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TITLE PAGE

Title: Individual differences in the relative reinforcing effects of 3,4-methylenedioxypyrovalerone (MDPV) under fixed and progressive ratio schedules of reinforcement in rats

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RUNNING TITLE PAGE

Running Title: Individual differences in MDPV self-administration

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Abbreviations used: dopamine transporter, **DAT;** norepinephrine transporter, **NET;** serotonin transporter, **SERT;** fixed ratio, **FR**; progressive ratio, **PR;** timeout, **TO;** conditioned stimulus, **CS;** 3,4-methylenedioxypyrovalerone, **MDPV;** 3,4-methylenedioxymethamphetamine, **MDMA**

Section: Behavioral Pharmacology

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ABSTRACT

The recreational use of designer drugs, including synthetic cathinones (bath salts), is associated with high levels of abuse and toxicity, and represents a growing threat to public health. MDPV (3,4-methylenedioxypyrovalerone) is a cocaine-like monoamine uptake inhibitor, and one of the most widely available and abused synthetic cathinones. The present study used male Sprague-Dawley rats to directly compare: (1) the acquisition of responding for MDPV and cocaine under a fixed ratio (FR) 1 schedule of reinforcement; (2) full dose-response curves for MDPV and cocaine under FR5; and (3) progressive ratio (PR) schedules of reinforcement. Self-administration of MDPV and cocaine was acquired at comparable rates, and by a similar percentage of rats. Compared to cocaine, MDPV was ~10-fold more potent and ~3-fold more effective at maintaining responding (PR; final ratio completed). Unlike cocaine, for which little variability was observed among rats, the FR5 dose-response curve for MDPV was shifted ~3-fold upward for a subset of rats (high-responders) relative to other rats with identical histories (low-responders). Compared to low-responding rats, high-responders also self-administered more cocaine under an FR5, and earned significantly more MDPV, cocaine, and methamphetamine under a PR schedule of reinforcement. In addition to functioning as a significantly more effective reinforcer than either cocaine or methamphetamine, MDPV also appears to be unique in its capacity to establish an enduring phenotype in rats, characterized by unusually high levels of drug intake. Although the factors underlying this high-responder phenotype are unclear, they might be related to individual differences in human drug-taking behavior.

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INTRODUCTION

The abuse of “designer drugs” including synthetic derivatives of cathinone represents a serious public health problem worldwide. The United Nations estimates that 25% of all new psychoactive substances are synthetic cathinones (UNODC, 2014), 13 of which have been classified as Schedule I by the US Drug Enforcement Administration. These compounds are marketed as *bath salts* or *research chemicals* and purported to be “safe” and “legal” alternatives to illicit stimulant drugs of abuse. Synthetic cathinones interact with monoamine transporters (dopamine [DAT]; norepinephrine [NET]; and serotonin [SERT]) and function as either cocaine-like inhibitors (inhibit uptake only), or amphetamine-like substrates (inhibit uptake and stimulate release) (e.g. Baumann et al., 2013; Simmler et al., 2013). *Bath salts* are primarily sold as powders or capsules and are administered by intranasal, oral, and intravenous routes for their euphoric and stimulant effects (Johnson and Johnson, 2014). Users report intense craving and frequently re-administer cathinones (Winstock et al., 2011), suggesting that cathinones function as powerful reinforcers. Mirroring increases in recreational use, poison control center calls and emergency room visits related to *bath salts* have also increased in recent years (AAPCC, 2016). As such, more research is urgently needed to better understand the abuse-related and toxic effects of synthetic cathinones.

Analysis of *bath salts* obtained in the US suggests that 3,4-methylenedioxypyrovalerone (MDPV) is one of the most widely available and abused cathinones (Kyle et al., 2011; Spiller et al., 2011; Borek and Holstege 2012; Murray et al., 2012; Shanks et al., 2012; Seely et al., 2013). Consistent with its capacity to inhibit monoamine uptake, MDPV is known to stimulate locomotor activity and produce discriminative stimulus effects similar to those of other stimulant drugs (e.g., cocaine, 3,4-methylenedioxymethamphetamine [MDMA], and methamphetamine) in rats and mice (Huang et al., 2012; Fantegrossi et al., 2013; Gatch et al., 2013; Gannon et al., 2016; Collins et al., 2016). Based in large part on structural similarities, early studies compared the reinforcing effects MDPV to methamphetamine (Aarde et al., 2013; Watterson et al., 2014),

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however, *in vitro* data have since indicated that MDPV is more similar to cocaine than methamphetamine, but MDPV is 100-500-fold more selective than cocaine for DAT relative to SERT (Baumann et al., 2013; Simmler et al., 2013).

The reinforcing effects of MDPV have been evaluated under fixed ratio (FR) and progressive ratio (PR) schedules of reinforcement, with two studies suggesting MDPV is a more effective reinforcer than methamphetamine (Aarde et al., 2013; Watterson et al., 2014), and a third suggesting the reinforcing effects of MDPV are comparable to those of cocaine (Schindler et al., 2016). Although Schindler and colleagues (2016) reported that experimentally naïve rats acquire responding for MDPV (0.03 mg/kg) and cocaine (0.5 mg/kg) at comparable rates, data from several behavioral assays suggest MDPV is 10-fold more potent than cocaine (Baumann et al., 2013; Gatch et al., 2013; Gannon et al., 2016; Schindler et al., 2016; Collins et al., 2016), raising the possibility the unit dose of cocaine was too large to provide an accurate comparison. Moreover, although two studies have used progressive ratio (PR) schedules of reinforcement to suggest MDPV is a more effective reinforcer than methamphetamine (a monoamine transporter substrate), a direct comparison of the relative reinforcing effectiveness of MDPV to cocaine (another monoamine transporter inhibitor) has not been made.

Thus, the present study aimed to directly compare: (1) the acquisition of responding for functionally equivalent doses of MDPV (0.032 mg/kg/inf) and cocaine (0.32 mg/kg/inf); (2) dose-response curves for the reinforcing effects of MDPV and cocaine under an FR5 schedule of reinforcement; and (3) a PR schedule of reinforcement (MDPV, cocaine, and methamphetamine). Together, these studies provide both qualitative and quantitative comparisons of the reinforcing effects of MDPV to two well-known stimulant drugs of abuse (cocaine and methamphetamine) and describe individual differences in drug-maintained behavior that was only observed in rats trained to respond for MDPV.

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MATERIALS AND METHODS

ANIMALS

Male Sprague-Dawley rats (275-300g) were obtained from Harlan (Indianapolis, IN) and singly housed with free access to water and rat chow in a temperature- (24°C) and light-controlled (10/14-h dark/light) environment. All procedures were conducted in accordance with the Institutional Animal Care and Use Committee of the University of Texas Health Science Center at San Antonio, and the Eighth Edition of the Guide for Care and Use of Laboratory Animals (National Research Council, 2011).

SURGERY

Rats were anesthetized with 2% isoflurane and prepared with chronic indwelling catheters in the left femoral vein as previously described (Collins et al., 2012a; 2012b; Collins and France, 2015). Catheters were passed under the skin and attached to a vascular access button placed in the mid-scapular region. Immediately following surgery, rats were administered Penicillin G (60,000 U/rat) subcutaneously to prevent infection. Rats were allowed 5–7 days to recover during which time catheters were flushed daily with 0.5 ml of heparinized saline (100 U/ml). Thereafter, catheters were flushed daily with 0.2 ml of saline prior to, and 0.5 ml of heparinized saline after the completion of self-administration sessions.

APPARATUS

Experimental sessions were conducted in standard operant conditioning chambers (Med Associates Inc., St. Albans, VT) housed inside sound attenuating cubicles. Each chamber was equipped with two response levers located 6.8 cm above the grid floor and 1.3 cm from the right

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or left wall. Visual stimuli were provided by two sets of green, yellow, and red LED lights, one located above each of the two levers, and a white house light located at the top center of the opposite wall. Illumination of the yellow LED above the active lever (left or right counterbalanced across rats) served as the discriminative stimulus. The conditioned stimulus (CS) consisted of the illumination of the green, yellow and red LEDs above the active lever, as well as the illumination of the houselight and was coincident with the start of the infusion. Drug solutions were delivered by a variable speed syringe pump through Tygon® tubing connected to a stainless steel fluid swivel and spring tether which was held in place by a counterbalanced arm.

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SELF-ADMINISTRATION STUDIES

Acquisition procedures

A total of 48 rats were allowed to respond for either MDPV (n=32) or cocaine (n=16) under a FR1:timeout (TO) 5-sec schedule of reinforcement during 10 daily 90-min sessions. Doses of MDPV (0.032 mg/kg/inf) and cocaine (0.32 mg/kg/inf) were chosen based on their relative position on the descending limb of FR dose-response curves (Collins and Woods, 2007; Aarde et al., 2013). The discriminative stimulus signaled drug availability and completion of the response requirement resulted in a drug infusion (0.1 ml/kg over ~1-sec), presentation of the CS, and initiation of a 5-sec timeout. The CS was present throughout the 5-sec timeout. Responses on the inactive lever, as well as responses on either lever during timeouts were recorded but had no scheduled consequence. Acquisition criteria were as follows: ≥20 infusions for two consecutive days with ≥80% responding on the active lever. Rats that failed to acquire due to low levels of responding (<5 inf per session) were mildly food restricted (15g) and active levers were baited with food until responding increased (usually 1-2 sessions); rats that failed to acquire because of stability or accuracy were provided additional sessions at FR1 until criteria

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were met. Response requirements were subsequently increased to an FR5 where they remained until stability criteria were met (±20% of the mean of 3 consecutive sessions and no increasing or decreasing trend).

Across Session Dose-Response Curves

Dose substitution (0.001-0.1 mg/kg/inf) was used to generate a full FR5:TO5-sec dose-response curve for MDPV in the first cohort (n=16) of the MDPV-trained rats. The first dose was always 0.032 mg/kg/inf, with remaining doses evaluated in a random order and until stability criteria were met.

These 16 rats (n=7, high-responders; n=9, low-responders) then transitioned to a PR schedule of reinforcement under which ratios incremented according to the following equation: Ratio=[5e^(inf#*0.2)]−5 (Richardson and Roberts, 1996). Sessions lasted a maximum of 12h but terminated if a ratio was not completed within 45 min (i.e., 45-min limited hold). Ratio completion resulted in delivery of a unit dose of MDPV (0.0032-0.32 mg/kg/inf), cocaine (0.032-1.78 mg/kg/inf), methamphetamine (0.0032-0.178 mg/kg/inf), or saline. Infusions were followed by a 5-sec timeout signaled by CS presentation during which responding was recorded but had no scheduled consequence. MDPV was always evaluated first, but substitute drugs were evaluated in random order, with all doses for a particular drug evaluated before moving to the next; 0.032 mg/kg/inf MDPV was re-evaluated before each substitute drug. Stability was defined as two consecutive sessions where the number of infusions obtained differed by ≤2.

Within-session Dose-Response Curves

The remaining 16 MDPV-trained (n=8, high-responders; n=8, low-responders) and 14 of the cocaine-trained rats transitioned to a multiple component, FR5:TO5-sec schedule that comprised five 20-min components, as described previously (Collins et al., 2012a). The discriminative stimulus signaled the start of each component, and completion of the response

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requirement resulted in delivery of the available unit dose, paired with CS presentation and a 5-sec timeout. During the first component, completion of the response requirement resulted in the CS presentation and 5-sec timeout, but no infusion. Under this procedure, the concentration of the drug in the syringe remained constant, and the infusion duration increased across components (0-5 sec) to deliver the desired unit doses of MDPV (0.0032, 0.01, 0.032, and 0.1 mg/kg/inf), cocaine (0.032, 0.1, 0.32, and 1.0 mg/kg/inf), or saline (duration/volume matched infusions). Each component was followed by a 5-min blackout period, where all visual stimuli were extinguished. Responses during timeouts or blackouts were recorded but had no scheduled consequence. Substitution tests were completed once responding stabilized for the training drug (three consecutive sessions with <20% variance in responding during each component), with cocaine (0.032-1.0 mg/kg/inf) substituted in MDPV-trained rats, and MDPV (0.0032-0.1 mg/kg/inf) substituted in cocaine-trained rats; saline was substituted in both groups. Substitutes were evaluated until stability criteria were met (at least 3 sessions), with rats returning to their training drug between substitution tests.

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DRUGS

Racemic MDPV was synthesized at the Chemical Biology Research Branch of the National Institute on Drug Abuse (NIDA) and National Institute on Alcohol Abuse and Alcoholism (NIAAA). (-)-Cocaine hydrochloride and (+)-methamphetamine hydrochloride were provided by the NIDA Drug Supply Program. All drugs were dissolved in 0.9% physiological saline and administered intravenously in a volume of 0.1 ml/kg, with the exception of infusion volumes for the multiple component schedule which are described above.

DATA ANALYSIS

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Acquisition data are shown as the mean ± standard error of the mean (S.E.M.) of the number of responses made on the active and inactive levers, as well as the percentage of rats that met acquisition criteria across the 10-day acquisition phase. Acquisition data for MDPV and cocaine were compared by two-way (drug and day) repeated measures (day) ANOVA followed by a Holm-Sidak's test for multiple comparisons. Mean number of days required to meet acquisition criteria between drugs were compared by unpaired, two-tailed t-test.

Data obtained during the transition from FR1 to FR5 are presented as of the mean (±S.E.M.) number of infusions of MDPV (0.032 mg/kg) or cocaine (0.32 mg/kg) obtained during the last three sessions under FR1 and the three days that satisfied stability criteria under the FR5 schedule of reinforcement. Two-way (drug and day) repeated measures (day) ANOVA with post-hoc Holm-Sidak tests were used to detect significant differences in the number of infusions obtained under FR1 and FR5 conditions for rats trained to respond for MDPV (low- and high-responders) and cocaine. Responding during post-infusion timeouts is expressed as the percent of active lever responding that occurred during the timeout relative to the total number of active lever responses and plotted as a frequency distribution using 10% bins. A Pearson's correlation test was performed to determine if percent timeout responding was related to the number of infusions (0.032 mg/kg MDPV) obtained.

Dose-response data (FR and PR) are presented as mean (±S.E.M.) of the number of infusions earned for each unit dose and analyzed by two-way (phenotype and dose) repeated measures (dose) ANOVA to detect differences between groups. The Holm-Sidak post-hoc test was used to detect differences in number of infusions between high- and low-responders under PR conditions.

Progressive ratio dose-response curves were used to obtain measures of reinforcing potency (dose estimated to produce a 50% effect [ED_{50}]) and effectiveness (maximal effect level; E_{max}) for individual subjects. The mean (±S.E.M.) E_{max} represents the dose-independent

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maximum number of infusions earned for a given drug and provides a measure of reinforcing effectiveness for each drug. ED_{50}s were obtained for individual subjects by normalizing dose-response curves for each drug to the E_{max} (E_{max}=100%) and analyzing all the data spanning the 20%-80% effect levels (including not more than one point above 80% and not more than one point below 20%) by linear regression. Potency data represent the mean ED_{50} ± 95% confidence intervals. Two-way (phenotype and drug) ANOVA followed by the Holm-Sidak test was used to detect differences in E_{max}, whereas one-way ANOVA followed by the Holm-Sidak test was used to detect differences in E_{max} values when data were collapsed across phenotype. ED_{50}s were considered statistically different if the confidence intervals did not overlap.

For within-session studies, responding during timeouts and blackouts are presented as the mean (±S.E.M.) percent of active lever responses made during post-infusion timeouts and mean (±S.E.M.) number of active lever responses made during each inter-component blackout. Two-way (group and dose/component) repeated measures (dose/component) ANOVA followed by the post-hoc Holm-Sidak test was used to detect differences in responding among groups of rats (MDPV-trained [low- and high-responders] and cocaine-trained). Prism 6 software (GraphPad Software, Inc., La Jolla, CA) was used to generate figures and conduct statistical analyses.

RESULTS

Acquisition

As shown in Figure 1, rats readily acquired responding for MDPV (0.032 mg/kg/inf) or cocaine (0.32 mg/kg/inf), with 87.5% (28/32) of rats acquiring responding for MDPV, and 75% (12/16) of rats acquiring responding for cocaine by the end of the 10-day period. The mean time to acquire was 5.1±0.4 days for MDPV and 5.1±0.6 days for cocaine. For both groups, the mean number of infusions earned increased as a function of day ($F[9,460]=3.5$, $p<0.001$) with cocaine

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maintaining slightly more infusions than MDPV (56.9±10.3 versus 32.1±2.6, respectively) by the end of the acquisition phase, but these differences were not significant. A two-way repeated measures ANOVA of the number of active lever responses made during acquisition revealed main effects of day ($F[9,414]=5.1$, $p<0.0001$) and an interaction between day and drug ($F[9,414]=2.3$, $p<0.05$), but no main effects of drug. When percent acquisition data were compared by two-way ANOVA, main effects of day ($F[9,9]=48.2$, $p<0.0001$) and drug ($F[1,9]=7.0$, $p<0.05$) were identified, however, post-hoc tests failed to identify group differences across the 10-day acquisition period.

Across Session FR5

Figure 2 (upper left panel) shows the number of infusions earned during the last three sessions in which MDPV (0.032 mg/kg) or cocaine (0.32 mg/kg) were available under the FR1:TO5-sec and FR5:TO5-sec schedules of reinforcement. Although the number of infusions did not vary as a function of response requirement (i.e., FR1 and FR5) when cocaine was available, a significant difference was observed when MDPV was available for infusion ($p<0.05$). This difference was due to a subset of rats (black squares) that significantly increased MDPV self-administration when the schedule of reinforcement changed from an FR1 to an FR5. While there were no differences in the number of infusions earned between high- and low-responding rats when MDPV was available under an FR1, post-hoc tests indicated high-responding rats self-administered significantly more MDPV than low-responding rats under an FR5 schedule of reinforcement ($p<0.05$).

Analysis of the across-session dose-response curves (figure 2; upper right panel) by two-way repeated measures ANOVA revealed significant main effects of responder phenotype ($F[1,14]=11.6$; $p<0.005$) and MDPV dose ($F[5,70]=14.0$; $p<0.0001$) on the number of MDPV infusions obtained; however, an interaction between responder phenotype and dose was not detected.

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The percent of active lever responding that occurred during the timeouts is also shown in Figure 2 (bottom panels). Similar to cocaine-trained (gray bars), approximately half of the MDPV-trained rats (white bars), made <20% of their active lever responses during post-infusion timeouts, whereas the remainder of the MDPV-trained rats (black bars) made >20% of their total active lever responses during the post-infusion timeout. Based on these data, MDPV-trained rats that made <20% of active lever responses during timeouts were operationally defined as "low-responders" (n=17), whereas MDPV-trained rats making >20% of their total active lever responses during timeouts were defined as "high-responders" (n=15). Because none of the cocaine-trained rats made >20% of their active lever responses during timeouts, they were viewed as a homogenous group (gray symbols). High rates of timeout responding were positively correlated ($r=0.7365$; $p<0.0001$) with the number of infusions of MDPV earned (Figure 2, lower right panel).

Multiple component FR5

Under this paradigm, MDPV, cocaine, and saline were evaluated across five sequential 20-min components (Figure 3, top panel). With respect to number of MDPV infusions, main effects of group ($F[2,27]=3.9$; $p<0.05$), dose ($F[4,108]=19.1$; $p<0.0001$), and a group by dose interaction ($F[8,108]=2.3$; $p<0.05$) were detected. Post-hoc tests revealed high-responders self-administered significantly more MDPV than low-responders or cocaine-trained rats at doses of 0.01 and 0.032 mg/kg/inf; cocaine-trained rats did not differ from low-responders at any dose. When cocaine was available, main effects of group ($F[2,27]=3.4$; $p<0.05$), dose ($F[4,108]=179.5$; $p<0.0001$), as well as dose by group interaction ($F[8,08]=4.1$; $p<0.0005$) were detected for the total number of infusions. Post-hoc tests revealed high-responders self-administered significantly more cocaine than low-responders and cocaine-trained rats at a dose of 0.1 mg/kg/inf (Figure 3, upper middle panel). All three groups of rats responded at low levels when saline was available, however, post-doc tests revealed that cocaine-trained rats responded more than low- and high- responders when the CS (alone) was presented ($p<0.05$)

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and more than low-responders ($p<0.05$) during the second saline component. Although potency differences were observed between MDPV and cocaine, the relative (MDPV was 10-fold more potent than cocaine) and absolute potency of MDPV (peak at 0.01 mg/kg/inf) and cocaine (peak at 0.1 mg/kg/inf) to maintain responding did not differ among the groups of rats (high-responders, low-responders, and cocaine-trained).

Similar to when a single unit dose was available throughout a 90-min session, differences in timeout responding were also observed under the multiple component procedure (Figure 3, middle row) with main effects of group (MDPV—$F[2,27]=20.0$; $p<0.0001$: cocaine—$F[2,27]=4.3$; $p<0.05$), dose (MDPV—$F[4,108]=10.4$; $p<0.0001$: cocaine—$F[4,108]=3.0$; $p<0.05$), and group by dose interactions (MDPV—$F[8,108]=15.3$; $p<0.0001$: cocaine—$F[8,108]=7.0$, $p<0.0001$) detected when either MDPV or cocaine were available. With regard to MDPV, post-hoc tests revealed that high-responders made significantly more timeout responses than low-responders and cocaine-trained rats at unit doses of 0.01-0.1 mg/kg/inf ($p<0.05$). Similar effects were observed when cocaine was available at unit doses of 0.1-1.0 mg/kg/inf ($p<0.05$). Although cocaine-trained rats exhibited a greater percentage of timeout responses than MDPV-trained rats when ineffective doses of MDPV (0.0032 mg/kg/inf) and cocaine (0.032 mg/kg/inf) or saline (second component) were available, this was likely due to the small number of total responses made under each of these conditions. With this exception, responding maintained by MDPV or cocaine did not differ between low-responders and cocaine-trained rats. Timeout responding when saline was available for infusion was comparable and low in all groups.

Responding during the inter-component blackouts was also compared across rats (Figure 4, bottom row), with a main effect of group and dose detected when either MDPV (group—$F[2,27]=4.4$, $p<0.05$; dose—$F[4,108]=5.5$; $p<0.0005$) or cocaine (group—$F[2,27]=3.6$, $p<0.05$; dose—$F[4,108]=7.0$, $p<0.0001$) were available for infusion. Interactions between dose and group were also detected when MDPV ($F[8,108]=3.3$; $p<0.005$) or cocaine ($F[8,108]=3.5$, $p<0.005$) were available. Similar to timeout responding, high-responders exhibited more

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blackout responses than both low-responders and cocaine-trained rats at unit doses of 0.01 and 0.032 mg/kg/inf MDPV ($p<0.05$) and 0.1 mg/kg/inf cocaine ($p<0.05$). When saline was available, blackout responding was low and not significantly different among the groups.

Progressive ratio

Dose-response curves for MDPV, cocaine, and methamphetamine generated under PR conditions are shown in Figure 4 (upper left panel), with ED_{50}s and E_{max} values provided in Table 1. E_{max} values obtained for MDPV were significantly greater than cocaine ($p<0.05$) or methamphetamine ($p<0.05$), whereas E_{max} obtained for methamphetamine and cocaine were not significantly different. Potency differences were also observed among the drugs, with MDPV being ~2-fold more potent than methamphetamine, and ~10-fold more potent than cocaine.

Although the potency of these drugs to reinforce behavior was not affected by responder phenotype, significant differences in E_{max} were observed between high- and low-responders for all three drugs ($p<0.05$). Although a two-way ANOVA revealed significant main effects of dose for MDPV ($F[6,84]=198.0$, $p<0.0001$), cocaine ($F[5,70]=144.0$, $p<0.0001$), and methamphetamine ($F[4,56]=98.3$, $p<0.0001$), a main effect of responder phenotype was only observed when MDPV was available for infusion ($F[1,14]=9.9$, $p<0.01$). Interactions between dose and responder phenotype were observed for MDPV ($F[6,84]=4.1$, $p<0.005$) and cocaine ($F[5,70]=2.8$, $p<0.05$), but not methamphetamine. Post-hoc tests indicated high-responders self-administered more infusions of 0.1-0.32 mg/kg MDPV ($p<0.05$) as well as more infusions of 1.0 mg/kg cocaine than low-responders ($p<0.05$).

DISCUSSION

Synthetic cathinone abuse has increased dramatically over the past decade, and MDPV is one of the most widely abused cathinones in the US. MDPV is readily self-administered by rats (e.g. Aarde et al., 2013; 2015; Watterson et al., 2014; Schindler et al., 2016), and mounting evidence suggests it is more reinforcing than methamphetamine (Aarde et al., 2013; Watterson

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et al., 2014), but direct comparisons of its reinforcing effectiveness to cocaine, the prototypical monoamine transporter inhibitor, have not been made. As such, the present study directly compared the reinforcing effects of MDPV and cocaine using three different endpoints: (1) acquisition of responding for functionally equivalent doses; (2) dose-response curves for MDPV and cocaine under an FR5 schedule of reinforcement with cross-substitution; and (3) a within-subject, quantitative analysis of PR dose-response curves for MDPV, cocaine, and methamphetamine. The results of this study not only provide additional evidence that MDPV is a more potent reinforcer, but also provide strong evidence that the relative reinforcing effectiveness of MDPV is significantly greater than cocaine or methamphetamine. Additionally, a subset of rats was identified that self-administered significantly more MDPV than all other rats, and these individual differences were also apparent when other stimulant reinforcers were available under either FR or PR schedules of reinforcement.

When evaluated under acquisition conditions, MDPV (0.032 mg/kg/inf) and cocaine (0.32 mg/kg/inf) did not differ with regard to the rate (days to acquire), level (number of infusions), or percent of rats that acquired responding. These data contrast those of Schindler et al. (2016) who reported MDPV maintained significantly more infusions than cocaine during acquisition; however, because larger unit doses generally maintain lower levels of FR responding, this discrepancy likely resulted from differences in the unit dose of cocaine evaluated in the previous (0.5 mg/kg/inf) and current study (0.32 mg/kg/inf). Upon transitioning from an FR1 to an FR5, all cocaine-trained rats exhibited proportional increases in their responding such that consumption of cocaine was similar under FR1 (~17 mg/kg/day) and FR5 (~16 mg/kg/day) schedules of reinforcement. Although all MDPV-trained rats (n=32) responded at rates sufficient to obtain ~1 mg/kg day when MDPV was available under an FR1 schedule of reinforcement, this level of intake was only maintained in 17 rats after the response requirement was increased to an FR5. Rather than simply increasing their responding 5-fold to meet the new response requirement, the remaining 15 rats exhibited a disproportionate (~12-fold) increase in

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responding, with daily levels of MDPV intake maintained at ~2.7 mg/kg/day. Such increases in responding are not common when drug reinforcers are evaluated using relatively short sessions and simple FR schedules and suggest there may be something unique about the effects of MDPV in this subset of rats.

Inter-subject variability in daily MDPV intake and time to meet stability criteria among these high-responders was large; however, all rats met stability criteria before transitioning into subsequent experiments. Interestingly, MDPV intake positively correlated with responding during timeouts. Aarde et al. (2015) previously reported that rats who intermittently engaged in ”binge-like” patterns of MDPV self-administration also made more timeout responses during these brief time periods; however, this observation was limited to a single dose (0.05 mg/kg/inf MDPV) available under a single schedule of reinforcement (FR1:TO20-sec). The present study extends this finding by examining patterns of responding maintained by a full range of MDPV doses, under FR5 (using both single and multiple component sessions) and PR schedules of reinforcement in an attempt to more fully characterize factors contributing to individual differences in MDPV intake. Although high levels of timeout responding may suggest a loss of stimulus control in these rats, others have alternatively argued that responding during periods of signaled drug unavailability is indicative of a compulsive pattern of responding that may contribute to addiction in humans (Deroche-Gamonet et al., 2004).

Examining the behavior of MDPV-trained rats under a variety of conditions allowed for several important observations. First, while the minimally effective dose of MDPV (0.0032 mg/kg/inf) was the same for both subsets of MDPV-trained rats under FR5 conditions, high-responders exhibited greater rates of responding when a range of MDPV doses (0.0032-0.1 mg/kg/inf) were available for self-administration, as compared to low-responders. Thus, the observed differences are not the result of differential sensitivities to the reinforcing effects of MDPV, but rather reflect vertical differences in the dose-response curves of MDPV between these subgroups of rats. Since the level of responding maintained by drugs under simple FR

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schedules of reinforcement can be influenced by a number of pharmacokinetic and pharmacodynamic properties, it is difficult to use these schedules to compare drugs with regard to their reinforcing effectiveness.

Full MDPV dose-response curves were therefore generated under a PR schedule of reinforcement which provides a more quantitative approach to assessing reinforcing effectiveness (Richardson and Roberts, 1996). Consistent with data obtained under the FR5 schedule, the potency (ED_{50}) of MDPV to maintain PR responding did not differ between the two subsets of rats; nevertheless, the maximum number of infusions earned (E_{max}) by high-responders was significantly greater than that of low-responders, suggesting MDPV is a more effective reinforcer in high- relative to low-responding rats. Because drug intake during the early portion of PR sessions, when response costs are low, may impact responding later in the session (i.e., when measures of effectiveness are determined), it will be important to demonstrate whether a similar result is obtained using behavioral economic procedures (e.g. demand curve analyses) that have been developed to limit the potential impact of drug intake early in the session (e.g., Hursh and Silberberg, 2008).

To determine whether the differences between high- and low-responders were specific to behavior maintained by MDPV, a series of within-subject substitution tests were performed under both FR5 and PR schedules of reinforcement. When cross-substituted for MDPV under a multiple-component FR5 schedule, cocaine maintained significantly higher rates of responding in MDPV-trained high-responders than in MDPV-trained low-responders or cocaine-trained rats. Similar to what was observed when MDPV was available, when high-responders were allowed to self-administer cocaine they made significantly more responses during post-infusion timeouts and inter-component blackouts, relative to MDPV-trained low-responders and cocaine-trained rats. Together with data obtained under standard FR5 conditions, these findings suggest that once established by MDPV self-administration, the high-responder phenotype (e.g., elevated drug intake, high rates of responding during periods of signaled unavailability [i.e., post-infusion

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timeouts and blackouts]) is persistent across time and transferable to other drug reinforcers. Because the current study was limited to drugs with similar mechanisms of action, whether the high-responder phenotype also transfers when responding is maintained by drugs from different pharmacological classes (e.g., opioids or ethanol) or by non-drug reinforcers will be important to determine.

In summary, acquisition of responding for MDPV (0.032 mg/kg/inf) and cocaine (0.32 mg/kg/inf) was comparable with regard to the number of infusions, days to meet acquisition criteria, and the proportion of rats acquiring. Consistent with potency differences reported for MDPV and cocaine based on elicited (i.e., locomotor activity) and operant behaviors (i.e., drug discrimination) in rats and mice (Marusich et al., 2012; Gatch et al., 2013; Gannon et al., 2016; Collins et al., 2016), as well as in vitro measures of inhibition of dopamine uptake (Baumann et al., 2013), MDPV was 10-fold more potent than cocaine at reinforcing responding under FR5 and PR schedules of reinforcement. In direct comparisons of reinforcing effectiveness, rats made ~3 times as many responses to obtain a single infusion of MDPV (final ratio ~2000) relative to cocaine or methamphetamine (final ratio ~700). These data are consistent with the literature on human patterns of MDPV use and abuse (Johnson and Johnson, 2014) and suggest MDPV is more reinforcing than two drugs widely abused by humans (cocaine and methamphetamine). Although the precise mechanism(s) that underlie the differences in the reinforcing effectiveness of these drugs are unclear, one explanation may be related to their selectivity for DAT over SERT (or DAT/SERT ratios; ~300-800 [MDPV], ~1.5-3 [cocaine], and ~10 [methamphetamine]) (Baumann et al., 2013; Simmler et al., 2013). Evidence showing a negative correlation between extracellular serotonin levels and the reinforcing effectiveness of monoamine transporter ligands as measured by PR self-administration (Wee and Woolverton 2006) appears to support this hypothesis; however, that wild-type and SERT knockout mice self-administer cocaine at comparable levels (Thomsen et al., 2009) argues against such a role for serotonin.

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The current study provides a comprehensive and direct comparison of the reinforcing effects of MDPV and cocaine and suggests MDPV is a significantly more effective reinforcer than cocaine. Additionally, MDPV appears to be unique in its capacity to establish a behavioral phenotype characterized by high levels of drug intake that are stable across time, apparent across a range of doses, and transferable to other drugs with similar mechanisms of action. Although the factors (e.g., behavioral, pharmacological, genetic, etc.) that contribute to the transition from low to high levels of MDPV self-administration are currently unknown, they may be related to individual differences in drug-taking behavior among human drug abusers.

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None

AUTHORSHIP CONTRIBUTIONS

Participated in research design: Collins

Conducted experiments: Gannon, Galindo, Collins

Contributed new reagents or analytic tools: Rice

Performed data analysis: Gannon, Collins

Wrote or contributed to the writing of the manuscript: Gannon, Rice, Collins

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FOOTNOTES

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FIGURE LEGENDS

Figure 1: Spontaneous acquisition of 0.032 mg/kg/inf MDPV (n=32, left) and 0.32 mg/kg/inf cocaine (n=16, middle) over the course of the 10 day acquisition phase in male Sprague-Dawley rats. Gray symbols are active lever responses and "X" are inactive lever responses. *Abscissa:* Numbers refer to consecutive days during the acquisition period. *Ordinate:* Total responses emitted on each lever during the active portion of the 90 minute session. Error bars represent ±S.E.M. *Right panel* – Percent of rats to acquire self-administration of 0.032 mg/kg/inf MDPV (squares) and 0.32 mg/kg/inf cocaine (circles) over the course of 10 days. *Abscissa:* Numbers refer to consecutive days during the acquisition period. *Ordinate:* Cumulative percent of rats within each training group to acquire self-administration of the training drug/dose.

Figure 2: *Upper panel – (left)* Self-administration of 0.032 mg/kg/inf MDPV (n=32, squares) and 0.32 mg/kg/inf cocaine (n=16, circles) during the last 3 days of an FR1 and FR5 schedule of reinforcement. *Abscissa:* Numbers refer to the final three sessions of FR1 and FR5 training. *(right)* Self-administration dose-response curves obtained under an FR5 schedule of reinforcement in MDPV-trained high-responders (n=7) and low-responders (n=9) using dose substitution. *Abscissa:* "SAL" represents infusions of saline, while numbers refer to dose of MDPV available during each session, expressed as mg/kg/inf on a log scale. *Ordinate:* Total infusions obtained during the 90-minute session. Error bars represent ±S.E.M. *Lower panel – (left)* Distribution of MDPV-trained (n=32) and cocaine-trained (n=16) rats based on timeout responding. *Abscissa:* percent of total responses emitted on the active lever occurring during the post-infusion timeout, presented in 10% bins. *Ordinate:* proportion of MDPV- or cocaine-trained rats. *(right)* Correlation between timeout responding and infusions obtained under FR5:TO5-sec. *Abscissa:* Average number of infusions of 0.032 mg/kg/inf MDPV obtained over the last 3 days of FR5 conditions. *Ordinate:* percent responses made on the active lever during

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timeouts versus total active lever responses. Asterisks and pound sign indicate statistical significance: *=p<0.05 vs low-responders during same FR, #=p<0.05 vs same group during FR1.

Figure 3: *Top row* – Self-administration dose-response curves obtained under a multi-component FR5 schedule of reinforcement for MDPV (left), cocaine (center), and saline (right) for high-responders (n=8), low-responders (n=8), and cocaine-trained (n=16) rats. *Ordinate:* Total infusions obtained during each 20-minute component. *Center row* –MDPV-(left), cocaine-(center), and saline- (right) induced timeout responding. *Ordinate:* Percent of active lever responses made during post-infusion timeouts versus the active session during each 20 minute component. *Bottom row* – MDPV-(left), cocaine-(center), and saline- (right) induced blackout responding. *Ordinate:* Active lever responses made during each 5-min inter-component blackout period. *Abscissa (for all panels):* "CS" represents data following presentation of only the injection-paired stimuli, while numbers refer to dose of MDPV (left column), dose of cocaine (center column) expressed as mg/kg/inf on a log scale or saline component number (right column). Error bars represent ±S.E.M. Asterisks and pound sign indicate statistical significance: *=p<0.05 as compared to cocaine-trained, **=p<0.05 as compared to low-responders and cocaine-trained, #=p<0.05 as compared to low-responders, ##=p<0.05 as compared to low-responders and high-responders.

Figure 4: Self-administration dose-response curves for MDPV (squares), cocaine (circles), and methamphetamine (diamonds) obtained under a PR schedule of reinforcement (upper left panel). Self-administration dose-response curves of MDPV (upper right panel), cocaine (lower left panel), and methamphetamine (lower right panel) in high-responders (n=7, black symbols) and low-responders (n=9, white symbols). *Abscissa:* "SAL" represents data obtained when

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saline was available for infusion, whereas doses refer to the unit dose of each drug available for infusion expressed as mg/kg/inf on a log scale. *Ordinate:* Total infusions obtained during the session. Error bars represent ±S.E.M. Pound signs indicate statistical significance as compared to low-responders: #=$p<0.05$.

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TABLES

Table 1: Measures of potency and reinforcing effectiveness of MDPV, cocaine, and methamphetamine

		MDPV	Cocaine	Methamphetamine
ED_{50} (± 95% CI)	High-responders	0.016* (0.01, 0.02)	0.15* (0.08, 0.23)	0.036* (0.03, 0.04)
	Low-responders	0.019* (0.01, 0.02)	0.17* (0.14, 0.20)	0.036* (0.03, 0.04)
	Grouped	0.017* (0.01, 0.02)	0.16* (0.11, 0.21)	0.036* (0.03, 0.04)
E_{max} ±S.E.M.	High-responders	31.2# ± 0.7	25.0# ± 0.9	25.4# ± 0.9
	Low-responders	26.3 ± 1.3	20.7 ± 1.4	21.2 ± 1.2
	Grouped	28.1* ± 1.0	22.3 ± 1.1	22.8 ± 1.0

Asterisk and pound sign indicate statistical significance: *=$p<0.05$ as compared to all other drugs, #=$p<0.05$ as compared to low-responders. Group sizes: n=7, high-responders; n=9, low-responders; n=16, grouped.

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Figure 1:

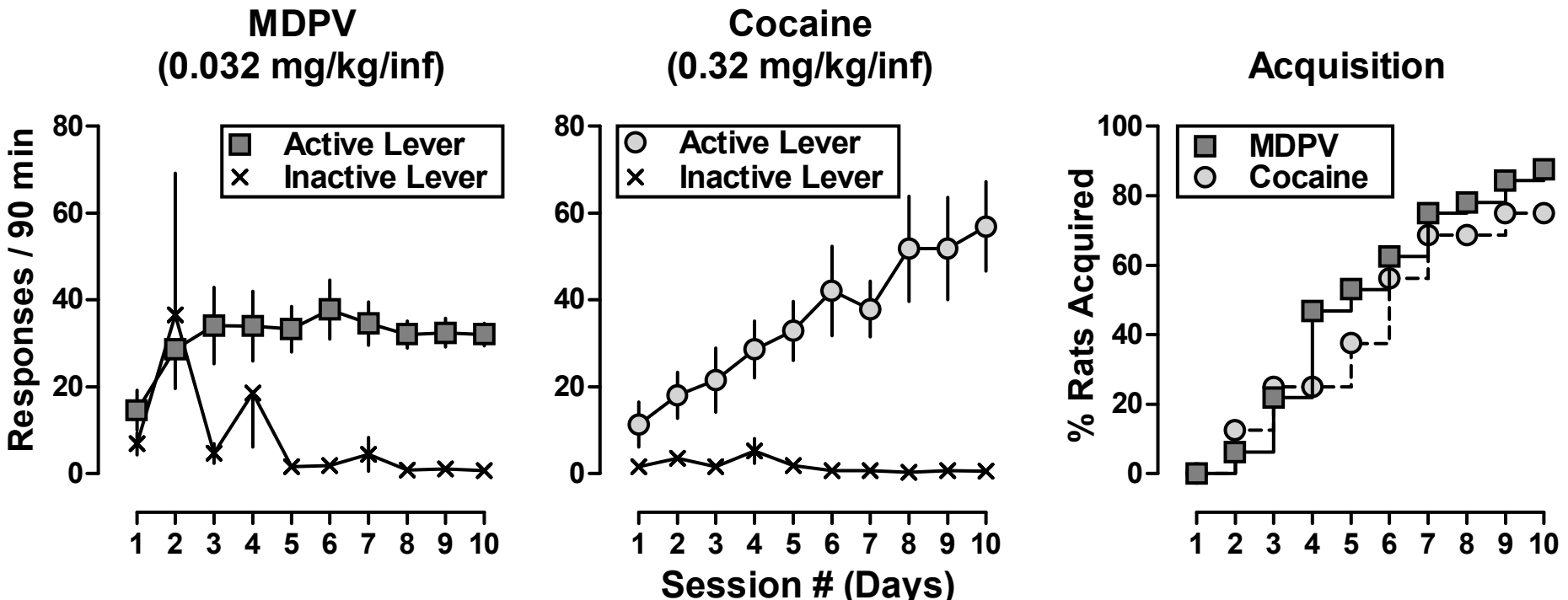

MDPV
(0.032 mg/kg/inf)
Cocaine
(0.32 mg/kg/inf)
Acquisition
Active Lever
Inactive Lever
Active Lever
Inactive Lever
MDPV
Cocaine
Responses / 90 min
% Rats Acquired
Session # (Days)
0
20
40
60
80
100
1
2
3
4
5
6
7
8
9
10

Figure 2:

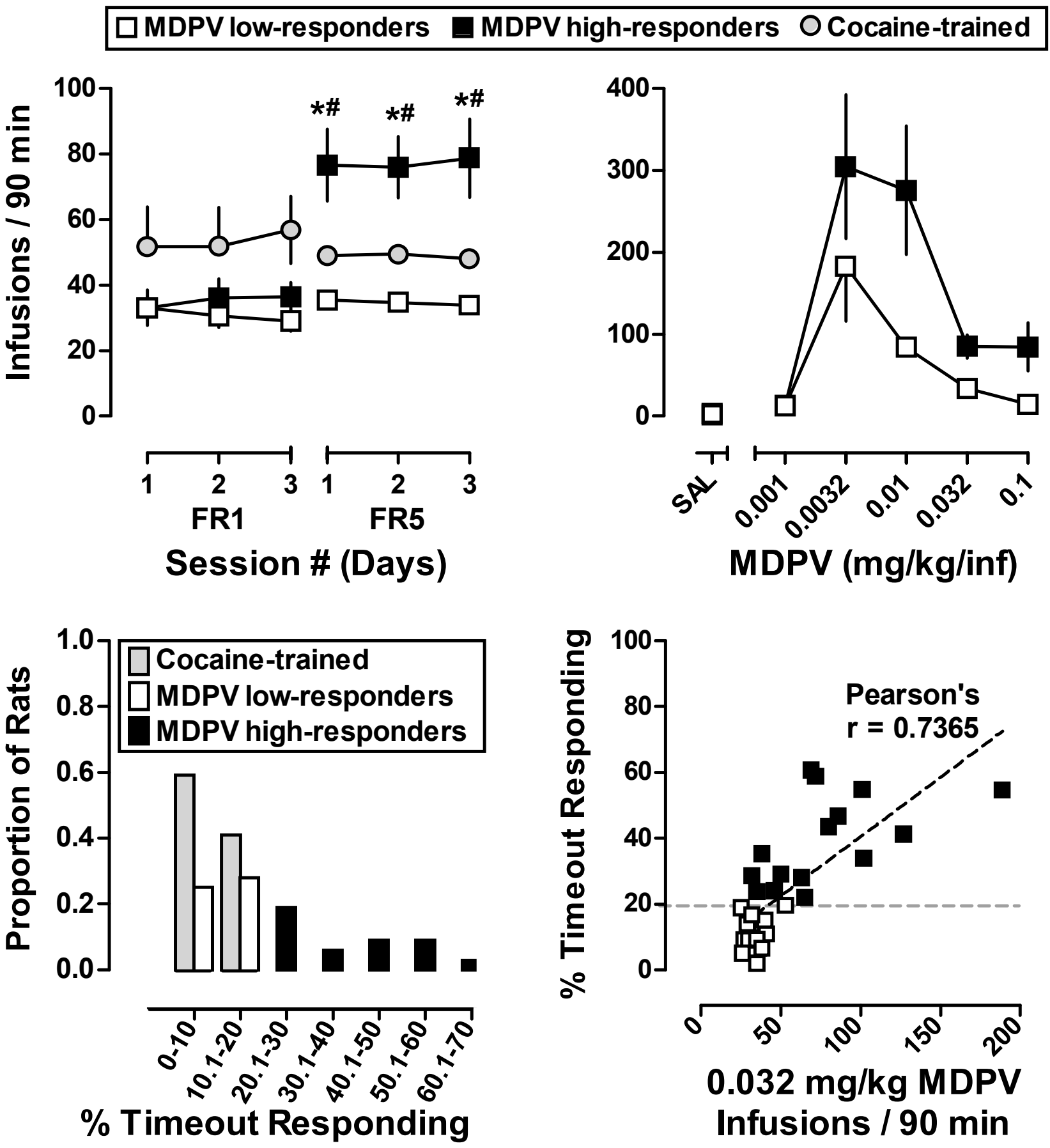

MDPV low-responders
MDPV high-responders
Cocaine-trained
Infusions / 90 min
100
80
60
40
20
0
*#
*#
*#
1
2
3
1
2
3
FR1
FR5
Session # (Days)
400
300
200
100
0
SAL
0.001
0.0032
0.01
0.032
0.1
MDPV (mg/kg/inf)
Proportion of Rats
1.0
0.8
0.6
0.4
0.2
0.0
Cocaine-trained
MDPV low-responders
MDPV high-responders
0-10
10.1-20
20.1-30
30.1-40
40.1-50
50.1-60
60.1-70
% Timeout Responding
% Timeout Responding
100
80
60
40
20
0
Pearson's
r = 0.7365
0
50
100
150
200
0.032 mg/kg MDPV
Infusions / 90 min

Figure 3:

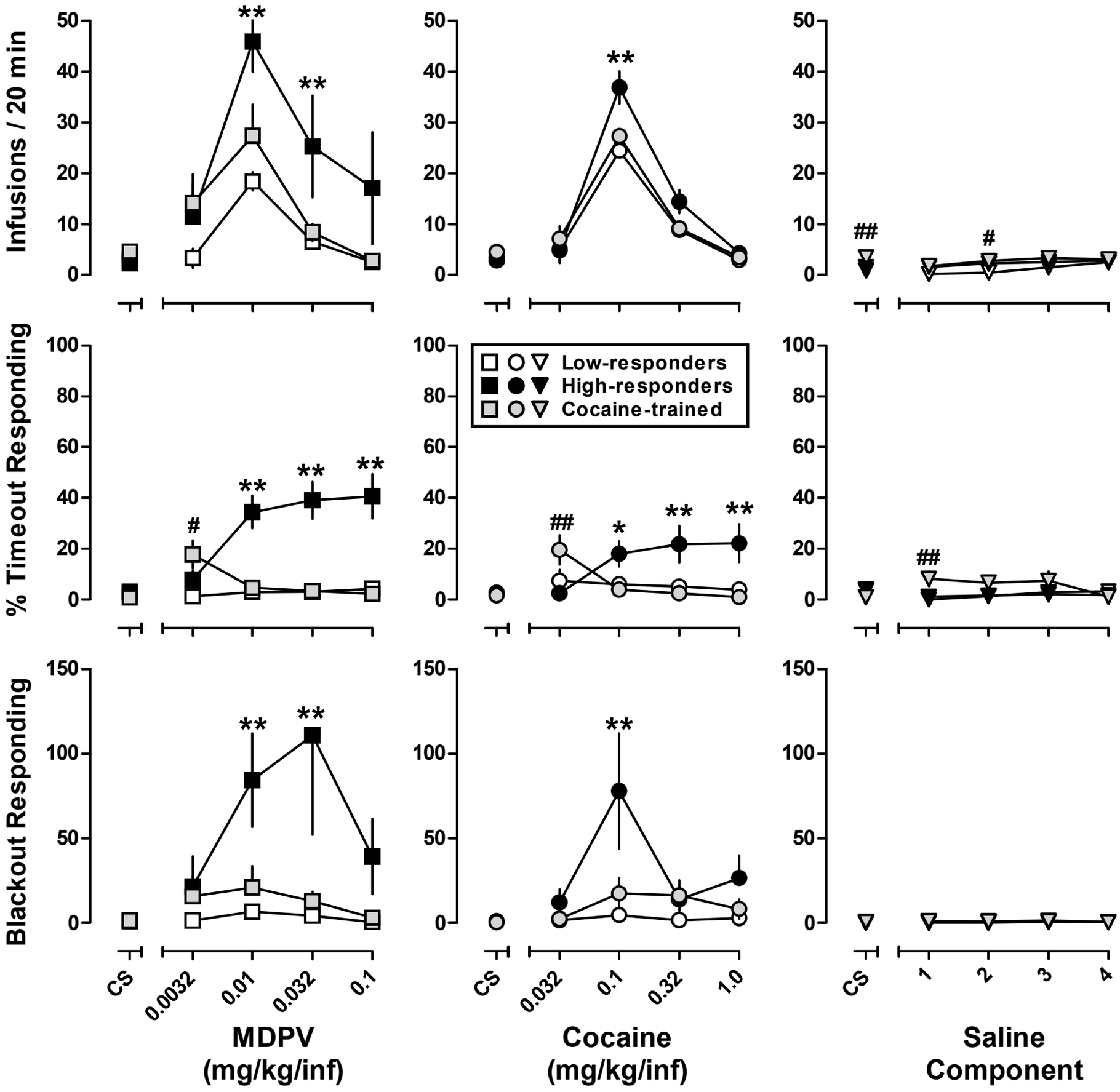
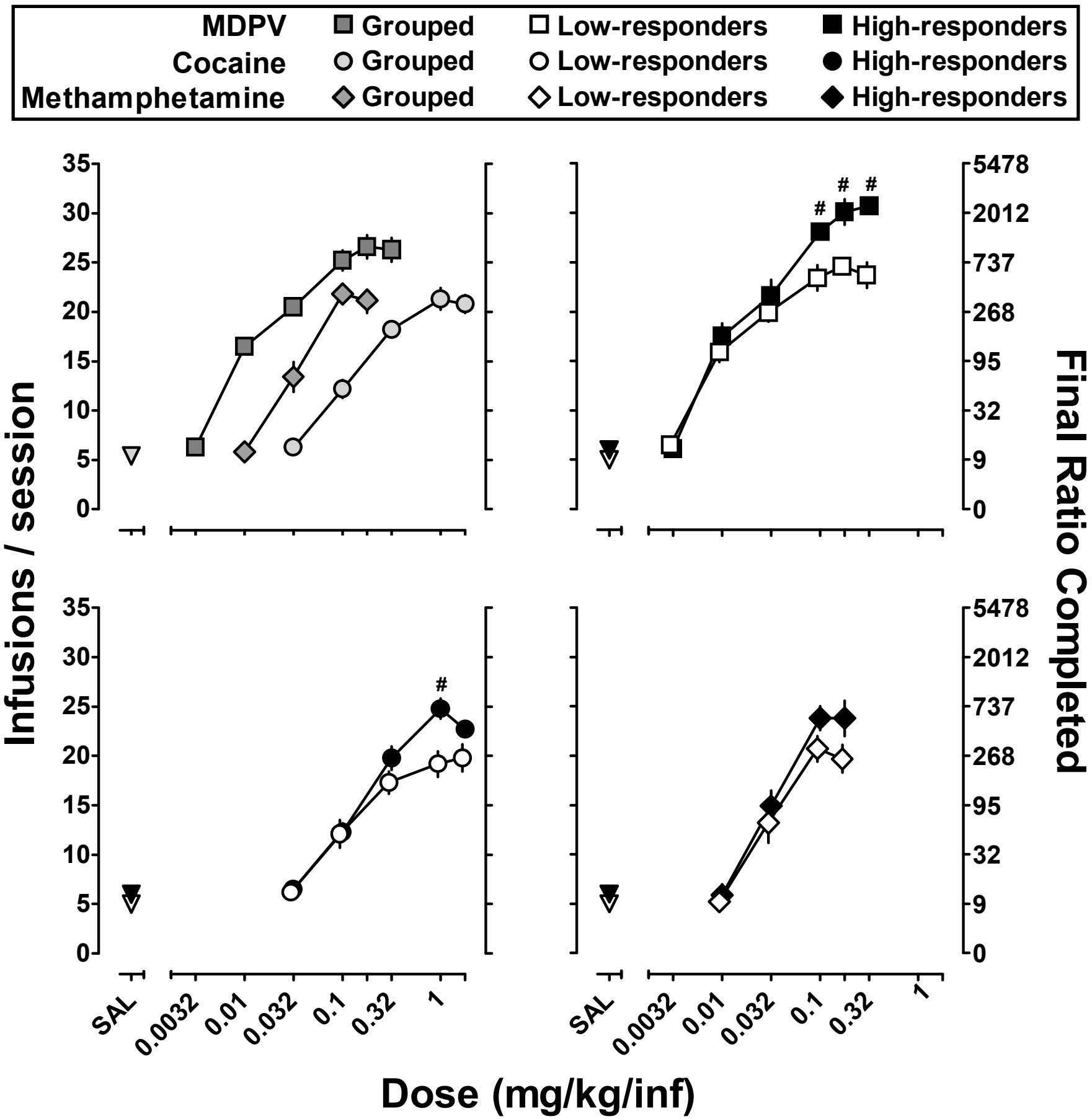

Infusions / 20 min
% Timeout Responding
Blackout Responding
Low-responders
High-responders
Cocaine-trained
CS
0.0032
0.01
0.032
0.1
0.032
0.1
0.32
1.0
1
2
3
4
MDPV (mg/kg/inf)
Cocaine (mg/kg/inf)
Saline Component

Figure 4:


MDPV
Grouped
Low-responders
High-responders
Cocaine
Grouped
Low-responders
High-responders
Methamphetamine
Grouped
Low-responders
High-responders
Infusions / session
Final Ratio Completed
35
30
25
20
15
10
5
0
5478
2012
737
268
95
32
9
0
#
SAL
0.0032
0.01
0.032
0.1
0.32
1
Dose (mg/kg/inf)