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Chronic variable stress and intravenous methamphetamine self-administration – role of individual differences in behavioral and physiological reactivity to novelty

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Abstract

Stress is a contributing factor to the development and maintenance of addiction in humans. However, few studies have shown that stress potentiates the rewarding and/or reinforcing effects of methamphetamine in rodent models of addiction. The present study assessed the effects of exposure to 14 days of chronic variable stress (CVS), or no stress as a control (CON), on the rewarding and reinforcing effects of methamphetamine in adult rats using the conditioned place preference (Experiment 1) and intravenous self-administration (Experiment 2) paradigms. In Experiment 2, we also assessed individual differences in open field locomotor activity, anxiety-like behavior in the elevated plus maze (EPM), and physiological responses to a novel environment as possible predictors of methamphetamine intake patterns. Exposure to CVS for 14 days did not affect overall measures of methamphetamine conditioned reward or reinforcement. However, analyses of individual differences and direct vs. indirect effects revealed that rats exhibiting high physiological reactivity and locomotor activity in the EPM and open field tests self-administered more methamphetamine and reached higher breakpoints for drug reinforcement than rats exhibiting low reactivity. In addition, CVS exposure significantly increased the proportion of rats that exhibited high reactivity, and high reactivity was significantly correlated with increased levels of methamphetamine intake. These findings suggest that individual differences in physiological and locomotor reactivity to novel environments, as well as their interactions with stress history, predict patterns of drug intake in rodent models of methamphetamine addiction. Such predictors may eventually inform future strategies for implementing individualized treatment strategies for amphetamine use disorders.

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1. Introduction

Stress is a significant contributing factor to the initiation and maintenance of psychostimulant use, craving, and relapse (Sinha et al., 2000). Several reports indicate that use of methamphetamine, a potent psychostimulant, is associated with stress in humans. For example, recent findings indicate that human methamphetamine users report a higher number of lifetime stressors compared to cocaine users as well as non-using controls (Mahoney et al., 2015). Human methamphetamine users also report high levels of psychological stress, which appear to perpetuate further use of the drug (Booth et al., 2010). Another study found that while acute stress reduced the subjective effects of methamphetamine, it also led to increased drug “wanting” in subjects without an extensive history of stimulant use (Soderpalm et al., 2003). Not surprisingly, there are high rates of co-morbidity between psychostimulant dependence and stress-related disorders such as major depression and post-traumatic stress disorder (Chen et al., 2011). One study of college students also identified an increase in prescription psychostimulant use during times of high stress (Moore et al., 2014). Thus, life stressors may impact psychostimulant use at a number of points along the transition between occasional and recreational use to more uncontrolled and habitual use. While increasing evidence suggests that chronic psychostimulant use progressively engages brain stress and anti-reward circuitry (Koob et al., 2014), the precise mechanisms and processes by which stress and methamphetamine addiction interact remain largely unclear.

Preclinical studies in rodents demonstrate that chronic or repeated stress can result in increased rates of acquisition of psychostimulant self-administration, binge-like cocaine intake, and motivation to self-administer amphetamine (Piazza et al., 1990, Goeders and Guerin, 1994, Haney et al., 1995, Goeders and Guerin, 1996, Miczek and Mutschler, 1996, Tidey and Miczek, 1997, Covington and Miczek, 2001, Kabbaj et al., 2001, Green et al., 2002, Covington and Miczek, 2005). However, preclinical investigations of the effects of chronic stress on methamphetamine self-administration are generally lacking. At the present time, two studies have examined the effect of chronic stress in adulthood on the acquisition of methamphetamine self-administration in rats. In one study, daily exposure to footshock or corticosterone (2 mg/kg i.p.) immediately prior to methamphetamine self-administration sessions failed to influence drug intake (Moffett and Goeders, 2005). In a study from our own laboratory, rats exposed to early life stress and then subjected to subsequent chronic stress in adulthood failed to increase methamphetamine self-administration (Lewis et al., 2016). These studies suggest the influence of stress on methamphetamine self-administration may not be particularly robust in rodent models, but they also do not account for other factors such as individual differences, which may also contribute to methamphetamine use.

There is a preponderance of evidence for individual differences in reactivity to stress and novelty that influence drug sensitivity and intake. In humans, a recent study identified that methamphetamine users differed from non-users in measures of not only the number of lifetime stressors, but also in sensation-seeking and impulsivity (Mahoney et al., 2015). Preclinical studies have corroborated the importance of individual differences in drug-related behaviors. For example, rats with high locomotor reactivity in a novel environment exhibit increased sensitivity to locomotor effects of stimulants including cocaine, amphetamine,

methamphetamine, and caffeine (Piazza et al., 1989, Hooks et al., 1992, Deroche et al., 1993, Bevins et al., 1997, Bevins and Peterson, 2004). In addition, such measures of individual variability predict self-administration of psychostimulants such as cocaine and amphetamines (Piazza et al., 1989, Pierre and Vezina, 1997, Marinelli and White, 2000, Piazza et al., 2000, Cain et al., 2006, Davis et al., 2008, Gancarz et al., 2011).

While the predictive nature of individual differences in the locomotor-stimulating and reinforcing properties of psychostimulants has been established, the interpretation of what the individual differences reflect is less clear. Some investigators consider that individual differences in locomotor reactivity are strongly related to stress reactivity (Bardo et al., 1996, Dellu et al., 1996, Piazza and Le Moal, 1996, Klebaur and Bardo, 1999) while others consider locomotor reactivity to be more related to novelty seeking (Dellu et al., 1996, Blanchard et al., 2009). Interestingly, chronic stress exposure can alter locomotor and physiological responses to novel environments and anxiety-like behavior (Monnikes et al., 1993, Bhatnagar and Dallman, 1998, Conrad et al., 1999, Ostrander, 2006, Jankord et al., 2010, Taylor et al., 2015). While anxiety profiles can predict cognitive deficits following chronic stress (Bellani et al., 2006), little is known regarding the relationships between chronic stress, responsivity to novelty, and anxiety-like behaviors and how these interactions affect methamphetamine reward and reinforcement.

The present study was designed to determine whether adult chronic variable stress (CVS) affects the rewarding and reinforcing effects of methamphetamine using the conditioned place preference and intravenous self-administration paradigms. To explore the contribution of individual differences to patterns of methamphetamine self-administration, we used linear regression analyses to determine the predictive ability of anxiety-like behavior, locomotor activity, and physiological reactivity in a novel environment on methamphetamine IVSA. Finally, structural equation modeling and path analyses were used to determine potential indirect effects of CVS on methamphetamine self-administration via these differences in measures of individual reactivity.

2. Experimental procedures

2.1 Animals

Adult male Sprague-Dawley rats (275-300 g upon arrival) were purchased from Charles River Laboratories (Wilmington, MA) and were pair-housed in a temperature and humidity controlled vivarium with a reversed light-dark cycle (12:12; lights off at 06:00 h). Rats were given one week to acclimate before any procedures were performed. Food and water were available *ad libitum*. Following implantation of intravenous catheters, rats were singly housed. All rats were weighed once a week throughout all phases of the study. Testing procedures were conducted during the dark phase of the light-dark cycle. All procedures were conducted according to the Guide for Care and Use of Laboratory Rats (National Research Council., 2011) and were approved by the Institutional Animal Care and Use Committee. All efforts were made to minimize animal pain and distress and to reduce the total number of animals used.

2.2 Drugs

(+)-Methamphetamine hydrochloride was obtained from Sigma-Aldrich (St. Louis, MO), dissolved in sterile saline, and brought to physiological pH (7.2–7.6) for both intravenous and intraperitoneal (i.p.) administration. All veterinary pharmaceuticals were obtained from Henry Schein Animal Care (Melville, NY).

2.3 Chronic Variable Stress (CVS)

Rats were exposed to two variable stressors/day for 14 consecutive days (hereby designated the CVS group). One stressor exposure occurred in the morning, and the other in the afternoon (Taylor et al., 2014). Briefly, the stressors used in this paradigm were: (1) restraint for 1 h with alternated wire mesh restrainer and standard, well-ventilated clear plastic restrainer, (2) restraint for 30 min as just described while being placed on an orbital shaker (RotoMix, Type 50800) at ~120 rpm, (3) social crowding (6–8 rats/cage) for 1 h while on an orbital shaker at ~120 rpm, (4) forced swim in warm (30°C; 20 min) or cold (18°C; 10 min) water, (5) tail pinch with a clothespin during restraint in the wire mesh restrainers for 10 min, (6) intermittent footshock for 15 min (Coulbourn Instruments, 0.5 mA; 5 cycles of: 5 s on/5 s off, repeated three times, then 155 s off) (7) overnight social crowding (6–8 rats/cage), and (8) overnight dirty bedding. Non-stressed control (CON) rats were left undisturbed in the home cage for the same amount of time. At the end of CVS procedures, both CON and CVS rats were handled for 5 days to acclimate them to the experimenters and procedure rooms. Effectiveness of CVS as a stressor was indirectly confirmed by measuring body weights at the beginning and end of CVS procedures, as we have previously shown that CVS treated rats demonstrate attenuated weight gain as compared to CON rats (Taylor et al., 2013, 2014).

2.4 Experiment 1: Effects of CVS on Methamphetamine Conditioned Place Preference

2.4.1 Apparatus—Photobeam Activity System (PAS)-Open Field stations with modified dark box inserts (41 cm W × 41 cm L × 38 cm H; San Diego Instruments, San Diego, CA) were used for conditioned place preference procedures. One compartment contained walls with vertical white stripes and a wire mesh floor, and the other was covered with dark paper containing large white circles and had a smooth floor. Animal location and locomotor activity was measured by photobeam breaks (2.5 cm apart). Between test sessions, compartments were cleaned with an odor eliminating cleanser (Quatricide, Pharmacal Research Laboratories, Naugatuck, CT).

2.4.2 Conditioned place preference (CPP) procedures—On the day after cessation of CVS procedures (Day 15, see Figure 1 timeline), all rats underwent testing for methamphetamine CPP. Based on our previously published work (Gass and Olive, 2009), rats were habituated to the testing apparatus during a single 30-min session with free access to both conditioning compartments. The next day, animals were subjected to a 30-min preconditioning test to determine any initial preference for one of the conditioning compartments. Each animal was assigned to receive methamphetamine either at a subthreshold dose (0.06 mg/kg) or a higher dose (0.25 mg/kg) (Zakharova et al., 2009, Thorn et al., 2012) in the initially nonpreferred compartment, and saline in the initially

preferred compartment. Next, animals underwent place conditioning in twice daily 30-min conditioning sessions. Saline conditioning sessions were conducted in the morning, and methamphetamine conditioning sessions were conducted approximately 4 h later in the afternoon to avoid drug carryover effects. Following five sequential conditioning days, animals were tested for CPP by allowing free access to both conditioning compartments for 30 min. Experimental groups were counterbalanced over time for both pre- and post-conditioning tests, which were conducted from mid-morning to early afternoon to minimize potential confounding effects of time of day. One animal in the CVS group that received 0.25 mg/kg methamphetamine failed to explore both compartments during the post-conditioning test and was removed from all analyses. It should also be noted that this experiment was conducted in two separate cohorts of rats, with $n=4$ in each experimental group for each cohort. A third cohort was also tested, but data from these animals (4 groups, $n=4$ per group) were excluded from analysis due to a potential miscalculation in the doses of methamphetamine administered, as evidenced by anomalous locomotor activity and complete lack of preference for the higher dose.

2.5 Experiment 2: Effects of CVS on Methamphetamine Self-Administration

2.5.1 Surgical Procedures—Jugular vein catheterization (JVC) surgery was performed the day after elevated plus maze (EPM) testing (see below and Figure 1) according to our previously published methods (Gass et al., 2009). Briefly, rats were anesthetized with isoflurane, and an incision was made in the skin to expose the jugular vein. Polyurethane catheters (Access Technologies, Skokie, IL) were inserted 3.0 cm into the vein, secured with braided silk sutures and the distal end of the catheter was externalized through a 3-mm dermal biopsy hole made between the scapulae. The catheter was connected to a threaded vascular access port (Plastics One, Roanoke, VA) and mounted on the dorsum. Access ports were capped with a silastic plug and flushed with 0.1 ml of antibiotic solution (66.6 mg/ml Timentin in 70 U/ml heparinized saline). Rats received one week of post-operative care with daily infusions of this antibiotic solution to maintain catheter patency and topical antibiotic ointments to protect against infection. In addition, carprofen (2.5 mg/kg; s.c.) was administered twice daily for the first 2 days of recovery to reduce post-surgical discomfort.

2.5.2 Apparatus—Intravenous drug self-administration was conducted in computer-interfaced and controlled operant conditioning chambers (Med Associates Model ENV-007; for further details, see (Gass et al., 2009, Watterson et al., 2014)). Chambers were equipped with a 2.5-cm retractable “active” lever as well as a non-retractable “inactive” lever. Each press on the active lever activated a syringe infusion pump (located outside of the self-administration chamber) to deliver a 0.05 mg/kg infusion of methamphetamine solution over 2 sec in a volume of 0.06 ml. Each drug infusion was accompanied by illumination of a white stimulus light located above the active lever for 2 sec, as well as a Sonalert speaker for presentation of a 2-sec auditory cue (~65 dB, 2900 Hz). A 20-sec timeout period followed each drug infusion, where additional active lever presses were recorded but had no programmed consequences. Each press on the inactive lever produced no programmed consequences at any time during the experiment. Chambers were located in melamine sound-attenuating cubicles equipped with a house light and ventilation fan to mask external noise and odors.

2.5.3 Acquisition and Maintenance Procedures—Following recovery (5-6 days) from surgery and after initial locomotor activity testing (see below and Figure 1), rats were allowed to spontaneously acquire methamphetamine self-administration in daily 2-h sessions. Self-administration sessions were conducted 7 days per week on a fixed ratio (FR) 1 schedule. Catheters were flushed with 0.1 ml of 66.6 mg/ml Timentin in 70 U/ml heparinized saline before and after each self-administration session to maintain catheter patency and minimize infection. Self-administration sessions were conducted once daily for 14 consecutive days. Acquisition of methamphetamine self-administration was defined as rats obtaining 10 or more infusions per session across two consecutive sessions.

2.5.4 Progressive Ratio IVSA procedures—Only animals that acquired methamphetamine self-administration under an FR1 schedule of reinforcement were subjected to two 10-h progressive ratio (PR) testing sessions to assess breakpoints for methamphetamine reinforcement. During PR sessions, the number of active lever presses required to obtain a single infusion of methamphetamine (0.05 mg/kg/infusion, in a volume of 0.06 ml over 2 s) was determined by the following equation: responses per reinforcer delivery = $[5 \times e^{(\text{injection number} \times 0.2)}] - 5$ (i.e., 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, etc.). Breakpoints were considered to be reached when the animal failed to emit any lever responses for 1 h. Following the first PR test, an additional 2-h FR1 session was conducted as described above to re-establish baseline levels of responding prior to a second identical PR session.

2.5.5 Assessment of Individual Differences

Elevated plus maze: The day after cessation of CVS procedures, all rats were tested on an elevated plus maze (EPM) for anxiety-like behaviors according to our previously published procedures (Huynh et al., 2011, Taylor et al., 2013). The maze was constructed of black acrylic and consisted of two open and two closed arms (40 cm walls), each of which was 50 cm × 10 cm. The maze was elevated 50 cm above the floor. Animals were first moved to an adjacent dark (<15 lux) holding room and allowed to acclimate for 30 min. Animals in each treatment group were balanced over the testing period to control for time of testing. For each 5 min test session, a rat was placed onto the center of the maze facing an open arm. A charge-coupled device camera mounted to the ceiling was used to record behavior. Behavior was manually scored from video recordings by an experimenter blinded to treatment. An arm was considered to be entered when at least 75% of the animal's body was located in the arm. The ratio of time in or entries into the open arms to total time in and entries into both arms (OTR) was calculated for analysis of anxiety-like behavior (Lapiz-Bluhm et al., 2008, Taylor et al., 2013).

Locomotor Activity: On the final day of recovery from surgical procedures (5-6 days post-surgery), all rats were tested for general locomotor activity in an open field arena (Bellani et al., 2006, Huynh et al., 2011, Taylor et al., 2011a, b). Animals were placed into PAS-Open Field stations (see CPP section above) and allowed to explore for 10 min. Locomotor activity was assessed by photobeam breaks.

Physiological reactivity to a novel environment: Prior studies have demonstrated that fecal boli elimination is an indirect measure of physiological stress reactivity and emotionality to novel environments and experiences (Hall, 1934, Monnikes et al., 1993). Consequently, fecal boli were counted following EPM and open field locomotor testing procedures (Bellani et al., 2006).

2.6 Statistical Methods

2.6.1 Analysis—Statistical analyses were conducted with SPSS (version 22), GraphPad (version 6), and MPlus (version 7.4). CPP data were analyzed via a two-way analysis of variance (ANOVA) and one sample t-tests, comparing difference scores to a null-hypothesis value of 0. Student's t-tests or Mann-Whitney tests were used to analyze percent weight gained during CVS exposure, EPM measures, locomotor activity, fecal boli, and PR responding. Repeated measures ANOVAs were used to analyze methamphetamine self-administration data. Significant main effects were followed with Bonferroni-corrected comparisons. Multiple regression was used to determine the predictive ability of anxiety-like behavior and locomotor activity on self-administration behavior. Fisher's exact test was used to determine whether the proportion of CVS and CON rats assigned to high- or low-reactivity groups differed. Indirect effects of stress group on active lever presses and methamphetamine infusions under fixed and progressive ratio were examined with path analysis, while also allowing for stressor group to have a direct effect on each of these dependent variables. For all statistical analyses, $p < 0.05$ was considered statistically significant.

2.6.2 Final Group Sizes—For Experiment 1, initial group sizes were $n = 8$ rats per group. One CVS rat in the 0.25 mg/kg group failed to explore both chambers in the pre-test and was excluded from analyses. For Experiment 2, initial group sizes were $n = 23$ -24 rats per group. For the EPM group differences analyses only, data from seven rats were excluded due to falling off the maze apparatus. After stress procedures, surgery, and training, the resulting group sizes were $n = 20$ CVS and $n = 19$ CON rats for acquisition analyses. Patency was lost in $n = 5$ CVS and $n = 7$ CON rats during PR testing, resulting in group sizes of $n = 14$ CVS rats and $n = 11$ CON rats for PR analyses.

3. RESULTS

3.1 Confirmation of stressor effectiveness

The effectiveness of the CVS paradigm as a stressor was confirmed by analysis of weight gain during the experiment. During the 14-day period of stressor administration rats exposed to CVS demonstrated attenuated percent weight gain compared to CON rats in both Experiment 1 ($9.1 \pm 1.53\%$ vs. $23.4 \pm 0.84\%$, $p < 0.001$) and Experiment 2 ($-17.3 \pm 3.3\%$ vs. $10.6 \pm 3\%$, $p < 0.001$).

3.2 Experiment 1: Effects of CVS on Methamphetamine Conditioned Place Preference

Contextual conditioning with methamphetamine did not produce a CPP for either CON or CVS rats at a dose of 0.06 mg/kg, but did establish a CPP for both CON and CVS rats at the 0.25 mg/kg dose. Analysis of CPP data revealed no significant preference for either

compartment in the CPP apparatus during habituation testing. Difference scores were calculated by subtracting the amount of time spent in the methamphetamine paired chamber during the pre-test from the amount of time spent in the methamphetamine paired chamber during the post-test. Prior to determining whether a conditioned preference for the methamphetamine paired chamber was developed, the effects of dose and stress were examined on difference scores with a two-way ANOVA. A main effect of dose was identified ($F_{1,27}=10.42$, $p<0.01$) on the difference scores but no main effect of stress ($F_{1,27}=0.312$, $p>0.05$) or interaction effect between stress and methamphetamine dose was observed ($F_{1,27}=0.45$, $p>0.05$; Figure 2). To determine whether the difference scores reflected a preference for the methamphetamine paired compartment after conditioning, each difference score was compared to a difference score of 0 (no preference, i.e., no increase in time spent in the methamphetamine paired chamber after conditioning) with a one sample t-test. No preference was identified in either CON or CVS rats for the subthreshold dose (0.06 mg/kg) of methamphetamine ($t_7=0.91$, $p>0.05$; $t_7=1.2$, $p>0.05$ respectively; Figure 2). On the other hand, both CON and CVS rats demonstrated a preference for the chamber paired with 0.25 mg/kg methamphetamine ($t_7=3.7$, $p<0.01$; $t_6=3.05$, $p<0.05$ respectively; Figure 2).

3.3 Experiment 2: Effects of CVS on Methamphetamine Self-administration

Analysis of IVSA data prior to accounting for individual differences (see below) showed that rats exposed to CVS failed to exhibit altered self-administration of methamphetamine compared to CON rats under both FR1 and PR schedules (Figure 3). Analysis of the first 14 days of FR1 methamphetamine IVSA revealed a significant main effect of day, such that active lever presses ($F_{13,481}=7.83$, $p<0.001$) and number of infusions obtained ($F_{13,481}=12.87$, $p<0.001$) per session increased as a function of time (Figure 3A and B). However, no main effect of stress history or interaction between stress history and day were identified for either active lever presses ($F_{1,37}=0.45$, $p>0.05$; $F_{13,481}=0.78$, $p>0.05$; Figure 3A) or infusions ($F_{1,37}=0.7$, $p>0.05$; $F_{13,481}=0.66$, $p>0.05$; Figure 3B) per session. Analysis of final response ratios completed under PR conditions also did not reveal significant differences between CVS and CON rats ($t_{23}=0.02$, $p>0.05$; Figure 3C).

No group differences were observed for the average number of days to reach acquisition criteria (at least 10 active lever presses on two consecutive days; CON 3.27 ± 0.66 vs. CVS 4.67 ± 0.86 ; $t_{24}=1.21$, $p>0.05$). Both CON and CVS rats exhibited the ability to discriminate between the active and inactive levers. One CVS and three CON rats demonstrated initial discrimination but then progressed to excessive (>100 per session) inactive lever presses due to methamphetamine-induced stereotypical behaviors after day four of training and high intake of methamphetamine. Data from these animals were excluded from this analysis only. Significant main effects of lever (CON: $F_{1,30}=4.48$, $p<0.05$; CVS: $F_{1,36}=9.35$, $p<0.01$) and day (CON: $F_{13,390}=2.13$, $p<0.05$; CVS: $F_{13,468}=4.36$, $p<0.0001$) were observed, as well as a significant interaction between lever and day (CON: $F_{13,390}=2.06$, $p<0.05$; CVS: $F_{13,468}=2.91$, $p<0.001$).

In order to examine the potential individual differences in effects of CVS on methamphetamine self-administration, we first performed linear regression on the aforementioned self-administration data to determine the extent to which anxiety-like

behavior, locomotor activity, and physiological reactivity to a novel environment contributed to the variances observed. Together these variables contributed to 54% of the variance in number of active lever presses ($R^2=0.54$, $F_{6,31}=4.9$, $p<0.01$), 57% of the variance in the number of infusions obtained ($R^2=0.57$, $F_{6,31}=5.46$, $p<0.01$) and 78% of the variance in PR breakpoints (final completed ratio; $R^2=0.78$, $F_{6,18}=7.1$, $p<0.01$). Interestingly, open field locomotor activity as well as elimination of fecal boli in the EPM and open field significantly predicted methamphetamine self-administration (standardized regression coefficients, t-statistics, and p-values for each predictor are summarized in Table 1). Consequently, raw data for significant predictors were converted into Z scores, which were then averaged to calculate an average predictive reactivity score, similar to our previous studies (Bellani et al., 2006, Huynh et al., 2011, Taylor et al., 2011a). Linear regression was again used to assess the contribution of the calculated predictive reactivity score to methamphetamine self-administration. These analyses revealed that the average predictive reactivity score contributed to 16% of the variance in active lever presses ($R^2=0.16$, $F_{1,38}=7.1$, $p<0.05$), 14% of the variance in number of infusions obtained ($R^2=0.14$, $F_{1,38}=6.22$, $p<0.05$) and 16% of the variance in PR breakpoints (final completed ratio; $R^2=0.16$, $F_{1,24}=4.27$, $p=0.05$). Standardized regression coefficients, t-statistics, and p-values for the average reactivity score are summarized in Table 1. Next, a median split (Bellani et al., 2006) was performed on average predictive reactivity scores to examine methamphetamine self-administration in rats demonstrating high ($n=19$) vs. low ($n=20$) reactivity. The median split resulted in $n=14$ CVS rats and $n=5$ CON rats being classified into the high reactivity group, and $n=6$ CVS rats and $n=14$ CON rats into the low reactivity group (Figure 4). Fisher's exact test was conducted to determine whether reactivity to novelty and stress history were independent of each other, and revealed a significant relationship between stress history and reactivity ($p<0.05$, Fishers exact test) such that exposure to CVS increased the proportion of rats exhibiting high reactivity, as determined by the z-score calculated from the EPM and open field (Figure 4).

Repeated measures ANOVAs using reactivity (high vs. low) as the between-subjects factor revealed a significant main effect of day, such that active lever presses ($F_{13,481}=8.10$, $p<0.001$) and infusions ($F_{13,481}=13.40$, $p<0.001$) per session increased over days (Figure 5A and B). In addition, a main effect of reactivity was identified such that active lever presses ($F_{1,37}=12.60$, $p<0.01$) and infusions ($F_{1,37}=10.95$, $p<0.01$) were significantly increased in rats with high vs. low reactivity (Figure 5A and B). High-reactivity rats exhibited significantly more active lever presses on days 2-4, 9-10, and 13-14 ($p<0.05$), and significantly more infusions on days 2-5, 7, 9-10, and 13-14 ($p<0.05$). No significant interactions between reactivity and day were identified (active lever presses: $F_{13,481}=1.47$, $p>0.05$, infusions: $F_{13,481}=1.71$, $p>0.05$, Figure 5A and B). Analysis of PR final ratio completed also revealed a significant difference between rats with high vs. low reactivity ($t_{23}=2.3$, $p<0.05$) such that rats in the high-reactivity group had increased PR breakpoints than rats in low-reactivity group (Figure 5C).

Both high- and low-reactivity rats exhibited the ability to discriminate between the active and inactive levers. As with the previous analysis, two high- and two low-reactivity rats were excluded from this analysis due to methamphetamine-induced stereotypical behaviors after day four of training and high intake of methamphetamine. Significant main effects of lever

(High: $F_{1,32}=13.34$, $p<0.001$; Low: $F_{1,36}=7.12$, $p<0.05$) and day (High: $F_{13,416}=2.17$, $p<0.05$; Low: $F_{13,442}=5.87$, $p<0.0001$) were observed, as well as a significant interaction between lever and day (High: $F_{13,416}=2.74$, $p<0.01$; Low: $F_{13,442}=2.34$, $p<0.01$).

Group differences between CON and CVS rats, as well as rats demonstrating high and low reactivity, were examined (Table 2). Student's t-test revealed that rats exposed to CVS exhibited decreased EPM locomotor activity (total entries), decreased time in the open arms of the EPM, and increased elimination of fecal boli in both the EPM and open field compared to CON rats ($p<0.05$, Table 2). The OTR for entries did not pass the D'Agostino & Pearson omnibus normality test, therefore a non-parametric test of independent samples (Mann-Whitney) identified that CVS rats entered the open arms significantly less than CON rats ($p<0.05$). However, no difference in locomotor activity in the open field was identified ($p>0.05$, Table 2). Similarly, Student's t-tests revealed that rats exhibiting high reactivity also demonstrated decreased EPM locomotor activity, decreased time in the open arms of the EPM, increased elimination of fecal boli in both the EPM and open field, as well as increased activity in the open field compared to low reactivity rats ($p<0.05$, Table 2). The Mann-Whitney test identified that high reactivity rats entered the open arms less than low-reactivity rats, though this did not reach significance ($p=0.058$).

Structural equation modeling using path analyses revealed no direct effects of CVS on each dependent variable of methamphetamine self-administration (active lever presses and infusions under fixed and progressive ratio schedules; Fig. 6A-C, all p 's >0.05). However, the CVS condition was associated with a higher likelihood of being in the high reactivity group (path coefficients 1.68 ± 0.50 , $p<0.05$), and being in the high reactivity group was positively associated with increased total active lever presses (path coefficient 130.06 ± 51.20), total infusions under fixed ratio conditions (path coefficient 2.02 ± 0.84), and breakpoints under progressive ratio conditions (path coefficient 9.04 ± 4.48). All indirect effects were significant with $p < 0.05$.

4. DISCUSSION

4.1 Summary of Data

When individual differences were not explicitly examined, exposure to CVS for 14 days did not affect overall measures of methamphetamine conditioned reward or reinforcement. Specifically, in Experiment 1, neither CVS nor CON rats exhibited a preference for the chamber paired with a subthreshold (0.06 mg/kg) dose of methamphetamine, while both CVS and CON groups demonstrated a similar preference for the chamber paired with a higher dose of methamphetamine (0.25 mg/kg). These findings indicate that CVS does not influence the threshold dose or increase the magnitude of the conditioned rewarding effects of methamphetamine. In Experiment 2, CVS treatment did not affect overall operant responding for methamphetamine, the total amount of methamphetamine self-administered, or the motivation to self-administer methamphetamine under conditions of increasing behavioral demand. However, when individual differences were factored into the analyses, physiological reactivity to both the EPM and open field and locomotor activity in the open field significantly influenced methamphetamine self-administration. Specifically, rats that exhibited greater locomotor activity in a novel open field environment and increased fecal

boli elimination in the EPM and open field were split into two groups based on median values of their standardized combined reactivity score. The resulting analyses revealed that, compared to rats with low reactivity scores prior to self-administration, rats with high reactivity scores demonstrated increased operant responding for and overall methamphetamine intake, and higher breakpoints for methamphetamine reinforcement under conditions of increasing behavioral demand. Moreover, novel findings that CVS exposure significantly increased the proportion of rats exhibiting high reactivity scores, and that CVS was indirectly, through increased reactivity, associated with increased operant responding for and intake of methamphetamine were also identified. Thus, the present study reveals a previously unknown relationship between CVS exposure, individual differences in reactivity to novel environments, and methamphetamine intake patterns.

4.2 Effects of CVS on Methamphetamine Conditioned Place Preference

Although numerous investigators have examined the effects of acute or chronic stress in early life or adolescence on the rewarding effects of amphetamine-type stimulants (for review see Aguilar et al., 2013) very few studies have examined the effects of chronic stress on methamphetamine CPP in adulthood. Our current findings show no effect of CVS on methamphetamine CPP in adults and are consistent with studies indicating that early life stress does not influence (Faure et al., 2009, Hensleigh and Pritchard, 2014) or even attenuate (Dimatelis et al., 2012) methamphetamine CPP. Our findings also confirm those of others in adult male Sprague Dawley rats, such that 0.06 mg/kg methamphetamine is a subthreshold dose for the development of methamphetamine CPP (Zakharova et al., 2009). However, the possibility remains that examination of the effects of a wider range of doses might reveal an effect of CVS on the conditioned rewarding properties of methamphetamine. Since open field locomotor activity and anxiety-like behavior in the EPM were not evaluated in the first experiment (see Figure 1), the contribution of individual differences to any potential effects of CVS on the conditioned rewarding effects of methamphetamine could not be examined, and are important directions for future studies.

4.3 Effects of CVS on Methamphetamine Self-Administration

CVS exposure in adulthood did not directly influence the acquisition of methamphetamine self-administration, the total intake of methamphetamine, or motivation to obtain methamphetamine under a progressive ratio schedule of reinforcement. The present findings are in line with previous data showing that neither the combination of early life stress with CVS in adulthood, nor injections of corticosterone nor non-contingent footshock in adulthood affected acquisition of methamphetamine self-administration (Moffett and Goeders, 2005, Lewis et al., 2016). Despite a lack of effect of stress in adulthood, evidence suggests chronic stress during critical early developmental periods does contribute to a facilitation of methamphetamine self-administration in adult rats (Lewis et al., 2013, 2016). Thus the interaction between chronic stress and the developing brain may be a key aspect of stress-induced vulnerability to methamphetamine abuse. Moreover, it is also possible that a longer duration of stress exposure is necessary to produce effects on methamphetamine self-administration. In the repeated restraint stress paradigm, changes in motivation occur in the second week of stress exposure and become more robust in the third week (Kleen et al., 2006), which is also similar to the timeline to observe cognitive impairments (McLaughlin et

al., 2007). Furthermore, only one unit dose of methamphetamine reinforcement (0.05 mg/kg/infusion) was utilized in this study, and it is possible that CVS might produce more profound group or individual effects in animals self-administering either higher or lower doses of the drug. Finally, a limitation of the current study is that circulating corticosterone levels were not measured, and thus assessment of HPA axis function was not available to corroborate attenuated weight gain as an assessment of stressor efficacy, although body weight gain is a validated marker for stressor effectiveness in male rats (Conrad, 2006). Future studies are needed to understand the relationship between the parameters by which the type and timing of chronic stress could potentially affect methamphetamine intake.

4.4 CVS Exposure, Individual Differences, and Methamphetamine Self-Administration

As expected, CVS exposure increased anxiety-like behavior on the EPM, decreased locomotor activity (total arm entries) in the EPM, and increased physiological reactivity to novel environments. Since CVS paradigms frequently result in attenuated weight gain, increased anxiety-like behavior, and altered reactivity to a novel or stressful environment (Monnikes et al., 1993, Bhatnagar and Dallman, 1998, Ostrander, 2006, Jankord et al., 2010, Taylor et al., 2015), our observations of these effects in the present study further confirm the effectiveness of the CVS paradigm. Interestingly, the present study did not identify a significant effect of CVS on locomotor activity in a novel open field environment. However, other investigators have shown that locomotor activity (total arm entries) and anxiety-like behavior on the EPM are not always predictive of locomotor activity in an open field environment (Bellani et al., 2006).

A significant relationship between individual differences and methamphetamine self-administration was identified by linear regression and path analyses. Locomotor activity in the open field, as well as fecal boli elimination in both the EPM and open field significantly predicted methamphetamine self-administration, while anxiety-like behavior and activity on the EPM did not. After rats were characterized as exhibiting high or low reactivity to novelty based on a median split of combined Z-scores of significant predictors, it was found that a significantly greater proportion of CVS rats were classified as high reactivity compared to CON rats. This finding suggests that CVS exposure increases the likelihood of high reactivity to novel environments, which in turn predicts and/or indirectly affects methamphetamine self-administration behaviors.

Additional analyses revealed a significant effect of reactivity to novelty on methamphetamine self-administration. High-reactivity rats exhibited increased operant responding for methamphetamine under fixed and progressive ratios of reinforcement compared to low-reactivity rats. These findings are consistent with the existing literature indicating that high responders (i.e., rats demonstrating high locomotor activity in a novel open field environment) exhibit increased operant self-administration for psychostimulants compared to low responders (Piazza et al., 1989, Pierre and Vezina, 1997, Marinelli and White, 2000, Piazza et al., 2000, Cain et al., 2006, Davis et al., 2008, Gancarz et al., 2011). In addition, Gancarz et al. (2011, 2012) found that rats with higher locomotor reactivity exhibited increased operant responding for methamphetamine reinforcement in combination with a visual stimulus compared to rats with lower reactivity. While the present study used a

higher unit dose of methamphetamine reinforcement (0.05 mg/kg/infusion) as compared to that utilized by Gancarz et al. (2011, 2012) who used 0.025 mg/kg/infusion, collectively these observations suggest that a range of doses can be used to detect the effects of individual differences on intravenous methamphetamine self-administration.

Individual differences in locomotor reactivity are thought to be either related to stress reactivity (Bardo et al., 1996, Dellu et al., 1996, Piazza and Le Moal, 1996, Klebaur and Bardo, 1999) or novelty seeking (Dellu et al., 1996, Blanchard et al., 2009). Interestingly, one study found that rats with high reactivity exhibit reduced anxiety-like behavior on the EPM, which suggests that these individual differences are related to novelty seeking rather than stress reactivity (Kabbaj et al., 2000). The present study found that when a composite predictive score of reactivity is examined, rats with high reactivity (and subsequent methamphetamine self-administration) exhibited increased anxiety-like behavior. Consequently, the findings presented here support the idea that individual differences predictive of methamphetamine self-administration may be indicative of stress reactivity rather than novelty seeking. This is also supported by the finding that chronic stress significantly increased the proportion of rats demonstrating high physiological and locomotor reactivity to a novel environment. However, the lack of effect of stress on locomotor activity in the open field and the inability of anxiety-like behavior to predict methamphetamine self-administration suggests that further research is warranted to fully understand the impact of chronic stress on individual differences in other factors predictive of methamphetamine self-administration.

5. Conclusions

In summary, we demonstrated that exposure to CVS for 14 days did not directly affect overall measures of methamphetamine conditioned reward or reinforcement. However, CVS exposure significantly increased the proportion of rats categorized as highly reactive based on physiological reactivity to the EPM and open field as well as locomotor activity in an open field. When animals were classified as exhibiting high or low reactivity based on individual differences in reactivity to novelty, distinct patterns of methamphetamine self-administration were observed. Consistent with previous reports with self-administration of other psychostimulants, we found that high-reactivity rats demonstrated increased self-administration of methamphetamine under fixed and progressive ratio schedules of reinforcement compared to low-reactivity rats. Thus, CVS was indirectly associated with increased methamphetamine self-administration. We conclude that assessment of individual differences in reactivity to novelty or stressful conditions is vital for future studies examining the relationship between chronic stress and methamphetamine intake in both animals and humans. Such measures may lead to increased understanding of the neural basis of stress effects on drug intake, as well as the potential development of individualized treatment approaches for amphetamine use disorders and identification of at-risk individuals.

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Highlights

- Chronic variable stress increases the proportion of rats exhibiting high-reactivity
- Reactivity phenotype predicts reinforcing properties of methamphetamine
- Chronic stress alone does not directly affect methamphetamine reward or reinforcement

Figure 1.

Diagram of the experimental procedures conducted in the present study.

Abbreviations: CON, control procedures; CVS, chronic variable stress procedures; FR, fixed ratio; IVSA, intravenous self-administration, METH, methamphetamine.

Figure 2.

Contextual conditioning with methamphetamine did not produce a CPP for either CON (n=8) or CVS (n=8) rats at a dose of 0.06 mg/kg, but did establish a CPP for both CON (n=8) and CVS (n=7) rats at the 0.25 mg/kg dose. No differences were observed between CVS and CON groups at either dose. * $p < 0.05$, ** $p < 0.01$ vs. 0 seconds (e.g., no preference for the methamphetamine paired chamber). Difference scores were calculated by subtracting the amount of time spent in the methamphetamine-paired chamber during the pre-test from the amount of time spent in the methamphetamine-paired chamber during the post-test. Data are presented as mean $\pm$ SEM.

Figure 3.

Patterns of methamphetamine self-administration in CVS and CON rats over 14 consecutive days. No differences between CVS and CON rats were observed for any dependent variable. A) Active lever presses under an FR1 schedule of reinforcement. B) Number of infusions obtained under an FR1 schedule of reinforcement. C) Final ratio completed and number of infusions obtained under a PR schedule of reinforcement. A-B) CVS (n=20) and CON (n=19) rats; C) CVS (n=14) and CON (n=11) rats. Data are presented as mean $\pm$ SEM.

Figure 4.

Proportion of rats classified as low- or high-reactivity based on Z-scores combining locomotor activity in the open field and physiological reactivity to the open field and EPM. The results suggest that CVS exposure increased the proportion of rats classified as high-reactivity rats (n=14 vs. CON n=4) and decreased the proportion of rats classified as low-reactivity (n=6 vs. CON n=14).

Figure 5.

Patterns of methamphetamine self-administration following classification of rats into high reactivity (n=14 CVS and n=5 CON rats) or low reactivity (n=6 CVS rats and n=14 CON rats) groups. High-reactivity rats exhibited a significant increase in all dependent variables compared to low-reactivity rats. A) Active lever presses under an FR1 schedule of reinforcement. B) Number of infusions obtained under an FR1 schedule of reinforcement. C) Final ratio completed and number of infusions obtained under a PR schedule of reinforcement. * $p < 0.05$ vs. low-reactivity group on corresponding day. Data are presented as mean $\pm$ SEM.

Figure 6.

Path analysis of direct and indirect influences of CVS on measures of methamphetamine self-administration. A-C) CVS did not directly influence the total number of active lever presses or methamphetamine infusions under a fixed ratio schedule, or breakpoints under progressive ratio conditions. However, CVS significantly influenced development of a high reactivity phenotype, which in turn directly influenced self-administration. Path coefficients are presented in the Results section. E = mean $\pm$ standard error of measurement. n.s., not significant

Table 1

Summary of predictors of methamphetamine self-administration

Predictors	Active Lever Presses			Total Infusions			PR Breakpoint			
	β	<i>t</i>	<i>p</i>	β	<i>t</i>	<i>p</i>	β	<i>t</i>	<i>p</i>	
EPM	OTR Time	-0.06	-0.25	0.80	-0.08	-0.36	0.72	0.39	0.98	0.35
	OTR Entries	-0.35	-1.89	0.07	-0.33	-1.81	0.08	-0.17	-0.38	0.71
	Total Entries	0.2	0.84	0.41	0.17	0.75	0.46	0.25	1.27	0.23
	Fecal Boli	0.76	3.29	<0.01	0.74	3.30	<0.01	1.26	4.96	<0.01
Open Field	Distance	0.39	2.63	<0.05	0.42	2.92	<0.01	0.34	2.08	0.06
	Fecal Boli	-0.49	-2.66	<0.05	-0.53	-2.97	<0.01	-0.59	-3.01	<0.05
Average Reactivity Score	0.40	2.67	<0.05	0.38	2.50	<0.05	0.49	2.67	<0.05	

Abbreviations: β , standardized regression coefficient beta; EPM, elevated plus maze; OTR, open to total ratio; PR, progressive ratio

Table 2

Summary of group differences for individual predictors

	Elevated Plus Maze				Open Field	
	<i>OTR Time</i>	<i>OTR Entries</i> *	<i>Total Entries</i>	<i>Fecal Boli</i>	<i>Distance (cm)</i>	<i>Fecal Boli</i>
CON	9.68 ± 2.1	13.92 ± 2.65	8.87 ± 1.11	1.2 ± 0.44	11049 ± 654	1.74 ± 0.54
CVS	2.18 ± 1.23	8.33 ± 5.15	1.59 ± 0.47	6.76 ± 0.6	9700 ± 868	4.95 ± 0.83
<i>df</i>	30	30	30	30	37	37
<i>t</i>	3.16	n. a.	6.29	-7.29	1.23	-3.21
<i>p</i>	<0.01	<0.05 *	<0.001	<0.001	0.23	<0.01
Low Reactivity	8.54 ± 2.0	13.95 ± 3.38	7.12 ± 1.22	1.71 ± 0.53	8997 ± 772	2.15 ± 0.58
High Reactivity	2.47 ± 1.35	7.54 ± 5.11	2.6 ± 0.90	6.93 ± 0.66	11788 ± 658	4.68 ± 0.89
<i>df</i>	30	30	30	30	37	37
<i>t</i>	2.43	n. a.	2.9	-6.227	-2.74	-2.41
<i>p</i>	<0.05	0.058 *	<0.01	<0.001	<0.01	<0.05

Note: data presented are mean ± SEM.

* denotes data were analyzed with Mann-Whitney test.

Abbreviations: CON, control procedures; CVS, chronic variable stress procedures; OTR, open to total ratio.