Published in final edited form as:
Exp Clin Psychopharmacol. 2011 August ; 19(4): 275–284. doi:10.1037/a0023897.

A Multivariate Assessment of Individual Differences in Sensation Seeking and Impulsivity as Predictors of Amphetamine Self-Administration and Prefrontal Dopamine Function in Rats

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Abstract

Drug abuse vulnerability has been linked to sensation seeking (behaviors likely to produce rewards) and impulsivity (behaviors occurring without foresight). Since previous preclinical work has been limited primarily to using single tasks as predictor variables, the present study determined if measuring multiple tasks of sensation seeking and impulsivity would be useful in predicting amphetamine self-administration in rats. Multiple tasks were also used as predictor variables of dopamine transporter function in medial prefrontal and orbitofrontal cortex, as these neural systems have been implicated in sensation seeking and impulsivity. Rats were tested on 6 behavioral tasks as predictor variables to evaluate sensation seeking (locomotor activity, novelty place preference, and sucrose reinforcement on a progressive ratio schedule) and impulsivity (delay discounting, cued go/no-go, and passive avoidance), followed by d-amphetamine self-administration (0.0056–0.1 mg/kg/infusion) and kinetic analysis of dopamine transporter function as outcome variables. The combination of these predictor variables into a multivariate approach failed to yield any clear relationship among predictor and outcome measures. Using multivariate approaches to understand the relation between individual predictor and outcome variables in preclinical models may be hindered by alterations in behavior due to training and thus, the relation between various individual differences in behavior and drug self-administration may be better assessed using a univariate approach in which a only a single task is used as the predictor variable.

Keywords

individual differences; impulsivity; sensation seeking; amphetamine; rats

Introduction

Drug abuse vulnerability has been linked to personality measured by various multi-trait inventories, such as the Five Factor Model (Costa and McCrae, 1992), the Eysenck Personality Questionnaire (Eysenck and Eysenck, 1985), the Tridimensional Personality

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The authors have no conflicts of interest.

Questionnaire (Cloninger et al., 1991) and the Zuckerman-Kuhlman Personality Questionnaire (Joireman and Kuhlman, 2004). Among the multiple traits identified in these comprehensive inventories, drug abuse vulnerability has been linked most closely to sensation seeking, a trait characterized by a general need for new and complex experiences, and the propensity to take risks in order to achieve these experiences (Zuckerman, 1979). Individuals who score high on sensation seeking or novelty seeking scales use and abuse drugs more often than low sensation seekers (Andrucci et al., 1989; Crawford et al., 2003; Kosten et al., 1994). High sensation seekers also are more sensitive to the reinforcing effects of drugs tested in a controlled laboratory setting (Kelly et al., 2006; Stoops et al., 2007).

Recent research suggests that sensation seeking and impulsivity are both correlated with drug abuse vulnerability (Dawe and Loxton, 2004; de Wit and Richards, 2004; Fillmore and Rush, 2002). Sensation seeking is generally defined as the motivation to engage in behaviors likely to produce rewarding outcomes, while impulsivity is generally defined as behavior occurring without foresight, or rash action (Dawe and Loxton, 2004). With respect to drug use, sensation seeking can be defined by greater sensitivity to the positive hedonic effect of the drug and greater attention toward drug-related cues. Impulsivity can be defined as a loss of control over drug-related behaviors, the inability to resist drug cravings, or lack of forethought about negative consequences (Dawe and Loxton, 2004). Although both sensation seeking and impulsivity are associated with drug abuse vulnerability, it is unclear to what extent these constructs precede or result from drug use, and the precise neural mechanisms underlying these associations remain to be elucidated. In this regard, animal models may allow for a rigorous examination of how these biologically-based constructs contribute to drug abuse vulnerability (Brady, 1991; Lynch et al., 2010; Olmstead, 2006).

Various experimental methods have been developed to examine sensation seeking using animal models. The most commonly used method is the locomotor activity test in which activity is measured in a novel, inescapable environment (Piazza et al., 1989). Animals with higher activity levels are considered high responders, and those with lower activity levels are considered low responders. High responder rats are more sensitive than low responder rats to the reinforcing effects of amphetamine (AMP) and cocaine, especially when administered at low unit doses (Cain et al., 2008; Klebaur et al., 2001; Mantsch et al., 2001; Piazza et al., 1989; Pierre and Vezina, 1997). An alternative method is the novelty place preference task, in which rats are given simultaneous access to a familiar and a novel environment. Animals tend to prefer the novel environment (Bardo et al., 1993; Hughes, 1968) and those highest in novelty preference are more likely to self-administer AMP, at least when a large sample size is used (Cain et al., 2005). Another method used to examine sensation seeking is the sucrose preference task. When given access to sucrose for a fixed period of time, high sucrose preferring rats self-administer more cocaine and AMP than low sucrose preferring rats (DeSousa et al., 2000; Gosnell, 2000). Progressive ratio schedules can also be used to evaluate the reinforcing effect of sucrose (Arnold and Roberts, 1997), and previous research has used responding on a progressive ratio schedule for sucrose as a screen for nicotine withdrawal effects (LeSage et al., 2006).

Various experimental methods also have been used to examine impulsivity using animal models. One of the most common methods of measuring impulsivity is the delay discounting task, in which animals choose between a small, immediate reward and a larger, delayed reward (Ainslie, 1975). High impulsive rats show a preference for the smaller, immediate option and are more likely to self-administer cocaine, AMP and methylphenidate compared to low impulsive rats, and are also more likely to acquire self-administration more quickly (Anker et al., 2009; Gipson and Bardo, 2009; Marusich and Bardo, 2009; Perry et al., 2005; 2008). An alternative method to measure impulsivity is the cued go/no-go task. In this task, animals are reinforced for responding during a go cue, and are not reinforced in the

presence of a no-go cue (Hellemans et al., 2005). This task differs from the 5-choice serial reaction time task in that the cued go/no-go task does not provide programmed negative consequences for responses during the no-go cue (Hellemans et al., 2005). In contrast, responses during the no-go cue in the 5-choice serial reaction time task produce a time out (Belin et al., 2008; Diergaarde et al., 2008). Another, albeit conceptually different, method of measuring impulsivity is passive avoidance. This task measures the latency to step down off a platform onto a surface previously paired with brief foot shock (Camacho et al., 1996). Animals low in impulsivity step off the platform more quickly than those high in impulsivity. While this method has not been used previously as a predictor of stimulant self-administration, it has been used in alcohol research (Santucci et al., 2004; 2008).

Even though a number of tasks measuring sensation seeking and impulsivity have been used as individual difference predictors of drug self-administration in laboratory animals, virtually all of this extensive work has been conducted using only a single task as the predictor variable (Cain et al., 2008; DeSousa et al., 2000; Gipson and Bardo, 2009; Gosnell, 2000; Marusich and Bardo, 2009; Piazza et al., 1989; Perry et al., 2008; Pierre and Vezina, 1997). This contrasts with experimental work in humans that often incorporates multiple traits as predictors of drug use (de Win et al., 2006; Ersche, et al., 2010; Jones and Lejuez, 2005; Magid et al., 2007; Perkins et al., 2008). In one exception, Belin et al. (2008) evaluated rats for individual differences in both locomotor activity and impulsive action in a 5-choice serial reaction time task. The locomotor activity predicted acquisition of cocaine self-administration, whereas impulsive action predicted the transition to compulsivity, i.e., persistence of cocaine self-administration in the presence of aversive outcomes. A factor analysis revealed that these two predictor variables were orthogonal to each other, suggesting a dissociation of the neural mechanisms involved; however, this study did not examine any specific neural mechanisms associated with each individual difference variable (Belin et al., 2008).

The purpose of the present study was to determine if measuring multiple behavioral tasks concomitantly would be useful for characterizing the constructs of sensation seeking and impulsivity as predictor variables for AMP self-administration in rats. Dopamine transporter (DAT) function was also analyzed for each rat in order to determine the role of the dopamine system in individual differences in sensation seeking and impulsivity. Since AMP is known to reverse the dopamine transporter (DAT; Goodwin et al., 2009; Kahlig et al., 2005; Sulzer et al., 2005), rats were examined for [^{3}H]DA uptake in medial prefrontal cortex (mPFC) and orbitofrontal cortex (OFC) using *in vitro* procedures. mPFC was examined due to its role in drug reward and reinstatement (Koya et al., 2006; Pentkowski et al., 2010), whereas OFC was examined due to its role in inhibitory control (Winstanley et al., 2004).

Method

Subjects

Subjects were 48 40-day-old male Sprague-Dawley rats (Harlan Industries, Indianapolis, IN) that were experimentally and drug naïve. During the six predictor tests, subjects were restricted to 15 g of food/day, delivered immediately after their daily session to provide motivation to lever press during food-maintained tasks. During the remainder of the experiment, subjects had free access to food in the home cage. Water was continuously available in the home cage. Subjects were housed individually in plastic, hanging home cages and were maintained on a 16/8 hr light/dark cycle (lights on at 6:00 am). Experimental protocols were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Kentucky, and followed the principles of laboratory animal care outlined in the *NIH Guide*.

Apparatus

Standard operant conditioning chambers for rats were used (28 cm × 24 cm × 25 cm; ENV-001; MED Associates, St. Albans, VT) and were housed inside sound-attenuating chambers (ENV-018M; MED Associates, St. Albans, VT). Chambers were equipped with a 28-V house light, two retractable levers (4.5 cm), and two white stimulus lights (28-V; 3 cm in diameter). Sugar-based 45 mg pellets (F0021 dustless precision pellet, Bio-Serve, Frenchtown, NJ) were dispensed individually from a pellet dispenser (ENV-203M-45; MED Associates, St. Albans, VT) into a recessed food receptacle (5 cm x 5 cm x 3 cm).

Locomotor chambers were equipped with photobeams and Versamax System software (AccuScan Instruments Inc., Columbus, OH). Inside each chamber was a horizontal 16 × 16 grid of photo beam sensors spaced 2.5 cm apart and 7.0 cm above the chamber floor. Locomotor activity was measured by photo beam breaks and was calculated as total distance traveled (cm).

A 3-compartment conditioned place preference (CPP) apparatus was used (ENV-256C, ENV-013, Med Associates, Inc., St. Alban, VT, USA). One side chamber was black with a metal bar floor, the other side was white with a wire mesh floor, and the center was grey with a solid Plexiglas floor. The two side chambers contained six photo beams, and the center chamber contained three photo beams.

A step-down foot shock chamber with a raised platform situated above a grid floor was used (Med Associates, Inc., St. Alban, VT, USA). The step measured 8 × 16 × 2.5 cm and was made of Plexiglas. The grid floor measured 20 × 16 cm and contained 4 photo beams. Photo beams were spaced in parallel, 4 cm apart. Rats had to break 3 beams in order to qualify as having stepped completely off the platform.

For all apparatus, experimental events were arranged and recorded by MED-PC software (Med-Associates, St. Albans, VT) on a computer located in the experimental room.

Procedures

Rats proceeded through the study in 4 cohorts of 12 rats per cohort, and rats within each cohort were exposed to all six predictor tests in a counterbalanced order. Rats were only exposed to one test per day, and they completed each test before being evaluated on the next test. The six predictor tests were as follows: (1) locomotor activity, which took 1 day; (2) novelty place preference, which took 3 days; (3) progressive ratio responding for sucrose, which took 21 days; (4) passive avoidance, which took 3 days; (5) delay discounting, which took 10 days; and (6) cued go/no-go, which took 21 days. Following completion of all six tests, the following outcome variables were determined: (1) acquisition of AMP self-administration; (2) AMP self-administration at varying unit doses (0.0056, 0.01, 0.03, 0.056, 0.1 mg/kg/infusion) or saline; and (3) DAT function in mPFC and OFC.

Locomotor activity (LA)—Rats were exposed to the novel locomotor chamber for one 60 min session. Total distance traveled was measured (Piazza et al., 1989).

Novelty Place Preference (NPP)—Rats were exposed to the place preference apparatus for assessment of novelty preference using previously published methods (Cain et al. 2005; 2006; 2008). Rats were confined to either the black or white compartment (counterbalanced within cohorts) of the conditioned place preference apparatus for 30 min on two consecutive days. On the third day, rats were placed in the grey center compartment, and given access to all compartments for 15 min. A preference ratio was calculated as the amount of time spent

in the novel compartment divided by the amount of time spent in the novel plus familiar compartment.

Progressive Ratio (PR)—Rats were exposed to the operant conditioning chambers for assessment of a lever pressing task for palatable food pellets available on a PR schedule of reinforcement using previously published methods (Marusich et al., 2010). Training began with three days of autoshaping which paired lever extension with pellet delivery (Brown and Jenkins, 1968), followed by fixed ratio (FR) training with pellets available on an FR 2, FR 4, FR 7, and FR 10 on four respective days. The stimulus light above the active lever was illuminated during FR and PR sessions except during brief timeouts in which pellets were dispensed. The side of the chamber containing the active lever was counterbalanced across subjects. Pellets were then available on a PR schedule which increased the response requirement for a pellet following each pellet delivery according to an exponential scale (1, 2, 4, 6, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178, 219, 268, etc; Richardson and Roberts, 1991). PR sessions were conducted for 14 consecutive days with 2-hr daily sessions; breakpoints did not necessarily represent the true breakpoints at which rats stopped responding completely, but instead simply referred to the final ratio value completed. The primary dependent measure was the average breakpoint across the last five days of training.

Passive Avoidance (PA)—On the first day, rats were placed on the raised platform in the chamber and the latency to step down from the platform onto the grid floor and break 3 photo beams (no foot shock) was recorded. On the second day, rats were placed on the platform, but stepping down onto the grid floor and breaking 3 photo beams resulted in presentation of a 2-s 0.4 mA foot shock. Animals could escape this shock by stepping back up onto the platform. On the third day, rats were placed on the platform and response latency to step down and break 3 photo beams was recorded. No shock was used during the third session (Camacho et al., 1996). The dependent measure was the difference in latency to step down on the third day compared to the first day.

Delay Discounting (DD)—Rats were exposed to the operant conditioning chambers for assessment of DD with an adjusting delay procedure for 10 consecutive daily sessions using previously published methods (Marusich and Bardo, 2009; Perry et al., 2005, Perry et al., 2008). A response on the immediate option produced one pellet immediately, and a response on the delayed option produced three pellets after an adjusting delay. Responses on the immediate option produced a 1-s decrease in the delay, and responses on the delayed option produced a 1-s increase in the delay. Stimulus lights were illuminated above the levers that were active, and therefore signaled food availability. Mean adjusted delays (MADs) were calculated at the end of each session by calculating the average adjusting delay. MADs were used as a measure of impulsive choice, with lower MADs indicating higher levels of impulsivity. The primary dependent measure was the average MAD across the last five sessions.

Cued go/no-go (CGNG)—Rats were exposed to the operant conditioning chambers for assessment of CGNG in a lever pressing task for food pellets using previously published methods (Hellemans et al., 2005). Training began with three days of autoshaping (Brown and Jenkins, 1968), followed by variable interval (VI) training with pellets available on a VI 4 s, VI 8 s, VI 14 s, and VI 20 s, on four consecutive days. Rats were then exposed to the CGNG procedure for 14 consecutive days in which 2-min periods of VI 20-s reinforcement alternated with 2-min periods of extinction during a 40-min session. Reinforcement (go trial) was signaled by illumination of the cue light above the lever, and extinction (no-go trial) was signaled by termination of the cue light. The side of the chamber containing the active lever was counterbalanced across subjects. The primary dependent measure was the ratio of

responses during go trials compared to no-go trials (go/no-go), averaged across the last five sessions.

Self-administration surgical procedure—Following completion of all 6 predictor tests, rats were given two to five days of free feeding, and then were surgically implanted with a chronic indwelling jugular catheter while under anesthesia using previously published methods (Marusich and Bardo, 2009; Marusich et al., 2010). One end of the catheter was inserted into the jugular vein, and the other end was attached to a metal cannula that exited the skin and was secured in a dental acrylic head mount adhered to the skull with metal jeweler screws. Catheter patency was maintained by daily 0.2 ml infusions of a mixture containing 20 ml saline, 0.6 ml heparin, and 0.2 ml gentamicin. Rats were given five to seven days of recovery, and were given free access to food in the home cage for the remainder of the experiment.

Acquisition of AMP self-administration—Following recovery from surgery, rats were exposed to the operant conditioning chambers for assessment of self-administration of AMP (0.03 mg/kg/infusion, 0.1 ml per infusion), available on an FR 1 schedule of reinforcement for 60-min daily sessions, as described previously (Marusich and Bardo, 2009). The side of the chamber containing the active lever was counterbalanced across subjects. AMP was infused over 5.9 s, followed by a 20-s time out signaled by the illumination of both stimulus lights during which lever pressing had no consequence. Rats were exposed to seven consecutive FR 1 sessions, followed by three sessions of FR 2, three sessions of FR 3, three sessions of FR 4, and seven sessions of FR 5.

AMP dose-effect determination—During the next phase of the experiment, rats were given access to different unit doses of AMP for self-administration on an FR 5 schedule of reinforcement, with each dose available for three consecutive sessions. Rats were tested with AMP doses in the following order: 0.01, 0.056, 0.1, and 0.0056 mg/kg/infusion, followed by seven sessions of saline substitution. The 0.03 mg/kg/infusion AMP dose was not reassessed, and data from the last three days of acquisition were used for this dose in the dose-effect curve.

Synaptosomal [^{3}H]dopamine (DA) uptake—From 1–3 days after the final operant conditioning session, rats were killed by rapid decapitation and DAT function in mPFC and OFC, which were isolated from an individual rat brain, were assessed by determining the kinetic parameters (V_{max} and K_m) of [^{3}H]DA uptake using a previously published method (Zhu et al., 2004). Synaptosomes of mPFC and OFC were resuspended in 2.2 ml of ice-cold Krebs-Ringer-HEPES buffer, pH 7.4, containing 0.1 mM pargyline, and 0.1 mM L-ascorbic acid. For saturation analysis, mPFC and OFC synaptosomes containing approximately 40 μg protein/100 μl and 50 μg protein/100 μl respectively, were incubated in a metabolic shaker for 5 min at 34°C and then incubated for 5 min at 34°C after adding one of 7 [^{3}H]DA concentrations (0.01–1 μM) in a 250 μl total volume. Incubation was terminated by the addition of 3 ml of ice-cold assay buffer, followed by immediate filtration through Whatman GF/B glass fiber filters (presoaked with 1 mM pyrocatechol for 3 h) on a cell harvester (Brandel Inc., Gaithersburg, MD). Filters were washed rapidly with 3 ml of ice-cold buffer and radioactivity bound to filter was determined by liquid scintillation spectrometry (PerkinElmer Life and Analytical Sciences). Nonspecific [^{3}H]DA uptake was determined in the presence of 10 μM nomifensine. Since DA is transported not only by DAT, but also by norepinephrine and serotonin transporters in PFC (Moron et al., 2002; Williams and Steketee, 2004), kinetic analysis of [^{3}H]DA uptake by DAT in mPFC and OFC were assessed in the presence of desipramine (5 nM) and paroxetine (5 nM) to prevent [^{3}H]DA

uptake into norepinephrine- and serotonin-containing nerve terminals, respectively, thereby isolating uptake of DA into DAT (Zhu et al., 2004).

Data analysis—During AMP self-administration, rats were removed from all subsequent phases of the study if their catheters malfunctioned, and all data from those rats were excluded from that phase of the experiment, and subsequent analyses. Additionally, some rats were excluded from neurochemical analyses due to experimental error because the neurochemical analyses failed to provide useable data. The different phases of the overall experiment were defined as: (1) predictor variables (all six tests); (2) self-administration acquisition (FR 1-FR 5); (3) dose-effect determination (including saline); and (4) neurochemical analyses. Data from 48 rats were included for predictor variables, 37 rats for acquisition, 33 rats for dose-effect determination, and 23 rats for neurochemical analyses.

Repeated measures analyses of variance (ANOVAs) were used to compare the numbers of active and inactive lever presses during exposure to different FR values. Only the last three days of exposure to each FR requirement were used for statistical analyses pertaining to acquisition. An additional repeated measures ANOVA was used to compare the number of infusions for each dose of AMP (including saline) from the dose-effect determination phase, with only the final two sessions of exposure to each dose used in the statistical analyses. Bonferroni adjusted pairwise comparisons were used as post-hoc tests. Statistical significance was defined by a p-value being less than 0.05 (after Bonferroni adjustment, if necessary). All ANOVAs were calculated with SPSS version 15.0.

Because some of the six predictor tests produced data that were highly positively skewed, all predictor test data were transformed by taking the hyperbolic tangent of the z score. This transformation can reduce skewness, and unlike a logarithmic or square root transformation, allowed negative values to be transformed, as well as positive values. Transformed data were used for all subsequent analyses. Pearson correlations were calculated for the six predictor variables with each other, as well as for the six predictor variables with the average number of infusions earned across the last three days of exposure to each FR value (acquisition), the average number of infusions earned across the last two days of exposure to each dose (dose-effect determination), and the outcomes of [^{3}H]DA uptake (neurochemical analyses). Moreover, Pearson correlations were calculated for the outcomes of the [^{3}H]DA uptake assays with the average numbers of infusions at different FR values (acquisition phase) and doses (dose-effect determination phase). Bonferroni adjustments were used to maintain a Type I error probability of 0.05 across each set of correlations pertaining to a specific predictor test variable and, for correlations involving neurochemical data, across each set of correlations pertaining to a specific FR value or dose. All correlations were calculated using GraphPad Prism version 4.0.

Since Pearson correlations describe bivariate relationships and an assessment of multivariate relationships was also desired, we fit four linear mixed models. Model I expressed the expected average number of infusions during acquisition as a linear function of the six predictor variables at each of the five FR values, with a random effect for each rat to account for correlations among that rat's average numbers of infusions at different FR values. Model II was similar but related the expected average number of infusions during dose-effect determination to the predictor variables at each of the six dose values. Model III expressed the expected V_{max} score as a linear function of the six predictor variables at each of the two brain regions, with a random effect for each rat. Model IV was similar, but related the expected K_m score to the predictor variables at each of the two brain regions. SAS version 9.2 was used to analyze the linear mixed models.

Results

Table 1 shows the correlations among all six of the predictor variables. LA was significantly correlated with NPP, indicating that rats showing more locomotor activity in a novel environment also showed a stronger preference for a novel environment. None of the other correlations among the predictor variables were significant. Table 2 shows the correlation of each predictor variable with acquisition of AMP self-administration at each FR value. No predictor variables correlated significantly with AMP self-administration on any FR schedule.

Figure 1a shows mean numbers of active and inactive lever presses across the incremental FR values. A repeated measures ANOVA showed a significant effect of FR schedule on the number of active lever presses [$F(4, 88) = 16.78$; $p < 0.001$], but not inactive lever presses, indicating that AMP served as a reinforcer. Additionally, there was a significant effect of dose on the number of infusions earned [$F(5, 140) = 41.46$; $p < 0.001$], and subsequent post hoc analyses showed that the number of infusions earned at each AMP dose was significantly different from the number earned at saline, except at the 0.01 mg/kg/infusion unit dose (Figure 1b); however, there were no significant differences across unit doses of AMP (0.0056–0.1 mg/kg/infusion). Note that the decrease in the number of subjects from the acquisition phase to dose-effect determination phase, and the exclusion of these subjects' data caused a difference in the mean number active lever presses for the 0.03 mg/kg/infusion unit dose (compare Figures 1a and 1b).

Table 3 shows the correlation of each predictor variable with AMP self-administration at each unit dose. No predictor variables correlated significantly with AMP self-administration at any unit dose. Table 4 shows the six predictor variables, numbers of AMP infusions during acquisition and numbers of AMP infusions during dose-effect determination correlated with the neurochemical measures obtained from mPFC and OFC. NPP was positively correlated with mPFC K_m, and number of infusions earned on the 0.03 mg/kg/infusion dose during dose-effect determination was positively correlated with OFC K_m.

For the linear mixed model analyses, Model I did not identify any one of the behavioral measures as significantly associated with number of infusions during the acquisition phase, controlling for all of the other behavioral measures. In contrast, Model II revealed that NPP [$F(6, 109) = 2.38$, $p < .05$] and DD [$F(6, 109) = 2.27$, $p < .05$] were significant predictors of number of infusions during the dose-effect determination phase. For NPP, the associations were positive at all doses except the highest, but only at the lowest AMP dose was the association significant [$t(109) = 3.02$, $p < .05$ after Bonferroni adjustment]. For DD, the associations were positive at all doses except saline, but at no dose was the association significant, suggesting that DD is modestly associated with the number of infusions generally instead of strongly associated with the number of infusions at any specific dose.

Model III did not identify any one of the behavioral measures as significantly associated with V_{max}, controlling for all of the other behavioral measures. In contrast, Model IV revealed that both NPP [$F(2, 12) = 8.02$, $p < .01$] and DD [$F(2, 12) = 6.68$, $p < .05$] were significant predictors of K_m. NPP was significantly positively associated with K_m in the mPFC region [$t(12) = 3.41$, $p < .05$ after Bonferroni adjustment] and non-significantly positively associated with K_m in the OFC region. DD was non-significantly negatively associated with K_m in the mPFC region but non-significantly positively associated with K_m in the OFC region.

Discussion

Based on the multivariate approach used often to assess personality traits associated with drug abuse vulnerability in humans, the current preclinical study was designed to implement a similar strategy to assess individual difference variables in rats. While a few preclinical studies have examined two different predictors of drug self-administration (Belin et al., 2008; Cain et al., 2005; 2006; 2008; Diergaarde et al., 2008; Hellemans et al., 2005), we are unaware of any previous preclinical research that has examined as many as six behavioral predictors using a within-subject design as in the current report. When assessed individually, several tests related to sensation seeking and impulsivity predict acquisition, maintenance and dose-effect measurements of drug self-administration, including the ones used in the present report, i.e., LA (Cain et al., 2008; Klebaur et al., 2001; Mantsch et al., 2001; Piazza et al., 1989; Pierre and Vezina, 1997), NPP (Cain et al., 2005), sucrose preference (DeSousa et al., 2000; Gosnell, 2000), and DD (Anker et al., 2009; Gipson and Bardo, 2009; Marusich and Bardo, 2009; Perry et al., 2005; 2008); however, the most important finding reported here is that combining these predictor variables into a multivariate approach failed to yield any significant correlations among predictor and outcome measures.

When the dose-effect data were analyzed using a linear mixed model (Model II), there was a significant relation between DD and AMP self-administration; unexpectedly however, high impulsive rats self-administered *less* AMP than low impulsive rats, a finding that contrasts with previous univariate studies showing that high impulsive rats based on DD show increased stimulant self-administration (Anker et al., 2009; Perry et al., 2005; 2008), including two reports from our own laboratory (Gipson and Bardo, 2009; Marusich and Bardo, 2009). The same linear mixed model (Model II) also showed that NPP was significantly correlated with AMP self-administration, a finding consistent with other work from our laboratory (Cain et al., 2005). Overall, these results suggest that multivariate tests may be relatively insensitive as reliable predictors of drug self-administration, which may represent a limitation of animal models for assessment of drug abuse vulnerability, at least under the conditions used in the current study.

The current multivariate approach also failed to yield any significant correlations among predictor variables and maximal velocity of dopamine uptake (V_{max}) in mPFC or OFC, prefrontal regions associated with drug reward and impulsivity (Koya et al., 2006; Pentkowski et al., 2010; Winstanley et al., 2004). There was a significant correlation between NPP and DAT affinity (K_m) in mPFC, suggesting that high novelty preferring rats had reduced DAT affinity (higher K_m). Nonetheless, the failure to observe significant relations among predictor variables and V_{max} contrast with a previous report showing that IN and NPP predict maximal dopamine uptake in prefrontal cortex (Zhu et al., 2004). The lack of association between multivariate predictor variables and DAT function parallels the findings obtained with AMP self-administration.

The LA test, when used as a univariate measure, is among one of the most reliable predictors of stimulant self-administration in rats (Cain et al., 2008; Klebaur et al., 2001; Mantsch et al., 2001; Piazza et al., 1989; 1991; Pierre and Vezina, 1997) and this relationship is thought to be mediated by individual differences in the stress axis (Piazza et al., 1991). Perhaps the differential handling and training histories in the current study may have altered habituation of the stress response across rats, thus negating the predictive validity of this test. As a further indication that these multivariate predictor tests interfered with subsequent performance on the outcome measures, there was little alteration in responding across the wide range of AMP unit doses tested (0.0056–0.1 mg/kg/infusion). Previous work has shown that rats typically adjust their responding within this AMP dose range (Cain et al., 2008; DeSousa et al., 2000; Klebaur et al., 2001), indicating that the

variable history across multiple operant and non-operant tasks also interfered with typical dose-dependent performance in AMP self-administration.

While there may be several potential reasons why the multivariate approach used here failed to provide predictive information about drug self-administration or DAT function, the most likely reason rests with the extensive and varied training history that occurred prior to assessment of AMP self-administration. In contrast to prior univariate studies, the current multivariate approach involved some transfer of training (interference) across both predictor and outcome tests. Inherent individual differences may have been obscured by the variable environmental history across rats due to the counterbalancing procedure used to measure the predictor variables. In particular, with the three operant conditioning predictor tests used (DD, CGNG and PR), it is reasonable to assume that there was carryover of performance from one test to the next, especially because the stimulus lights were used in all three tasks to indicate pellet availability. These stimulus lights were later paired with a drug infusion during the self-administration phase. Individual differences in the amount of handling and training also occurred with the two predictor tests involving exposure to a novel context (LA and NPP). Another related potential problem was that limited training was used for each operant procedure due to the time constraints imposed with testing so many procedures in each animal. It is difficult to determine if subjects were given adequate exposure to each procedure in order to properly assess sensation seeking and impulsivity. Due to the shorter life span of rodents compared to humans, it is difficult to conduct multivariate tests with many different measures, particularly when using operant procedures that require extensive training.

Many of the limitations of the present experiment could be examined in future research. Using different measures of sensation seeking or impulsivity that require less training and therefore shorten the overall length of the study would be useful. Another approach would be to use a different format of drug self-administration to better assess the reinforcing efficacy of the drug such as self-administration on a PR schedule, or examination of self-administration escalation with extended access. Additionally, examining different training doses of AMP could also produce a different outcome. Larger scale studies would also be capable of providing a more powerful assessment. The number of subjects included in the present experiment allowed for detection of moderate to large effects, but was not sufficient to detect small effects, which represents a limitation compared to many investigations that use a larger number of human subjects.

While animal models have many benefits for drug abuse research (Brady, 1991; Haney and Spealman, 2008; Lynch et al., 2010), limitations remain. Not only is behavior and neurobiology more complex in humans than in laboratory animals, multi-faceted human traits such as sensation seeking and impulsivity may not be modeled completely in laboratory animals; however, problems in measuring traits or constructs is not unique to animal models. For example, in humans, various personality questionnaires and behavioral measures that putatively measure the construct of “impulsivity” (e.g., balloon analogue risk task, Bechara gambling task, CGNG and DD) are often unrelated (Jones and Lejuez, 2005; Lejuez et al., 2003; Perkins et al., 2008). Future research should examine which abuse-related predictor variables generalize most robustly across human and non-human animals, regardless of whether univariate or multivariate approaches are used.

Acknowledgments

The authors thank Kristin Alvers, Joshua Beckmann, Emily Denehy, Kate Fischer, Cassandra Gipson, Luke Holderfield, A. Chip Meyer, and Justin Yates for assistance. Research supported by NIH Grants P50 DA05312 and T32 DA007304.

References

Ainslie G. Specious reward: A behavioral theory of impulsiveness and impulse control. Psychological Bulletin. 1975; 82:463–496. [PubMed: 1099599]

Andrucci G, Archer RP, Pancoast DL, Gordon RA. The relationship of MMPI and Sensation Seeking Scales to adolescent drug use. Journal of Personality Assessment. 1989; 53:253–266. [PubMed: 2786070]

Anker JJ, Perry JL, Gliddon LA, Carroll ME. Impulsivity predicts the escalation of cocaine self-administration in rats. Pharmacology, Biochemistry, and Behavior. 2009; 93:343–348.

Arnold JM, Roberts DCS. A critique of fixed and progressive ratio schedules used to examine the neural substrates of drug reinforcement. Pharmacology, Biochemistry, and Behavior. 1997; 57:441–447.

Bardo MT, Bowling SL, Robinet PM, Rowlett JK, Lacy M, Mattingly BA. Role of D1 and D2 receptors in novelty-maintained place preference. Experimental and Clinical Psychopharmacology. 1993; 1:101–109.

Belin D, Mar AC, Dalley JW, Robbins TW, Everitt BJ. High impulsivity predicts the switch to compulsive cocaine-taking. Science. 2008; 320:1353–1354.

Brady JV. Animal models for assessing drugs of abuse. Neuroscience and Biobehavioral Reviews. 1991; 15:35–43. [PubMed: 2052196]

Brown PL, Jenkins HM. Auto-shaping of the pigeon's key-peck. Journal of the Experimental Analysis of Behavior. 1968; 11:1–8. [PubMed: 5636851]

Cain ME, Denehy ED, Bardo MT. Individual differences in amphetamine self-administration: the role of the central nucleus of the amygdala. Neuropsychopharmacology. 2008; 33:1149–1161. [PubMed: 17568395]

Cain ME, Dotson WF, Bardo MT. Individual differences in the effect of novel environmental stimuli prior to amphetamine self-administration in rats (Rattus norvegicus). Experimental and Clinical Psychopharmacology. 2006; 14:389–401. [PubMed: 16893281]

Cain ME, Saucier DA, Bardo MT. Novelty seeking and drug use: contribution of an animal model. Experimental and Clinical Psychopharmacology. 2005; 13:367–375. [PubMed: 16366767]

Camacho F, Smith CP, Vargas HM, Winslow JT. α_2-Adrenoceptor antagonists potentiate acetylcholinesterase inhibitor effects on passive avoidance learning in the rat. Psychopharmacology. 1996; 124:347–354. [PubMed: 8739550]

Cloninger C, Przybeck TR, Svrakic DM. The Tridimensional Personality Questionnaire: U.S. normative data. Psychological Reports. 1991; 69:1047–1057. [PubMed: 1784653]

Costa, PT.; McCrae, RR. Revised NEO Personality Inventory (NEO-PI-R). Odessa FL: Psychological Assessment Resources; 1992.

Crawford A, Pentz MA, Chou CP, Li C, Dwyer JH. Parallel developmental trajectories of sensation seeking and regular substance use in adolescents. Psychology of Addictive Behaviors. 2003; 17:179–192. [PubMed: 14498812]

Dawe S, Loxton NJ. The role of impulsivity in the development of substance use and eating disorders. Neuroscience and Biobehavioral Reviews. 2004; 28:343–351. [PubMed: 15225976]

DeSousa NJ, Bush DEA, Vaccarino FJ. Self-administration of intravenous amphetamine is predicted by individual differences in sucrose feeding in rats. Psychopharmacology. 2000; 148:52–58. [PubMed: 10663417]

de Win MM, Schilt T, Reneman L, Vervaeke H, Jager G, Dijkink S, Booij J, van den Brink W. Ecstasy use and self-reported depression, impulsivity, and sensation seeking: a prospective cohort study. Journal of Psychopharmacology. 2006; 20:226–235. [PubMed: 16510480]

de Wit, H.; Richards, JB. Dual determinants of drug use in humans: reward and impulsivity. In: Bevins, RA.; Bardo, MT., editors. Motivational factors in the etiology of drug abuse. University of Nebraska Press; Lincoln, NE: 2004. p. 19-55.

Diergaarde L, Pattij T, Poortvliet I, Hogenboom F, de Vries W, Schoffelmeer AN, De Vries TJ. Impulsive choice and impulsive action predict vulnerability to distinct stages of nicotine seeking in rats. Biological Psychiatry. 2008; 63:301–308. [PubMed: 17884016]

Ersche KD, Turton AJ, Pradhan S, Bullmore ET, Robbins TW. Drug Addiction Endophenotypes: Impulsive Versus Sensation-Seeking Personality Traits. Biological Psychiatry. 2010; 68:770–773. [PubMed: 20678754]

Eysenck, HJ.; Eysenck, MW. Personality and Individual Differences: A Natural Sciences Approach. New York: Plenum Press; 1985.

Fillmore MT, Rush CR. Impaired inhibitory control in chronic cocaine abusers. Drug and Alcohol Dependence. 2002; 66:265–273. [PubMed: 12062461]

Gipson CD, Bardo MT. Extended access to amphetamine self-administration increases impulsive choice in a delay discounting task in rats. Psychopharmacology. 2009; 207:391–400. [PubMed: 19784636]

Goodwin JS, Larson GA, Swant J, Sen N, Javitch JA, Zahniser NR, De Felice LJ, Khoshbouei H. Amphetamine and methamphetamine differentially affect dopamine transporters in vitro and in vivo. The Journal of Biological Chemistry. 2009; 284:2978–2989. [PubMed: 19047053]

Gosnell BA. Sucrose intake predicts rate of acquisition of cocaine self-administration. Psychopharmacology. 2000; 149:286–292. [PubMed: 10823410]

Haney M, Spealman R. Controversies in translational research: Drug self-administration. Psychopharmacology. 2008; 199:403–419. [PubMed: 18283437]

Hellemans KGC, Nobrega JN, Olmstead MC. Early environmental experience alters baseline and ethanol-induced cognitive impulsivity: relationship to forebrain 5-HT_{1A} receptor binding. Behavioural Brain Research. 2005; 159:207–220. [PubMed: 15817184]

Hughes RN. Behavior of male and female rats with free choice of two environments differing in novelty. Animal Behavior. 1968; 16:92–96.

Joireman, J.; Kuhlman, DM. The Zuckerman-Kuhlman personality questionnaire: origin, development and validity of a measure to assess an alternative five-factor model of personality. In: Stelmack, RM., editor. On the Psychobiology of Personality: Essays in Honor of Marvin Zuckerman. Amsterdam: Elsevier; 2004. p. 49-64.

Jones HA, Lejuez CW. Personality correlates of caffeine dependence: the role of sensation seeking, impulsivity, and risk taking. Experimental and Clinical Psychopharmacology. 2005; 13:259–266. [PubMed: 16173890]

Kahlig KM, Binda F, Khoshbouei H, Blakely RD, McMahon DG, Javitch JA, Galli A. Amphetamine induces dopamine efflux through a dopamine transporter channel. Proceedings of the National Academy of Sciences USA. 2005; 102:3495–3500.

Kelly TH, Robbins G, Martin CA, Fillmore MT, Lane SD, Harrington NG, Rush CR. Individual differences in drug abuse vulnerability: amphetamine and sensation-seeking status. Psychopharmacology. 2006; 189:17–25. [PubMed: 16972106]

Klebaur JE, Bevins RA, Segar TM, Bardo MT. Individual differences in behavioral responses to novelty and amphetamine self-administration in female and male rats. Behavioural Pharmacology. 2001; 12:267–275. [PubMed: 11548112]

Kosten T, Ball SA, Rounsaville BJ. A sibling study of sensation seeking and opiate addiction. The Journal of Nervous and Mental Disease. 1994; 182:284–289. [PubMed: 10678310]

Koya E, Spijker S, Voorn P, Binnekade R, Schmidt ED, Schoffelmeer AN, De Vries TJ, Smit AB. Enhanced cortical and accumbal molecular reactivity associated with conditioned heroin, but not sucrose-seeking behaviour. Journal of Neurochemistry. 2006; 98:905–915. [PubMed: 16787418]

Lejuez CW, Aklin WM, Jones HA, Richards JB, Strong DR, Kahler CW, Read JP. The Balloon Analogue Risk Task (BART) differentiates smokers and nonsmokers. Experimental and Clinical Psychopharmacology. 2003; 11:26–33. [PubMed: 12622341]

LeSage MG, Burroughs D, Pentel PR. Effects of nicotine withdrawal on performance under a progressive-ratio schedule of sucrose pellet delivery in rats. Pharmacology, Biochemistry, and Behavior. 2006; 83:585–591.

Lynch WJ, Nicholson KL, Dance ME, Morgan RW, Foley PL. Animal models of substance abuse and addiction: Implications for science, animal welfare, and society. Comparative Medicine. 2010; 60:177–188. [PubMed: 20579432]

Magid V, Maclean MG, Colder CR. Differentiating between sensation seeking and impulsivity through their mediated relations with alcohol use and problems. Addictive Behaviors. 2007; 32:2046–2061. [PubMed: 17331658]

Mantsch JR, Ho A, Schlussman SD, Kreek MJ. Predictable individual differences in the initiation of cocaine self-administration by rats under extended-access conditions are dose-dependent. Psychopharmacology. 2001; 157:31–39. [PubMed: 11512040]

Marusich JA, Bardo MT. Differences in impulsivity on a delay discounting task predict self-administration of a low unit dose of methylphenidate in rats. Behavioural Pharmacology. 2009; 20:447–454. [PubMed: 19696657]

Marusich JA, Beckmann JS, Gipson CD, Bardo MT. Methylphenidate as a reinforcer for rats: Contingent delivery and intake escalation. Experimental and Clinical Psychopharmacology. 2010; 18:257–266. [PubMed: 20545390]

Moron JA, Brockington A, Wise RA, Rocha BA, Hope BT. Dopamine uptake through the norepinephrine transporter in brain regions with low levels of the dopamine transporter: evidence from knock-out mouse lines. The Journal of Neuroscience. 2002; 15:389–395. [PubMed: 11784783]

Olmstead MC. Animal models of drug addiction: Where do we go from here? Quarterly Journal of Experimental Psychology. 2006; 59:625–653.

Perkins KA, Lerman C, Coddington SB, Jetton C, Karelitz JL, Scott JA, Wilson AS. Initial nicotine sensitivity in humans as a function of impulsivity. Psychopharmacology. 2008; 200:529–544. [PubMed: 18604520]

Perry JL, Larson EB, German JP, Madden GJ, Carroll ME. Impulsivity (delay discounting) as a predictor of acquisition of IV cocaine self-administration in female rats. Psychopharmacology. 2005; 178:193–201. [PubMed: 15338104]

Perry JL, Nelson SE, Carroll ME. Impulsive choice as a predictor of acquisition of iv cocaine self-administration and reinstatement of cocaine-seeking behavior in male and female rats. Experimental and Clinical Psychopharmacology. 2008; 16:165–177. [PubMed: 18489020]

Piazza PV, Deminiere JM, Le Moal M, Simon H. Factors that predict individual vulnerability to amphetamine self-administration. Science. 1989; 245:1511–1513. [PubMed: 2781295]

Piazza, PV.; Deminiere, JM.; Maccari, S.; Le Moal, M.; Mormede, P.; Simon, H. Individual vulnerability to drug self-administration: Action of corticosterone on dopaminergic systems as a possible pathophysiological mechanism. In: Willner, P.; Scheel-Kruger, J., editors. The mesolimbic dopamine system: From motivation to action. New York: Wiley; 1991. p. 473-495.

Pierre PJ, Vezina P. Predisposition to self-administer amphetamine: the contribution of response to novelty and prior exposure to the drug. Psychopharmacology. 1997; 129:277–284. [PubMed: 9084067]

Pentkowski NS, Duke FD, Weber SM, Pockros LA, Teer AP, Hamilton EC, Thiel KJ, Neisewander JL. Stimulation of medial prefrontal cortex serotonin 2C (5-HT(2C)) receptors attenuates cocaine-seeking behavior. Neuropsychopharmacology. 2010; 35:2037–2048. [PubMed: 20520599]

Richardson NR, Roberts DCS. Fluoxetine pretreatment reduces breaking points on a progressive ratio schedule reinforced by intravenous cocaine self-administration in the rat. Life Sciences. 1991; 49:883–840.

Santucci AC, Cortes C, Bettica A, Cortes F. Chronic ethanol consumption in rats produces residual increases in anxiety 4 months after withdrawal. Behavioural Brain Research. 2008; 188:24–31. [PubMed: 18061285]

Santucci AC, Mercado M, Bettica A, Cortes C, York D, Moody E. Residual behavioral and neuroanatomical effects of short-term chronic ethanol consumption in rats. Brain Research Cognitive Brain Research. 2004; 20:449–461. [PubMed: 15268922]

Stoops WW, Lile JA, Robbins CG, Martin CA, Rush CR, Kelly TH. The reinforcing, subject-rated, performance, and cardiovascular effects of amphetamine: influence of sensation-seeking status. Addict Behaviors. 2007; 32:1177–1188.

Sulzer D, Sonders MS, Poulsen NW, Galli A. Mechanisms of neurotransmitter release by amphetamine: a review. Progress in Neurobiology. 2005; 75:406–433. [PubMed: 15955613]

Williams JM, Steketee JD. Characterization of dopamine transport in crude synaptosomes prepared from rat medial prefrontal cortex. Journal of Neuroscience Methods. 2004; 137:161–165. [PubMed: 15262056]

Winstanley CA, Theobald DE, Cardinal RN, Robbins TW. Contrasting roles of basolateral amygdala and orbitofrontal cortex in impulsive choice. The Journal of Neuroscience. 2004; 24:4718–4722. [PubMed: 15152031]

Zhu J, Green T, Bardo MT, Dwoskin LP. Environmental enrichment enhances sensitization to GBR 12935-induced activity and decreases dopamine transporter function in the medial prefrontal cortex. Behavioural Brain Research. 2004; 148:107–117. [PubMed: 14684252]

Zuckerman, M. Sensation seeking: beyond the optimal level of arousal. Erlbaum; Hillsdale CA: 1979.

Figure 1a.

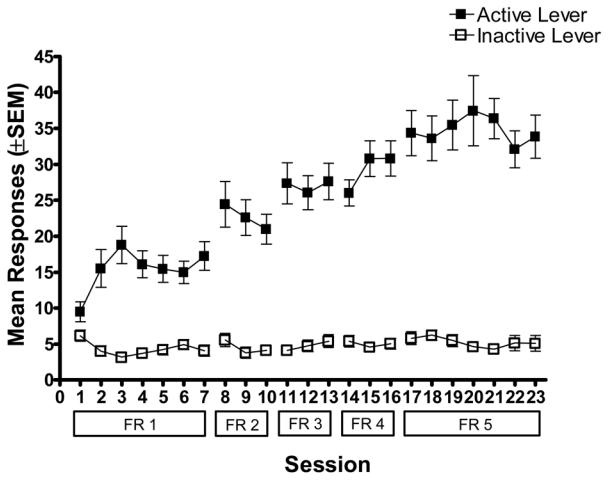

Figure 1b.

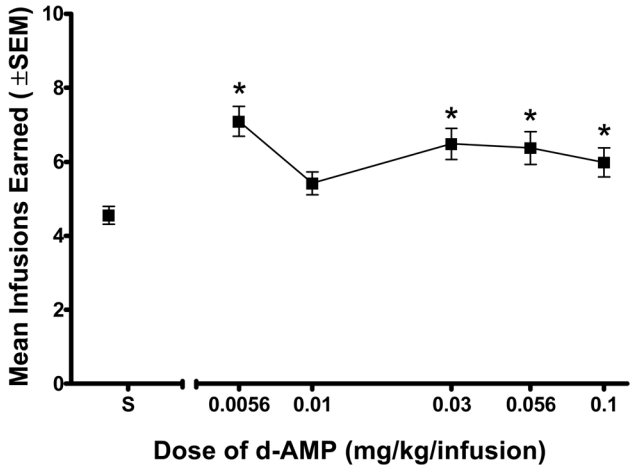

Figure 1.

Figure 1a. Mean numbers of active and inactive lever presses plotted as functions of session during acquisition of AMP self-administration.

Figure 1b. Mean number of infusions earned plotted as a function of AMP dose (log scale). Asterisk (*) denotes doses at which numbers of infusions earned differed significantly compared to at saline (S).

Table 1

Correlations between the six predictor variables.

	NPP	PR	PA	CGNG	DD
LA	0.4148*	0.0530	−0.3779	0.0356	−0.0135
NPP		−0.0552	−0.0722	0.1628	−0.0095
PR			−0.0696	−0.0611	0.1210
PA				0.0212	−0.2402
CGNG					0.0515

Asterisk (*) denotes correlation that is significant. Bonferroni adjustments were used to maintain a Type I error probability of 0.05 across each row. LA=locomotor activity; NPP=novelty place preference; PR=progressive ratio responding for sucrose pellet; PA=passive avoidance; CGNG=cued go/no-go; DD=delayed discounting.

Table 2

Correlations between the six predictor variables, and the number of infusions earned at each FR value during AMP acquisition.

	FR 1	FR 2	FR 3	FR 4	FR 5
LA	0.0274	0.0310	−0.0997	−0.0003	0.0382
NPP	0.1425	−0.0191	−0.1280	0.1847	0.2109
PR	0.2638	0.2852	0.2930	0.0848	0.1902
PA	−0.1833	−0.1833	0.0126	0.0883	−0.2406
CGNG	−0.0565	−0.0530	0.1245	0.1903	−0.0524
DD	0.3024	0.1571	0.2848	0.2789	0.3787

Table 3

Correlations between the six predictor variables, and the number of infusions earned at each dose of AMP (expressed as mg/kg/infusion) during dose-effect determination.

	Sal	0.0056	0.01	0.03	0.056	0.1
LA	0.0486	−0.0841	−0.1233	0.1786	0.1600	−0.0093
NPP	0.1657	0.2751	0.2307	0.3964	0.2439	−0.0461
PR	−0.2712	−0.1665	0.0490	0.1126	−0.0723	−0.1290
PA	0.2397	−0.0101	0.0571	−0.2074	−0.0097	0.0393
CGNG	0.1291	−0.0530	−0.0129	0.0076	−0.0757	0.0509
DD	−0.1137	0.2438	0.3797	0.3467	0.4225	0.3289

Table 4

The six predictor variables, the number of infusions earned at each FR value, and the number of infusions earned at each dose of AMP correlated with neurochemical data.

	mPFC V_{max}	mPFC K_m	OFC V_{max}	OFC K_m
LA	−0.0585	0.2292	−0.0186	−0.0271
NPP	−0.2195	0.5068*	−0.3781	0.0565
PR	0.1415	−0.2129	0.2423	−0.1379
PA	−0.2334	−0.3759	−0.3463	−0.1306
CGNG	−0.3647	−0.3749	−0.1988	−0.1647
DD	0.2434	−0.1047	0.1714	0.1820
FR 1	0.1255	0.0012	0.2403	0.1448
FR 2	0.2003	−0.1185	0.3177	0.0370
FR 3	0.0210	−0.4331	0.1970	−0.0517
FR 4	0.1397	−0.1773	0.2258	0.1283
FR 5	0.0182	0.1363	0.1089	0.4648
Sal	−0.2621	−0.1741	−0.4000	−0.0233
0.0056	−0.0844	0.0496	−0.0227	0.1276
0.01	0.1321	−0.0696	0.1750	0.2772
0.03	0.0179	0.1507	0.1322	0.4952*
0.056	0.1849	0.1003	0.1817	0.2543
0.1	−0.0209	−0.0966	0.0173	0.3043

Asterisk (*) denotes correlations that are significant. Bonferroni adjustments were used to maintain a Type I error probability of 0.05 across each row.