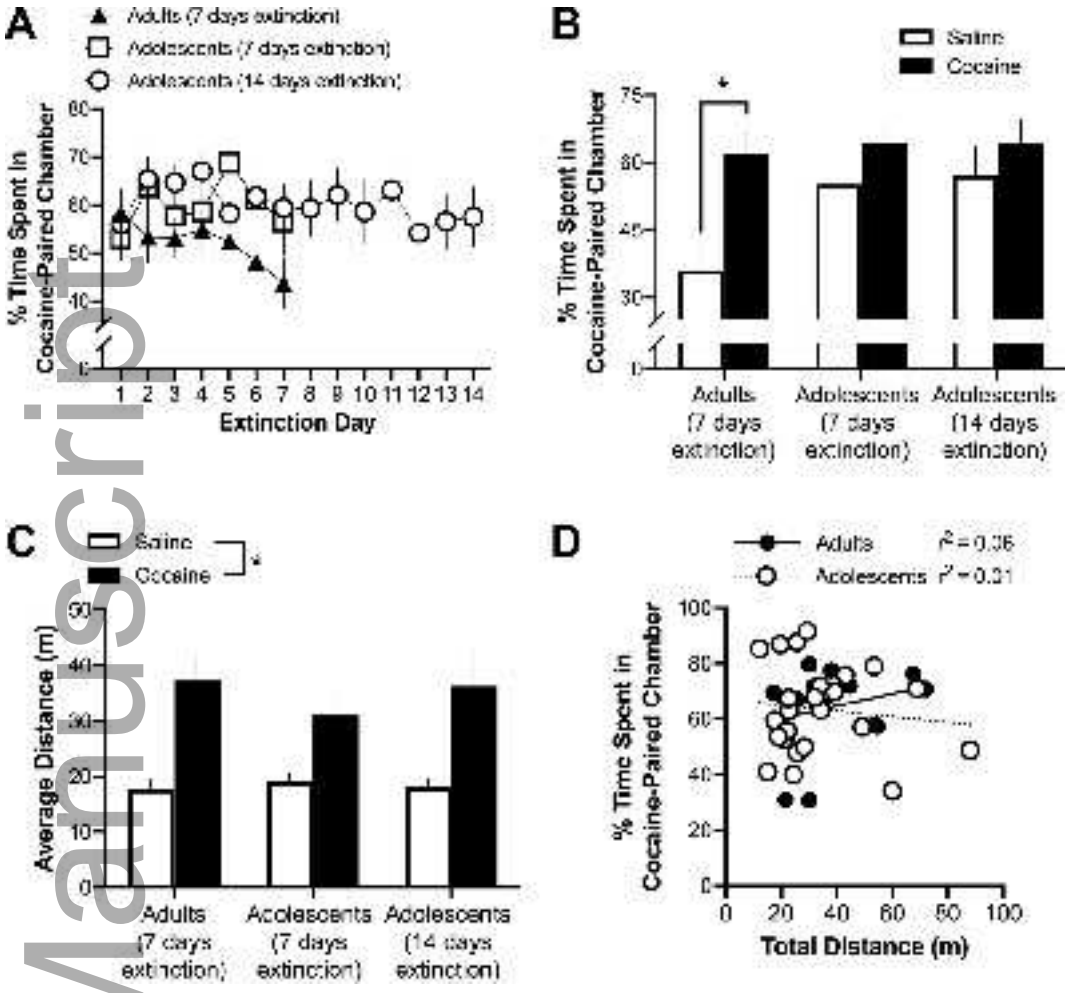

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