ORIGINAL INVESTIGATION

Escalation of cocaine self-administration in adulthood after social defeat of adolescent rats: role of social experience and adaptive coping behavior

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Abstract

Background The link between adolescent social stress and substance abuse is modeled in social defeat of adolescent male rats, at an age when social experiences are essential for neurobehavioral maturation.

Objective We investigated the role of social experience and social defeat stress during adolescence on social behavior and cocaine self-administration (CocSelfAd) in early adulthood.

Methods We manipulated social experience by housing male rats in pairs (PH) or singly (SH) on postnatal day (P) 21. In addition, rats were subjected to social defeat from P35–44. Social behavior was measured during the first and last social defeat in PH and SH adolescents and PH adults. After assessing the behavioral response to novelty and cocaine (P57–61), intrajugular catheters were implanted and CocSelfAd was analyzed.

Results Residents were less aggressive toward PH adolescent intruders compared to PH adult intruders. Adults were submissive and defensive when attacked, whereas PH adolescents froze. In the course of repeated defeats, adolescent PH rats increased freezing, while SH rats decreased freezing. Longer attack-induced freezing after repeated defeats predicted escalated CocSelfAd in adulthood. PH controls acquired CocSelfAd more slowly than PH defeated and SH rats. Defeated PH rats increased CocSelfAd during progressive ratio schedules of reinforcement and during a 24-h continuous access binge compared to PH controls and SH defeated rats.

Conclusions Social defeat in adolescence of PH rats caused persistent increases in adult CocSelfAd. Adolescent PH rats coped with attacks adaptively by increasing freezing behavior after repeated social defeats, a measure that predicted CocSelfAd in adulthood.

Keywords Addiction · Cross-sensitization · Juvenile · Psychostimulant · Isolation rearing · Social stress · Progressive ratio · Binge · Subjugation

Introduction

Thirty percent of youths experience peer victimization, and about 10 % are victimized on a regular basis (Nansel et al. 2001; Newman et al. 2005). Experiencing the negative consequences of victimization, such as low self-esteem, increases severity of psychopathologies and substance abuse. Social defeat in laboratory rodents, in which the subjects (termed intruders) are confronted with aggressive conspecifics (termed residents) may model victimization in humans (Bjorkqvist 2001) and may help to identify neural mechanisms that cause stress-induced human pathologies, such as substance use disorder (Buwalda et al. 2005). Social defeat is an ethologically and etiologically relevant stressor for the rat (Miczek et al. 1991; Miczek et al. 2008) that elicits an intense glucocorticoid stress response (Koolhaas et al. 1997) that does not habituate over repeated confrontations (Covington and Miczek 2005; Watt et al. 2009). Episodes of social defeat in adulthood reliably increase cocaine self-administration in adult male rats (Covington and Miczek 2005; Miczek et al. 2011). As adolescence is a particularly sensitive period for social development,

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we measured cocaine self-administration following social defeat during adolescence.

Neural and behavioral development is frequently investigated in rodents during adolescence between postnatal days (P) 21 and 59 (reviewed in Burke and Miczek 2014). Adolescence is considered a period of hypersensitivity to stressful events as illustrated by slower return to baseline of stress-stimulated plasma corticosterone compared to adults (Goldman et al. 1973; Romeo 2010). Adolescent-typical social behaviors, such as play fighting, are essential for the development of normal adult social behaviors (Hall and Perona 2012; Lukkes et al. 2009c). The long-term effects of adolescent social deprivation and adolescent social defeat overlap because both sensitize the mesocorticolimbic dopamine system (Burke et al. 2010; Whitaker et al. 2013) and increase measures of psychostimulant reward (Burke et al. 2011; Howes et al. 2000). The interaction between social deprivation and defeat during adolescence was investigated in the present experiment.

Analysis of aggressive resident behavior during social defeat of socially housed male mice and female rats suggest that residents are less aggressive toward adolescents compared to adult intruders (Garcia-Pardo et al. 2014; Ver Hoeve et al. 2013). Further, social and nonsocial behaviors of socially housed adolescent intruders when confronted with an adult resident are different from adult intruder behaviors (Garcia-Pardo et al. 2014; Ver Hoeve et al. 2013). We now provide a detailed analysis of both resident and intruder behavior during adolescent and adult social defeat of pair-housed (PH) male rats.

As social deprivation during adolescence has short- and long-term effects on social behavior (Lukkes et al. 2009b; Panksepp et al. 1984), and single housing (SH) intensifies the negative effects of adult social defeat (de Jong et al. 2005; Nakayasu and Ishii 2008; Ruis et al. 1999; Von Frijtag et al. 2000), we investigated the role of housing conditions during adolescent social defeat on adult cocaine self-administration. Adolescent PH rats adopt submissive postures more quickly over repeated social defeat encounters exhibiting conditioned defeat behavior (Watt et al. 2009). We conducted a detailed analysis of adolescent rat behavior during the first and fourth social defeats to discover changes in social behaviors and investigated their relationship with adult behavioral phenotypes because individual differences in conditioned behavior during adolescence might predict adult behaviors.

A frequently documented consequence of peer victimization is increased illicit drug use (Hoffmann et al. 2000; Tharp-Taylor et al. 2009). Victimization of adolescents by adults also increases substance abuse (Nelson et al. 1995). Animal studies demonstrate that stress history in adulthood increases measures of drug reinforcement, preference, and locomotion, but capturing this link in adolescent rodents is investigated less (Burke and Miczek 2014). A history of brief intermittent

social defeat episodes in adult rats promotes greater voluntary self-administration of cocaine according to fixed or progressive ratio schedules of reinforcement (PR), and particularly during a 24-h continuous access binge (Covington and Miczek 2005; Miczek et al. 2011). Adolescent social defeat stress increases amphetamine-stimulated locomotion and conditioned place preference in rats (Burke et al. 2013; Burke et al. 2011). In the present study, we investigated cocaine self-administration under several schedule conditions after adolescent social defeat. Single housing increases measures of drug self-administration, but only when the rat is isolated from weaning onward (Lopez et al. 2011; Robbins et al. 1996; Schenk et al. 1990). We investigated how housing conditions interacted with social defeat during adolescence and their effects on cocaine self-administration in adulthood using limited and extended access conditions.

The current study aimed to identify the long-term effects of social deprivation and social defeat during adolescence on novelty and cocaine-stimulated locomotion, acquisition of cocaine self-administration, motivation for cocaine, and cocaine self-administration during a 24-h binge in early adulthood. Furthermore, we sought to compare and contrast adolescent social defeat behaviors as a function of age and housing conditions. The overarching hypotheses were that adolescent social deprivation and social defeat would increase cocaine self-administration and pair housing would serve as a social buffer against the negative effects of social defeat stress.

Experimental methods

Animals and environment

Long-Evans rats from Charles River Laboratories (Wilmington, MA) were housed in a vivarium that was maintained at 21 ± 1 °C with 35–40 % humidity on a reverse light/dark cycle (lights on 2000 to 0800 hours). Non-littermate male rats ($N=96$, eight separate one-day shipments over 18 months) arrived on P20 or P21 and were housed singly or in pairs in standard polycarbonate cages ($45\times24\times20$ cm) with wood chip and Crink-l'Nest (The Andersons, Maumee, OH) bedding. Adult non-littermate rats arrived on P58 ($N=12$) and were pair housed (PH). Food (Purina laboratory rodent chow) and tap water were available ad libitum. Reliably aggressive adult male (500–700 g) rats ($Ns=20$) were housed with females in large stainless steel cages ($71\times46\times46$ cm) filled with the same bedding as intruders in a separate room, but within the same vivarium. All adolescent and adult intruder rats were handled and weighed Monday–Friday from P32 onward. Facilities and procedures were approved by the Tufts Institutional Animal Care and Use Committee in adherence with the guidelines established by the National Institutes of Health (National Research Council (US). Committee for the

Update of the Guide for the Care and Use of Laboratory Animals et al. 2011).

Social stress

Residents were confronted once per day for 12 days with non-experimental rats prior to experimental social defeat to identify the most reliably aggressive residents. The six most consistently aggressive residents were selected based on latency to attack, aggressive postures, and frequency of attacks/bites. Adolescents were defeated four times over 10 days, following the intermittent episodic social defeat design of previous experiments in adults (Boyson et al. 2014; Covington and Miczek 2005), occurring specifically on P35, P38, P41, and P44 (Fig. 1). Controls remained undisturbed during each defeat procedure. Cage-mates of defeated and control rats had the same treatment. The females and any pups were removed a few minutes before the defeat procedure. The experimenter recorded the latency and frequency of bites and supine postures, the duration of each supine posture, and the total duration of the interaction (termed latency to submission). The interaction was terminated 5 min after the first attack bite or earlier if the intruder displayed a submissive supine posture for greater than 4 s or if more than 12 attack bites occurred. The intruder was then removed and placed in a protective mesh cage (20×30× 20 cm), which was then placed inside the resident's home cage for 10 min. Intruders were exposed to a different resident during each episode of defeat. If no attack bite was observed within 5 min, the intruder was placed into a different resident's cage. A group of PH adult rats underwent the same procedure and were exposed to the same residents used for adolescent defeats but at different ages (P65, P68, P71, and P74).

Rat behavior was measured using The Observer XT v9.0.436 (Noldus Information Technology, The Netherlands) that allowed for recording of simultaneous and overlapping events. A trained observer (intra-observer reliability $r>0.93$) measured previously described intruder and resident behaviors (Buwalda et al. 2013; Koolhaas et al. 1980; Plyusnina et al. 2011) during separate observations. Nonsocial exploration (walking, rearing, and sniffing) and nonambulatory motor behavior were quantified for all rats. The following resident behaviors were quantified: attack bite (usually directed at rump and flank and included weaker nips), aggressive posture (pin), other aggression (foreleg attack, hind leg attack, dragging), frontal threat, lateral sideways threat, pursuit, allogrooming, and anogenital investigation. Intruder behaviors quantified were supine posture, escape from supine, upright defensive posture, freezing posture, walking slowly, tail rattle, follow and crawl under resident, approach resident, and social investigation (sniffing resident). Since the duration of the fights (latency to submission) varied, all Observer-coded data were expressed as a percentage of total time or number per minute. A time lag of −3 to +5 s from the attack bite was used to determine the probability of intruder behaviors occurring in response to an attack bite.

Adult locomotion testing

All locomotor testing occurred in the vivarium, but in a separate room. Previously defeated rats were undisturbed except for handling and weighing until the open field test on P57. The open field test was conducted under red lighting between 0900 and 1600 hours in plastic cages that measured 54.0×35.5× 48.5 cm or 48.3×29.3×43.2 cm. Two different-sized open fields were used because identical replacements were unavailable mid-experiment. A computer equipped with EthoVision XT v.8.5.614 (Noldus) measured motor behavior. A center rectangle (37.5×21.6 cm larger cage; 31.8×17.1 smaller cage) was outlined with EthoVision. The dependent variables "distance moved" and "time in center" were closely similar in the two different open fields ($p>0.05$). Immediately after the open field test, rats were placed into custom-built acrylic chambers (30× 30.5×24.5 cm; one rat per chamber), which served as the home cage until cocaine self-administration was initiated.

From P57 to P59, all rats received daily saline injections (1 ml/kg, i.p.). On P60, each rat was injected with saline and returned to the home cage illuminated by red lighting, and then a tracking software (HomeCageScan v3.0, CleverSys Inc., Reston, VA, USA) was started a few seconds later. After 10–12 min, the rat was injected with cocaine (10 mg/kg, i.p.) and placed back in the home cage. Cocaine hydrochloride (Research Technology Branch of the National Institute on

Overview	Arrival Housing	Social Defeat Stress	Novel Test	Re-house	Saline Injections	Cocaine Behavior	Surgery	IV Cocaine Self-Administration			
								Acquisition	Maintenance	Progressive Ratio	Binge
Details	Pair or single standard housing	Confronted with new aggressor every 3rd day ≈15 mins per day	Open field 60 mins	Individual housing non-standard cages	Habituation to saline injections (homecage) ip.	Saline then cocaine 10 mg/kg ip. (homecage) 40 mins	Jugular catheter implantation	FR1 0.75 mg/kg/inf. max 15 inf. or 5 hrs. per day	FR1-FR5 0.75 mg/kg/inf. behavioral shaping max 15 inf.	PR 0.30 mg/kg/inf. avg. of 3 days interrupted by FR5 sessions	FR5 0.30 mg/kg/inf. 24 h. unlimited access
Age (days)	21	35 38 41 44	57	57	57 58 59	60	61-63	≈65 66 67	≈68 - 77	≈77 79 81	≈84
Stage	Weaning Adolescence					Adulthood					

Fig. 1 Experimental timeline and summary of procedures. Abbreviations: Fixed ratio schedule of reinforcement (*FR*), progressive ratio schedule of reinforcement (*PR*), intravenous (*IV*), infusion (*inf.*), average (*avg.*)

Drug Abuse, Rockville, MD, USA) was dissolved in sterile 0.9 % saline for all experiments. The behaviors defined by CleverSys "walk to the left" and "walk to the right" from 6 to 10 min after i.p. injection were combined into one measurement and used as the dependent variable, "walking duration (s)". Walking for 6 to 10 min after i.p. saline was compared with the corresponding 5 min time bin after i.p. cocaine consistent with previous studies (Covington and Miczek 2005; Miczek et al. 2011).

Cocaine self-administration

Surgery Rats were anesthetized with a mixture of ketamine (100 mg/kg) and xylazine (6 mg/kg). An indwelling catheter (Silastic silicone tubing, Dow Corning, ID 0.63 mm, OD 1.17 mm) was implanted into the right jugular vein as previously described (Covington and Miczek 2001). Following 5 days of recovery, rats were placed in a different custom-built chamber (30×30.5×24.5 cm) within a sound- and light-attenuating enclosure.

Procedures All self-administration procedures and equipment were identical to previous experiments using adult rats (Boyson et al. 2014; Covington and Miczek 2001; Miczek et al. 2011). MED-PC IV (Med Associates Inc., St. Albans, VT) controlled experimental events and recorded operant responding.

Acquisition protocol Immediately after the rats were placed into the self-administration chamber, the experimental session was started, and rats were allowed to self-administer cocaine (0.75 mg/kg per infusion) according to a fixed ratio (FR) 1 schedule with only the active lever present. The session was terminated after 5 h or 15 infusions, whichever came first. Rats were considered to have acquired the task when they obtained 15 infusions in two consecutive sessions, after which the FR was increased. If rats did not achieve this requirement within 3 days/15 h of access to cocaine, the experimenter shaped the rat's behavior by placing female urine on the active lever. Palatable food was used as a secondary technique. No behavioral shaping was used during measurement of dependent variables. Ninety-six percent of PH controls, 73 % of the PH defeat group, 55 % of SH controls, and 43 % of the SH defeat group required shaping. After acquisition, the FR was slowly increased to 5, and after three stable days of FR5 responding, the motivation to self-administer cocaine was studied according to a PR. Responses per minute were calculated by dividing the total responses while cocaine was available by the duration of the session for all procedures.

Progressive ratio schedule of cocaine reinforcement Rats were given access to cocaine on a PR that required an increasing number of responses to be reinforced by an infusion of cocaine (0.3 mg/kg/infusion). The schedule permitted all rats to reach their breakpoint within 5 h, using the following progression 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 118, 145, 178, …, 402, 492, 603….etc. (Richardson and Roberts 1996). A lower dose of cocaine was used for this phase (0.3 mg/kg/infusion) based on previous studies suggesting that reliable augmented self-administration following adult social defeat is more consistently observed at this lower dose (Covington and Miczek 2001; 2005). The session terminated when no cocaine infusion was delivered for 60 min. Three PR sessions were conducted on different days separated by FR5 maintenance sessions.

Twenty-four-hour binge protocol After stable FR5 responding was observed following the third PR session, rats were given access to cocaine (0.3 mg/kg/infusion) for 24 h on an FR5 schedule.

Statistical analysis

Intruder behaviors in adult PH rats were compared to intruder behaviors of adolescent PH rats using a one-way ANOVA because there were no significant effects of time for adults. If one group had no variance because the behavior was not observed, Kruskal-Wallis ANOVA on ranks was performed. Intruder behaviors by adolescents were analyzed with a repeated measures (RM) two-way ANOVA (housing [pair vs. single]×time [P35 first vs. P44 fourth defeat]). The average weight from P53–56 (early adult post stress) minus the average weight from P32–35 (pre defeat) for each individual calculated body weight gain, which was analyzed with a two-way ANOVA (housing×treatment [social defeat vs. control]). Open field behavior was analyzed with a two-way ANOVA (housing×treatment). Walking in response to i.p. injections was analyzed with a three-way ANOVA (housing×treatment×drug [saline vs. cocaine]) followed by two-way ANOVAs for each housing condition and post hoc pairwise comparisons using the Holm-Sidak method. Acquisition of the operant response for cocaine was analyzed by comparing the proportion of rats to have reached our criterion for acquisition. Kaplan-Meier 1-survival analysis of the first 15 h of access followed by log-rank (Mantel-Cox) tests which determined differences between treatment groups. Rats that performed zero responses during the first 15 h of access were excluded from this analysis (N=19, 13 in PH and 6 in SH) because cocaine availability was unknown. The total number of cocaine infusions obtained under the PR was averaged over the three PR sessions. For the 24-h binge, the total number of cocaine infusions obtained was the dependent variable. Two-way ANOVAs (housing×treatment) were applied to PR and binge data. Significant main effects and interactions were followed up by pairwise comparisons (Holm-Sidak). Separate linear regression analyses were used to assess

potential relationships between defeat behavior and cocaine self-administration. Change in attack-induced freezing was calculated by subtracting the probability of freezing during an attack bite on P35 from the same measure on P44. Separate Grubbs' tests were applied to data sets where appropriate (resident behavior, intruder behavior, and locomotion) to identify outliers (Grubbs 1969). Statistical outliers were removed only if the value was >2.25 standard deviations above or below the mean for the PR ($n=1$) and binge ($n=1$). Survival analysis and log ranks were performed using IBM SPSS Statistics v21 (IBM Corp.). Sigma Plot v11 (Systat Software Inc.) was used for the remainder of statistical tests. The alpha level was always 0.05 for statistical significance.

Results

Social defeat behaviors

Adult vs. adolescent defeat (statistics in Table 1) One-way ANOVA indicated that residents attacked adult intruders faster than adolescent intruders (Table 1). The rate of attack bites per minute was significantly higher when confronted with adults versus adolescent intruders. The adult residents spent more time in the lateral sideways threat posture when confronting an adult intruder compared to an adolescent. In contrast, the adult resident engaged in significantly more frontal threat postures when confronted with an adolescent intruder. The latency for the first attack bite, supine posture, and full submission was significantly longer when the resident was

confronted with an adolescent intruder. Adult intruders spent a significantly greater percentage of their time in the supine posture, while adolescents spent a significantly greater percent of time following/crawling under the resident and approaching the resident. In response to an attack bite, adult intruders were significantly more likely to adopt the supine and upright defensive posture, whereas the adolescent intruders were more likely to freeze or remain immobile.

Resident behaviors with PH vs. SH adolescent rats (statistics in Table 2) Residents spent significantly more time engaged in aggression (attack bite and other aggressive behaviors) and sniffing the SH rats (Table 2). However, residents spent significantly more time engaged in frontal threat postures, allogrooming, and anogenitally investigating the PH compared to SH rats. The latency to attack bite declined significantly from the first to the last defeat. The percent of time engaged in anogenital investigation of PH intruders on the P35 defeat was significantly greater than the last defeat and also greater than in SH intruder on P35. The percent of time engaged in nonsocial exploration reduced over the four defeats when confronted with PH rats, such that nonsocial exploration was lower than when confronted with SH rats by P44.

PH vs. SH adolescent intruder behavior (statistics in Table 2) During the 10 days of repeated confrontations with aggressors, the number of escapes from supine, upright defensive postures, and immobility increased, while latency to submission, social investigation of the resident, and nonsocial exploration decreased (statistics in Table 2). The latency to

Table 1 Mean (± SEM) social defeat behaviors, averaged across all observations for each individual

	Adult	Adolescent	Statistics	Change
Resident behaviors				
Attack bite (number/min)	3.50±0.65	1.16±0.16	$p<0.001$	↓
Lateral sideways threat (% of time)	5.71±2.48	0.07±0.04	$p<0.001$	↓
Frontal threat (% of time)	0.19±0.12	1.14±0.17	$p=0.002$	↑
Latency to attack bite (s)	31.7±1.5	88.4±6.4	$p<0.001$	↑
Intruder behaviors				
Supine (% of time)	17.65±2.69	4.49±0.98	$p<0.001$	↓
Follow and crawl under resident (% of time)	0.89±0.50	2.03±0.36	$p=0.005$	↑
Approach resident (number/min)	0.00±0.00	0.42±0.04	$p<0.001$	↑
Latency to supine (s)	42.6±3.8	131.7±12.5	$p<0.001$	↑
Latency to submission (s)	76.9±8.2	272.4±18.6	$p<0.001$	↑
Probability of behavior after attack bite				
Supine	0.33±0.04	0.16±0.02	$p<0.001$	↓
Upright defensive	0.27±0.03	0.06±0.02	$p<0.001$	↓
Freezing	0.09±0.03	0.24±0.02	$p<0.001$	↑
Nonambulatory motor behavior	0.15±0.04	0.40±0.02	$p<0.001$	↑

Table 2 Mean (± SEM) social defeat behaviors of pair- and single-housed intruders during the first defeat (P35) and fourth defeat (P44)

	Pair P35	Pair P44	Single P35	Single P44	Statistics
Resident behaviors					
Attack bites (number/min)	1.06±0.19	1.22±0.16	1.73±0.36	2.30±0.34	Housing: $F(1,38)=7.65$, $p=0.008$
Latency to attack bite (s)	112.4±18.3	68.9±8.9	116.6±16.0	70.9±8.6	Day: $F(1,45)=11.21$, $p=0.002$
Aggressive posture (number/min)	0.63±0.13	1.21±0.30	0.68±0.20	0.90±0.24	Not significant
Other aggression (number/min)	0.25±0.04	0.29±0.06	0.77±0.14	0.99±0.16	Housing: $F(1,37)=24.95$, $p<0.001$
Frontal threat (% of time)	1.19±0.22	1.10±0.22	0.71±0.30	0.49±0.19	Housing: $F(1,46)=5.15$, $p=0.028$
Lateral sideways threat (% of time)	0.02±0.015	0.12±0.069	0.01±0.007	0.01±0.005	Not significant
Pursuit (% of time)	2.99±0.55	3.43±0.83	3.15±0.62	1.71±0.42	Not significant
Allogroom (% of time)	2.926±0.644	2.188±0.440	0.575±0.208	0.289±0.074	Housing: $F(1,37)=26.42$, $p<0.001$
Sniff intruder (% of time)	11.23±1.45	13.86±1.67	21.31±2.72	19.64±2.59	Housing: $F(1,46)=13.31$, $p<0.001$
Anogenital investigation (% of time)	12.42±2.33[ab]	6.85±1.15	6.98±0.99	5.02±0.81	Day: $F(1,40)=4.34$, $p=0.044$; Housing: $F(1,40)=4.76$, $p=0.034$
Nonsocial exploration (% of time)	21.85±1.33[a]	16.74±1.16[c]	20.87±1.55	21.83±1.59	Housing×day: $F(1,45)=5.44$, $p=0.024$
Nonambulatory motor behavior (% of time)	69.2±2.5	73.2±2.1	72.4±2.1	71.9±2.0	Not significant
Intruder behaviors					
Supine (% of time)	1.06±0.27	5.66±1.31	6.45±1.69	5.07±1.17	Not significant
Escape (number/min)	0.21±0.05	0.58±0.19	0.23±0.08	0.74±0.21	Day: $F(1,45)=9.15$, $p=0.004$
Upright defensive (number/min)	0.10±0.04	0.37±0.13	0.06±0.04	0.34±0.13	Day: $F(1,45)=7.77$, $p=0.008$
Latency to supine (s)	136.2±19.6	62.3±10.5	91.1±14.4	83.7±12.9	Not significant
Latency to submission (s)	327.5±35.0	253.4±29.9	289.3±12.1	244.7±31.2	Day: $F(1,45)=5.17$, $p=0.028$
Latency to freeze (s)	114.9±17.8[a]	45.3±6.8[c]	81.3±11.8	65.2±10.2	Day: $F(1,41)=13.32$, $p<0.001$; Housing×day: $F(1,41)=8.33$, $p=0.006$
Freezing (% of time)	5.59±0.94[a]	10.12±1.80[c]	7.34±1.51	4.10±0.50	Housing×day: $F(1,42)=8.47$, $p=0.006$
Walk slowly (% of time)	0.292±0.099	0.116±0.049	0.067±0.055	0.158±0.071	Housing: $F(1,46)=5.91$, $p=0.019$
Tail rattle (% of time)	0.183±0.083	0.471±0.203	0.570±0.363	0.289±0.224	Not significant
Follow and crawl under resident (% of time)	2.48±0.64	2.78±0.79	5.31±1.10	4.45±0.88	Housing: $F(1,45)=4.84$, $p=0.033$
Approach (number/min)	0.51±0.08	0.34±0.07	0.88±0.16	0.65±0.16	Housing: $F(1,45)=9.12$, $p=0.004$
Social investigation (% of time)	5.35±1.03	3.20±0.65	6.31±1.15	3.61±0.55	Day: $F(1,45)=6.25$, $p=0.016$
Nonsocial exploration (% of time)	35.23±2.72	31.81±2.77	39.88±2.45	28.84±1.29	Day: $F(1,46)=10.71$, $p=0.002$
Nonambulatory motor behavior (% of time)	57.6±3.0	61.0±2.3	54.8±2.3[a]	64.5±1.6	Day: $F(1,41)=10.89$, $p=0.002$; Housing×day: $F(1,41)=4.33$, $p=0.044$
Probability of behavior after attack bite					
Supine	0.16±0.03	0.16±0.03	0.17±0.03	0.20±0.03	Not significant
Upright defensive	0.03±0.02	0.08±0.04	0.01±0.01	0.04±0.02	Not significant
Freezing	0.20±0.03[b]	0.29±0.03[c]	0.29±0.03[a]	0.18±0.02	Housing×day: $F(1,46)=10.11$, $p=0.003$
Nonambulatory motor behavior	0.46±0.04	0.37±0.02	0.37±0.03	0.37±0.03	Not significant

[a] Different from P44 in the same treatment group ($p<0.05$)

[b] Different from single on P35 ($p<0.05$)

[c] Different from single on P44 ($p<0.05$)

freeze significantly decreased from P35 to P44 in PH rats only, and on P44, PH rats were quicker to freeze than SH intruders. The percent of time engaged in freezing behavior significantly increased from P35 to P44 in the PH intruders only, and on P44, PH rats spent more time freezing than SH intruders (Table 2 and Fig. 2). Single-housed intruders spent more time following/crawling under the resident and approaching the resident. In response to an attack bite, PH rats were less likely to freeze during the first defeat, but significantly more likely to freeze during the fourth defeat, both compared to SH intruders (Table 2 and Fig. 2). Single-housed intruders actually reduced freezing in response to an attack bite on P35 compared to P44. Figure 2 highlights selected data from Table 2. In general, from the first to the last social defeat, PH rats showed an increased probability of freezing in response to an attack bite, increased overall

Fig. 2 Summary of selected adolescent intruder behaviors from Table 1. The relative probability of freezing after an attack bite is represented by the thickness of the *arrow* between "*Bite*" and "*Freeze*". The size of the *gray circle* represents the relative percent of time adopting the freezing posture. The length of the *dotted black lines* (latency to freezing posture) and *gray lines* (latency to supine posture) represent the relative amount of time from introduction to the resident until the behavior was first observed
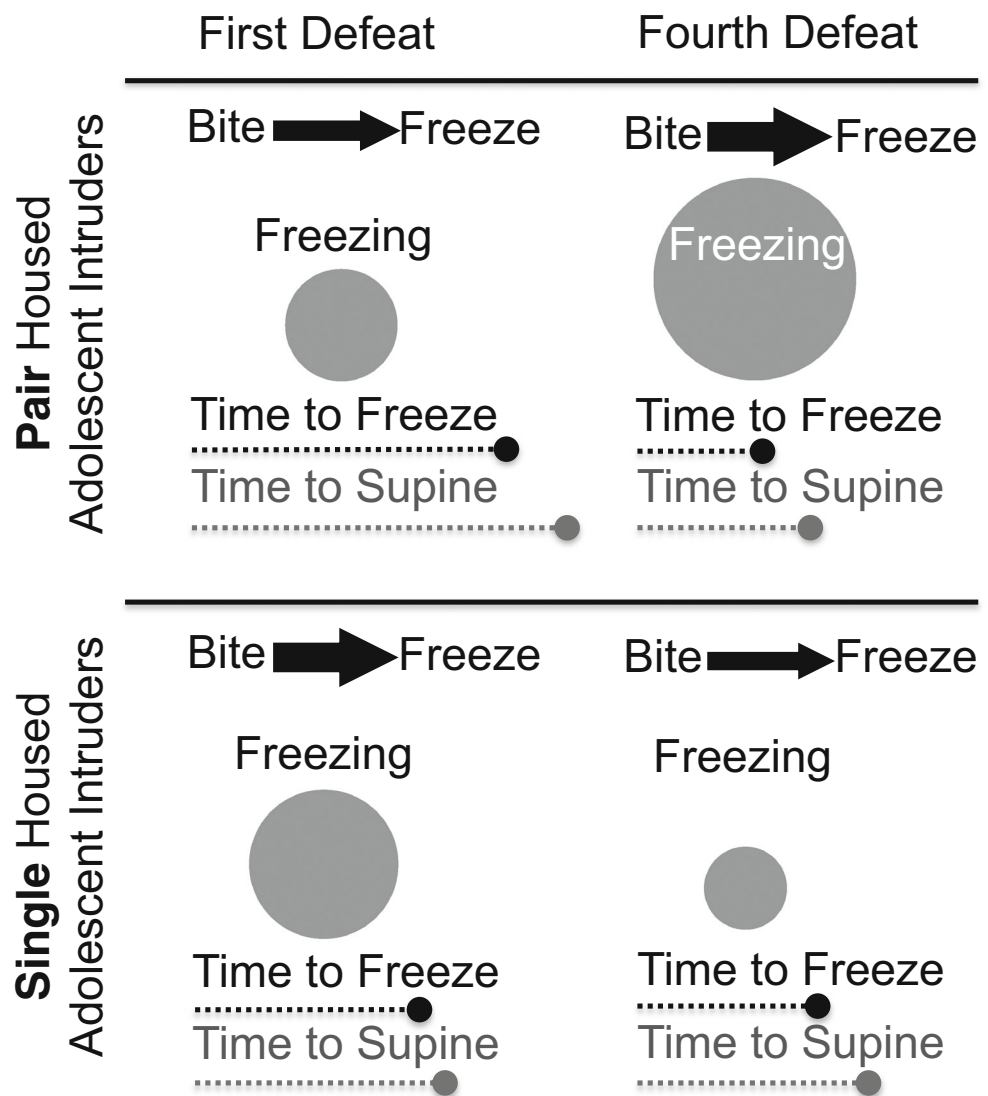
freezing, and decreased latencies to freeze and supine posture, none of which were observed in SH rats.

Body weight

From P32 to P61, the PH control group gained 190±4.4 g, PH stress group gained 198±5.6 g, SH control 188±3.7 g, and SH stress 182±2.2 g (N=22–26/group). Two-way ANOVA revealed significantly less weight gain in SH compared to PH rats ($F_{(1,91)}$=4.364, p=0.039), an effect that appears driven differences in weight gain among stressed rats.

Locomotion

Pair-housed rats moved less in the open field ($F_{(1,92)}$=22.56, p<0.001) and spent more time in the center of the open field ($F_{(1,92)}$=16.09, p<0.001; Fig. 3a) in early adulthood (P57) compared to SH rats. Cocaine increased walking in PH controls (p=0.004), PH defeated rats (p=0.026), and SH controls (p=0.003), but not in SH defeated rats as indicated by post hoc tests based on the a priori hypothesis that i.p. cocaine increases walking compared to i.p. saline. There was a significant main effect of housing ($F_{(1181)}$=10.488, p=0.001) and drug ($F_{(1181)}$=20.28, p<0.001; 3 B).

Cocaine self-administration

Pair-housed defeated rats, SH controls, and SH defeated rats acquired the iv. cocaine self-administration task significantly faster in adulthood compared to PH controls (respectively, χ^2=4.5, p=0.034; χ^2=10.1, p=0.001; χ^2=16.5, p<0.001; Fig. 4). Furthermore, a greater proportion of SH defeated rats acquired the task than PH defeated rats (χ^2=5.5, p=0.019). There was no difference between rats that acquired with or without behavioral shaping in subsequent measures of cocaine taking (p>0.05). There was no effect of housing or treatment

Fig. 3 **a** Total distance moved (mean±SEM) and time spent in the center of the 60-min open field test on P57 for social defeat (stress) and control treatment groups that were pair-housed or single-housed since P21 ($N=22$–26/group). *Significant effect of housing ($p<0.001$). **b** Total duration of walking (mean±SEM) 5–10 min after i.p. injection of saline and cocaine (10 mg/kg; $N=23$–26/group). #Significantly different from saline of same treatment group ($p<0.01$)

A. Open Field

B. Cocaine Challenge
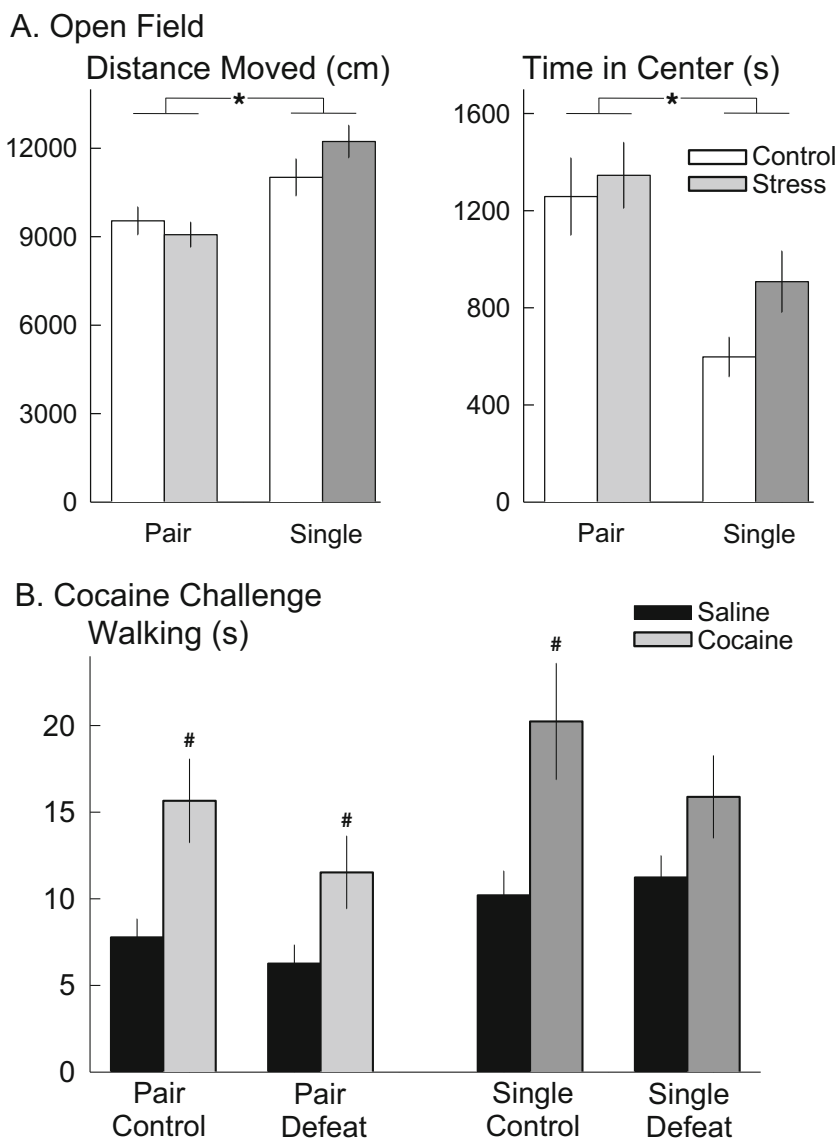
on maintenance as measured by the average responses per minute during the three FR5 sessions prior to the first PR session ($p>0.05$).

For the infusions obtained during PR, there was a significant main effect of housing ($F(1,61)=5.01$, $p=0.029$) and a significant interaction between housing and treatment ($F(1,61)=6.445$, $p=0.014$; Fig. 5). Post hoc tests showed that defeat increased cocaine infusions in PH rats ($p=0.043$). Among the defeated rats, PH rats took more infusions than SH rats ($p=0.002$).

There was a significant interaction between housing and stress treatment for total binge infusions obtained ($F(1,46)=4.55$, $p=0.038$; Fig. 5). Post hoc tests showed that in pair-housed rats, social defeat in adolescence increased the number of binge infusions ($p=0.038$). Furthermore, PH defeated rats obtained more cocaine infusions than SH defeated rats ($p=0.019$).

The average proportion of freezing in response to an attack bite on P44 (fourth defeat) was positively correlated with the responses per minute during the binge ($p=0.007$, $r^2=0.30$; Fig. 6, top) and during PR ($p=0.012$, $r^2=0.020$; data not shown). The change in freezing from P35 to P44, calculated by subtracting the freezing during attack bite on P35 from the corresponding value on P44, was positively correlated with total infusions obtained during the 24-h binge ($p=0.032$, $r^2=0.20$; Fig. 5, middle) and during PR ($p=0.005$, $r^2=0.25$; data not shown). Cocaine self-administration data were split at zero into rats that increased freezing from P35 to P44 and those that decreased freezing, and rats that increased freezing over repeated social defeats obtained more cocaine infusions during the binge ($F(1,21)=4.789$, $p=0.040$) and PR ($F(1,29)=6.069$, $p=0.020$) compared to decreased freezing group (Fig. 5, bottom).
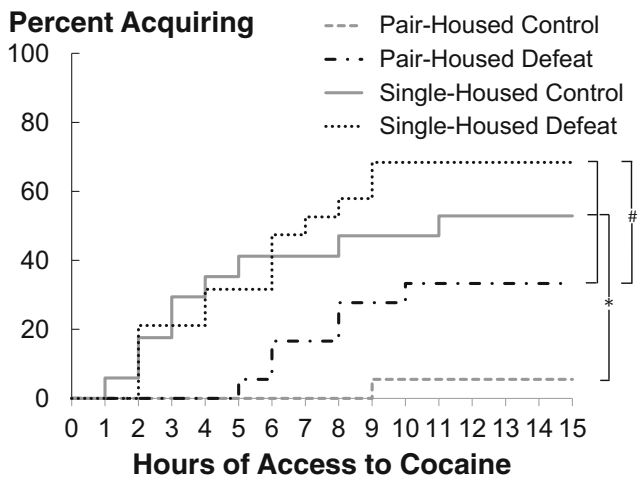
Percent Acquiring

Fig. 4 One minus survival plot of the cumulative proportion of pair control (N=18), pair defeat (N=18), single control (N=17), and single defeated rats (N=19) reaching the acquisition criterion during the first 15 hours (over 3 days) of access to cocaine (0.75 mg/kg/infusion). *Significant difference from pair-housed control group (p<0.05). #Significant difference between pair- and single-housed defeated rats (p<0.05)

Discussion

This is the first study to demonstrate that episodes of social defeat during adolescence escalate cocaine intravenous self-administration in adulthood. Defeated PH rats consumed more cocaine compared to SH defeated rats, suggesting that social experience plays a pivotal role escalation of cocaine self-administration in socially defeated adolescents. PH controls acquired cocaine self-administration more slowly than PH defeated and SH rats. Cocaine self-administration in adulthood was predicted by greater attack-induced freezing after repeated social defeats in adolescence, suggesting a

relationship between adolescent social behavior and adult cocaine taking. We confirmed and expanded upon recent studies by showing that residents engaged in a higher attack rate toward adult versus adolescent intruders (Garcia-Pardo et al. 2014; Ver Hoeve et al. 2013; Zou et al. 2014). Intruders coped with the aggressor differently depending on age and housing conditions. For example, adult intruders were more likely to adopt the supine posture after an attack bite, whereas adolescent intruders were more likely to freeze. Notably, PH rats modified their behavior from the first to the last defeat by increasing freezing, whereas SH rats actually decreased freezing in response to an attack bite.

The greater probability of freezing in response to an attack bite of adolescent rats is specific to social stress because adolescents freeze to a similar extent to adults in nonsocial contexts (Broadwater and Spear 2013). Single-housed rats exhibited more freezing when confronted with the resident for the first time on P35 in agreement with other tests conducted in adult rats after adolescent SH (Lukkes et al. 2009a; Lukkes et al. 2009b; van den Berg et al. 1999). Adolescent PH rats increased attack-induced freezing, total freezing behavior, and were quicker to freeze or adopt supine postures, which was in sharp contrast to SH rat behavior (summarized in Fig. 2). The PH adolescent's behavior may be considered to be adaptive because for adult rats increased freezing in the presence of the resident is thought to reduce the probability of further attacks (Buwalda et al. 2012; Nocjar et al. 2012; Paul et al. 2011). Rats with a genetic predisposition for anxiety exhibit greater freezing when confronted with an aggressor, perhaps driven by a high-level anticipatory anxiety (Frank et al. 2006). The change in freezing during adolescence over repeated confrontations may be interpreted as an adaptive fear or anxiety response, and this adaptive coping behavior during adolescence

Fig. 5 Left, number of cocaine infusions (mean±SEM) obtained by pair-housed control (N=16), pair-housed defeat (N=13), single-housed control (N=18), and single-housed defeated rats (N=19) averaged across progressive ratio (PR) schedule of reinforcement sessions. Right, number of cocaine infusions (mean± SEM) accumulated by pair control (N=14), pair defeat (N=11), single control (N=13), and single defeated rats (N=13) during the 24-h binge. *Significantly different from pair-housed control group. #Significantly different from single-housed defeat group
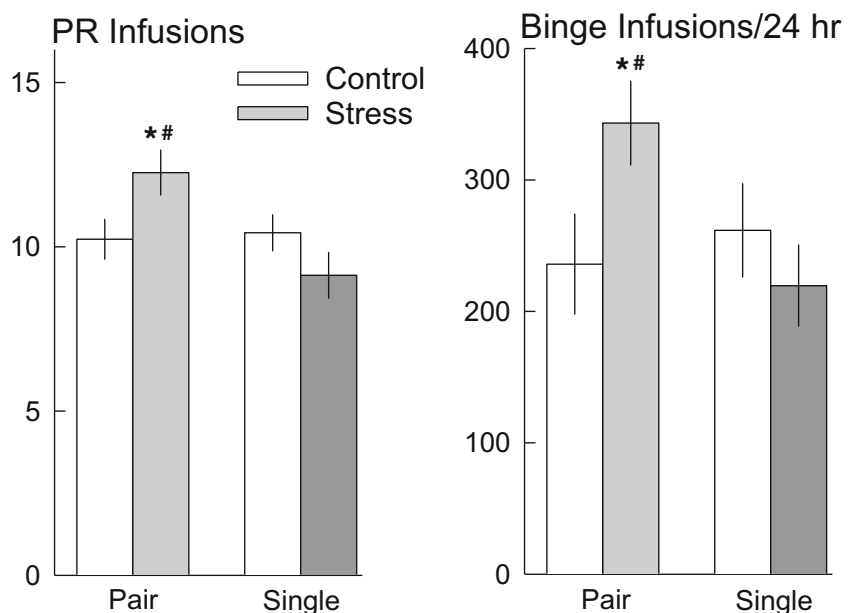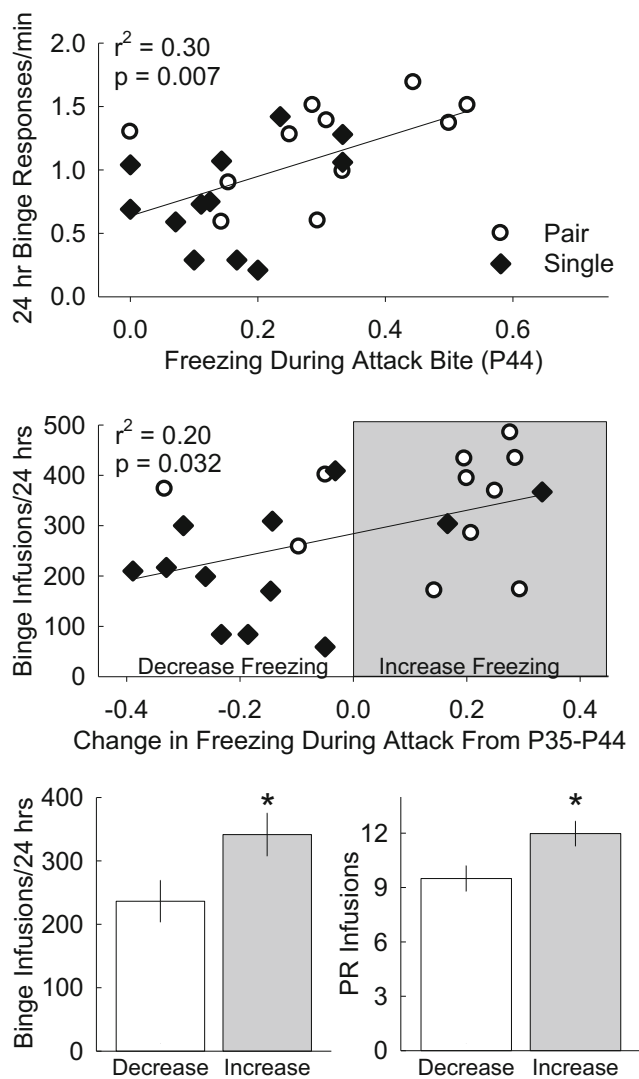
Fig. 6 *Top panel*, the probability that an intruder freezes during an attack bite on P44 is positively correlated with responses per minute during the 24-h continuous access binge ($p<0.01$). *Middle panel*, the change in the probability that an intruder freezes during attack bite from first (P35) to fourth (P44) defeat is positively correlated with total cocaine infusions during a 24-h binge ($p<0.05$). Values above zero were termed increased freezing and below zero, decreased freezing. *Bottom panel*, individuals that increased freezing from P35 to P44 demonstrated increased cocaine self-administration during the binge and under a progressive ratio schedule of reinforcement (PR) ($p<0.05$)

predicts some cocaine self-administration behaviors approximately 40 days later in early adulthood.

The increase in social exploration of residents in adulthood following adolescent SH is thought to provoke further attacks from aggressors (van den Berg et al. 1999). The increased approaching, following, and crawling under the resident observed in SH intruders confirms previously reported SH effects on social behavior of adolescent rats (Buwalda et al. 2013; Varlinskaya and Spear 2008) and is one possible explanation for increased attacks and other aggression directed

toward SH intruders. Thus, we interpret the reduced attack-induced freezing over repeated defeats observed in SH adolescents as a maladaptive behavior.

Defeat in adolescence did not cause hyperactivity in a novel locale, which conflicts with our previous studies in Sprague Dawley rats (Burke et al. 2010; Burke et al. 2011; Watt et al. 2009). A different social defeat schedule, strain of rat, novel apparatuses, and different treatment of controls may account for the conflicting results. The increased locomotion for SH rats compared to PH rats agrees with other studies during adolescence (Levine et al. 2007; Meng et al. 2010) and adulthood (Chappell et al. 2013; Lapiz et al. 2003; Powell et al. 2002; Wright et al. 1991). Less time in the center of the open field of SH rats suggests greater anxiety (Prut and Belzung 2003) and replicates a previous report (Meng et al. 2010). There was no significant correlation between novelty- or cocaine-stimulated locomotion and cocaine self-administration ($p>0.05$; data not shown), in agreement with a previous report (Thomsen and Caine 2011). The current dose of cocaine (10 mg/kg, i.p.) causes cross-sensitization after adult social defeat (Covington et al. 2005; Covington and Miczek 2001; Miczek et al. 2011). Social defeat in adolescence did not enhance cocaine hyperactivity, similar to some earlier results with amphetamine (Burke et al. 2013; Burke et al. 2010). Overall, brief adolescent social defeat did not impact behavior in the novel open field, but social deprivation throughout adolescence caused hyperactivity and anxiety-like behavior. There might be age differences in social defeat cross-sensitization to a cocaine challenge.

Single housing during adolescence increased the acquisition of cocaine self-administration in adulthood, confirming several previous studies (Baarendse et al. 2014; Bardo et al. 2001; Ding et al. 2005; Howes et al. 2000). More than 20 days after four episodes of social defeat applied to PH adolescent rats, we observed an increased rate of acquisition of cocaine self-administration. Adult social defeat only increases acquisition a few days after repeated defeats (Haney et al. 1995; Kabbaj et al. 2001; Tidey and Miczek 1997), but not a couple weeks later (Covington et al. 2005; Covington and Miczek 2001). These data support the hypothesis that social deprivation and social defeat during adolescence both increase acquisition of cocaine taking in early adulthood.

Adolescent social defeat of PH rats increased the motivation to lever press for cocaine during the PR and compulsive cocaine taking during a 24-h binge compared to PH controls and SH defeated rats. A similar intermittent social defeat protocol applied to adult rats also increases these same measures (Covington and Miczek 2005; Covington et al. 2008), suggesting social defeat increases cocaine taking under these parameters regardless of age. In these previous experiments, the adult rats were reared in groups by the supplier until early adulthood and then SH upon arrival and throughout the social defeat experiences. The effect of adult social defeat on cocaine

self-administration of socially housed rats remains to be investigated. In the current study, PH could be interpreted as social buffer against drug taking only during the acquisition phase of cocaine self-administration.

The current data confirm the observation that SH during adolescence increases acquisition of intravenous self-administration without effects on subsequent measures of drug taking (Lu et al. 2003). This was the first study that investigated the possible role of housing conditions on the escalation of drug taking caused by social defeat. We expected synergism between SH and adolescent defeat based on previous studies in adults suggesting reduced stress reactivity in socially defeated PH rats (de Jong et al. 2005; Nakayasu and Ishii 2008; Ruis et al. 1999; Von Frijtag et al. 2000). However, there was no compounding effect of SH and adolescent defeat on cocaine self-administration during PR or the 24-h binge. Some social behaviors, which peak during rat mid-adolescence, are rewarding (Trezza et al. 2010), and SH adolescents exhibit increased preference for social contact and engage in social behavior more than group-housed adolescents (Douglas et al. 2004; Varlinskaya et al. 1999; Yates et al. 2013). Thus, social interaction with the resident might be less aversive for SH intruders in the current study to explain the absence of escalated cocaine taking in adulthood. Some of our data support this hypothesis; SH rats exhibited more social behaviors during social defeat than PH rats, such as approach, follow, and crawl under the resident. Single-housed rats also failed to increase fear- and anxiety-related behaviors over the course of repeated social defeats (summarized in Fig. 2).

Social defeat of PH adolescents increased all measures of cocaine self-administration in early adulthood, while social deprivation during adolescence increased only the acquisition of cocaine self-administration. Behavior during repeated confrontations with an aggressor of PH rats was adaptive, while the behavior of SH rats was maladaptive based on the higher rate of aggression toward SH intruders. Social defeat stress procedures provide episodes of discrete salient events that activate stress-related neurocircuitry including the mesocorticolimbic dopamine system, while social deprivation does not (reviewed in Burke and Miczek 2014). We have identified an adolescent social experience (PH defeat) that increased cocaine taking in early adulthood and may serve useful for identifying stress-induced neural mechanisms and possible interventions for social stress escalated substance abuse. Our ongoing studies investigate the corticotropin releasing factor system in the adolescent ventral tegmental area, which is implicated in escalated cocaine taking after adult social defeat (Boyson et al. 2014; Boyson et al. 2011)

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