A RODENT MODEL OF PHENOTYPIC VARIATION IN SWEET PREFERENCE,
ADDICTION VULNERABILITY, AND PHARMACOLOGICAL TREATMENT
SENSITIVITY

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Dedication

Dedicated to the memory of my father, Hubert Holtz.

Abstract

        Sweet preference is a stable, genetically-mediated trait that is associated with vulnerability to drug dependence in both human and non-human animals. For instance, rats that have been selectively bred for high intake of a saccharin solution (HiS) are more drug-prone than rats bred for low saccharin intake (LoS). The experiments detailed in this review investigated whether the HiS and LoS phenotypes would display differential effects of pharmacological agents on cocaine self-administration and cocaine-seeking behavior. Treatment effects (e.g., baclofen, progesterone, allopregnanolone, or histamine) were examined between the HiS and LoS rats during the following phases: escalation during long access (LgA) to the drug [Experiments 1 (baclofen) and 3 (progesterone)], including pre- and post-escalation short-access (ShA) periods, dose-response assessment, and cocaine-primed reinstatement of extinguished drug-seeking behavior [Experiments 2 (baclofen) and 4 (allopregnanolone)]. The last experiment investigated the effect of pharmacological treatment of steady-state cocaine self-administration; specifically, we used a punishment paradigm in which histamine was added to the i.v. cocaine self-administration solution (Experiment 5). We found that baclofen and progesterone decreased cocaine intake in LoS animals but increased cocaine intake in HiS animals. Baclofen attenuated cocaine-primed reinstatement of drug-seeking behavior in both HiS and LoS rats equally. In contrast, allopregnanolone potentiated low-dose cocaine-primed reinstatement in LoS rats, whereas it attenuated this response following moderate-dose cocaine priming injections in the HiS rats. Histamine was equally effective in punishing cocaine self-administration in both phenotypes;

however, LoS rats exhibited a delay in reaching baseline levels of self-administration following histamine punishment. By demonstrating that phenotypic variance in sweet preference can predict treatment sensitivity in substance-dependent individuals, these data can inform more effective, personalized treatment strategies.

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* work previously published

*Experiment 2: Effects of baclofen on the reinstatement of cocaine-seeking (relapse) in HiS and LoS rats

Holtz NA, Carroll ME (2011) Baclofen has opposite effects on escalation of cocaine self-administration: increased intake in rats selectively bred for high (HiS) saccharin intake and decreased intake in those selected for low (LoS) saccharin intake. Pharmacol Biochem Behav 100: 275-83

* Experiment 3: Effects of progesterone on the escalation (bingeing) of i.v. cocaine self administration in HiS and LoS rats

Anker JJ, Holtz, NA, Carroll, ME (2012) Effects of progesterone on escalation of intravenous cocaine self-administration in rats selectively bred for high or low saccharin intake. Behav Pharmacol 23: 205-10

Experiment 4: Effects of allopregnanolone on the reinstatement of cocaine-seeking (relapse) in HiS and LoS rats

Experiment 5: Effects of histamine punishment on cocaine self administration in HiS and LoS rats

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Introduction

A central question in drug abuse research pertains to why some people become addicts while others do not. It is clear that there is no single explanation; addiction vulnerability is the product of multiple interacting factors such as cultural/socioeconomic conditions, psychiatric liability, and genetic predisposition (Agrawal et al. 2012; Swendsen and Le Moal 2011). However, results from experiments designed to address this question are of little utility unless they ultimately guide more effective preventative and therapeutic measures. The introduction to this review will provide background on the contribution of genes to substance use disorders, and how this has been established in the human and non-human animal literature. It will then discuss the heritability of sweet preference and its relationship to addiction vulnerability, leading to the main research question addressed by this review: can sweet preference serve as a predictor for sensitivity to pharmacological interventions directed at cocaine self-administration and cocaine-seeking behaviors?

Human Studies

In humans, vulnerability to drug dependence is heterogeneous, as its determinants vary widely between individuals (Swendsen and Le Moal 2011). Of these determinants, genetic disposition has substantial influence. Initial studies showed that adopted children born from individuals with histories of drug dependence were more likely to also become drug dependent than those born from non-dependent parents (Cadoret et al. 1986; Goodwin et al. 1973). Subsequent comparisons of drug dependence between

monozygotic and dizygotic twins have provided a range of heritability estimates, accounting for between 33% to 79% of the variance in vulnerability between individuals (for review, see Agrawal and Lynskey 2008). Furthermore, different licit and illicit drugs offer unique estimates (Agrawal and Lynskey 2006; Kendler et al. 1999; Kendler and Prescott 1998; Prescott and Kendler 1999). Adoption and twin studies have elucidated a role for genes in dependence liability, although either approach has methodological limitations that may inflate heritability at the cost of environmental estimates (Miller et al. 2000; Richardson and Norgate 2005). Animal experiments, on the other hand, can control environmental factors, thus they offer convergent evidence for genetic influence in addiction vulnerability.

Animal Models and Selection of Behavioral Traits

Observations of drug use vulnerability in non-human mammals precede recent developments in the systematic application of animal models of drug self-administration (Higgins 2003). For example, Darwin noted in <u>The Descent of Man, and Selection in Relation to Sex</u> (Darwin 1874) that while "many kinds of monkeys have a strong taste for tea, coffee, and spirituous liquor… An American monkey, an Ateles, after getting drunk on brandy, would never touch it again, and thus was wiser than many men." Current research has expanded on this prescient observation using a variety of methodologies. One approach in the recent animal literature involves assessing variability in drug-related measures (e.g., self-administration, locomotor activity, etc.) in rats from genetically heterogeneous, outbred stocks (see Table 1). Since it is assumed that these rats are

derived from more or less random mating procedures, this strategy has the advantage of face validity by approaching the heterogeneity found in human populations. Deroche-Gamonet et al. (2004), for example, modeled multiple criteria for addiction diagnoses based on the DSM-IV (American Psychiatric Association. and Task Force on DSM-IV. 1994) in humans using an array of procedures, such as progressive-ratio responding (motivation), reinstatement (relapse), and resilience of shock-punished cocaine self-administration (persistence despite negative consequences). Outbred rats were the subjects in this study, and each rat underwent all procedures. The authors found great variability between the animals in meeting the various criteria, with distributions closely modeling human epidemiological trends such that only a minority met all three (National Center for Health Statistics (U.S.) and National Center for Health Statistics (U.S.). 2011). A more recent study has confirmed this pattern, showing that only a small percentage of rats prefer drugs to other non-drug rewards, regardless of drug-taking history (Cantin et al. 2010). As environmental conditions were equivalent between animals, these data support the role of genes in addiction vulnerability.

Table 1 **Summary of studies on selected individual differences and their effects on drug- and food- motivated behavior**

Selection criterion (High vs. Low vulnerability)	Drug-related behavior	Drug/Reinforcer	Reference
Novel environment reactivity (HR vs. LR)	Drug-induced locomotor activity (HR>LR)	Amphetamine	Piazza et al. (1990)
		Morphine	Deroche et al. (1993)
	Self administration (HR>LR)	Amphetamine	Piazza et al. (1990)
Impulsivity (delay discounting; HiI vs. LoI)	Acquisition (HiI>LoI)	Cocaine	Perry et al. (2008)
	Acquisition (HiI>LoI)	Ethanol	Poulos et al. (1995)
	Escalation (HiI>LoI)	Cocaine	Anker et al. (2009b)
	Reinstatement (HiI>LoI)	Cocaine	Perry et al. (2008)
Impulsivity (5CSRTT; HiI vs. LoI)	Acquisition (HiI>LoI)	Cocaine	Dalley et al. (2005)
	Self-administration (HiI>LoI)	Cocaine	Dalley et al. (2005)
	Reinstatement (HiI>LoI)	Cocaine	Economidou et al. (2009)
Incentive salience (ST vs. GT)	Self-administration (ST>GT)	Cocaine	Saunders and Robinson (2011)
	Reinstatement (ST>GT)	Cocaine	Saunders and Robinson (2011)
	Locomotor sensitization (ST>GT)	Cocaine	Flagel et al. (2008)
	Impulsivity (2CSRTT; GT>ST)	Food	Lovic et al. (2011)
Wheel Running (HiR vs. LoR)	Self administration (HiR>LoR)	Cocaine	Larson and Carroll (2005)
	Reinstatement (HiR>LoR)	Cocaine	Larson and Carroll (2005)

HR and LR, high and low novelty responders; HiI and LoI, high and low impulsive; ST and GT, sign-trackers and goal-trackers; HiR and LoR, high and low wheel-runners

Furthermore, variability in drug-seeking behavior has been associated with individual differences in other stable, genetically-mediated traits associated with addiction liability in humans (see Table 1). For example, activity level in a novel environment is a stable behavior that corresponds with human characteristics linked to drug abuse liability or resilience (Blanchard et al. 2009; Davis et al. 2008), such as sensation seeking (Howard et al. 1997), stress response (Lovallo 2006), and aspects of impulsivity such as behavioral disinhibition (Iacono et al. 2008) and the discounting of delayed rewards (Mackillop et al. 2011).

In a seminal study by Piazza et al. (1989), outbred Sprague-Dawley rats exhibited significant variation in locomotor activity induced by a novel environment, and this behavior was positively correlated with amphetamine-induced locomotor activity and amphetamine self-administration. The results from this study have been expanded across multiple drug classes and animal models of addiction, with the high novelty reactive rats, or high responders (HR), displaying drug-prone profiles and the low novelty responders (LR) showing drug-resilient profiles (Kabbaj 2006).

Additional investigators have screened rats on behavioral measures that do not directly assess drug use or response and have established a positive relationship with measures that involve drugs. Typically, there is a direct relationship between assessment of certain drug (e.g., self-administration) - and nondrug (e.g., novel response)-related behaviors, such that high measures on one parameter predict high measures on the other. For example, rats screened for high impulsivity using a delay discounting task for food also acquired cocaine self administration (Perry et al. 2005) and escalated cocaine intake

at faster rates (Anker et al. 2009b) compared to rats screened for low impulsivity. Using

another measure of impulsivity, the five choice serial reaction time task (5-CSRTT),

Economidou et al. (2009) showed that rats screened for high impulsivity reinstated drug-

seeking behavior despite punishment for longer periods than rats screened for low

impulsivity. This relationship holds for other procedures that model traits that vary

between individuals, such as attribution of incentive salience to reward-related stimuli

(sign trackers – ST) vs. attention focused on reward location (goal trackers – GT)

(Saunders and Robinson 2011) and varying interest in natural rewards such as high (HiR)

or low (LoR) levels of physical activity (Larson and Carroll 2005).

Selective Breeding of Behavioral Traits in Animals

Another approach to investigating genetically-mediated differences in addiction

vulnerability involves breeding rodents based on bidirectional behavioral criteria (see

Table 2). By mating animals that exhibit extremely high or low measures, these studies

illustrate that the behavior of interest is under genetic influence because successive

generations show more stable and robust corresponding behaviors. A prominent example

of this procedure is found in alcohol research in which breeding for differential drug

intake was conducted by selecting animals on measures of high and low ethanol intake

(Mardones et al. 1953). Since then, multiple rodent strains have been produced based on

varied behavioral and physiological responses to alcohol in humans (Crabbe 1984; Li et

al. 1993). Such criteria have included alcohol consumption (Foroud et al. 2000; McBride

and Li 1998), sensitivity to withdrawal (Crabbe et al. 1985), ethanol-induced

hypothermia (Cunningham et al. 1991), and locomotor reactivity (Phillips and Dudek 1991).

Table 2 **Summary of studies on selectively-bred individual differences and their effects on drug- and food- motivated behavior**

Selective-breeding criterion (High vs. Low vulnerability)	Drug-related behavior	Drug/ Reinforcer	Reference
Ethanol consumption (HAC vs. LAC)	Impulsivity (delay discounting; HAC>LAC)	Saccharin	Oberlin and Grahame (2009)
	Impulsivity (probabilistic discounting; HAC>LAC)	Sucrose	Wilhelm et al. (2007)
	Impulsivity (response disinhibition; HAC>LAC)	Sucrose	Wilhelm et al. (2007)
	Exercise (HAC>LAC)	Wheel-running	Riley et al. (1977)
Novel environment reactivity (HR vs. LR)	Acquisition (HR>LR)	Cocaine	Davis et al. (2008)
Active-avoidance learning (RHA vs. RLA)	Acquisition (RHA>RLA)	Cocaine	Fattore et al. (2009)
	Locomotor Sensitization (RHA>RLA)	Cocaine	Pisula (2003)
	Impulsivity (delay discounting; RHA>RLA)	Food	Moreno et al. (2010)
	Impulsivity (response disinhibition; RHA>RLA)	Food	Moreno et al. (2010)
	Novel environment reactivity (RHA>RLA)	—	Pisula (2003)
N/A (inbred strains; LEW vs. F334)	Acquisition	Cocaine	Kosten et al. (1997)
	Self-administration	Cocaine	Haile et al. (2001)
		Ethanol	Suzuki et al. (1988)
	Locomotor sensitization	Cocaine	Kosten et al. (1994)
	Reinstatement	Cocaine	Kruzich and Xi (2006)

HAC and LAC, high and low alcohol consumers; HR and LR, high and low novelty responders; RHA and RLA, Roman high and low avoidance; LEW and F344, Lewis and Fischer F344

The selective breeding approach has also been used to differentiate animals by their avidity for certain drugs, such as diazepam (Gallaher et al. 1987), opiates (Belknap et al. 1983), methamphetamine (Atkins et al. 2001; Wheeler et al. 2009), nicotine (Smolen et al. 1994), and cocaine (He et al. 2008). Similar to studies that selected outbred animals for behaviors directly or indirectly related to the behavioral effects of drugs, animals selected for high and low addiction vulnerability also display related features in the behavioral measures related to drug abuse vulnerability. For example, animals bred for high (vs. low) alcohol consumption also displayed greater impulsivity following delay and probabilistic discounting procedures (Oberlin and Grahame 2009; Wilhelm et al. 2007), as well as impaired response inhibition (Wilhelm et al. 2007). High alcohol-consuming animals also showed greater avidity for natural rewards such as physical activity (Riley et al. 1977).

Furthermore, the selective breeding procedure has also been applied to behaviors related to addiction. For example, one bidirectional criterion is novelty induced-locomotor activity, the same measure that had a positive relationship with response to amphetamine by Piazza et al. (1990) in outbred rats. Rats selectively bred for high activity in this paradigm acquired cocaine self-administration more rapidly than those bred for low activity (Davis et al. 2008). Another example of selected lines that exhibit behavioral dimensions linked to drug abuse vulnerability are the Roman high (RHA) - and low (RLA)-avoidance rat lines. This selection criterion is based on the learning rate of active avoidance in a shuttle box, and it has been proposed to model differential emotional coping patterns to aversive events (Giorgi 2006). High-avoidance rats also display an array of behaviors indicative of drug abuse vulnerability, such as high

impulsivity (Moreno et al. 2010) and high responsivity to a novel environment (Pisula 2003) relative to the low avoidance line. Accordingly, the high-avoidance rats showed greater behavioral sensitization to cocaine (Pisula 2003), more robust responding during the acquisition of cocaine self-administration, stronger resistance to extinction, and greater reacquisition to a low dose of cocaine compared to their low-avoidance counterparts (Fattore et al. 2009). Interestingly, the high-avoidance rats also showed greater response to natural rewards, such as environmental enrichment (Fernandez-Teruel et al. 2002).

Another model that warrants discussion involves the Lewis (LEW) and Fischer 344 (F344) inbred rat strains. While not selectively bred for drug-related behaviors, this model has been extensively studied in relation to genetically-mediated drug abuse vulnerability and related biochemical markers. Thus, it serves as an important reference (Carroll et al. 2008; Kosten and Ambrosio 2002). Some drug-related behaviors in which these strains differ (LEW>F334) are rates of cocaine self-administration acquisition (Kosten et al. 1997), maintenance of cocaine self-administration (Haile et al. 2001), sensitization to cocaine's behavioral effects (Kosten et al. 1994), reinstatement of cocaine-seeking following extinction (Kruzich and Xi 2006), and alcohol consumption (Suzuki et al. 1988).

The fundamental principle unifying the literature described so far is that addiction vulnerability and certain types of behaviors reliably co-vary, and it is probable that they are mediated by common underlying mechanisms. This principle has been well-studied with a selective breeding procedure employing differential response to sweets.

Sweet Preference and Addiction: Human Studies

Avidity for sweet consumption is positively related to substance use disorders in human populations. For instance, those who abuse cocaine (Janowsky et al. 2003), nicotine (Pepino and Mennella 2007; Pomerleau et al. 1991), opioid (Weiss 1982), and alcohol (Kampov-Polevoy et al. 1997; Kampov-Polevoy et al. 2001; Wronski et al. 2007) experience greater hedonic effects of sweetened dietary substances than those who do not abuse these drugs. Similar to drug addiction vulnerability, variability in response to sweets is a stable trait that also has a heritable influence (Desor and Beauchamp 1987; Keskitalo et al. 2007a; Keskitalo et al. 2008; Keskitalo et al. 2007b; Reed et al. 1997; Uhl et al. 2009). It has been proposed that these differences in sweet preference are not predominantly mediated by genetic variation in coding for peripheral taste processing (i.e., taste receptors); rather, differences in reward processing are related to the central nervous system (Bachmanov et al. 2011; Lu et al. 2005). Further, both alcohol-naïve individuals and alcoholics with familial histories of alcoholism display greater sweet preference than those without family histories of alcoholism (Kampov-Polevoy et al. 2003). This effect is attributable to sensitivity to sweets and not necessarily increased sensitivity to tastes (e.g., bitterness) in general. For instance, alcohol-naïve children of alcoholics are less likely to be sensitive to the bitter taste of 6-n-propylthiouracil (PROP) (Pelchat and Danowski 1992) than children from nonalcoholic families. Individuals who are less sensitive to PROP find alcohol less bitter and consume greater amounts of it (Pelchat and Danowski 1992). Together, these findings suggest that addiction vulnerability and sweet preference are at least partially determined by common genetic factors that code for reactivity to rewarding nondrug events.

Sweet Preference and Addiction: Animal Models

Many of the animals screened or selectively bred to show divergence in drug seeking or drug response also show divergence in avidity for sweet substances, as summarized in Table 3. For instance, the selected HR rats respond more robustly for sucrose (Klebaur et al. 2001) compared to LR rats, while rats selected for high impulsivity using the 5-CSRTT show more operant responding for sucrose compared to low impulsive rats (Diergaarde et al. 2009). Additionally, rats selectively-bred for high rates of active avoidance learning (RHA) consume more sweetened dietary substances, such as saccharin solution, compared to those selectively-bred for low rates of avoidance learning (RLA) (Fernandez-Teruel et al. 2002; Razafimanalina et al. 1996). Rodents selected from outbred stocks or selectively bred for high and low alcohol consumption ingest high and low amounts of sucrose and saccharin solutions, respectively (Grahame et al. 1999; Overstreet et al. 1993; Sinclair et al. 1992; Stewart et al. 1994). Conversely, rats screened for high consumption of sweet substances ingest more ethanol (Gahtan et al. 1996), amphetamine (DeSousa et al. 2000), and morphine (Gosnell et al. 1995) than rats screened for low ethanol intake. However, it should be noted that rats screened for high and low impulsivity with the delay-discounting task do not display a difference in saccharin intake (Perry et al., unpublished data), suggesting that sweet preference does not completely overlap with all drug vulnerability factors. Nonetheless, taken together with human research, results from these models display a clear relationship between sweet intake and addiction vulnerability that strongly implicates some shared, genetically-mediated biological mechanisms in these behaviors.

Table 3 **Summary of studies on selected individual differences for sweet preference and their effects on drug- and food- motivated behavior**

Selective-breeding/selection criterion (High vs. Low vulnerability)	Behavior	Sweet Substance/Drug	Reference
Novel environment reactivity (HR vs. LR)[b]	Operant responding (HR>LR)	Sucrose	Klebaur et al. (2001)
Impulsivity (5CSRTT; HiI vs. LoI)[b]	Operant responding (HiI>LoI)	Sucrose	Diergaarde et al. (2009)
Active-avoidance learning (RHA vs. RLA)[a]	Consumption (RHA>LHA)	Saccharin	Fernandez-Teruel et al. (2002)
Ethanol consumption (HAC vs. LAC)[a]	Consumption (HAC>LAC)	Saccharin	Grahame et al. (1999)
	Consumption (HAC>LAC)	Saccharin	Sinclair et al. (1992)
	Consumption (HAC>LAC)	Sucrose	Stewart et al. (1994)
Ethanol consumption (HAC vs. LAC)[b]	Consumption (HAC>LAC)	Saccharin	Overstreet et al. (1993)
Sweet preference (SL vs. SDL)[b]	Consumption (SL>SDL)	Ethanol	Gahtan et al. (1996)
	Self-administration (SL>SDL)	Amphetamine	Desousa et al. (2000)
	Self-administration (SL>SDL)	Morphine	Gosnell et al. (1995)

HR and LR, high and low novelty responders; HiI and LoI, high and low impulsivity; HAC and LAC, high and low alcohol consumers; SL and SDL, sweet likers and sweet dislikers
[a] Selectively bred
[b] Selected from outbred stocks

Selective Breeding for High (HiS) and Low (LoS) Saccharin Intake

While investigating genetic influence on variability in response to sweets, Nachman (1959) was the first to illustrate the heritability of saccharin preference by employing a selective breeding program in which rats were mated based on consumption of a saccharin solution relative to water in a 2-bottle choice test. Subsequent experiments using inbred strains of mice further supported the heritability of saccharin preference (Capeless and Whitney 1995; Lush 1989; Pelz et al. 1973; Ramirez and Fuller 1976); however, none of these studies investigated additional behavioral features in these animals. Later, Dess and Minor (1996b) selectively bred rats (Holtzman Sprague-Dawley, Indianapolis, Indiana, USA) for high and low saccharin intake using a 2-bottle choice task detailed in the General Methods section.

Originally, the high and low saccharin-consuming lines, now called Occidental (Occidental College, Los Angeles, CA) HiS and LoS rats, were used to investigate the interaction between genetically mediated sweet preference and measures of emotionality. Subsequently, Dess and colleagues investigated ethanol intake between the HiS and LoS rats, since rats selectively bred for high and low ethanol consumption also consumed high and low amounts of saccharin, respectively (Overstreet et al. 1993; Sinclair et al. 1992). As predicted, the HiS rats consumed more alcohol than LoS rats in free-choice and forced-consumption tests (Dess et al. 1998), and these results prompted further investigation into differences between the lines with regard to other drugs of abuse.

Thus, a second colony was established from the Occidental HiS and LoS lines at the University of Minnesota, in which the primary interest was cocaine-self

administration across various phases of the addiction model, although other drugs (e.g., heroin) and assays of drug-vulnerability (e.g., delay discounting, drug-induced locomotor activity, etc.) have been employed. These experiments have largely shown the HiS and LoS rats to have drug-prone and -resilient profiles, respectively. Furthermore, the HiS and LoS rats also display differential behavioral profiles on other addiction-related measures, such as novelty reactivity (Dess and Minor 1996b) as well as motor (Anker et al. 2008) and choice (Perry et al. 2007) impulsivity. Given that these are common features in human and animal addiction vulnerability research, the HiS and LoS rats provide an exemplary animal model of genetically mediated addiction proneness and resilience. A review of experiments conducted until 2007 in both colonies of the Occidental lines substantiated the drug-proneness and resilience of the HiS and LoS rats, respectively, and also showed that these characterizations may interact with other vulnerability factors such as age and sex (Carroll et al. 2008). A selective summary of studies investigating drug-related behavior in these lines is given in Table 4.

Table 4 **Summary of results from studies on selectively bred HiS and LoS rats and drug-related behavior**

Behavioral Model	Drug/Reinforcer	Phenotype effects	Sex differences	Reference
Acquisition	Cocaine	HiS>LoS	M only	Carroll et al. (2002)
Escalation	Cocaine	HiS>LoS	F only	Perry et al. (2006)
Impulsivity (Delay discounting)	Food	HiS>LoS	F>M	Perry et al. (2007)
Impulsivity (Response disinhibition)	Cocaine	HiS>LoS	F>M	Anker et al. (2008)
Reinstatement	Cocaine	HiS>LoS	F only	Perry et al. (2006)
Drug-induced locomotor activity	Cocaine	HiS>LoS	F>M (HiS)	Carroll et al. (2007)
Drug-induced locomotor sensitization	Cocaine	HiS>LoS (F)	F>M	Carroll et al. (2007)

F, female; HiS, high saccharin; LoS, low saccharin; M, male

Table 5 shows reported saccharin scores from studies conducted after 2007 and group means from the Carroll and Dess laboratories through 2008, as well as scores from 2010-2012. Group means were derived by averaging reported scores from each experiment from either laboratory within the noted timeframes. Scores have remained relatively consistent between previous and recent studies conducted in the Carroll laboratory, although scores from the Dess laboratory tend to be more divergent between the phenotypes. Because many procedures used in the Carroll laboratory involve surgical i.v. catheter implantation, older (90-120 days) rats are used compared to those used in the Dess laboratory (60-90 days), and many rats have experience with cocaine before saccharin testing. These factors may account for inter-laboratory differences.

Table 5 **Mean saccharin phenotype scores from recent studies in groups of HiS and LoS rats**

Females		Males		
HiS	LoS	HiS	LoS	References
—	—	24.3	4.6	Gosnell et al. (2010)
21.5	7.9	—	—	Anker et al. (2009)
47.0	4.0	—	—	Yakovenko et al. (2011)
23.1	8.0	—	—	Anker and Carroll (2011a)
36.6	16.9	12.6	9.8	Radke et al. (submitted) (exp 1)
27.6	22.2	11.7	11.6	Radke et al. (submitted) (exp 2)
43.0	26.7	13.4	6.9	Radke et al. (submitted) (exp 3)
24.1	10.4	—	—	Holtz and Carroll (2012) (exp 1)[a]
18.1	0.9	—	—	Holtz and Carroll (2012) (exp 2)[b]
20.2	6.5	—	—	Holtz et al. (in preparation) (adults)
36.9	32.7			Holtz et al. (in preparation) (adolescents)
24.6	13.9	—	—	Holtz and Carroll (submitted) (adults)
38.4	20.1	—	—	Holtz and Carroll (submitted) (adolescents)
19.9	10.1	—	—	Regier et al. (2012)
21.5	8.1	—	—	Anker et al. (2012)[c]
21.1	8.7	—	—	Holtz and Carroll (in preparation)[d]
28.5	22.6	—	—	Holtz et al. (in preparation)[e]
28.3	13.7	15.5	8.2	Group means from 2010-2012
35.1	10.1	18.8	4.7	Group means from Carroll Lab through 2008
44.1	5.1	31.5	6.1	Group means from Dess Lab through 2008

HiS, high saccharin; LoS, low saccharin; a, b, c, d and e correspond with Experiments 1,2,3,4 and 5 in this review

One important consideration pertains to the specificity of phenotypic selection between the HiS and LoS lines. That is, the greater sweet consumption, drug-seeking and self-administration behavior observed in the HiS animals may not be the result of selective breeding for differential sensitivity to rewarding (or aversive) events per se, but may result from differential breeding for high and low general activity levels. Thus, while it appears that progenitor HiS rats and their offspring are more prone to consume highly-palatable substances compared to the LoS rats, such differentiation may simply be a cumulative artifact of increased, nonspecific movement within that phenotype. Contrary to this notion, the very criterion of selective breeding is a choice between a bottle containing water and one containing a liquid solution that is preferred to water under normal conditions. If the hypothesis were true that the HiS rats consume more saccharin compared to LoS rats because drinking behavior falls under the rubric of general activity, we would also expect the HiS rats to consume more water in general. In this case, we would see no differences in saccharin phenotypes scores (which account for water consumption; saccharin score determination detailed in General Methods section). However, it may be argued that both lines have an equivalent preference for saccharin solution compared to water baseline, and the putatively increased general activity levels of the HiS rats would result in a shorter latency of initial saccharin consumption within the preference test. In this case, HiS rats would effectively have more time to consume saccharin within a test, resulting in the divergent scores. Given the length of access to saccharin solution (24 h) this scenario seems unlikely, but as of yet it is unexamined.

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Distinguishing the underlying cause of the HiS and LoS phenotypes may best be resolved by their performance on a task in which the outcome measure is independent of rate or latency, namely a discrete, operant choice task (Bitterman 1962). The delay discounting procedure is such a task that yields an outcome measure considered to be a metric of choice impulsivity, and high-ratings of impulsivity are often associated with greater drug vulnerability (Perry and Carroll 2008). During the discrete experimental trial within the delay discounting session, a choice is presented in which the animal can press only one of two levers to receive either a small, immediate reward (e.g., one food pellet) versus a larger, more delayed reward (e.g., three food pellets). In this design, the lever associated with the greater, more delayed reinforcer alternates between experimental sessions. HiS rat have shown stable, low mean average delays (MAD) when averaged across the trials during a session, and are considered to display higher impulsivity (i.e., more sensitive to temporal discounting of a larger reward), while LoS rats display high MADs indicative of lower impulsivity (Perry et al. 2007). Since the average delay measure depends on the animal's choice and not its rate or latency of response, we can say that the phenotypic differentiation of the HiS and LoS lines shows greater specificity than simple variation in general activity levels (e.g., response rate, latency, etc.).

***Pharmacological Treatment Sensitivity in Rats with Differential Drug-Seeking
Behaviors***

Biologically determined individual differences play a role in pharmacological
treatment sensitivity in animal models of drug abuse (see Table 6). For instance,
spiradoline, a κ-opioid agonist, produced a greater reduction in cocaine-induced
locomotor activity in female mice compared to males (Sershen et al. 1998), and a similar
drug, bremazocine, decreased phencyclidine self-administration under a fixed ratio
schedule of reinforcement more robustly in female rhesus monkeys compared to males
(Cosgrove and Carroll 2004). Furthermore, the $GABA_B$ agonist, baclofen, had a greater
effect of lowering acquisition rates of i.v. cocaine self-administration in female rats
compared to male rats (Campbell et al. 2002), and ketoconazole, a corticosterone
synthesis inhibitor, suppressed heroin self-administration more in female than in male
rats (Carroll et al. 2001). Sex differences in treatment sensitivity also extend to non-
pharmacological interventions. For example, access to a running wheel decreased
cocaine self administration more in female rats compared to males (Cosgrove et al.
2002), and access to saccharin reduced phencyclidine intake more in female compared to
male rhesus monkeys (Cosgrove and Carroll 2003). While the scope of individual
differences in these examples is limited to sex, together they offer an experimentally
tractable link between neurobiological differences that underpin both addiction severity
and treatment sensitivity.

Table 6 **Sex differences in pharmacological and behavioral treatment effects**

Behavioral Model	Drug	Treatment	Treatment Effect	Reference
Acquisition	Cocaine	Baclofen	F>M	Campbell et al. (2002)
Drug-induced locomotor activity	Cocaine	Spiradoline	F>M	Sershen et al. (1998)
Self-administration	Heroin	Ketoconazole	F>M	Carroll et al. (2001)
Self-administration	Phencyclidine	Bremazocine	F>M	Cosgrove et al. (2004)
Self-administration	Cocaine	Running wheel	F>M	Cosgrove et al. (2002)
Self-administration	Phencyclidine	Saccharin	F>M	Cosgrove et al. (2003)

F, female; M, male

Similar to sex, variance in autosomal genomic regions is likely a strong determinant in substance abuse behaviors, and pharmacological treatment outcomes for addiction may likewise be influenced by genetic factors. This assumption drives the developing field of pharmacogenetics, which proposes that polymorphisms within an individual's genome may predict treatment outcome and can therefore guide treatment choices. The application of pharmacogenetics has been investigated for a number of psychiatric illnesses (Costa 2012), such as schizophrenia (Burdick et al. 2010), depression (Gvozdic et al. 2012; Schosser and Kasper 2009), attention-deficit/hyperactivity disorder (Kieling et al. 2010), and substance abuse disorders (Sturgess et al. 2011). For instance, initial clinical evidence showed that polymorphisms of the Asp40 allele in the gene encoding for the μ-opioid receptor (OPRM1) predicted the efficacy of naltrexone administration on abstinence and reduction in alcohol craving in alcoholics (Anton et al. 2008; Oroszi et al. 2009), as well as the reduction of positive

subjective response to and self-administration of alcohol in individuals not diagnosed with alcoholism (Setiawan et al. 2011). These findings have also been supported by preclinical work in which a single nucleotide polymorphism in the OPRM1 gene was associated with the dose-dependent effects of naltrexone on the reduction of alcohol intake in rhesus monkeys (Vallender et al. 2010).

Importantly, the convergence of behavioral, neurobiological, and genetic assessment is a promising and rapidly developing strategy for the treatment of psychiatric illnesses like substance dependence (Costa 2012; Hulse 2012). For instance, while genotype may predict naltrexone's efficacy in treating alcoholism, associated phenotypic behavioral markers can also be informative. Garbutt et al. (2009) and Laaksonen (2011) showed that individuals with a preference for high concentrations of sucrose respond more favorably to naltrexone treatment of alcoholism compared to those preferring lower concentrations. As previously stated, sweet preference is under heritable influence, and an ideal animal model would promote analysis of the relationship between this genetically-mediated behavioral marker of addiction vulnerability, differences in treatment sensitivity, as well as underlying neurobiological functions and genetic information.

The HiS and LoS rats fit these criteria for such a model, and the following experiments are examples that establish evidence of differential pharmacological treatment sensitivity between the phenotypes. Similar to the Vallender et al. (2010) study with rhesus monkeys, one eventual goal of these investigations is to elucidate relevant homologous genetic indicators and related neurobiological functions that may inform

pharmacogenetic approaches to addiction. The major focus of addiction pharmacogenetics has been on alcoholism; however, given that there is no approved pharmacological treatment for stimulant addiction, the present studies focused on treatment interventions using models of cocaine addiction. Thus, the fundamental question investigated in these studies is whether phenotypic differences in sweet preference and substance dependence vulnerability can be used to predict pharmacological treatment sensitivity.

The effects of pharmacological treatments (baclofen, progesterone, allopregnanolone, or histamine) were examined between the HiS and LoS rats during the following phases: escalation during long access (LgA) to the drug [Experiments 1 (baclofen) and 3 (progesterone)], including pre- and post-escalation short-access (ShA) periods, dose-response assessment, and cocaine-primed reinstatement of extinguished drug-seeking behavior [Experiments 2 (baclofen) and 4 (allopregnanolone)]. The last experiment investigated the effect of pharmacological treatment of steady-state cocaine self administration; specifically, we used a punishment paradigm in which histamine was added to the i.v. cocaine self-administration solution (Experiment 5). Table 7 summarizes the recent differences in treatment response found between the HiS and LoS phenotypes.

Table 7 **Differences in pharmacological treatment effects between rats selectively bred for high (HiS) and low (LoS) saccharin intake**

Behavioral Model	Drug/Reinforcer	Treatment	Treatment Effect	Reference
Escalation	Cocaine	Baclofen	LoS>HiS	Holtz and Carroll (2011)[a]
		Progesterone	LoS>HiS	Anker et al. (2012)[c]
Reinstatement	Cocaine	Baclofen	HiS=LoS	Holtz and Carroll (2011)[b]
		ALLO	HiS>LoS	Holtz and Carroll (in preparation)[d]
Fixed and Progressive Ratio	Sucrose	Naltrexone	HiS=LoS	Gosnell et al. (2010)
	Cocaine	Histamine	LoS>HiS	Holtz et al. (in preparation)[e]

ALLO, allopregnanolone; HiS, high saccharin; LoS, low saccharin; a, b, c, d and e correspond with Experiments 1,2,3,4 and 5 in this review

General Methods

Subjects

A total of 124 HiS and LoS female rats approximately 90–120 days old served as subjects in the following experiments. These rat lines were derived from a selective breeding program originating from Occidental College (Los Angeles, CA) that was subsequently propagated at the University of Minnesota (Minneapolis, MN). Rats were bred and pair-housed in plastic cages with ad libitum access to rat chow (Purina Mills, Minneapolis, MN, USA) and water prior to the experiments. Phenotypic differences in avidity for sweet substances were the primary interest in the these experiments, and HiS and LoS females were used exclusively because they display a wider range of saccharin intake and other behavioral measures compared to HiS and LoS males (Carroll et al.,

2008). After surgical implantation of i.v. cannulae, rats were placed in operant-

conditioning chambers and allowed to recover for 3 days (including the day of surgery).

The rats remained in the chambers for the duration of the self-administration studies,

wherein they continued to have free access to water and were fed 16 g of ground food

(Purina Laboratory Chow) daily following experimental sessions to maintain consistency

in food intake across subjects. Following self-administration procedures, animals were

removed from operant-conditioning chambers and returned to plastic cages for 14 days

with ad libitum access to food and water. After this period, saccharin preferring

phenotypes were confirmed using the saccharin preference test described below (see

Table 5 for saccharin scores). Humidity, temperature (21–23 ◦C), and light–dark cycle

(12–12 h; lights on at 6:00 a.m.) were regulated throughout all housing conditions. All

procedures followed guidelines established by the National Research Council (National

Research Council (U.S.). Committee for the Update of the Guide for the Care and Use of

Laboratory Animals. et al. 2011) and were approved by the University of Minnesota

Institutional Animal Care and Use Committee under protocol # 1008A87754. Animal

housing and laboratory facilities were accredited by the Association for the Assessment

and Accreditation of Laboratory Animal Care (AAALAC).

Saccharin Intake Measurement

Saccharin phenotype scores were derived from a 48-hr, 2-bottle preference test.

To obtain an estimate of water intake that was not influenced by the availability of

saccharin in a 2-bottle test, rats only had access to one bottle containing water during the

first 24-hr period, and water consumption during this period constituted the 24-hr water

intake baseline. Over the next 24 hrs, rats had access to both a water bottle and a bottle containing a 0.1% (w/v) saccharin solution. This was to allow free choice of saccharin intake that was not influenced by thirst, and saccharin consumption over this period constituted the 24-hr saccharin intake measure. The following equation illustrates how a saccharin phenotype score is derived from these measures:

$$\text{Saccharin phenotype score} = \frac{\text{24-hr saccharin intake (mL)} - \text{24-hr water intake (mL)}}{\text{Body weight (g)}} \times 100$$

Saccharin intake less baseline water intake is divided by the animal's body weight to account for differences in overall fluid intake mediated by factors that determine animal weight, such as sex and age. A positive score indicates a preference for saccharin, a negative score indicated a saccharin aversion, and a score of zero indicated no preference.

Apparatus

Cocaine Self-Administration

Following surgery and throughout the experiment, rats were individually housed in custom-made octagonal operant conditioning chambers with alternating Plexiglas and stainless steel walls. Every operant chamber had an active lever and an inactive (control) lever. Above each lever (Coulborn Instruments, Lehigh Valley, PA) there were corresponding 4.6-W, tri-colored (red, green, yellow) stimulus lights, and one white 4.6-W house light was positioned at the top of the chamber to signal the start of session. A

press on the active lever resulted in the intravenous delivery of cocaine solution and the illumination of the stimulus light directly above the lever for the duration of the infusion. A press on the inactive lever resulted in the illumination of the stimulus light directly above that lever but had no further consequences. Special inserts in the chamber walls allowed access to a food receptacle and a drinking spout. All operant chambers were enclosed in melamine-coated, wooden, sound-attenuating boxes that included a fan for ventilation. During the experimental sessions, response-contingent cocaine infusions were delivered by an infusion system consisting of a syringe pump (PHM-100; Med Associates, St. Albans, VT) and a 30-mL syringe with a blunted 22-gauge needle. The syringe was connected to a swivel (050-0022, Alice King Chatham, Hawthorne, CA) that was attached to the top of the operant chamber via Tygon tubing line (1.52 mm o.d., 0.52 mm i.d.; Fischer Scientific, Springfield, NJ). The swivel was connected to a tether (C31CS; Plastics One, Roanoke, VA), enclosed in a protective metal spring that extended into the operant chamber, and attached to a cannula affixed atop an infusion harness (Instech Laboratories, Plymouth Meeting, PA). The infusion harness was placed on the rat directly following surgery. The bottom end of the cannula that extended through within the infusion harness connected to an i.v. catheter implanted in the rat's jugular vein. Programming, data collection, and data storage for all experimental sessions were handled by Med-PC software and PCs equipped with a Med-PC interface (Med Associates, St. Albans, VT).

Drugs

     The cocaine solution (1.6 mg/mL) was prepared by dissolving cocaine HCL (National Institute of Drug Abuse, Research Triangle Institute, Research Triangle Park, NC) in sterile saline (0.9% NaCl) and adding heparin (5 USP units/mL) to prevent catheter thrombus formation. The cocaine infusion rate was 0.025 mL/s, and the solution was dispensed at an interval of 1 s/100 g body weight resulting in 0.4 mg/kg per infusion (i.v.) for all fixed-ratio (FR1) experimental components, except where noted. Baclofen (Sigma Aldrich, St. Louis, MO) was dissolved in saline and administered at a dose of 2.5 mg/kg (i.p.). Equivalent volumes of saline (0.9% NaCl) were administered as a control injection for baclofen. Progesterone (0.5 mg/kg, Sigma Aldrich) and allopregnanolone (15 mg/kg, Sigma Aldrich) were dissolved in peanut oil and delivered subcutaneously. Equivalent volumes of peanut oil were administered subcutaneously as control injections for progesterone and allopregnanolone. Histamine dihydrochloride (16 mg/mL, Sigma Aldrich) was dissolved directly into the 1.6 mg/mL cocaine solution and delivered at a dose of 4 mg/kg/infusion (i.v.). Baclofen, allopregnanolone, progesterone doses in the present experiments were chosen because they have previously revealed optimal individual differences in treatment effects (Anker et al. 2009a; Anker et al. 2007; Campbell et al. 2002). The histamine dose was chosen based on unpublished pilot data from our laboratory that established dose-response functions of histamine punishment on cocaine self-administration.

Procedures

Surgery

Rats were anesthetized with i.p. injections of both ketamine (60 mg/kg) and xylazine (10 mg/kg). Atropine (0.15 mL/rat i.p.) and doxapram (5 mg/kg i.p.) were also administered to prevent bradycardia and to assist respiration. The surgical procedure involved implanting one end of a polyurethane catheter in the rat's right jugular vein and subcutaneously tunneling the other end dorsally before exiting through a medial incision approximately 1 cm rostral to the scapulae. The free end of the catheter was connected to the bottom of a cannula embedded in the infusion harness which remained capped off throughout the 3-day post surgical recovery period in which the animals were housed in their operant conditioning chambers. Over the recovery period, 4 total subcutaneous injections of buprenorphine (0.05 mg/kg) were administered approximately every 12-h for analgesia along with heparinized saline (20 USP units/mL, 0.3 mL/rat) and gentamicin (2 mg/kg i.v.) to prevent catheter occlusion and infection. The same dose of heparinized saline was administered daily 15-min prior to experimental sessions to further ensure catheter patency. Animals were weighed and catheter patency was assessed weekly with a combination of ketamine (100 mg/mL), midazolam (5 mg/mL), and saline (3:3:14 ratio, 0.10 mL/rat). Patency was established by loss of the righting reflex following injection of this combination. If catheters were not patent, a second catheter was implanted in the left jugular vein following the surgical procedures described above, and the rat was again allowed to recover for 3 days before resuming experimental sessions.

Cocaine self-administration training

Rats were trained to self-administer cocaine infusions 3 days following surgery under a fixed ratio-1 (FR 1) schedule of reinforcement. At the beginning of every daily 2-h training session (9:00 a.m. to 11:00 a.m.), rats were given 3 non-contingent cocaine infusions and the left, active (cocaine-associated) lever was baited with a small amount of peanut butter (0.5-1.0 g). Rats were considered to have acquired cocaine self-administration when they administered ≥ 25 infusions for 3 consecutive days without priming infusions or peanut butter, and they were required to have an active to inactive (right) lever response ratio of at least 2:1. The ratio requirement was established to confirm drug-seeking behavior as opposed to non-drug conditioned reinforcement (i.e., responding for stimulus lights) or incidental lever responses due to cocaine-induced locomotor activity.

Escalation of cocaine self-administration

Following self-administration training, rats were allowed to self-administer cocaine under an FR 1 schedule for 3 sessions from 9:00 a.m. to 11:00 a.m. (short-access period, ShA) to establish baseline rates of stable responding (no increasing or decreasing trend) prior to the long-access (LgA) phase.

Next, rats self-administered cocaine under an FR 1 schedule for 21 consecutive days (Experiments 1 and 3), during which session length was increased to 6 h (9:00 a.m. to 3:00 p.m.). On the first day of LgA, rats were randomly assigned within each

phenotype group to receive either treatment (baclofen, Experiment 1; progesterone Experiment 3) or control injections at 8:30 a.m. before every session.

To investigate the effects of treatment and phenotype differences during extended access on ShA intake, pre-session treatment and control injections were discontinued, and session length was returned to 2 h (9:00 a.m. to 11:00 a.m.) for 3 days following LgA.

Maintenance, extinction, and reinstatement of cocaine-seeking behavior

Following self-administration training described above, rats began a maintenance period (Experiments 2 and 4) in which they self-administered cocaine under an FR 1 schedule for 14 daily 2-h sessions (9:00 a.m. to 11:00 a.m.).

Following the maintenance phase, a 21-day extinction phase began where the same procedure described in the maintenance phase was followed with the exception that cocaine was replaced with saline. After the extinction period, a 3-day pre-reinstatement phase began in which the house light and lever-paired lights were unplugged and remained unplugged for the remainder of the experiment.

The effects of baclofen (Experiment 2) and allopregnanolone (Experiment 4) pretreatment on the reinstatement of cocaine-seeking behavior were investigated during daily 2-h sessions (9:00 a.m.-11:00 a.m.) over a 13-day period. Treatment or control injections were administered one-half hour before session, and 3 doses of cocaine (C) priming injections were administered (C; 5, 10, or 15 mg/kg, presented in random order) at the beginning of successive sessions that were separated by a session when saline or

vehicle priming injections were given. Each dose of cocaine was administered twice for each animal; once following control injections and once following pretreatment. During the cocaine-priming sessions, animals received control injections one-half hour before and saline again at the onset of session. There was also an additional session in which animals received treatment injections 30-min prior to session onset and saline priming injections.

Experiment 1: Effects of baclofen on the escalation (bingeing) of i.v. cocaine self-administration in HiS and LoS rats

Background

The escalation model is invaluable for understanding one of the most important aspects of addiction, yet only a few studies have addressed the use of treatment agents during this phase (e.g., Hansen and Mark 2007; Specio et al. 2008), and none have evaluated individual differences using selectively bred animals with varying potential to develop cocaine-seeking behavior. Therefore, in this study, HiS and LoS rats were compared on escalation of cocaine intake while treated with a pharmacological intervention, baclofen, a potent agonist at the $GABA_B$ receptor that has been used to treat alcohol and cocaine dependence (for review, see Karila et al. 2008; Leggio et al. 2010; Roberts 2005; Smith et al. 2004). In rats, baclofen dose-dependently attenuated discrete contextual cue- and cocaine-primed reinstatement (Campbell et al. 1999; Filip and Frankowska 2007), cocaine sensitization (Frankowska et al. 2009), maintenance of i.v. cocaine self-administration (Campbell et al. 1999), and cocaine-induced dopamine release in the nucleus accumbens (Fadda et al. 2003). In another study, baclofen

pretreatment reduced the reinstatement of cocaine-primed behavior in baboons (Weerts et al. 2007).

In contrast, the effectiveness of baclofen in human populations has been mixed. In a preliminary open-label trial, baclofen reduced cocaine craving and use compared to placebo (Ling et al. 1998). In another study baclofen reduced cocaine self-administration, but it failed to alter cocaine's positive subjective effects in non-opioid dependent volunteers (Haney et al. 2006). Despite the mainly promising results of these earlier studies, a more recent multi-site, double-blind trial failed to show an effect of baclofen on self-reported abstinence or negative urine screens compared to placebo in individuals suffering from severe cocaine addiction (Kahn et al. 2009). One possible reason for incongruent results from experiments that test treatment drugs for stimulant addiction, like baclofen, is that treatment sensitivity may be genetically-mediated.

Methods

Subjects

Thirty five experimentally naïve adult female rats selectively bred at the University of Minnesota (Carroll et al. 2002) from Occidental HiS and LoS lines (Occidental College, Los Angeles, CA) were used in Experiment 1.

Procedure

Rats underwent the experimental procedures for cocaine self-administration training and escalation described in the General Methods section. HiS and LoS rats were

treated with either baclofen [HiS+B ($n = 8$) or LoS+B ($n = 9$), respectively] or saline

[HiS+S ($n = 8$) or LoS+S ($n = 10$), respectively] once daily 30-min prior to LgA sessions.

Data Analysis

Cocaine infusions and responses on the infusion-paired lever served as the

dependent measures for the LgA and ShA periods. Responses and infusions earned

during LgA were averaged into 3 blocks of 7 days and were subsequently analyzed using

three-way mixed factor analyses of variance (ANOVA; treatment×phenotype×block)

with block as a repeated measure. Three-way, repeated measures ANOVA

(treatment×phenotype×phase) were used to analyze comparisons of the 3-day average of

infusions self-administered during the pre- and post-ShA periods for the HiS and LoS

groups. The mean number of days to reach acquisition criteria was compared using a

two-way ANOVA (treatment×phenotype). All post hoc analyses were made using

Fischer's least-significant-difference *t* tests. Results were considered significant if $p <$

0.05. All statistical analyses were performed with GB Stat software (Dynamic

Microsystems, Silver Spring, MD).

Results

LgA Phase

There were significant main effects of phenotype, ($F_{1, 30} = 10.581$, p < .01) and

block, ($F_{2, 60} = 6.785$, $p < .01$) in responses during this phase. There were also significant

interactions between phenotype and treatment, ($F_{1, 68} = 12.421$, $p < .005$) as well as

treatment and block, ($F_{2, 60} = 7.714$, $p < .005$). Post hoc comparisons indicated that

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LoS+Sal responses were greater than the LoS+B group across all 7-day blocks ($p < .01$; response data not shown). The first 7-day block of responses for the HiS+B group was significantly less than the second ($p < .01$) and third 7-day blocks ($p < .01$) for that same group. The second 7-day block of responses for the HiS+B group were also significantly less ($p < .01$) than the third block of responses for that group. The third 7-day block of responses for the HiS+B group were significantly greater than the HiS+Sal group ($p < .01$), while There were no significant differences in responses made between the HiS+Sal and LoS+Sal groups across any of the 7-day blocks.

Figure 1 shows the mean ($\pm$ *SEM*) number of cocaine infusions (0.4 mg/kg) under a FR 1 schedule of reinforcement for each daily session (6 h/day) during LgA (for LoS (a) and HiS (b) groups). On day 1 of LgA mean infusions ranged from 34 to 108 infusions per 6-h session across the four groups. The HiS+Sal and the LoS+B groups increased by 28% and 49%, respectively; whereas, the cocaine infusions self-administered for the HiS+B group increased 113% by the last day of LgA. There were significant main effects of treatment, ($F_{1, 29} = 2.11$, p $< .01$), and block, ($F_{1, 30} = 10.58$, $p < .01$). There were also significant interactions in infusions between phenotype and treatment, ($F_{1, 66} = 6.93$, $p < .02$), block and phenotype, ($F_{2, 58} = 4.45$, $p < .02$), as well as block and treatment, ($F_{2, 58} = 3.79$, $p < 0.03$). Post hoc comparisons showed that the LoS+B group earned significantly fewer infusions than the LoS+Sal group across all 7-day blocks ($p < .01$, Figure 1a). The LoS+Sal group did not increase infusions when comparing block 1 with block 3 (Figure 1a). Post hoc comparisons also showed that the HiS+B group showed a significant increase (escalation) in the number of cocaine infusions from the first block of 7 days to the last ($p < .01$), and during the last block of 7

days (Figure 1b), the HiS+B group self-administered significantly more cocaine infusions compared to the HiS+Sal group ($p < 0.01$). Baclofen treatment was associated with escalation of cocaine intake in the HiS group and overall attenuation of cocaine self-administration in the LoS group. The HiS phenotype was associated with greater cocaine intake in the last block of LgA compared to the LoS phenotype.
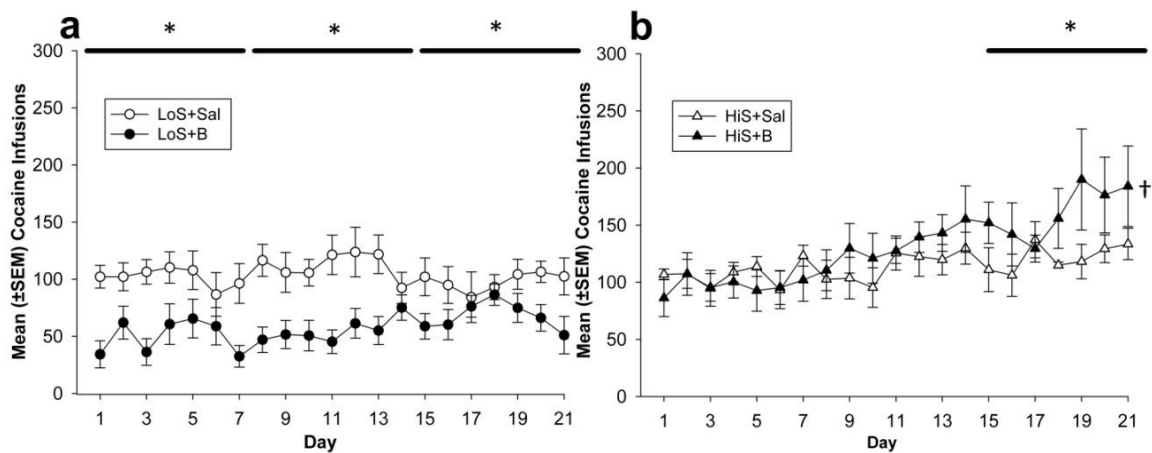
Figure 1 Panels A and B show infusions self-administered by the LoS and HiS rats (respectively) each day throughout the LgA (6-h sessions) period. Open circles represent the saline-treated LoS rats (LoS+Sal) and filled circles indicate baclofen-treated LoS rats (LoS+B). Open triangles represent the saline treated HiS rats and filled triangles indicate baclofen (B)-treated HiS rats. The * indicates that there was a significant difference in infusions between treatment groups ($p < .05$) for the HiS (HiS+B > HiS+Sal) in last 7-day interval and throughout all 3 7-day intervals for the LoS rats (LoS+Sal > LoS+B). Infusions earned by the HiS+B group were significantly higher during the last 7-day block than the first and second blocks ($p < .05$), as indicated by the †. Reprinted with permission from: *Holtz NA, Carroll ME (2011) Baclofen has opposite effects on escalation of cocaine self-administration: increased intake in rats selectively bred for high (HiS) saccharin intake and decreased intake in those selected for low (LoS) saccharin intake. Pharmacol Biochem Behav 100: 275-83 (Figure 1, pg. 279)*

Figure 2 shows that the LoS+Sal group also self-administered fewer cocaine infusions during the last 7-day block compared to the HiS+Sal group ($p < 0.05$); however, there were no significant differences between these groups for the first and second block of 7 days. There were no significant differences in inactive lever responses across groups during the LgA phase.
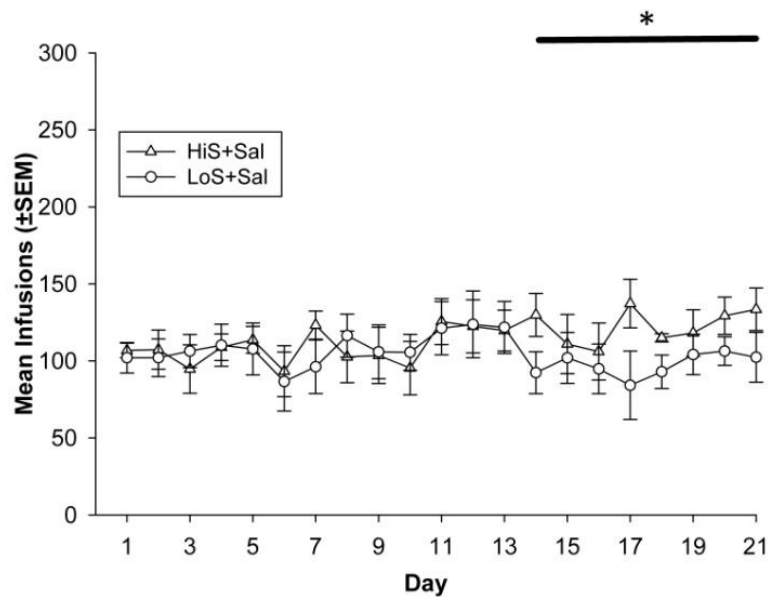
Figure 2 Mean (±SEM) cocaine infusions are presented for the LoS+Sal (open circles) and HiS+Sal groups (open triangles) during the LgA (6-h sessions) period. The * indicates that there was a significant difference in infusions between groups (HiS+Sal > LoS+Sal) in the last 7-day interval ($p < .05$). Reprinted with permission from: *Holtz NA, Carroll ME (2011) Baclofen has opposite effects on escalation of cocaine self-administration: increased intake in rats selectively bred for high (HiS) saccharin intake and decreased intake in those selected for low (LoS) saccharin intake. Pharmacol Biochem Behav 100: 275-83 (Figure 2, pg. 279)*

ShA cocaine self-administration pre- versus post- LgA

Figure 3 shows the mean (± *SEM*) cocaine infusions self-administered by all groups during the ShA phases before and after the LgA period. There was a main effect of phenotype, ($F_{1,31} = 13.23$, $p < .01$) and significant interactions between phenotype×treatment, ($F_{1,31} = 4.55$, $p < .05$) as well phenotype×phase (pre- or post-LgA) ($F_{1,31} = 13.25$, $p < .005$). Post hoc comparisons showed no significant differences in infusions administered between any of the groups during the pre-LgA phase. The HiS+B group showed a significant difference in infusions when comparing the pre-LgA and post-LgA phases ($p < .05$). Specifically, the HiS+B group earned more infusions during the post-LgA phase vs. pre-LgA.
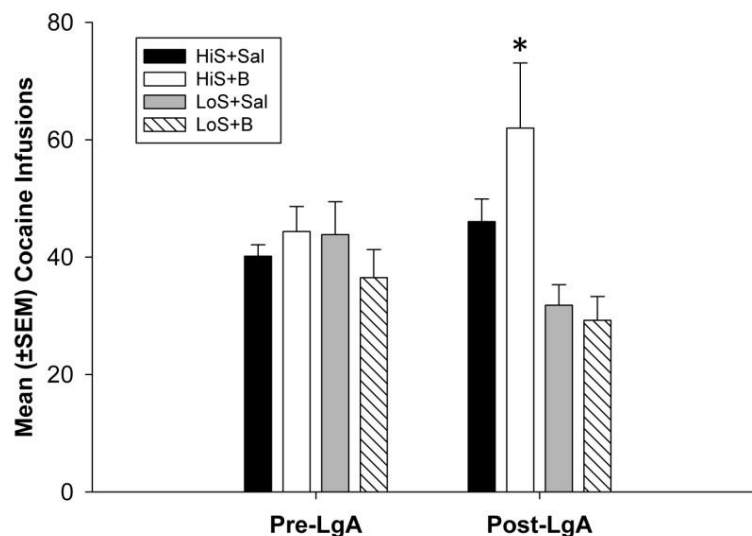
Figure 3 Mean (±SEM) cocaine infusions are presented for the 4 experimental groups, HiS+Sal (filled bar), HiS+B (open bar), LoS+Sal (gray bar), and LoS+B (hatched bar) during ShA (2-h sessions) pre- (left) and post-LgA (right). The * indicates a significant increase ($p < .01$) of cocaine infusions self–administered for the HiS+B group between pre-LgA and post-LgA. Reprinted with permission from: *Holtz NA, Carroll ME (2011) Baclofen has opposite effects on escalation of cocaine self-administration: increased intake in rats selectively bred for high (HiS) saccharin intake and decreased intake in those selected for low (LoS) saccharin intake. Pharmacol Biochem Behav 100: 275-83 (Figure 3, pg. 279)*

Discussion

The purpose of Experiment 1 was to examine the effects of baclofen treatment on the escalation of cocaine-seeking behavior in HiS and LoS rats. With a procedure modified from Ahmed and Koob (Ahmed and Koob 1998; 1999) and previously used in our laboratory (for review, see Carroll et al. 2009), we showed that baclofen potentiated the escalation of cocaine-seeking in HiS rats and decreased responding in the non-escalating LoS rats throughout LgA. Also, when comparing within-subject infusions earned between ShA periods pre- and post-LgA, only the HiS+B group increased intake. While the HiS+Sal group administered more cocaine during the last 7 days of the LgA phase compared to the LoS+Sal group, neither group escalated intake during this phase. This finding is inconsistent with a previous study in which HiS and LoS rats escalated their intake over a 21-day period (Perry et al. 2006). However, in the previous study, 12-h (vs. 6-h) sessions were used. Six-hour sessions were chosen in Experiment 1 because we have previously found this length to be appropriate for determining treatment effects (Larson et al. 2007). Further, baclofen is rapidly metabolized (Wuis et al. 1990), and supplemental injections necessitated by 12-h sessions may have disrupted the experiment and introduced stress or other confounds. It is important to note that the principal aim of this experiment was not to replicate the findings of Perry et al. (2006), but to provide a setting to examine differential treatment effects. Consequently, we demonstrated that baclofen treatment exaggerated the latent traits of high- and low-vulnerable animals under conditions that otherwise engender marginal phenotypic differences in cocaine intake. This novel finding is an initial step that needs further study, such as investigating

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the effects of baclofen on HiS and LoS rats with longer access conditions and over protracted periods.

Additionally, it is important to note that baclofen and other GABA receptor agonists can decrease the elevated locomotor activity associated with cocaine use (Frankowska et al. 2009). Thus, the differential treatment sensitivity between the HiS and LoS rats during escalation may have been due to genetic variability in baclofen's effects on cocaine-induced locomotor activity. However, there were no differences in inactive lever pressing during this phase, suggesting similar locomotor activity between the groups. Further, previous studies have shown no effect of baclofen on food-maintained lever responding up to two times the dose use in the present study (Brebner et al. 2000; Roberts and Andrews 1997), suggesting that 2.5 mg/kg baclofen is unlikely to elicit differential rate-decreasing effects in locomotor activity between the HiS and LoS rats.

One interpretation of the differential effects of baclofen on LgA responding between the lines relates to the inhibitive action of baclofen on the mesolimbic dopamine reward system through $GABA_B$ agonist activity. The rewarding effects of cocaine are primarily attributed to its inhibition of the dopamine transporter and consequent increase in synaptic dopamine (Ritz et al. 1987). The elevated proclivity for cocaine self-administration in the HiS rats may have driven them to surmount the inhibitory effects of baclofen on this increase in dopamine by self-administering more cocaine. Such an increase in intake in the HiS+B rats, compared to the HiS+Sal rats, may have potentiated the upward shift of hedonic set points, a process proposed to be fundamental in the

transition from regulated drug use to binge-patterns and addiction (Koob and Kreek 2007). This concept is supported by the finding that only HiS+B rats showed an increase in post-LgA responding when treatment was discontinued compared to HiS+Sal rats, reflecting a potential deviation from baseline reward functioning. To further investigate this phenomenon, baseline intracranial self-stimulation reward thresholds between the phenotypes could be compared to thresholds following chronic baclofen treatment.

The genetically-determined neurobiological differences between the HiS and LoS rats and their contribution to the differential drug-seeking and treatment sensitivity patterns in the present and previous studies are not yet well understood. One possible explanation for the effective attenuation of cocaine self-administration throughout the LgA period in the LoS rats but not HiS rats could be attributed, in part, to a differential functionality or distribution of GABAergic neurons, thus the variable inhibitory action of baclofen on cocaine reinforcement. Future studies more directly investigating variations in GABAergic functioning is necessary to better characterize the behavioral differences seen in these lines, such as using *in vivo* microdialysis to quantify GABA and dopamine in mesolimbic areas during chronic baclofen and cocaine treatment in the HiS and LoS animals. Such data could have translational impact, as a recent study has shown that a GABA receptor polymorphism is associated with variance in cocaine dependence in humans (Smelson et al. 2012).

These results may provide valuable insight into the current lack of available treatment drugs for cocaine addiction and for preventing one of its most critical phases, escalation. Future genetic investigations into the HiS and LoS lines could inform recent

pharmacogenetic research into treatment agents for stimulant abuse (Haile and Kosten 2009). Data may point to homologous human genetic markers indicative of phenotypic vulnerability differences, potentially resulting in more informative clinical trials. For instance, recent efforts in alcoholism research have suggested that genes encoding for GABA receptor subtypes are possible areas of focus. Ooteman et al. (2009) showed that alcoholics with a polymorphism in the GABRA6 gene displayed greater reduction in craving with acamprosate treatment, a drug that facilitates $GABA_A$ transmission and reduces alcohol intake (McNeely and Sherman 2011). The opposite effects of baclofen in HiS vs. LoS rats may explain the relatively small effect of baclofen in human cocaine addicts, as it might be expected that the recruited participants had considerable genetic diversity.

The findings in our HiS rats in Experiment 1 are in contrast to results of other experiments that have largely been successful in reducing drug intake in pharmacological interventions during escalation on outbred rats. For example, Specio et al. (2008) showed that corticotropin-releasing factor antagonists blocked the escalation of cocaine self-administration under periods of extended access. Hansen and Mark (2007) found similar results with the nicotinic acetylcholine receptor antagonist, mecamylamine. However, the application of a mutant albumin-butyrylcholinesterase in rats potentiated responding for cocaine during the first 4 days of extended access (Carroll et al. 2010). Experiments investigating pharmacological treatments using an animal model of escalation may produce more informative results if high- and low-vulnerability lines are included.

Experiment 2: Effects of baclofen on the reinstatement of cocaine seeking (relapse) in HiS and LoS rats

Background

Similar to the escalation model, reinstatement (relapse) is a phase of human drug addiction that has yielded individual differences in responding. For example, female (vs. male) rats (Lynch and Carroll 2000), rats screened for high (vs. low) measures of impulsivity using a delay discounting procedure (Perry et al. 2008), HiS (vs. LoS) rats (Perry et al. 2006), and rats selected for high (HiR) vs. low (LoR) wheel running (Larson and Carroll 2005) showed elevated reinstatement of cocaine-seeking behavior. Also, like escalation, reinstatement models a critical phase of human drug addiction in which individual differences in drug-abuse vulnerability have not been compared with respect to treatment sensitivity. Thus, the aim of experiment 2 was to extend the findings of experiment 1 to the reinstatement model. We hypothesized that baclofen would attenuate cocaine-primed reinstatement responding more in LoS vs. HiS animals.

Methods

Subjects

Twenty-seven experimentally naïve adult female rats selectively bred at the University of Minnesota (Carroll et al. 2002) from Occidental HiS and LoS lines (Occidental College, Los Angeles, CA) were used in Experiment 2.

Procedure

Rats underwent the experimental procedures for cocaine self-administration training, maintenance, extinction and reinstatement described in the General Methods section. HiS ($n = 14$) and LoS ($n = 13$) rats were treated with baclofen or saline once daily 30-min prior to session onset, and priming injections were administered as described above.

Data Analysis

Dependent measures were the mean number of lever presses made during maintenance, extinction, and reinstatement. For maintenance and extinction, mean responses were analyzed using a two-way, mixed factorial ANOVA (phenotype×day) with day as a repeated measure. Cocaine infusions achieved during maintenance were also examined using a two-way, mixed factorial ANOVA (phenotype×day) with day as the repeated measure. For reinstatement, mean responses were analyzed using a three-way, mixed factorial ANOVA (phenotype×pretreatment×cocaine dose) with cocaine dose being a repeated measure. As detailed below, they were subsequently analyzed with a two-way, mixed factorial ANOVA (pretreatment×cocaine dose) with pretreatment being a repeated measure. All post hoc analyses were made using Fischer's least-significant-difference *t* tests. Results were considered significant if $p < 0.05$. All statistical analyses were performed with GB Stat software (Dynamic Microsystems, Silver Spring, MD).

Results

Maintenance

Figure 4a shows the mean responses made (± *SEM*) on the cocaine-paired lever during the maintenance phase. There was a significant main effect of phenotype, ($F_{1, 403}$ = 10.72, $p < .05$), and an interaction between phenotype and day, ($F_{13, 403} = 8.42$, $p < .05$). Post hoc analysis revealed that the HiS group made significantly more responses than the LoS group during days 3, 5, 7, and 9-14 ($p < .05$). Figure 4b shows the mean infusions self-administered (± *SEM*) during maintenance. There was a significant main effect of phenotype, ($F_{1, 403} = 11.89$, $p < .01$), and an interaction between phenotype and day, ($F_{13, 403} = 9.09$, $p < .05$), with more infusions in days 3, 5, 9, 10, and 12-14 ($p < .05$).
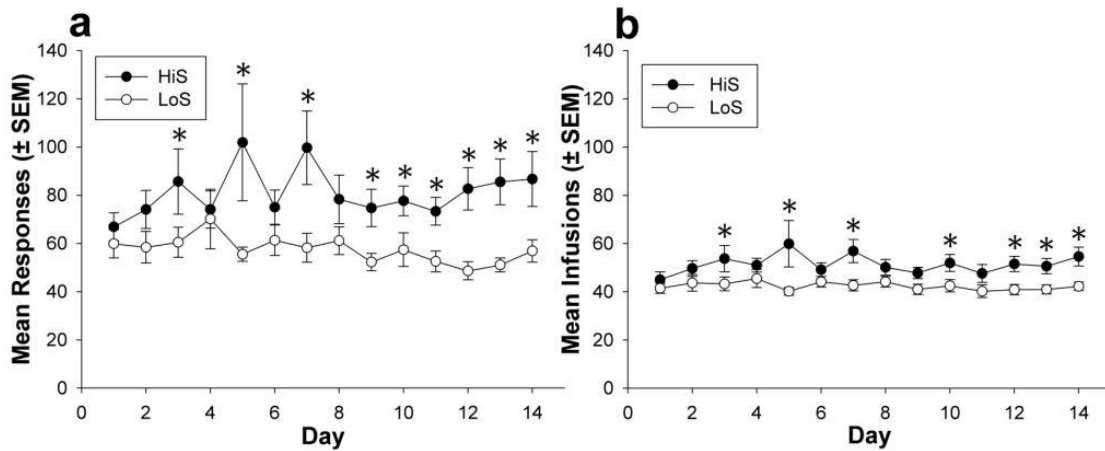
Figure 4 Mean (±SEM) cocaine-paired lever responses (left panel) and cocaine infusions self-administered (right panel) are presented for the 2 experimental groups, HiS (filled circles), and LoS (open circles) during the maintenance phase. The * indicates that the HiS group made more responses or self-administered more cocaine infusions than the LoS group on that day ($p < .05$). Reprinted with permission from: *Holtz NA, Carroll ME (2011) Baclofen has opposite effects on escalation of cocaine self-administration: increased intake in rats selectively bred for high (HiS) saccharin intake and decreased intake in those selected for low (LoS) saccharin intake. Pharmacol Biochem Behav 100: 275-83 (Figure 4, pg. 280)*

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Extinction

Figure 5 shows the mean responses (± *SEM*) on the lever previously paired with cocaine during the extinction phase when saline was replaced with cocaine. There were no significant phenotype differences in responding during this phase.
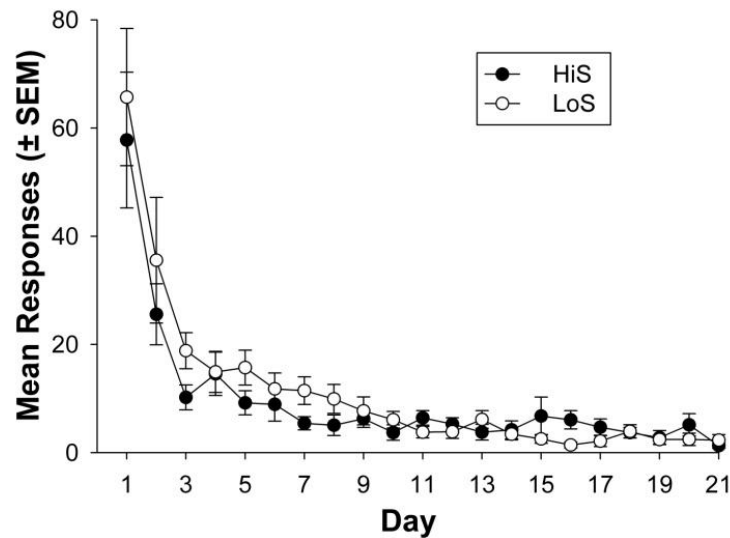
Figure 5 Mean (±SEM) responses on the previously cocaine-paired lever are presented for the 2 experimental groups, HiS (filled circles), and LoS (open circles) during the extinction phase. There were no significant differences between groups during this phase. Reprinted with permission from: *Holtz NA, Carroll ME (2011) Baclofen has opposite effects on escalation of cocaine self-administration: increased intake in rats selectively bred for high (HiS) saccharin intake and decreased intake in those selected for low (LoS) saccharin intake. Pharmacol Biochem Behav 100: 275-83 (Figure 5, pg. 280)*
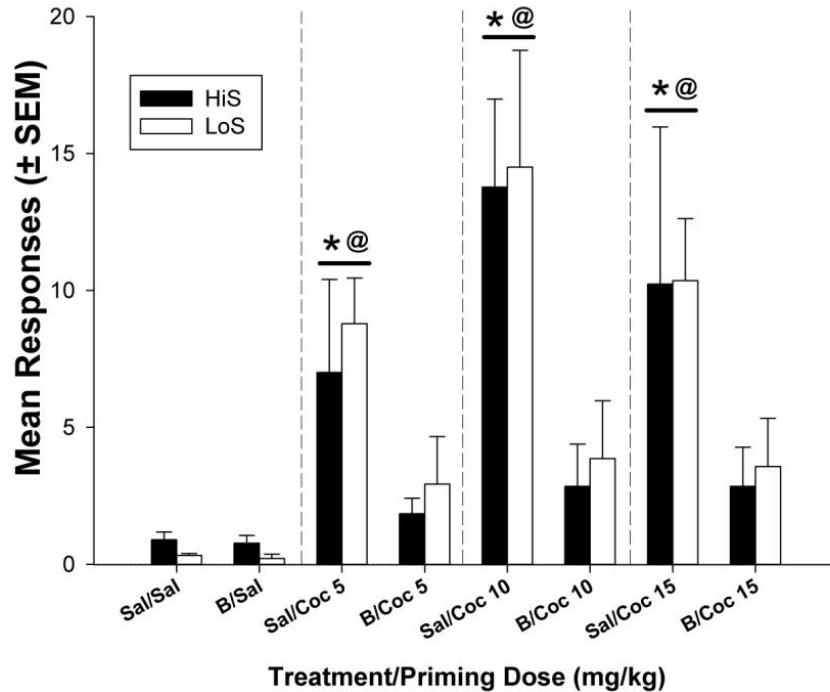
Reinstatement

Figure 6 shows responses (± SEM) on the lever previously paired with cocaine during the reinstatement phase. These responses were originally analyzed with a three-way, mixed factorial ANOVA (phenotype×pretreatment×cocaine dose); however, no significant three-way interaction was found. Responses were then combined between the HiS and LoS rats and analyzed using a two-way, mixed factorial ANOVA (pretreatment×cocaine dose). There were significant main effects of treatment ($F_{1, 162} = 15.72$, $p < .001$), dose ($F_{3, 156} = 10.45$, $p < .001$), and a significant interaction between treatment and dose ($F_{3, 156} = 4.18$, $p < .01$). Post hoc analyses showed that rats receiving 5, 10, and 15 mg/kg cocaine priming injections following vehicle pretreatment had significantly more responses than when they received a saline priming injection following vehicle pretreatment, ($p < .01$). Rats made significantly fewer responses when they were pretreated with baclofen compared with vehicle following the 5 ($p < .05$), 10 ($p < .01$), and 15 ($p < .01$) mg/kg cocaine doses. When administered alone as a priming injection, baclofen did not induce significantly more responding than after saline priming injections, nor where there phenotype differences in these conditions.

Figure 6 Responses made on the previously cocaine-paired lever were combined between groups as indicated by the bars during the reinstatement phase. The * indicates a significant ($p < .05$) difference between the 5, 10, and 15 mg/kg dose priming injections that were preceded by Sal injections and saline priming injections that were preceded by Sal treatment. The @ indicates a significant ($p < .05$) difference between the 5, 10, and 15 mg/kg dose cocaine priming injections preceded by Sal treatment and the corresponding 5, 10, and 15 mg/kg dose cocaine priming injections preceded by B treatment. Reprinted with permission from: *Holtz NA, Carroll ME (2011) Baclofen has opposite effects on escalation of cocaine self-administration: increased intake in rats selectively bred for high (HiS) saccharin intake and decreased intake in those selected for low (LoS) saccharin intake. Pharmacol Biochem Behav 100: 275-83 (Figure 6, pg. 280)*

Discussion

The results from Experiment 2 show that baclofen attenuated cocaine-primed

reinstatement of drug-seeking behavior equally well in both the HiS and LoS lines.

However, there were no differences between the phenotypes in reinstatement responding

following cocaine priming injections preceded by saline. It is noteworthy that this result

does not concur with Perry et al. (2006), in which HiS rats responded more than LoS rats during extinction and following a 15 mg/kg cocaine injection. Repeated exposure to the same cocaine dose in the present experiment may have produced a floor effect and obscured phenotype differences in responding following cocaine-priming injections. Further, in the Perry et al. (2006) study there were 10 days of maintenance and 14 days of extinction compared to 14 days of maintenance and 21 days of extinction used in the present study. This extended protocol was used because Anker et al. (2009a) found individual (sex) differences in treatment effects during cocaine-primed reinstatement using a similar design. However, it is possible that differences in cocaine-primed reinstatement and baclofen treatment effects might exist under different reinstatement conditions that may reduce the occurrence of floor effects. These issues could be addressed through future work investigating a range of treatment doses.

The attenuating effects of baclofen on cocaine-induced reinstatement of drug-seeking in both the HiS and LoS rats corroborates previous research investigating baclofen's effects on the rodent model of relapse (Campbell et al. 1999; Filip and Frankowska 2007). In contrast to its differential effects on LgA responding (Experiment 1), baclofen attenuated cocaine-primed reinstatement in both the HiS and LoS animals during the reinstatement condition, supporting its efficacy in preventing relapse. The differential results between the LgA and reinstatement paradigms may be because intermittent baclofen and cocaine injections were acutely administered at much lower overall doses during reinstatement (Experiment 2), while cocaine and baclofen were chronically administered throughout the LgA phase of Experiment 1. These results illustrate the importance of considering acute vs. chronic treatment effects. Also, it is

possible that the animal model of escalation is more sensitive to divergent treatment effects compared to reinstatement due to the large amounts of cocaine that are consumed (e.g., a rate-dependent effect). For example, extended access to drugs provides an opportunity for the animal to attempt surmounting baclofen's effects on self-administration (e.g., HiS escalation of cocaine intake), while during reinstatement the total cocaine dose is smaller and not controlled by the animal. Phenotype differences may be subtle and more or less sensitive to different behavioral assays. Further translational research comparing the two phases with regard to treatment sensitivity and individual differences may support the utility of investigating the efficacy of pharmacological agents during escalation.

Experiment 3: Effects of progesterone on the escalation (bingeing) of i.v. cocaine self administration in HiS and LoS rats

Background

Female rats (Roth and Carroll 2004) and monkeys (Carroll et al. 2005) escalate drug intake more than males, and escalation is a defining factor in the progression toward drug abuse in humans (Ahmed et al. 2002). Preclinical and clinical work indicate that this difference between sexes is largely attributable to the gonadal hormones estrogen and progesterone (Anker and Carroll 2010b; 2011b; Carroll and Anker 2010; Carroll et al. 2004), as estrogen enhanced, and progesterone inhibited the escalation of cocaine self-administration in female rats (Larson et al. 2007). In clinical studies with women, estrogen was associated with increased cocaine-induced positive subjective effects (Evans et al. 2002; Sofuoglu et al. 1999); whereas, pretreatment with progesterone

attenuated these responses (Evans and Foltin 2006; Sofuoglu et al. 2002; Sofuoglu et al. 1999; Sofuoglu et al. 2004).

Given the possible therapeutic value of progesterone during bingeing phases of drug abuse, the extent to which it may reduce escalation in an animal model of cocaine intake warrants investigation. Thus, the purpose of Experiment 3 was to compare the effects of systemic progesterone on the escalation of i.v. cocaine self-administration in the HiS and LoS rats that differ widely in drug abuse vulnerability and motivation to seek drugs of abuse (Carroll et al. 2008). In light of the results in Experiment 1, we expected that progesterone would reduce cocaine self-administration in LoS rats but not HiS rats.

Methods

Subjects

Thirty-four experimentally naive adult female rats, selectively bred at the University of Minnesota from Occidental HiS and LoS lines (Occidental College, Los Angeles, California, USA), served as subjects in this study.

Procedure

The procedures for this escalation experiment where modified from those described in the General Methods section. Rats were trained to self-administer cocaine (0.8 mg/kg/infusion) under an FR1 schedule of reinforcement during 2-h sessions daily. Throughout training and the rest of the experiment, HiS and LoS rats received subcutaneous injections of either progesterone [HiS P (progesterone-treated), $n = 9$; LoS

P, $n = 7$] or vehicle [peanut oil; HiS VEH (vehicle-treated), $n = 9$; LoS VEH, $n = 9$] 30

min before each session. Rats were studied for a total of approximately 50 days. The

estrous cycle was not controlled in the present experiment, as previous work has shown

that cocaine, when self-administered under the LgA conditions of the present study,

disrupts the cycle phase (Larson et al. 2007). Once rats acquired stable cocaine self-

administration at 0.8 mg/kg/infusion of cocaine, they were allowed to self-administer

each of three doses of cocaine that were given in mixed order (0.2, 0.4, and 1.6

mg/kg/infusion) for three sessions of stable responding. The session length was then

extended to 6 h (LgA; 09:00–15:00 h) for 21 days, and subsequently, cocaine intake was

reassessed under the ShA dose–response condition (0.2, 0.4, 0.8, and 1.6

mg/kg/infusion).

Data Analysis

Responses and infusions served as the primary dependent measures. Responses

and infusions during LgA were averaged into seven blocks of 3 days each and analyzed

using three-factor repeated-measures analyses of variance (ANOVA) with phenotype

(LoS vs. HiS) and treatment (progesterone vs. vehicle) as the between-group factors, and

day (LgA) as the repeated measure. The number of responses and infusions during the

pre-LgA and post-LgA dose–response conditions was analyzed with separate three-factor

repeated-measures ANOVA with phenotype and treatment as the between-subject factors,

and dose as the repeated measure. Additional three-factor, repeated measures ANOVAs

with phenotype as the between-subject factor, and dose and access condition (pre-LgA

vs. post-LgA) as repeated measures were conducted. After a significant main effect, post-hoc tests were conducted using Fisher's least significant difference protected t-tests. Statistical analyses were conducted using GB Stat (Dynamic Microsystems Inc., Silver Spring, Maryland, USA).

Results

Pre-long-access dose–response condition

There were no significant effects of treatment (progesterone vs. vehicle) on cocaine-maintained responses or cocaine infusions during the pre-LgA dose–response condition; however, HiS rats had more responses ($F_{1, 135} = 8.43$, $p < 0.01$) (not shown) and infusions ($F_{1,135} = 8.67$, $p < 0.01$) (Figure 7c and d vs. Figure 7a and b) than LoS rats.
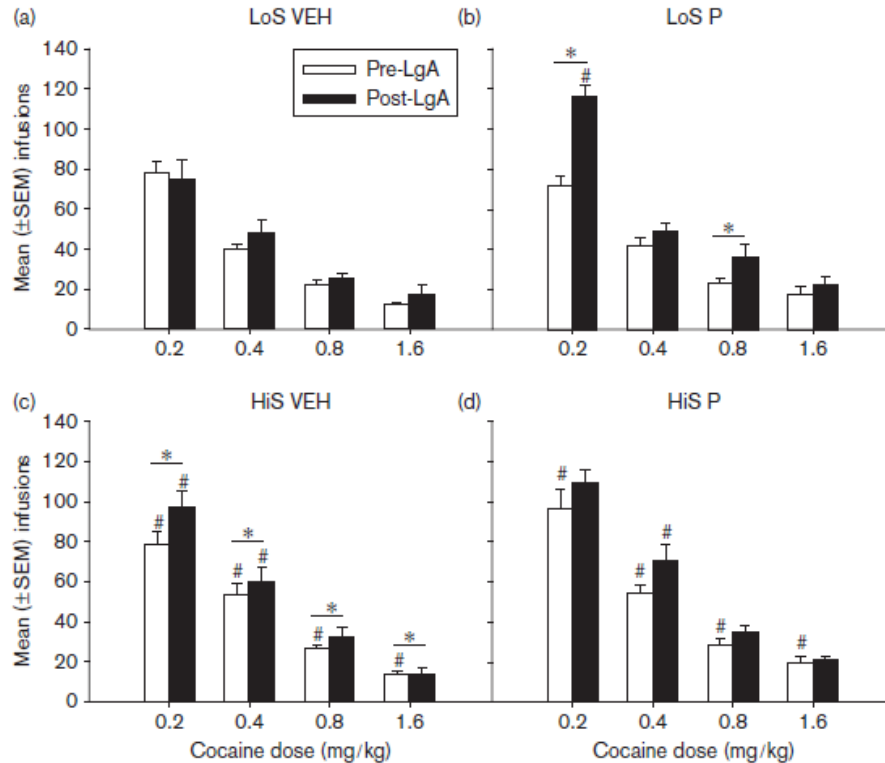
Figure 7 Mean (±SEM) cocaine infusions obtained under the pre-LgA and post-LgA dose–response conditions for the four groups: (a) LoS VEH; (b) LoS P; (c) HiS VEH; (d) HiS P. #Significant phenotype difference for the respective dose and LgA (pre or post) condition ($p < 0.05$). *Significant within-subject difference between pre-LgA versus post-LgA conditions ($p < 0.05$). Reprinted with permission from: *Anker JJ, Holtz, NA, Carroll, ME (2012) Effects of progesterone on escalation of intravenous cocaine self-administration in rats selectively bred for high or low saccharin intake. Behav Pharmacol 23: 205-10 (Figure 1, pg. 207)*

Long access

Similar to the pre-LgA condition, there was no significant effect of progesterone treatment on cocaine-reinforced lever responses during LgA, but HiS rats had more responses than LoS rats ($F_{1, 237} = 7.04$, $p < 0.05$) (not shown). For infusions, there was a significant main effect of phenotype ($F_{1, 237} = 24.13$, $p < 0.01$) and a significant treatment×phenotype×day interaction ($F_{6, 237} = 2.25$, $p < 0.05$). A subsequent post hoc

analysis indicated that both HiS groups (HiS VEH and HiS P) earned significantly more infusions during their last 3-day block compared with their first 3-day block ($p < 0.05$) (Figure 8a, b, and d), indicating escalation of cocaine intake. In contrast, LoS rats (LoS VEH and LoS P) did not escalate their drug intake (Figure 8a–c). HiS VEH rats earned more infusions than LoS VEH rats during blocks 4–7 ($p < 0.05$) (Figure 8a), whereas HiS P rats earned more cocaine infusions than LoS P across all LgA blocks ($p < 0.05$) (Figure 8b). The HiS P group earned more infusions during blocks 2 and 3 than the HiS VEH group ($p < 0.05$) (Figure 8d). In contrast, LoS P rats earned significantly fewer cocaine infusions than LoS rats treated with the vehicle during blocks 2 and 3 ($p < 0.05$) (Figure 8c).
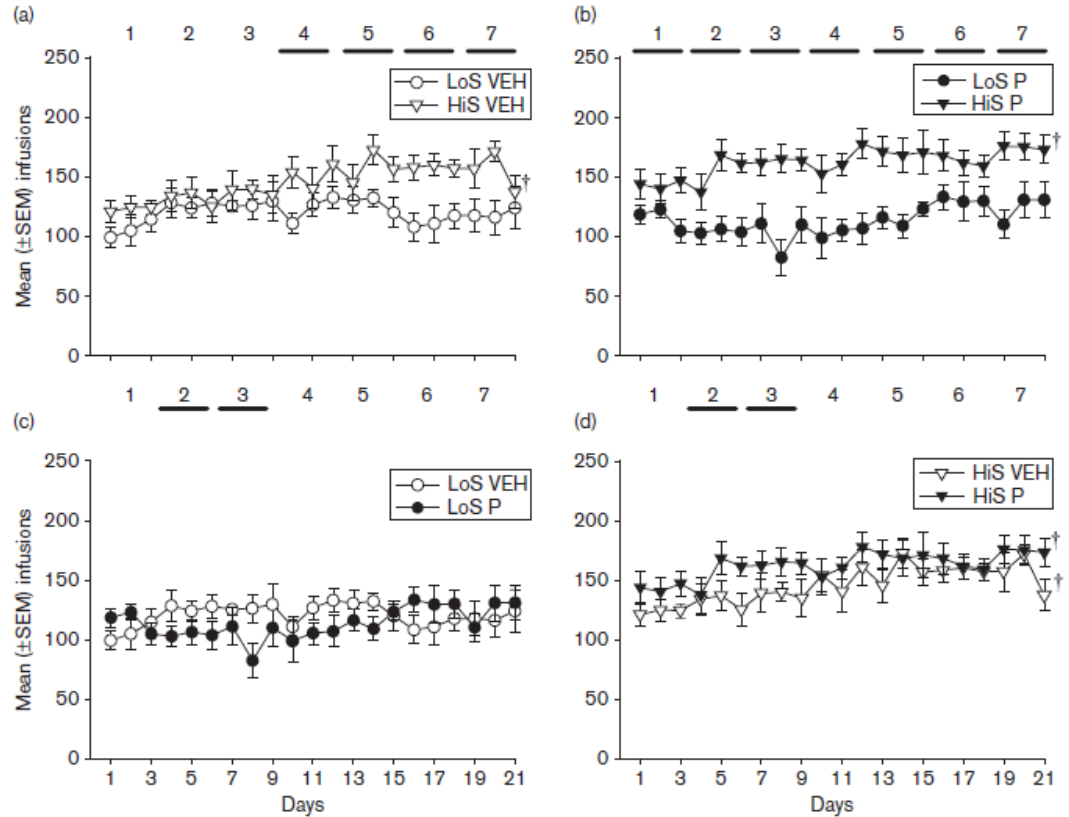
Figure 8 Mean (±SEM) cocaine infusions (0.4mg/kg) are presented for each day of the LgA phase (6 h). (a–d) Horizontal lines indicate the 3-day intervals during which there were significant group differences in responses or drug deliveries ($p < 0.05$). † Significant within-group difference in infusions during interval 7 (days 19–21) compared with interval 1 [days 1–3; (a), (b), and (d)]. Reprinted with permission from: *Anker JJ, Holtz, NA, Carroll, ME (2012) Effects of progesterone on escalation of intravenous cocaine self-administration in rats selectively bred for high or low saccharin intake. Behav Pharmacol 23: 205-10 (Figure 2, pg. 208)*

Post-long-access dose–response condition

After LgA, rats were retested under the fixed-ratio 1 condition. Analyses of active lever responses (not shown) indicated a significant main effect of phenotype ($F_{1,115}$ = 15.75, $p < 0.01$) and dose ($F_{3,115}$ = 151.53, $p < 0.01$). For infusions, there were significant main effects of treatment ($F_{1,115}$ = 14.51, $p < 0.01$), phenotype ($F_{1,115}$ = 4.49, $p < 0.01$), and dose ($F_{3,115}$ = 299.25, $p < 0.01$), and a significant treatment×phenotype×dose

interaction ($F_{3,115} = 3.27$, $p < 0.01$). Post hoc comparisons indicated that, at the two lowest doses of cocaine (0.2 and 0.4 mg/kg) HiS VEH rats earned more cocaine infusions than LoS VEH rats ($p < 0.05$) (Figure 7a and c), and the LoS P group earned more 0.2 mg/kg infusions than the LoS VEH group ($p < 0.05$) (Figure 7b). LoS rats treated with progesterone earned fewer 0.4 mg/kg cocaine infusions than HiS P rats ($p < 0.05$) (Figure 7b and d). Comparison of cocaine intake under the dose–response conditions before and after LgA for each group indicated that HiS VEH rats earned more infusions post-LgA compared with pre-LgA ($F_{1,63} = 6.14$, $p < 0.05$) (Figure 7c), and LoS P rats earned more infusions of 0.2 and 0.8 mg/kg cocaine after LgA compared with that before LgA ($p < 0.05$) (Figure 7b).

Discussion

The primary finding of experiment 3 was that a pharmacological treatment (i.e., progesterone) enhanced the drug-prone and drug-resistant behavioral profiles inherent in the HiS and LoS rats under the LgA condition (as is illustrated when comparing Figure 8a and b). Specifically, on days 3–9, progesterone (vs. vehicle) potentiated cocaine self-administration in the HiS rats, whereas it suppressed intake in the LoS rats. That the effects of progesterone on cocaine self-administration occurred from only days 3–9 suggests that the animals may have become tolerant to the effects of progesterone on cocaine during LgA. Altering the dosing regimen such that progesterone was administered every other day may have prolonged its effect on LgA cocaine self-administration. Additionally, the lack of a treatment effect in HiS rats on days 10–21

may be due to a ceiling effect. For example, HiS rats have a greater capacity to continue to escalate cocaine intake at the end of a 21-day LgA condition (Perry et al. 2006). In addition, the lack of an effect on days 1–3 may have been the result of the change in session length from 2 to 6 h. Results from several studies indicate that treatment effects and individual differences in LgA cocaine intake often emerge after several days of cocaine self-administration (Anker et al. 2010; Gipson et al. 2011; Larson et al. 2007; Mantsch et al. 2008). In contrast to the results in the LgA condition, progesterone enhanced cocaine intake at the lowest cocaine concentration in the LoS rats after the LgA period, illustrating a complex interaction between phenotype, treatment, phase, and dose. Regardless of treatment status (progesterone or vehicle), HiS rats escalated cocaine self-administration during the LgA condition; whereas, LoS rats maintained a lower steady rate of cocaine intake. These findings agree with previous reports indicating that HiS (vs. LoS) rats are more likely to escalate their cocaine intake under similar LgA conditions (Carroll et al. 2007; Perry et al. 2006). Overall, these findings confirms that the HiS and LoS rat lines are useful genetic models of drug abuse vulnerability and resilience and differential sensitivity to treatment (Carroll et al. 2008).

Two factors that need to be considered when comparing results from the present and previous studies are strain and selective breeding. Heretofore, the attenuating effect of progesterone on cocaine self-administration has almost exclusively been demonstrated in female Wistar rats, whereas in the present study Sprague–Dawley rats were used (Anker and Carroll 2010b). Furthermore, findings indicate that sensitivity to drug abuse treatment is strongly influenced by strain. For example, Haile and Kosten (2001) demonstrated that, the effects of several D2 and D1 agonists and antagonists differed

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between the inbred Lewis and Fischer 344 rats, and similar to the findings with progesterone, administration of one treatment (SKF 38393) increased cocaine self-administration in Fischer 344 rats (drug abuse resilient) and decreased it in Lewis rats (drug abuse prone). An additional consideration when comparing the present results to previous findings is that rats in the present study were selectively bred for differential saccharin intake over successive generations. With each generation, the behavioral phenotype (and the underlying genes) between HiS and LoS rats has increasingly diverged. Thus, the fact that progesterone treatment facilitated cocaine self-administration in HiS rats and had an opposite effect in LoS and Wistar rats could be explained by the contribution of genetic influences associated with strain (in the case of heterogeneous Wistar rats) and selective breeding (in the case of LoS rats).

In sum, the attenuating effects of progesterone on i.v. cocaine self-administration in the LoS rats have been reported in our laboratory under similar LgA conditions (Larson et al. 2007) and during other phases of drug abuse that are modeled in outbred Wistar female rats (Anker and Carroll 2010b; 2011b; Carroll and Anker 2010; Carroll et al. 2004). Results from clinical work provide cross-species support to these findings by indicating that progesterone treatment decreased positive subjective ratings of smoked and i.v. cocaine in women (Evans and Foltin 2006; Sofuoglu et al. 2002; Sofuoglu et al. 1999; Sofuoglu et al. 2004). Similar to the differential effects of baclofen in Experiment 1, the results of the present study suggest that although progesterone may be efficacious in decreasing responses to cocaine, this treatment effect may depend on the individual's drug abuse vulnerability.

Experiment 4: Effects of allopregnanolone on the reinstatement of cocaine seeking (relapse) in HiS and LoS rats

Background

The attenuating effects of progesterone on drug seeking-behavior discussed in Experiment 3 have largely been attributed to its metabolite, allopregnanolone (ALLO), and these effects appear to be sex-specific (Anker and Carroll 2010b). For example, ALLO was effective at reducing cocaine-primed reinstatement of drug seeking behavior in female but not male rats (Anker et al. 2009a). This study also showed that the attenuating effects of progesterone on drug-primed reinstatement decreased when its metabolite, ALLO, was inhibited by finasteride. Like baclofen, this neuroactive steroid enhances GABA transmission; however, in contrast to baclofen's agonist-properties at the $GABA_B$ receptor, ALLO acts as a positive allosteric modulator at the $GABA_A$ receptor. Additional studies have shown that ALLO attenuates the escalation of cocaine self-administration (Anker et al. 2012) and stress (yohimbine)-induced reinstatement of drug-seeking behavior in female rats (Anker and Carroll 2010a), suggesting that ALLO may serve as an effective therapy for relapse prevention in cocaine-dependent females.

The purpose of Experiment 4 was to extend the findings of Experiment 2 by investigating possible differences in sensitivity to the attenuating effects of this potential pharmacotherapy for substance dependence using the cocaine-primed reinstatement procedure. Given the results of the previous experiments, we hypothesized that ALLO would attenuate cocaine-primed reinstatement of drug-seeking behavior either equally well between the phenotypes, or to a greater degree in the LoS animals.

Methods

Subjects

Nineteen experimentally naïve adult female rats, selectively bred at the University of Minnesota (Carroll et al. 2002) from Occidental HiS and LoS lines (Occidental College, Los Angeles, CA), were used in Experiment 4.

Procedure

Rats underwent the experimental procedures for cocaine self-administration training, maintenance, extinction and reinstatement described in the General Methods section. HiS ($n = 9$) and LoS ($n = 10$) rats were treated with allopregnanolone or peanut oil once daily 30 min prior to session onset and priming injections as described above.

Data Analysis

Dependent measures were the mean number of lever presses made during maintenance, extinction, and reinstatement. For maintenance and extinction, mean responses were analyzed using a two-way, mixed factorial ANOVA (phenotype×day) with day as a repeated measure. Cocaine infusions achieved during maintenance were also examined using a two-way, mixed factorial ANOVA (phenotype×day) with day as the repeated measure. For reinstatement, mean responses were analyzed using a two-way, mixed factorial ANOVA (phenotype×treatment) with treatment being a repeated measure. All post hoc analyses were made using Fischer's least-significant-difference *t*

tests. Results were considered significant if $p < 0.05$. All statistical analyses were performed with GB Stat software (Dynamic Microsystems, Silver Spring, MD).

Results

Maintenance

Figure 9a shows the mean responses (± *SEM*) on the cocaine-paired lever during the maintenance phase. There were no significant differences in responses made on the active lever during this phase. Figure 9b shows the mean infusions self-administered (± *SEM*) during maintenance. For this measure, there was a significant interaction between phenotype and day, ($F_{13, 293} = 2.29$, $p < .01$). Post hoc analyses indicated that HiS rats self-administered more infusions than LoS rats on days 9-11 ($p < .05$).
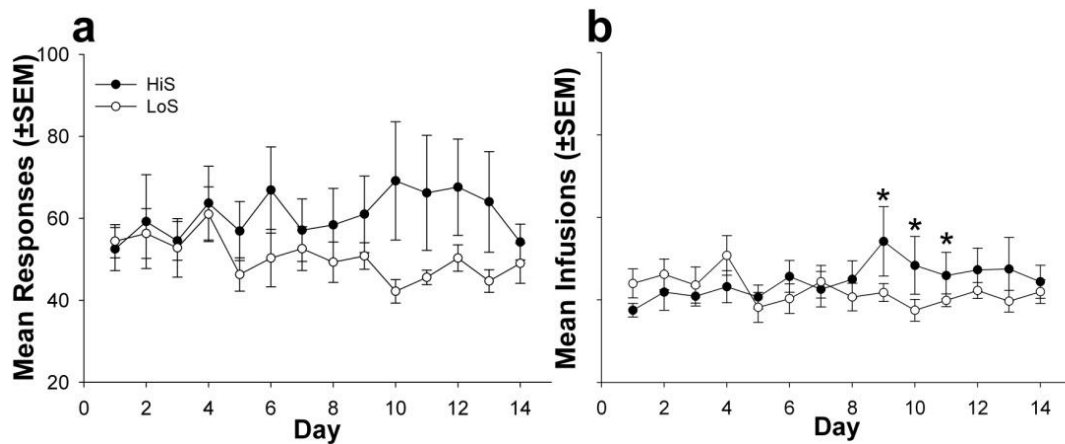
Figure 9 Mean (±SEM) cocaine-paired lever responses (left panel) and cocaine infusions self-administered (right panel) are presented for the 2 experimental groups, HiS (filled circles), and LoS (open circles) during the maintenance phase. The * indicates that the HiS group self-administered more cocaine infusions than the LoS group on that day ($p < .05$).

Extinction

Figure 10 shows the mean responses (± *SEM*) on the lever previously paired with cocaine during the extinction phase when saline was replaced with cocaine. For this measure, there was a significant main effect of day, $(F_{20, 380} = 11.38, p < .01)$, and an interaction between phenotype and day, $(F_{20, 440} = 1.60, p < .05)$. Post hoc analysis revealed that the LoS group made significantly more responses than the HiS group during days 1, 16, and 18.
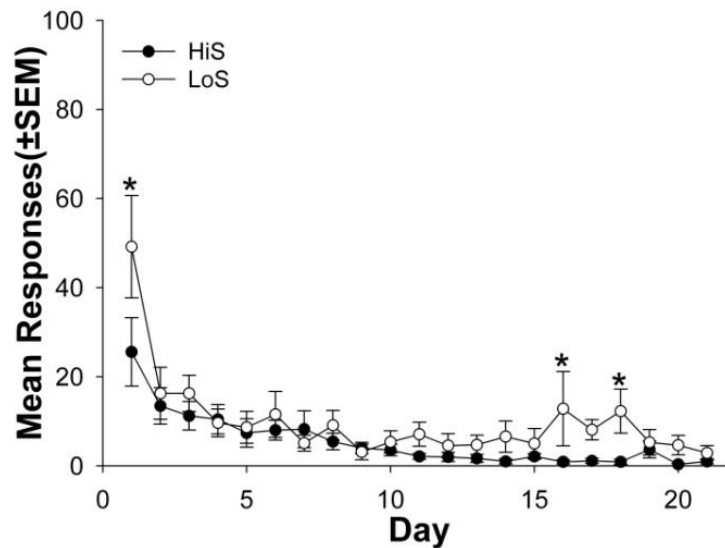
Figure 10 Mean (±SEM) lever responses (left panel) on the previously cocaine-paired lever are presented for the 2 experimental groups, HiS (filled circles), and LoS (open circles) during the extinction phase. The * indicates that the LoS group made more responses than the HiS group on that day $(p < .05)$.

Reinstatement

Figure 11 shows responses (± SEM) on the lever previously paired with cocaine during the reinstatement phase. For this phase, there was a significant main effect of treatment ($F_{1, 162} = 15.72, p < .001$) and a significant interaction between treatment and phenotype ($F_{3, 156} = 4.18, p < .01$). Post hoc analyses showed that, compared to saline priming injections (Sal) preceded by peanut oil treatment (VEH), HiS rats responded more on the lever previously paired with cocaine following all pretreatment priming conditions ($p < .01$) with the exception of the condition in which Sal injections were preceded by allopregnanolone (ALLO) and when the 10 mg/kg cocaine (Coc) priming injections were preceded by ALLO treatment. Compared to the condition in which saline priming injections were preceded by peanut oil treatment (VEH), LoS responding was not significantly different than the condition in which ALLO preceded Sal and the condition in which Coc (5 mg/kg) was preceded by VEH. However, compared to the VEH/Sal condition, LoS rats responded more on the previously cocaine-paired lever following all other pretreatment priming conditions ($p < .05$). HiS rats responded more than LoS rats when VEH treatment was followed by the 5 mg/kg Coc priming injection. LoS rats showed more responding when the 5 mg/kg Coc dose was preceded by ALLO compared to when it was preceded by VEH. When administered before Sal priming injections, ALLO did not induce significantly more responding than VEH treatment.
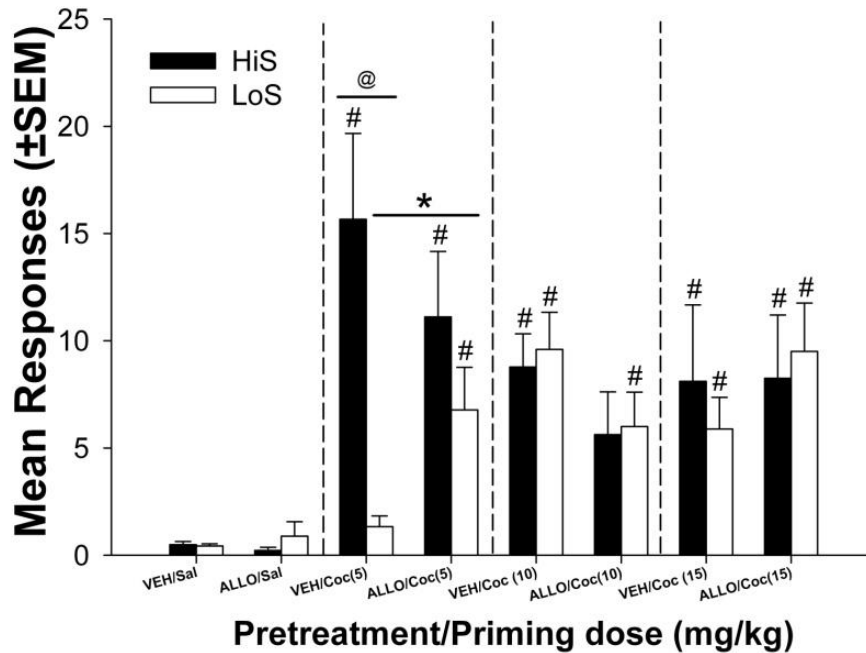
Figure 11 Responses made on the previously cocaine-paired lever between groups during the reinstatement phase. The # indicates a significant ($p < .05$) difference between pretreatment priming dose conditions compared to the condition in which Sal was preceded by VEH. The @ indicates that HiS rats responded more than the LoS rats when the 5 mg/kg Coc priming injection was preceded by VEH ($p < .01$). The * indicates that LoS rats responded more when the 5 mg/kg Coc dose was preceded by ALLO treatment compared to when it was preceded by VEH ($p < .05$).

Discussion

In Experiment 4 we found that, compared to control pretreatment, ALLO potentiated the reinstatement of drug-seeking behavior primed by the smallest dose of cocaine tested (5 mg/kg) in LoS animals. HiS rats responded more following the 5 mg/kg cocaine priming injection preceded by VEH compared to LoS rats. This was similar to results found previously by Perry et al. (2006) with a 15 mg/kg cocaine priming injection. In contrast to its potentiating effects in LoS rats, ALLO attenuated reinstatement responding primed by the 10 mg/kg dose in the HiS animals, insofar as responding in this condition was not significantly different than saline priming injections preceded by VEH.

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There was no effect of ALLO for either phenotype on reinstatement responding primed with the highest cocaine dose (15 mg/kg).

These results ran counter to our hypothesis that ALLO would have a greater effect in the LoS animals compared to the HiS animals. It should be noted that while the potentiating effect of ALLO on reinstatement following the smallest dose of cocaine was statistically significant, the difference in responding between the ALLO and VEH conditions amounted to only a few lever presses over the 2-h reinstatement session. As such, these results need to be interpreted with caution. While the effects are modest, they reveal an interaction between cocaine dose, paradigm, and treatment, especially within the context of results from Experiments 1-3. For instance, while progesterone was more effective at decreasing cocaine self-administration in LoS animals during periods of extended drug access in a manner similar to baclofen, it potentiated self-administration of the lowest dose of cocaine during the final short-access phase in this phenotype. This effect was not found in the HiS rats.

Previous work has indicated that progesterone metabolite ALLO increases the release of dopamine in response to drugs of abuse (Rouge-Pont et al. 2002) and has anxiolytic properties (Brot et al. 1997). ALLO may also enhance the rewarding effects of small doses of cocaine and decrease its aversive (i.e., anxiogenic) effects, thereby facilitating its capacity as a reinforcer and priming injection in the LoS animals. This is particularly relevant, as LoS animals are more sensitive to aversive events and less sensitive to rewarding events than HiS animals (Carroll et al. 2008; Dess et al. 2000; Dess and Minor 1996a; Dess et al. 2005). Furthermore, recent work by Regier et al.

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(Regier et al. 2012) found that LoS animals have more neuronal activity as measured by c-Fos expression in the nucleus accumbens shell following a single cocaine injection compared to HiS rats. Because this area is considered part of the "extended amygdala", a network of brain structures implicated in the aversive aspects of drugs (Koob 2009), these results support the notion that ALLO (as a metabolite of progesterone) may ameliorate cocaine's aversive properties in the LoS animals, thereby driving greater low-dose cocaine-primed reinstatement and self-administration.

Overall, Experiment 4 illustrates that phenotypic treatment sensitivity is a complex and subtle phenomenon. Given the potential therapeutic relevance of this model, it will be important to address several important questions. For example, do the effects of ALLO treatment on extended access to smaller doses of cocaine vary in the HiS vs. LoS animals, and are there phenotype differences in ALLO's effects on PR responding for multiple doses of cocaine? Different reinstatement priming conditions could be examined, such drug-associated cues and stress. Furthermore, as ALLO's effects on dopamine activity in the striatum are influenced by estrous cycle (Laconi et al. 2007), future studies may investigate the interaction of fluctuations in gonadal hormones and phenotypic sensitivity to ALLO treatment across the various animal models of addiction.

Experiment 5: Effects of histamine punishment on cocaine self administration in HiS and LoS rats

Background

According to the DSM-IV (American Psychiatric Association. and Task Force on DSM-IV. 1994), one criterion for the diagnosis of a substance dependence disorder is the regular, continued use of a drug despite aversive consequences (e.g., health complications, hangovers, social repercussions, etc.). One way this aspect of compulsive drug use is modeled in the animal laboratory is by applying electric shock to an organism whenever it makes an operant response for a drug delivery. For instance, Deroche-Gamonet et al. (2004) showed that a small subset of rats in a large heterogeneous population continued to press a lever for i.v. cocaine infusions despite the delivery of shock after the lever-press response. The results of this study have translational relevance, because as in the case with rats, only a similarly small subset of humans who try drugs end up meeting the criterion of dependence (i.e., drug use despite aversive consequences) (National Center for Health Statistics (U.S.) and National Center for Health Statistics (U.S.). 2011). Indeed, Vogel-Sprott et al. (1965) showed that alcoholics were less sensitive to punishment by electric shock compared to non-alcoholics. Electric shock has further been used to illustrate individual differences in punished drug seeking behavior and to investigate other aspects of substance dependence (Barnea-Ygael et al. 2012; Cooper et al. 2007; Dworkin et al. 1989). For instance, Belin et al. (2011) showed that drug-prone rats selected for high novelty preference (HNP) self-administered more cocaine in spite of electric shock following the lever-press response compared to drug-resilient rats with a low preference for novelty (LNP). Based on these findings, we were

interested in comparing the effects of punishment on cocaine self-administration in the HiS and LoS rats.

For Experiment 5, we used a different form of punishment than the studies previously described: i.v. histamine administration. This method is effective at reducing drug self-administration and other reward-driven behaviors in nonhuman primates (Negus 2005; Woolverton et al. 2012). We used histamine in order to improve the face validity of punishment-resistant drug seeking by modeling self-inflicted punishment in non-human animals. That is, while electric shocks serve as effective punishing events (i.e., discrete administration, rapid onset/offset, etc.), they are qualitatively different than the negative consequences of drug use typically encountered by the human addict (i.e., hangovers, anxiety, anhedonia, etc.). Histamine (via i.v. infusion), on the other hand, is fast acting and rapidly metabolized (Middleton et al. 2002; Rose and Browne 1938), and approaches the discrete, immediate effects of shock on drug self-administration through its action on H_1 receptors in the periphery (Goldberg 1980). In this regard, i.v. histamine administration may better model one aversive consequence of cocaine (and opioid) use in humans, namely neurological pruritus. This condition is characterized by aversive, delocalized itching sensations throughout the body; however, the phenomenon is mediated by mu and kappa opioid receptors (Weisshaar et al. 2009).

Additionally, we were interested in modeling a particular pharmacological approach to substance dependence treatment in which the treatment drugs enhance the aversive aspects of drug consumption by interfering with drug metabolization, thereby punishing drug intake. For example, disulfiram is a pharmacological agent that has been

investigated as a treatment for alcoholism and cocaine addiction that acts, in part, by enhancing the aversive, interoceptive aspects of the drugs (Jorgensen et al. 2011). Disulfiram's efficacy in treating cocaine dependence in humans has recently been demonstrated to be mediated by genetic variance (Kosten et al. 2012). Therefore, we were interested in showing if phenotype may mediate sensitivity to the effects of such a pharmacological treatment intervention, however by adding an aversive agent directly to the drug itself in this instance. In the present experiment, we were primarily interested in isolating the aversive effects of this type of pharmacological strategy. To do this, we used histamine because it does not readily cross the blood brain barrier when administered i.v. (Halpern et al. 1959), whereas systemic (i.p.) disulfiram administration has complex interactions with drugs of abuse within the brain (Devoto et al. 2012). This strategy is in contrast to those investigated in Experiments 1-4, in which the treatment agents are largely thought to moderate the rewarding aspects of cocaine.

In this experiment, animals were allowed to self-administer cocaine to establish a baseline of operant response. We then added histamine directly into the cocaine solution and measured its effects on self-administration compared to baseline. We were also interested in potential differences in reacquisition of baseline self-administration rates in the absence of punishment, so the histamine and cocaine solution was then replaced with cocaine-only solution, and the rats' operant responding was again measured and compared to baseline.

Subjects

Nineteen experimentally naïve adult female rats, selectively bred at the University

of Minnesota (Carroll et al. 2002) bred from Occidental HiS and LoS lines (Occidental

College, Los Angeles, CA), were used in Experiment 5.

Procedure

HiS ($n = 9$) and LoS ($n = 10$) rats were trained to self-administer i.v. cocaine as

described in the General Methods section. Once stability criteria were met, rats

continued to self-administer cocaine (0.4 mg/kg/infusion) for 10 daily, 2-h sessions (9:00

a.m.-11:00 a.m.). This constituted the Pre-Histamine phase. Next, during the Histamine

phase, histamine hydrochloride (4 mg/kg/infusion) was added directly into the cocaine

solution, and rats continued to self-administer for 10 more sessions. Last, the

histamine/cocaine solution was replaced with a cocaine-only solution (0.4

mg/kg/infusion) and the rats continued to self-administer drug for 20 more sessions.

These sessions were considered to comprise the Post-Histamine phase.

Data Analysis

For each subject, infusions were averaged into 5-day blocks (2 pre-histamine

blocks, 2 histamine blocks, and 4 post-histamine blocks; see Figure 12). These blocks of

average infusions served as the dependent measure in Experiment 5. Data were analyzed

with a mixed linear model (MIXED procedure) using SAS software (SAS Institute Inc., Cary, NC). Fixed effects in this analysis consisted of phenotype (HiS vs. LoS), block (1-8), as well as a phenotype×block interaction. Blocks of infusions were further analyzed using preplanned within- and between-subjects contrasts. Changes in self-administration were also analyzed during the Histamine and Post-Histamine phases relative to Pre-Histamine cocaine self-administration by computing percent change of average infusions self-administered during blocks 3-8 compared to block 2 ([block 2 infusions - block x infusions] / [block 2 infusions] X100). Percent changes for each Histamine and Post-Histamine block were compared between HiS and LoS animals using Student's t-tests. Results were considered significant if $p < 0.05$.
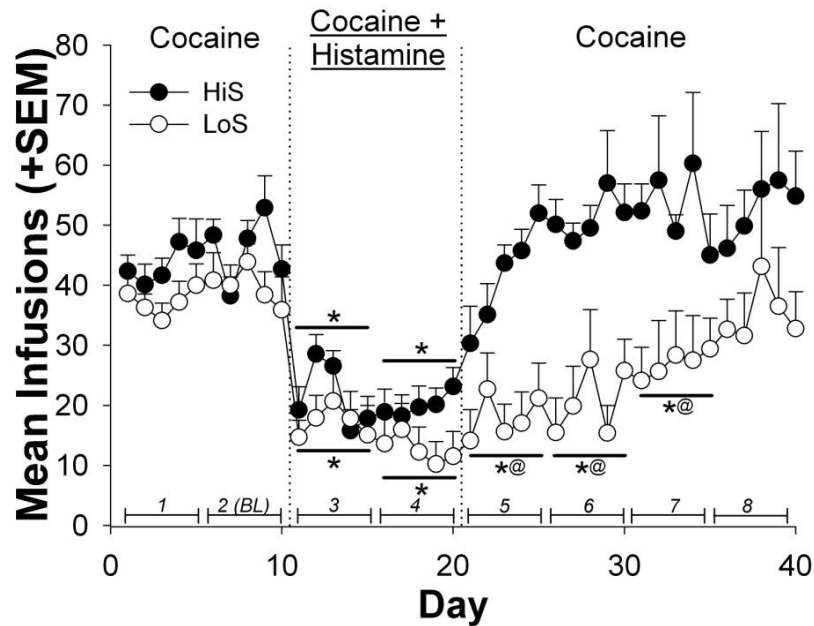
Figure 12 Mean infusions self-administered by HiS and LoS rats. Line segments with italicized numbers directly above the horizontal axis illustrate blocks of 5-day infusion averages. Blocks 1 and 2 constituted the Pre-Histamine phase, blocks 3 and 4 the Histamine phase, and blocks 5-8 the Post-Histamine phase. Block 2 served as the baseline reference measure (BL) of cocaine self-administration prior to histamine exposure. The * indicates a significant difference from baseline (BL, block 2) within each phenotype. The @ indicates phenotype differences in infusions self-administered between the phenotypes ($p < .05$).

Results

As illustrated in Figure 12, analyses of 5-day average blocks of infusions indicated significant fixed effects for phenotype ($F_{1, 17} = 20.26$, $p < .001$) and block ($F_{7, 110} = 26.43$, $p < .001$), as well as a phenotype×block interaction ($F_{7, 110} = 3.03$, $p < .01$). Both HiS and LoS rats showed a significant decrease of infusions self-administered within the Histamine phase compared to their respective baselines ($p < .05$). LoS rats maintained significantly reduced cocaine intake during blocks 5, 6, and 7 of the Post-Histamine phase compared to baseline ($p < .01$). HiS animals showed no differences

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during the Post-Histamine phase compared to baseline and self-administered more

infusions than the LoS animals during blocks 5, 6 and 7 ($p < .01$) of the Post-Histamine

phase.

When comparing percent changes in infusions from baseline, results indicated

that LoS rats had a greater percent decrease compared to HiS animals during blocks 5 and

6 of the Post-Histamine phase ($p < .05$; see Figure 13). There were no differences

between the phenotypes in percent change of infusions from baseline during the

Histamine phase. Thus, the phenotype differences emerged only during the early Post-

Histamine (10 day) recovery phase.
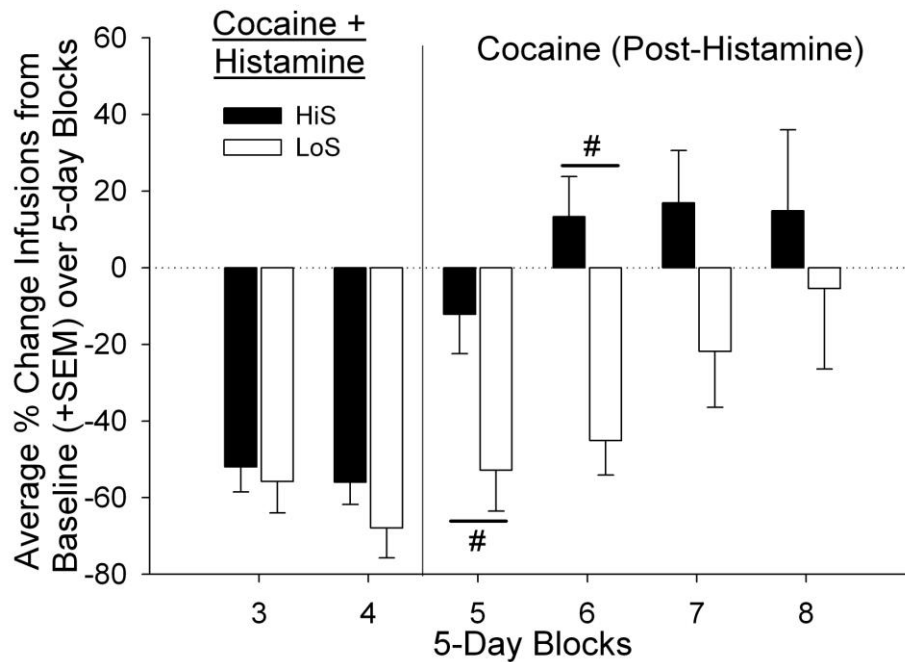
Figure 13 Percent change of infusions self-administered by HiS and LoS rats averaged
into 5-day blocks compared to a 5-day baseline average. The # indicates phenotype
differences in percent change of infusions self-administered between the phenotypes
compared to baseline ($p < .05$)

Discussion

Results from Experiment 5 showed that histamine was an effective punisher of cocaine self-administration in both HiS and LoS animals. However, LoS rats exhibited a delay in reestablishing baseline rates of cocaine self-administration during the Post-Histamine phase. In contrast, self-administration rates were not different from baseline during any portion of the Post-Histamine phase in the HiS animals. While it cannot be ruled out that the HiS and LoS animals metabolize histamine differently, or that it may elicit differential subjective effects between the lines, the absence of phenotype differences in the direct punishing effects of histamine (during the Histamine phase) suggests that histamine served equally well as a punishing agent (has similar, aversive subjective effects) for both lines. Importantly, histamine had an enduring suppressant effect on cocaine self-administration in LoS but not HiS rats after its removal. Future studies could further support this notion by assessing the pharmacokinetic and discriminative stimulus properties of i.v. histamine between the phenotypes.

One interpretation of the delayed return to baseline in the LoS rats is that they were more sensitive to the long-term punishing effects of histamine on cocaine self-administration. Recent research has also established that aversive events are more effective at reducing reward-driven behaviors in LoS (vs. HiS) rats. For instance, Radke et al. (in preparation) showed that LoS rats had reduced operant responding for chocolate-flavored food pellets during withdrawal from chronic cocaine administration compared to HiS rats. However, Experiment 5 is the first study showing the deleterious effects of pairing an aversive event with a positively reinforced response that can persist after its removal in the LoS but not HiS animals. Future work could look at the possible effects

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of histamine punishment on devaluing the reinforcing properties of cocaine by assessing post-histamine PR ratio responding for cocaine. While histamine is rapidly metabolized and short acting (Middleton et al. 2002; Rose and Browne 1938), we also need to rule out the possibility that the decrease in Post-Histamine intake in the LoS animals is the result of some lingering, differential, nonspecific somatic effect (e.g., malaise, peripheral inflammation, etc.) of histamine exposure on general motor activity or motivation. Future studies may look at the effects of non-contingent histamine administration on operant responding in the HiS and LoS rats to address this issue.

The results from Experiment 5 suggest that, similar to experiments 1 and 3, LoS animals may be more sensitive to punishment of ongoing drug self-administration, albeit through pairing the reinforcer with noxious stimuli in the present example. Thus, high and low sweet preference may be a valuable behavioral predictor of sensitivity to treatments, such as disulfiram, that in effect punish drug consumption in humans. Future studies with the HiS and LoS animals may provide additional justification for such clinical trials by directly examining the effects of disulfiram administration on cocaine and ethanol consumption.

General Discussion

The main goal of the experiments detailed in this review was to investigate differences in the effects of pharmacological treatments on i.v. cocaine self-administration and cocaine-seeking behavior using an animal model of phenotypic variance in sweet consumption and substance dependence vulnerability. Experiment 1 showed that baclofen, a $GABA_B$ agonist that has been investigated as a treatment for substance dependence, had opposite effects on cocaine self-administration during periods of extended drug access. Specifically, baclofen attenuated cocaine self-administration in the LoS line, while it potentiated the escalation of cocaine intake in the HiS line. Experiment 2 was conducted to extend this investigation to an animal model of cocaine-induced relapse (reinstatement). However, baclofen attenuated cocaine-primed reinstatement equally well in either phenotype. Experiment 3 demonstrated that another potential GABAergic treatment for substance dependence, the neuroactive steroid progesterone, had effects similar to those found in Experiment 1; namely, progesterone decreased cocaine intake in the LoS rats, but it increased intake in the HiS rats. In contrast, Experiment 4 showed that allopregnanolone, a neuroactive metabolite of progesterone, potentiated the reinstatement of drug-seeking behavior following a low-dose cocaine priming injection in LoS rats and attenuated reinstatement responding in HiS rats following a moderate cocaine priming dose. Experiment 5 demonstrated that i.v. histamine punishment was equally effective at reducing cocaine self-administration between the lines; however, LoS rats exhibited a delay in reaching baseline rates of cocaine self administration when histamine punishment was removed. The following

discussion will address these findings within the context of clinical practice and implications for future experiments.

Clinical Implications

Our understanding of how to predict addiction severity and customize treatments based on individual vulnerability should lead to better management of drug abuse treatments. For example, as treatment completion is positively associated with subsequent abstinence (Tzilos et al. 2009), one major clinical obstacle is lack of patient compliance and dropout (Baekeland and Lundwall 1975; Laudet et al. 2009). Furthermore, the more rapidly these treatment efforts produce positive outcomes, the more likely patients are to continue treatment (Baekeland and Lundwall 1975; Stark 1992). Given the heterogeneity in pharmacological response, quick, noninvasive measures of stable behavioral markers that predict sensitivity to a given agent could result in more rapid, positive treatment outcomes and thus greater long-term adherence. In addition to the results from the present experiment, two recent clinical studies suggest that one noninvasive marker could be sweet preference. For instance, Garbutt et al. (2009) and Laaksonen et al. (2011) demonstrated that alcoholics with a high sweet-preference respond better to naltrexone than those with a lower sweet preference. The results from our studies showing differential pharmacological treatment (i.e., baclofen, progesterone, allopregnanolone) sensitivity between rats that have been selectively bred for high vs. low sweet response offer translational support for the use of sweet preference as a clinical predictor. In this regard, sweet preference may be considered an

endophenotype, or a measurable intermediary between a disease (i.e., substance dependence) and its heritable biological mechanisms (Gottesman and Gould 2003).

A potential mechanism driving the ingestion of sweet substances is its activation of μ-opioid receptors within GABAergic interneurons in the ventral tegmental area (VTA) (Lemon et al. 2004), which facilitates the encoding of reward information (Taha et al. 2006) by attenuating the inhibitive action of these interneurons on dopaminergic neurons in the VTA and striatum (Johnson and North 1992; however, see Margolis et al. 2012), a network that is also a major locus of action for drugs of abuse. Thus, as sweet preference is a relatively stable trait (Desor and Beauchamp 1987), these studies suggest that it may be a viable probe into the functioning of the endogenous opioid system, and thus an endophenotype of addiction liability and sensitivity to treatments that target the GABA and opioid systems (e.g., naltrexone, baclofen, progesterone, allopregnanolone). Recent imaging and pharmacogenetic work has corroborated this notion, showing that μ-opioid receptor binding was predictive of behavioral treatment sensitivity in cocaine- or alcohol-dependent individuals (Ghitza et al. 2010; Mann and Hermann 2010).

It is likely that sweet preference alone may not be sensitive enough a measure for predicting response to all pharmacological treatment options, and may need to be refined and used in combination with other assessments, such as genotyping. However, it has been argued that searching for genetic variants with liability for complex behavioral diseases, such as addiction, is substantially less effective than the application of environmental modifications (e.g., public health initiatives, behavioral interventions, etc.) due to the high number of genetic variants, each with small effect size (Merikangas and

Risch 2003). The HiS/LoS model offers additional value, in that the experimentally

delineated genomes (via selective breeding) in these animals may also help elucidate

salient predictive variants that could guide clinical pharmacogenetic efforts. As

suggested by Agrawal et al. (2012), the future of effective substance dependence therapy

should involve personalized treatments shaped by the patients' endophenotypes

(electroencephalogram activity, Stroop task performance, neuroimaging outcomes, etc.),

biomarkers, addiction severity, and genotype.

Sweet preference may also serve as a probe of affective traits that could guide

treatment choices (Dess and Edelheit 1998), such as those that enhance the aversive

aspects of drug intake as explored in Experiment 5. Previous work has shown that LoS

rats display greater negative reactivity, or emotionality, compared to HiS rats under

stressful conditions (Dess et al. 2000; Dess and Minor 1996b). LoS rats show greater

latency of emergence and increased defecation in the novel open field, more stress-

induced anorexia (Dess and Minor 1996a) and analgesia than HiS rats (Dess et al. 2000).

LoS rats also display more emotional reactivity (e.g., subordination, hyperthermia) when

competing with weight-matched HiS rats for food (Eaton et al. 2012). Further, compared

to HiS rats, LoS animals displayed increased reactivity of the hypothalamic-pituitary-

adrenal (HPA) axis, a system that is integral to the stress response and linked to a

multitude of psychiatric disorders including drug addiction (Evans et al. 2011; Sinha

2008). LoS rats also display greater withdrawal symptoms following chronic (but not

acute; Radke et al., submitted) drug administration (Dess et al. 2005). As stress and

emotionality are powerful liability factors in many aspects of substance dependence

(Ungless et al. 2010), the HiS and LoS model may have special utility in investigating a

more broad construct of emotionality that can, along with results from other high- and low-vulnerable animal models, provide an overarching framework with predictive and translational value. For instance, aversive response to drugs of abuse has been extensively studied between the inbred Lewis and Fischer rats. These strains show similarities with the HiS/LoS lines in respect to many aspects of drug abuse vulnerability (Lewis>Fischer) and stress response (Fischer>Lewis) (Kosten and Ambrosio 2002). Interestingly, the less addiction-prone Fischer rats show greater conditioned taste aversion following administration of drugs that are reinforcing under other conditions (e.g., self-administration) (Kosten and Ambrosio 2002). Table 8 illustrates the divergent responses to negative events in rats that vary in avidity for rewarding events.

Table 8 **Relationship between high and low responders for drug and nondrug rewards and the positive effects of drugs and response to aversive conditions (adapted from Carroll et al. 2009)**

Groups	Positive effects (drug seeking/drug-taking)	Negative effects	Reference
HiI vs. LoI (impulsivity)	HiI>LoI	LoI>HiI Cocaine extinction	Perry et al. (2008)
		LoI>HiI Heroin withdrawal Cocaine withdrawal	Dalley et al. (2007) Dalley et al. (2007)
HiS vs. LoS (saccharin intake)	HiS>LoS	LoS>HiS Ethanol withdrawal Glucose withdrawal	Dess et al. (2005) Yakovenko et al. (2011)
		HiS>LoS Acute morphine withdrawal	Radke et al. (submitted)
		LoS>HiS Food deprivation- induced wheel running	Dess et al. (2000)
		LoS>HiS Food deprivation + methylphenidate- induced wheel running	McLaughlin et al. (2011)
		LoS>HiS Acoustic startle	Dess et al. (2000)
		LoS>HiS Food-deprivation + methylphenidate- induced startle	McLaughlin et al. (2011)
		LoS>HiS Social subordination	Eaton et al. (2012)
HR vs. LR (novelty reactivity)	HR>LR	LR>HR Fear, anxiety and emotionality	Dellu et al. (1996) Kabbaj et al. (2000) Stead et al. (2006)
HAC vs. LAC (ethanol consumption)	HAC>LAC	LAC>HAC Withdrawal: Ethanol	Chester et al. (2003)
LEW vs. F344 (inbred strains)	LEW>F344	F344>LEW Fear, anxiety, emotionality	Kosten and Ambrosio (2002)
		F344>LEW Taste aversion (morphine)	Riley (2011)

HiI and LoI, high and low impulsivity; HiS and LoS, high and low saccharin; HR and LR, high and low responders ; HAC and LAC, high and low alcohol consumers; LEW and F344, Lewis and Fischer F344

Future Directions

While animal research using high and low vulnerable phenotypes has enhanced our understanding into the etiology of substance dependence, this work may be of marginal translational value unless it ultimately improves treatment outcomes. Thus, a goal of the present research is to conduct parallel experiments with the other differential phenotypes described in the Introduction section. In the preclinical and clinical literature, the various phenotypic traits (i.e., sweet preference, impulsivity, novelty-seeking) likely produce non-homogenous mechanisms of addiction vulnerability or resilience. For instance, while the HiS and LoS animals display high and low impulsivity, respectively, rats screened for high and low impulsivity do not show differential saccharin-preference scores (Perry et al., unpublished data). Recently, we also failed to show a difference in saccharin-preference scores between the addiction-prone and -resilient Lewis and Fischer rats (Holtz et al., in preparation). As such, we cannot infer that these high and low-vulnerable animals would display differential treatment response to agents such as baclofen or progesterone, as was the case with HiS and LoS rats in Experiments 1 and 3.

In addition, we need to investigate how independent these predictors are and how they interact. For example, clinical studies have shown that sweet-liking only predicts alcoholism if it is comorbid with sensation-seeking (Kampov-Polevoy et al. 1998; Lange et al. 2010). Insofar as sweet preference predicts treatment sensitivity in human studies, questions remain regarding whether the predictive value of this trait in the attenuating effects of naltrexone on alcohol consumption is less powerful than sensation-seeking phenotype, how these factors interact, and how they are moderated by other phenotypic

variables, such as impulsivity. This may be relevant, as impulsivity is emerging as a predictor of behavioral and pharmacological treatments for nicotine and cocaine dependence in humans (MacKillop and Kahler 2009). Clearly, assessing sweet preference and pharmacological treatment sensitivity in the HR/LR and HiI/LoI models (see Table 8), amongst others, could help justify and guide clinical investigations to address these issues.

Furthermore, it is important that the hypotheses of these particular review studies are also examined in the clinical laboratory. If baclofen, for instance, were more effective in reducing cocaine intake in sweet-likers compared to sweet-dislikers, as was found with the effects of naltrexone on alcohol intake (Garbutt et al. 2009; Laaksonen et al. 2011), more experiments would be needed to understand the translational limitations of our model. Future research should also investigate phenotype differences in sensitivity to the various treatments for dependence to multiple classes of drugs. While the present studies investigated pharmacological agents that reduce the rewarding effects of drugs or punish drug self-administration, agonist replacement therapy is an effective treatment strategy for opioid dependence (e.g., methadone; see Bart 2012) that is also showing promise for treating stimulant dependence (e.g., modafinil; see Herin et al. 2010). Future research should investigate how phenotypes differing in drug abuse vulnerability respond to this approach.

A more recent pharmacological development for substance dependence is the orexin antagonist and hypnotic, Almorexant (Steiner et al. 2012). The orexins are neuropeptides that originate from cell groups segregated to the lateral (LH) hypothalamus

and perifornical area (PFA) that project to brain regions associated with reward processing (Peyron et al. 1998). Importantly, orexin mediates behaviors related to both drug and non-drug rewards, such as sweet substances (Cason et al. 2010; Thompson and Borgland 2011). For example, orexin receptor antagonism reduces feeding behavior (Rodgers et al. 2001), and attenuates ethanol consumption, reinstatement of cocaine- and ethanol-seeking behavior (James et al. 2011; Shoblock et al. 2011; Srinivasan et al. 2012), and motivation for cocaine as assessed by a progressive-ratio schedule of reinforcement in rodents (Espana et al. 2010). Recently, we demonstrated that HiS rats display greater orexin expression in the LH and PFA compared to LoS rats (Holtz et al. 2012), suggesting that phenotype may also predict response to drugs that target the orexin system. Additionally, a study by Moorman and Aston-Jones (2009) demonstrated that an orexin receptor antagonist was more effective at reducing ethanol consumption in outbred rats selected for high (vs. low) ethanol consumption. While providing another line of evidence implicating orexin in the heterogeneity of addiction vulnerability, these data also suggest that, while orexin system may be a potential mechanism for pharmacological interventions, such effects may be moderated by vulnerability phenotype. Therefore, we are currently investigating the effects of orexin antagonist on drug self-administration in the HiS and LoS animals.

In summary, this review has provided evidence that phenotypic variance in sweet-intake may be a valid predictor of vulnerability to the development of substance abuse, as well as sensitivity to pharmacological treatments for this disorder. Baclofen and progesterone attenuated cocaine self-administration during periods of extended drug access in the LoS animals, but increased cocaine consumption in the HiS animals.

Baclofen attenuated cocaine-primed reinstatement of drug-seeking behavior in both HiS and LoS rats equally. In contrast, allopregnanolone potentiated low-dose cocaine-primed reinstatement in LoS rats; whereas, it attenuated this response following moderate-dose cocaine priming injections in the HiS rats. Histamine was equally effective in punishing cocaine self-administration in both phenotypes. However, LoS rats exhibited a delay in reaching baseline levels of self-administration following histamine punishment. These data may inform therapeutic strategies by illustrating that phenotypic traits can aid in matching substance-dependent patients with optimal pharmacological interventions.

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