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Initial locomotor sensitivity to cocaine varies widely among inbred mouse strains

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Abstract

Initial sensitivity to psychostimulants can predict subsequent use and abuse in humans. Acute locomotor activation in response to psychostimulants is commonly used as an animal model of initial drug sensitivity and has been shown to have a substantial genetic component. Identifying the specific genetic differences that lead to phenotypic differences in initial drug sensitivity can advance our understanding of the processes that lead to addiction. Phenotyping inbred mouse strain panels are frequently used as a first step for studying the genetic architecture of complex traits. We assessed locomotor activation following a single, acute 20 mg/kg dose of cocaine (COC) in males from 45 inbred mouse strains and observed significant phenotypic variation across strains indicating a substantial genetic component. We also measured levels of COC, the active metabolite, norcocaine and the major inactive metabolite, benzoylecgonine, in plasma and brain in the same set of inbred strains. Pharmacokinetic (PK) and behavioral data were significantly correlated, but at a level that indicates that PK alone does not account for the behavioral differences observed across strains. Phenotypic data from this reference population of inbred strains can be utilized in studies aimed at examining the role of psychostimulant-induced locomotor activation on drug reward and reinforcement and to test theories about addiction processes. Moreover, these data serve as a starting point for identifying genes that alter sensitivity to the locomotor stimulatory effects of COC.

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Keywords

Cocaine; inbred strains; locomotor; mice; pharmacokinetics

The most recent survey of drug use in the US indicates that there were 2.8 million new drug users in 2012 (SAMHSA 2013). However, only a subset of individuals who try drugs go on to develop addiction. Predisposition to develop addiction is controlled, in part, by genetic factors (Kreek *et al.* 2012) and thus, identifying genetic differences that alter addiction liability is an active area of biomedical research.

The progression from initial drug exposure to subsequent use and abuse is thought to be dependent on many factors including physiological or neurobiological changes following exposure (Badiani & Spagnolo 2013; Russo *et al.* 2010). There is evidence in both humans and animals that initial response to an addictive substance can predict subsequent use and abuse (De Wit & Phillips 2012; Fergusson *et al.* 2003; Haertzen *et al.* 1983; Lambert *et al.* 2006; Lundahl & Lukas 2007; Schafer & Brown 1991). In humans, initial positive responses to cocaine (COC) and other stimulants predict latency to second use (Davidson *et al.* 1993) and increased risk for dependence and abuse (Lambert *et al.* 2006). Initial sensitivity in animal models is often measured as acute locomotor activation following drug administration. The extent to which this behavior predicts active drug-seeking behaviors such as self-administration and conditioned place preference varies across studies. Early studies on rats provided evidence that elevated lomocotor activation was associated with higher levels of self-administration (Deminiere *et al.* 1989; Piazza *et al.* 1989). This finding has been replicated in more recent studies (Allen *et al.* 2007; Fattore *et al.* 2009; Giorgi *et al.* 2005) but not in others (Mandt *et al.* 2008; Mantsch *et al.* 2001). These data indicate that associations between initial locomotor sensitivity to COC and drug reward and reinforcement behaviors are complex and likely depend on many factors including genetic background.

A wide range of untapped phenotypic and genetic diversity exists among inbred mouse strains and may be useful for dissecting the biological and genetic mechanisms that influence addiction-related behaviors. Inbred strain surveys are an effective method for analyzing the genetic architecture of complex phenotypes and may be helpful in dissecting the biological and genetic mechanisms that influence addiction-related behaviors. We tested male mice from 45 inbred strains, including both standard and wild-derived, for locomotor activation following an acute dose of COC and observed significant strain effects on behavior indicating substantial genetic influence. Furthermore, we tested levels of COC and two metabolites in both brain and plasma in the same set of strains at two time points after COC administration. Correlational analysis of behavior and pharmacokinetic (PK) measures indicate that PK explains some, but not all, of the phenotypic variation observed among inbred strains.

These data represent the largest inbred strain survey and the first reported analysis of wild-derived strains for COC-induced locomotor response and PK. The strain differences reported here serve as a starting point for more detailed analysis of genetic and PK

influences on COC-induced activation and the role of initial locomotor sensitivity on addictive behaviors in mice.

Materials and methods

Animals

A total of 839 males from 45 inbred mouse strains were studied for locomotor response to COC and a separate set of 306 mice from the same set of strains were tested for COC PK. Strains were chosen to maximize genetic diversity and included both standard laboratory and wild-derived strains. The complete list of strains and number of mice tested for each strain are available in Table S1. For both behavioral and PK studies, mice were purchased from the Jackson Laboratory (Bar Harbor, ME, USA) and allowed to acclimate in an animal holding room in the vivarium at the University of North Carolina for at least 10 days prior to testing. Strains were randomly assigned to a batch such that all animals in a given strain were spread across multiple batches. For behavior, an average of 18 mice per strain (range: 12–22) were tested in 42 batches across 17 months. A total of 52 C57BL/6J (B6) mice were tested across 21 of the 42 batches to serve as a temporal control. At least two B6 mice were tested per batch with the exception of one batch in which only one B6 mouse was tested. For PK studies, a total of six male mice per strain (three mice per time point) were tested across 23 batches. Mice were group housed (2–4 per cage) in ventilated caging (Tecniplast, Buguggiate, Italy) on a 12-h light/dark cycle (lights on at 0700 h). Food (Purina RMH 3000; Purina, St. Louis, MO, USA) and water were provided *ad libitum*. Mice were approximately 67 days of age (±6.4 days) at the onset of testing. Behavioral testing was conducted between the hours of 0800 and 1200 h during the light part of the animals' light/dark cycle. All procedures were approved by the Institutional Animal Care and Use Committee of The University of North Carolina.

Behavior

Mice were tested over three consecutive days. On Days 1 and 2, mice received an intraperitoneal (IP) injection of physiological saline in a volume of 0.01 ml/g immediately prior to placement in an open field (OF; Med Associates ENV515-16, St. Albans, VT, USA) for 30 min. On Day 3, mice received an IP injection of 20 mg/kg COC HCl (Sigma-Aldrich, St. Louis, MO, USA) immediately prior to a 30-min test session in the OF. The OF was a 17 × 17″square arena with a white Plexiglas floor and clear Plexiglas walls surrounded by infrared beams spaced 1″ apart on the *X*, *Y* and *Z*-axes to detect both horizontal and vertical movement. Measures in the OF included distance traveled (in centimeters), number of ambulatory (directed) movements, rearing behavior, average velocity and percent time resting. Data were collected and analyzed using Med-Associates Windows-based Open field activity software (v5). Individual animals' behaviors were calculated as the sum of the behavior across the entire 30-min test period. Habituation to the novel OF was calculated by subtracting Day 2 activity from Day 1 activity. Locomotor response to COC, changes in average velocity and rearing were calculated as a difference score by subtracting Day 2 phenotypes after saline injection from Day 3 phenotypes after COC injection. Latency to peak locomotor response was also assessed.

Pharmacokinetics

Naïve mice were administered a single 20 mg/kg dose of IP COC HCl, anesthetized with isoflurane at 10 and 30 min following COC administration and 50 μl of blood was collected by cardiac puncture, placed in a heparinized tube, centrifuged and the resulting plasma was removed and snap-frozen in liquid nitrogen. Whole brain was collected, rinsed, weighed and snap-frozen in liquid nitrogen. Plasma and brain samples were stored at −80°C until analyzed. Plasma and brain concentrations of COC, norcocaine (NOR) and benzoylecgonine (BZE) were quantified using liquid chromatography tandem mass spectrometry (Slawson *et al.* 2002).

Plasma and brain concentrations are expressed as ng/ml and ng/g, respectively. The area under the concentration vs. time curve (AUC) in plasma and brain was calculated for each animal using a linear-up log-down function (Winnonlin v5.2; Pharsight, St. Louis, MO, USA).

Statistical analysis

Basic statistics were performed using SPSS 16.0 for Mac (Chicago, IL, USA) and SAS software SAS v. 9.2 (SAS Institute Inc., Cary, NC, USA). We performed hierarchical cluster analysis to investigate how behavior phenotypes are correlated with each other. We used 1-absolute (Pearson correlation) as the measure of dissimilarity between pairs of phenotypes and the complete linkage for clustering.

Intrastrain (V_E) and interstrain ($V_G + V_E$) variance were used to calculate genetic variance (V_G) and broad sense heritability was calculated using the following formula: $H^2 = V_G/(V_G + V_E)$.

Difference scores describing habituation to the novel OF (Day 1 – Day 2) and behavioral response to COC (Day 3 – Day 2) for locomotor behavior, rearing and average velocity were treated as dependent variables and analyzed by one-way analysis of variance (ANOVA) to determine strain effects. An analysis of covariance (ANCOVA) model that also includes Day 2 behavior as a covariate was used for COC response. Brain and plasma concentrations of COC, NOR and BZE were also analyzed by ANOVA to determine the effects of strain and time (10 and 30 min post-drug administration). For any phenotype with a significant strain by time interaction, we performed *post hoc t*-tests to investigate which strains have significant time effects and *P*-values were adjusted using the Bonferroni correction for multiple tests (45 comparisons). Corrected *P*-values <0.0011 are reported as significant.

The relationship between behavioral responses to COC and PK was investigated with a linear regression model where difference scores are response variables and PK AUC are covariates.

Results

Stability of behavior

Statistical analysis of B6 behavior across batches indicated that average velocity ($P = 0.022$) and rearing behavior ($P < 0.0001$) have significant batch effects. However, for average

velocity, the batch effect is no longer significant after multiple testing correction. No batch effects were observed for distance traveled, ambulatory episodes and percent time resting.

Open field behaviors

Hierarchical cluster analysis indicated that total distance traveled, ambulatory episodes and percent time resting are tightly clustered compared with rearing and average velocity on each of the three testing days (Fig. S1). Based on this result, we describe here the data for total distance only, along with rearing behavior and average velocity. Strain means for all variables are provided in Tables S2 and S3 and on the Mouse Phenome Database (http://phenome.jax.org/).

Open field behavior on Days 1 and 2

Significant strain differences were observed for habituation to the OF for distance traveled ($F_{(44,831)} = 7.7$; $P < 0.001$), rearing ($F_{(44,831)} = 4.6$; $P < 0.001$) and average velocity ($F_{(44,831)} = 2.0$; $P < 0.001$). In general, most strains showed a decrease in locomotor activity and rearing on Day 2 vs. Day 1 (Fig. 1a, b). Average velocity also decreased across the first two days of testing for many strains, but 14 strains showed either no change or an increase in average velocity on Day 2 vs. Day 1 (Fig. 1c).

Acute COC and open field behavior

Inbred strains varied significantly in their response to a single dose of COC. There were significant strain effects on locomotor activity ($F_{(44,837)} = 15.1$; $P < 0.001$), rearing ($F_{(44,837)} = 11.4$; $P < 0.001$) and average velocity ($F_{(44,837)} = 9.7$; $P < 0.001$) after controlling for these behaviors on Day 2. Locomotor activity and average velocity increased in most strains following exposure to COC, although some strains showed little or no increase in locomotor activity (Fig. 2a) and average velocity decreased in five strains (Fig. 2b). Rearing decreased following COC administration in most strains, although a subset of eight strains exhibited increased rearing (Fig. 2c).

Broad-sense heritabilities of total distance, rearing and average velocity difference scores were calculated at 0.53, 0.37 and 0.30, respectively.

Mean latency to peak locomotor response across strains was 12.9 min (range: 2.5–21.1) with significant strain differences observed ($F_{(44,821)} = 4.3$; $P < 0.001$; Fig. S2a). However, latency did not correlate with locomotor response to COC ($r_{(45)} = 0.12$; $P > 0.40$).

Locomotor activity and response to COC

Mice that showed lower habituation scores (difference score of Day 1 – Day 2) showed a trend toward increased COC locomotor activation, although the correlation was not significant ($r_{(45)} = -0.27$; $P = 0.071$). A comparison of locomotor response to novelty on Day 1 and COC locomotor difference scores (Day 3 – Day 2) indicated that Day 1 locomotor behavior was significantly correlated with COC-induced locomotor activation ($r_{(45)} = 0.43$; $P < 0.01$; Fig. S3).

Pharmacokinetic analysis

Cocaine—Plasma COC concentration decreased significantly between 10 and 30 min in all strains ($F_{(1,305)} = 354.7$; $P < 0.001$) and mean COC concentration in the plasma differed significantly across strains ($F_{(44,305)} = 2.5$; $P < 0.001$) (Fig. S4a). No strain by time interaction effects were observed ($F_{(44,305)} = 1.2$; $P > 0.05$).

Brain concentrations of COC also decreased significantly between 10 and 30 min time points in all strains ($F_{(1,305)} = 245.7$; $P < 0.001$) and mean COC concentration in the brain varied significantly across strains ($F_{(44,305)} = 2.5$; $P < 0.001$; Fig. 3a, Table S4). No strain by time interaction effects were observed ($F_{(44,305)} = 1.2$; $P > 0.05$)

Norcocaine—Plasma NOR concentration decreased significantly between 10 and 30 min ($F_{(1,305)} = 117.8$; $P < 0.001$). Mean plasma NOR concentration varied across strains ($F_{(44,305)} = 6.5$; $P < 0.001$) and was lower than brain concentration at both time points (Fig. S4b). No strain by time interaction effects were observed ($F_{(44,305)} = 1.4$; $P > 0.05$).

Brain NOR concentration was approximately 10 times lower than COC concentration at both 10 and 30 min and NOR levels in the brain also decreased significantly between 10 and 30 min ($F_{(1,305)} = 203.1$; $P < 0.001$) and varied significantly by strain ($F_{(44,305)} = 10.5$; $P < 0.001$). A significant strain by time effect was observed ($F_{(44,305)} = 1.9$; $P < 0.01$) and *post hoc t*-tests by strain indicated that the decrease in brain NOR concentration was significant for four strains ($P < 0.001$). (Fig. 3b, Table S4).

Benzoylecgonine—Plasma BZE levels increased between 10 and 30 min ($F_{(1,305)} = 133.0$; $P < 0.001$), varied significantly across strains ($F_{(44,305)} = 7.0$; $P < 0.001$) and showed a significant strain by time interaction ($F_{(44,305)} = 2.0$; $P < 0.01$). *Post hoc t*-tests by strain indicated that two strains showed a significant increase ($P < 0.001$) in plasma BZE while the remainder showed no significant increase (Fig. S4c).

Benzoylecgonine levels in the brain were significantly lower than concentrations of both COC and NOR at both 10 and 30 min. However, unlike both COC and NOR, levels of BZE increased significantly between 10 and 30 min ($F_{(1,305)} = 798.5$; $P < 0.001$). Significant strain differences in brain concentrations of BZE were also observed ($F_{(44,305)} = 7.8$; $P < 0.001$) as well as a strain by time interaction effect ($F_{(44,305)} = 3.1$; $P < 0.001$). *Post hoc t*-tests by strain indicate that there are 17 strains for which brain BZE concentrations are significantly increased ($P < 0.001$) at 30 min (Fig. 3c, Table S4).

Ratios of brain to plasma exposure—The mean ratios of brain to plasma concentrations of COC, NOR and BZE at 10 min after administration were 5.2, 4.0 and 0.04 and at 30 min were 5.6, 3.6 and 0.07, respectively (Table S4). The ratio of brain to plasma concentrations of COC varied significantly across strains at both 10 min ($F_{(44,166)} = 5.2$; $P < 0.001$) and 30 min ($F_{(44,137)} = 3.4$; $P < 0.001$). Similarly, ratio of brain to plasma concentration of NOR varied significantly across strains at 10 min ($F_{(44,167)} = 5.0$; $P < 0.001$) and 30 min ($F_{(44,137)} = 5.2$; $P < 0.001$) as did the ratio of brain to plasma concentration of BZE at 10 min ($F_{(44,167)} = 2.3$; $P < 0.001$) and 30 min ($F_{(44,137)} = 5.2$; $P < 0.001$) (Table S4).

Pharmacokinetic and behavioral correlations

Behavior was measured over a 30-min period and PK data were collected at 10 and 30 min time points after administration of COC. Linear regression of the AUC representing brain and plasma concentrations of COC, NOR and BZE over the entire 30 min on distance, average velocity and rearing difference scores indicated that COC concentration in the brain was significantly and positively correlated with both locomotor activation ($t_{(44)} = 2.45$; $P < 0.05$) and average velocity ($t_{(44)} = 2.87$; $P < 0.01$). Norcocaine concentration in the brain showed a similar pattern with both locomotor activation ($t_{(44)} = 2.53$; $P < 0.05$) and average velocity ($t_{(44)} = 2.32$; $P < 0.05$) (Fig. 4). COC and NOR concentrations in the brain did not predict rearing behavior. Benzoylecgonine concentrations in the brain did not affect locomotor activity, rearing or average velocity. Plasma levels of COC, NOR and BZE did not have an effect on any behavior (Table S5).

Discussion

Inbred strain surveys have been utilized for decades to examine the genetic architecture of complex traits (Rodgers & Mc 1962). However, phenotypic assessment of numerous strains has only recently been carried out for many complex phenotypes. One impetus for these efforts has been the perception of a ‘phenotype gap’ or the lack of access to a full range of phenotypes that model human disease thereby impeding functional annotation of genes (Bullard 2001; Paigen & Eppig 2000). Moreover, the rapidly increasing amount of genomic data across hundreds of inbred strains expands the utility of such efforts (Baker *et al.* 2011; Keane *et al.* 2011; Kirby *et al.* 2010; Szatkiewicz *et al.* 2008; Williams & Mulligan 2012). The data presented herein represent the largest inbred strain survey for locomotor and PK responses to COC using both standard and wild-derived strains, thereby increasing the range of phenotypic and genetic diversity currently available in the literature. These data provide a starting point from which to examine genetic architecture and strain differences and provide a rich resource for planning further experiments and analyses aimed at expanding knowledge of how genetic background and, ultimately, specific genes influence initial sensitivity to COC and whether these genes are involved in the rewarding and reinforcing properties of psychostimulants.

Significant phenotypic variation for locomotor response to COC was observed across the 45 inbred strains. Similar strain patterns have been observed in surveys of COC-induced locomotor activation using fewer strains. A/J and 129S1 have been classified as low responding, C3H, SJL and DBA have intermediate responses and B6 mice have a robust response (Eisener-Dorman *et al.* 2011; Ruth *et al.* 1988; Seale & Carney 1991; Thomsen & Caine 2011; Zombeck *et al.* 2010). B6-related strains, C57BR/cdJ and C57L/J, have previously been shown to exceed the response of B6 mice at 20 mg/kg and most other doses (Downing *et al.* 2003). Differences among closely related strains can be exploited for fine-mapping and gene identification as these strains carry larger segments of shared haplotype (Bailey *et al.* 2008; Eisener-Dorman *et al.* 2010; Kumar *et al.* 2013). The concordance between strain phenotypes described in this study and those reported by others indicates that at moderate doses, strains at the extreme ends of the phenotypic distribution are stable and replicable across laboratories. This is an important observation because behavioral traits can

be labile and vary under individual laboratory conditions making it difficult to generalize across studies (Crabbe *et al.* 1999; Sorge *et al.* 2014; Wahlsten *et al.* 2006).

Based on the literature suggesting that locomotor response to novelty predicts initial sensitivity to the locomotor effects of psychostimulants (Deroche *et al.* 1993; Hooks *et al.* 1991; Kosten & Miserendino 1998; Mantsch *et al.* 2001), we examined the correlation between locomotor response to the novel OF on Day 1 and locomotor response to COC. A significant positive correlation was detected indicating that strains with increased locomotor response to novelty also had a greater locomotor response to COC. This result is in agreement with similar observations of increased psychostimulant-induced locomotor response in rodents selected for response to novelty (Giorgi *et al.* 1997; Hooks *et al.* 1991; Kabbaj 2006; Piazza *et al.* 1989).

We also examined the role of locomotor habituation on response to COC by comparing the difference score between locomotor activity on Day 1 vs. Day 2 and COC-induced locomotor activation. A negative and borderline significant correlation was observed between habituation and COC-induced locomotor activation and indicated that strains with lower habituation scores were more activated by COC. The inability to habituate across testing sessions may reflect inherent strain differences in anxiety-like behavior or deficits in memory (Bolivar 2009; Muller *et al.* 1994). Interestingly, several of the strains that do not show habituation, including DBA/2J, BALB/c, CBA/J and BUB/BnJ, have been described as performing poorly in standard learning and memory tests such as the Morris water maze, fear conditioning and the eight-way radial arm maze (Crawley *et al.* 1997). It is interesting to speculate that strains with little or no habituation may perceive the OF as novel on both Days 1 and 2, and by extension, on Day 3, when they receive the drug and that this may result in higher locomotor response to the drug. Enhancement of psychostimulant-induced locomotor response in novel vs. familiar environments has been reported (Badiani *et al.* 1995a,1995b) and has important implications for models of relapse which depend on environmental cues (Badiani & Spagnolo 2013).

These data represent the first report of COC-induced locomotor activation in wild-derived strains. Wild-derived mice present challenges for conducting behavioral assays due to general ‘wildness’ (Wahlsten *et al.* 2003) defined as ease of capturing mice for testing and the ability of the animal to stay within a behavioral phenotyping enclosure. However, the added genetic diversity they provide has proven useful for identifying genomic loci involved in behavior (Logan *et al.* 2013). The wild-derived strains used in this study did not cluster at the high end of the phenotypic distribution for locomotor activity on either Day 1 or Day 2. These results are consistent with what has previously been reported (Wahlsten & Crabbe 2014) and indicate that wild-derived mice are not more active in the OF compared with standard inbred strains. Wild-derived strains are also not among the highest or lowest responders to COC. Upon closer examination, however, wild-derived strains cluster by subspecific background. The *Mus musculus domesticus* strains (Zalende/EiJ, WSB/EiJ and PERA/EiJ) cluster together near the lower end of the phenotypic range while the five wild-derived strains with a predominantly *Mus musculus musculus* background (Yang *et al.* 2011) cluster at the higher end of the phenotypic range. Interestingly, subspecific clustering was not observed for rearing behavior. PWD/PhJ (*musculus*) and Zalende/EiJ (*domesticus*) mice

exhibited the most extreme increase in rearing response to COC, contrary to the decrease observed for many other strains. It should be noted that rearing was the only behavior for which we saw significant batch effects. Because of our randomized design, we do not expect that batch effects highly biased the estimation of strain effects. However, we cannot fully eliminate this possibility.

The observation that COC and NOR levels in the brain correlate significantly, but modestly, with locomotor activation and average velocity has been reported by others (Benuck *et al.* 1987; Festa *et al.* 2004; Reith *et al.* 1987; Wiener & Reith 1990; Zombeck *et al.* 2010) and indicates that PK alone does not fully explain strain differences in COC-induced locomotor activation. Benzoylecgonine does not readily pass the blood brain barrier, but has been observed at very low levels in the brain of rats (Misra *et al.* 1974; Nayak *et al.* 1976) and mice (Benuck *et al.* 1987). It has been postulated that brain concentrations of BZE following subcutaneous injections of 20 mg/kg COC result from some passage of BZE into the brain as well as metabolism of COC that is present in the brain (Misra *et al.* 1975). However, previous studies have shown that BZE is inactive toward the dopamine transporter (Reith *et al.* 1986) and based on inactivity and low brain concentrations reported here, it is not surprising that BZE levels did not correlate with behavior.

The relatively low level of genetic correlation between brain concentration of COC and NOR and COC-induced locomotor behavior and average velocity across all strains indicates that the position of a strain on the phenotypic distribution can be explained only partially by PK parameters. It is likely that some strains sharing a similar behavioral response may not do so because of the same underlying mechanisms. For example, WSB and PERA mice have similar average locomotor responses to COC when activity data are summed across the entire 30 min. However, WSB mice exhibit a peak behavioral response immediately after drug administration while locomotor activation in PERA mice does not peak until 22 min post-drug administration (Fig. S2b). Interestingly, PERA mice have one of the highest levels of NOR in the brain at 10 min and a relatively low concentration of COC at the same time point. Norcocaine administered IP at doses similar to COC does not increase locomotor activity (Bedford *et al.* 1980; Elliott *et al.* 1987) and has even been shown to inhibit locomotor behavior in BALB/cByJ mice when administered systemically (Reith & Lajtha 1986; Reith *et al.* 1985). Therefore, it is possible that the high levels of NOR along with the low level of COC diminishes locomotor activation at earlier time points. Further necessary steps required for understanding these data and other mechanisms that determine location in the phenotypic distribution include assessing behavior and PK parameters with an expanded dose range and additional time points to accurately capture peak concentrations of COC and its metabolites in the brain. This study measures PK at only two time points. The initial 10-min time point was based on peak behavioral response from literature and observations in our laboratory. However, actual peak concentrations of COC in the brain likely occur at a much earlier time point (Benuck *et al.* 1987). Therefore, our calculation of AUC does not accurately capture the complete PK profile that might explain differences in behavior over the entire 30-min test.

This study is limited to a single dose but provides a starting point for further genetic, behavioral and pharmacological studies. Strains at the extreme ends of the phenotypic

distribution can be examined more closely to test specific hypotheses regarding the link between locomotor activation and the rewarding and reinforcing effects of COC (Orsini *et al.* 2004) or the relationship between drug reward and stress response pathways (Koob 2008). Phenotypically divergent strains can also be used for standard quantitative trait locus studies to identify loci that contribute to COC-induced locomotor response. Assessing complete PK profiles in strains for which PK differences appear to play a more prominent role in initial locomotor response will also provide information regarding the role of drug and metabolite disposition in behavioral differences. Finally, data collected in genetic reference populations, such as inbred mouse strains, can be drawn upon repeatedly as technology and resources expand, providing a lasting legacy on which to develop and test new hypotheses.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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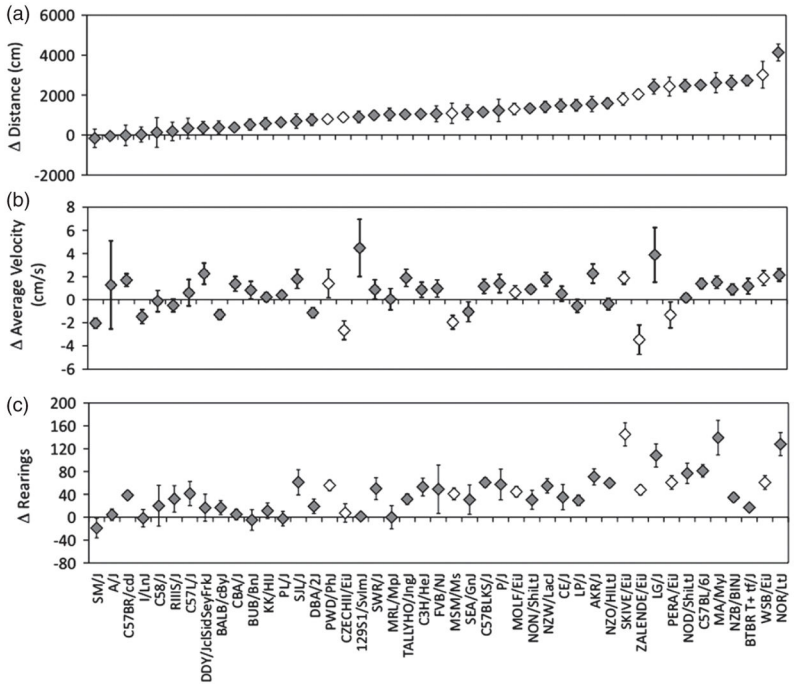

Figure 1. Open field habituation

Day 2 minus Day 1 difference scores for distance (a), average velocity (b) and rearing behavior (c). Each data point is the strain mean and error bars are SEM. Strains are sorted from lowest to highest for locomotor activity habituation. Open diamonds denote wild-derived strains and filled diamonds represent classical inbred strains.

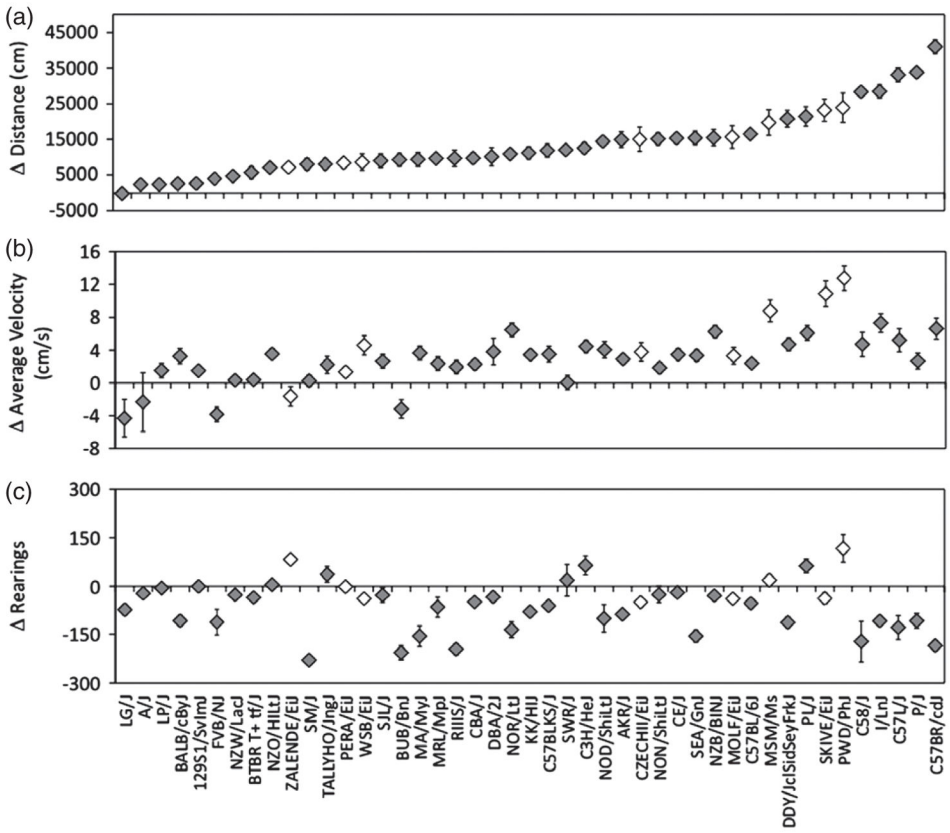

Figure 2. COC-induced OF behavior

Day 3 minus Day 2 difference scores for distance (a), average velocity (b) and rearing behavior (c). Each data point is the strain mean and error bars are SEM. Strains are sorted from lowest to highest for COC-induced locomotor activation. Open diamonds denote wild-derived strains and filled diamonds represent classical inbred strains.

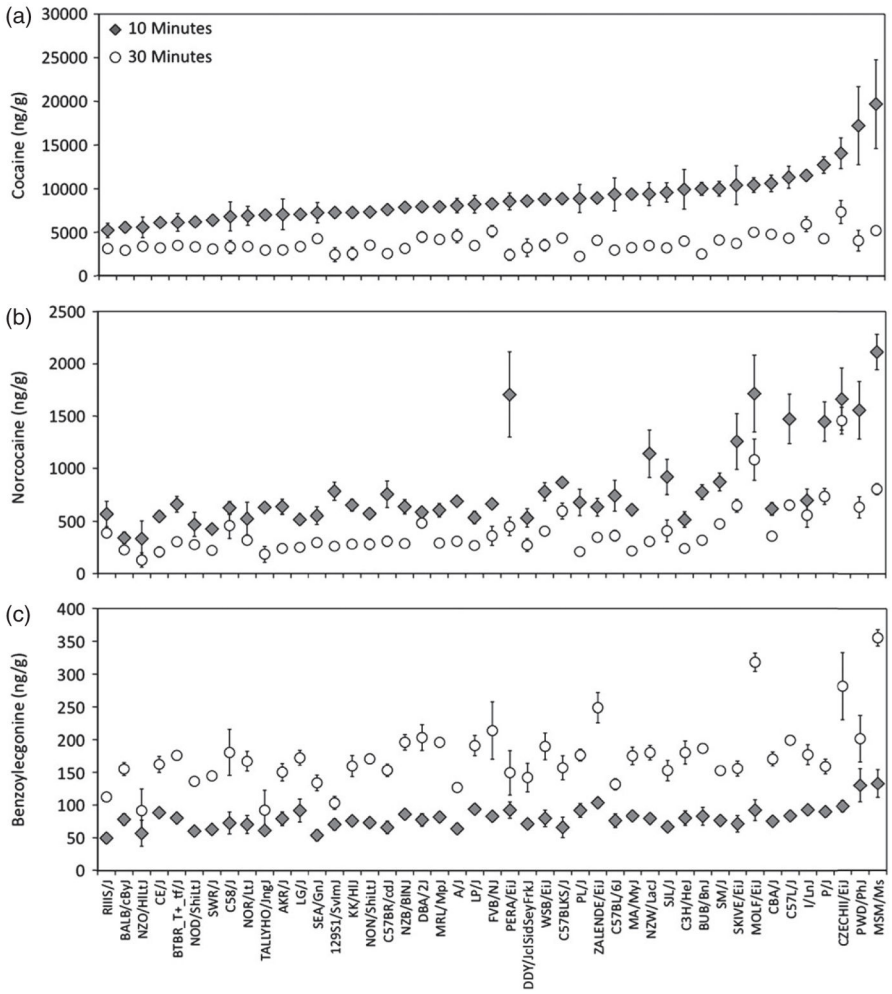

Figure 3. Brain pharmacokinetics

Brain concentrations of COC (a), NOR (b) and BZE (c) for all strains at 10 (gray diamonds) and 30 (open circles) minutes post-drug administration. Error bars are SEM.

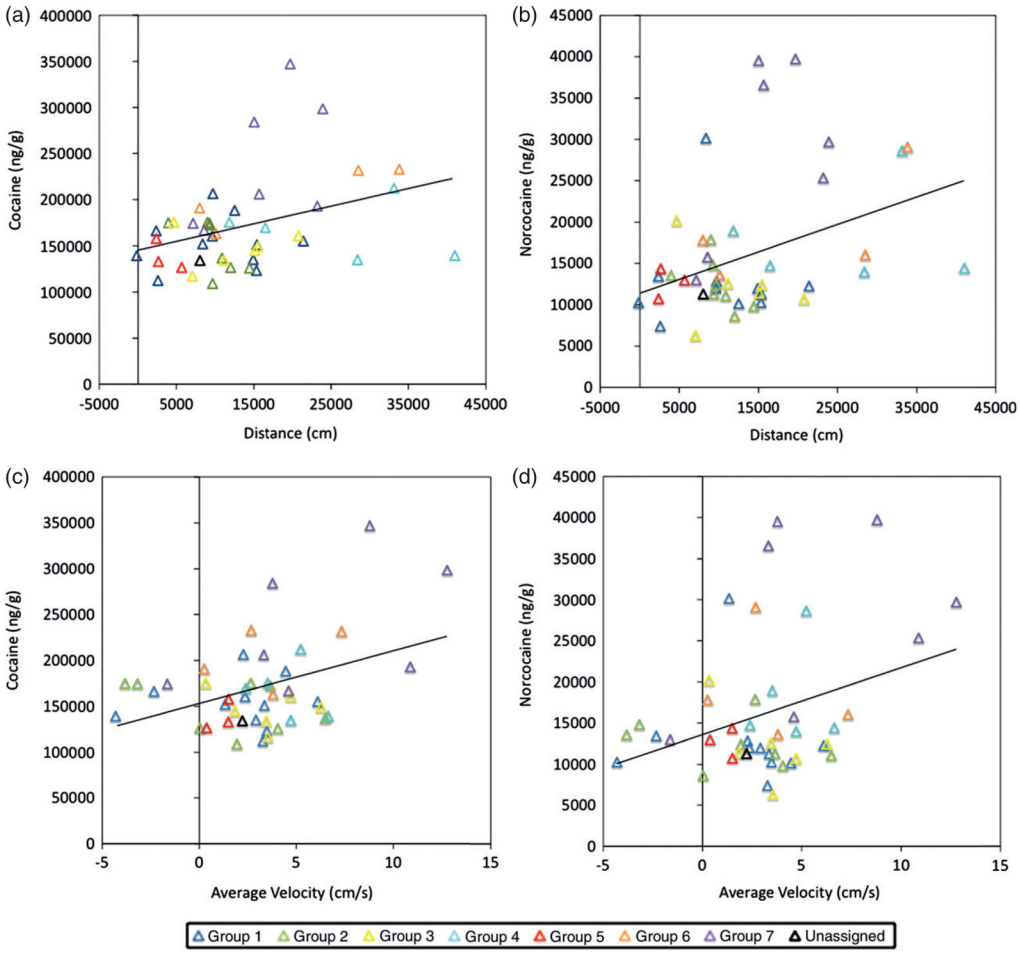

Figure 4. PK and behavior correlations

Correlation of brain concentrations of COC (a, b) and NOR (c, d) with locomotor activation and average velocity. Brain concentrations of COC and NOR are expressed as area under the curve as determined by a linear-up log-down function. Each triangle is a strain mean and triangles are color coded based on strain location in the Petkov (Petkov *et al.* 2004) phylogenetic tree.