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ACOUSTIC STARTLE AND COCAINE USE IN RATS

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An Abstract of
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Abstract

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Marina Georgia Wheeler

Cocaine addiction is the compulsive use of cocaine despite the negative consequences of doing so and a desire to abstain. Identifying individual factors that can predict vulnerability to abuse cocaine is an important aim of scientific investigation and can be used to develop early interventions directed towards at risk populations as well as for the development of animal models which more appropriately reflect the disorder. Interestingly, men addicted to cocaine but no longer using cocaine exhibit markedly diminished acoustic startle amplitudes. These decreased startle amplitudes could precede or be a consequence of cocaine use however the evidence available does not suggest that they are the consequence. There is no evidence for or against the alternative, that low acoustic startle precedes cocaine use. This dissertation project investigates the potential value of acoustic startle amplitudes in predicting vulnerability to abuse cocaine using a rat intravenous model of cocaine self-administration. Results show that rats with drug naïve low startle amplitudes are more sensitive to cocaine, motivated, persistent in cocaine seeking and more likely to reinstate extinguished cocaine seeking compared to high startle rats. Low startle rats also exhibit a significant preference for novel environments, a behavioral phenotype previously shown to predict habitual cocaine self-administration in rats. These data suggest that low startle may precede cocaine abuse and therefore predict the likelihood to abuse cocaine.

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CHAPTER 1

General Introduction

The acoustic startle response is a reflexive reaction to an unexpected auditory stimulus observed in many species (Davis, 1980; Koch & Schnitzler, 1997). It is mediated in rats and presumably humans by a simple neural pathway from cochlear root neurons in the auditory nerve projecting to the nucleus reticularis pontis caudalis with projections onto motor neurons in the facial motor nucleus and spinal cord (Davis, Gendelman, Tischler, & Gendelman, 1982; Lee, Lopez, Meloni, & Davis, 1996). The short latency eye-blink component of the acoustic startle response is measured in humans (Grillon, 2002) and the whole body response is measured in rats (Davis, 1980). Acoustic startle amplitude is a highly heritable trait (Anokhin, Heath, Myers, Ralano, & Wood, 2003; Cadenhead, Swerdlow, Shafer, Diaz, & Braff, 2000) with high inter-individual variability within a population. Although the elicitation of acoustic startle does not require forebrain structures (Davis & Gendelman, 1977), several forebrain structures can modulate the acoustic startle response. For example, pharmacological manipulations of cortical and sub-cortical brain regions have been shown to alter acoustic startle (Davis, 1980). Dopamine is a modulatory neurotransmitter and agonists of the dopaminergic system enhance acoustic startle amplitudes in rats (Davis, 1980; Davis & Aghajanian, 1976) while dopaminergic antagonists attenuate it (Kehne & Sorenson, 1978; Schwarzkopf, Bruno, & Mitra, 1993).

Cocaine addiction is the compulsive use of cocaine despite its negative consequences and the desire to abstain from use (*Diagnostic and Statistical Manual of Mental Disorders*, 2000). In 2009 the Substance Abuse and Mental Health services reported that cocaine was the third most popular illicit drug abused by Americans. In 2000, Efferen and colleagues reported that cocaine addicted men not currently abusing

cocaine had markedly diminished acoustic startle amplitudes compared to non-cocaine using controls. This effect was replicated on a different group of men in which the low levels of startle remained for up to one year of abstinence, the longest time tested (Cocoran et al., 2011). This intriguing pair of observations leads to the question of whether these low levels of startle precede and hence are a risk factor for cocaine abuse or instead are a result of chronic cocaine intake.

Cocaine, Dopamine and Acoustic Startle

Cocaine's primary reinforcing effect is through its actions on the mesolimbic-cortical dopaminergic system (D. C. Roberts, Corcoran, & Fibiger, 1977; D. C. Roberts & Koob, 1982; Wise, 1984). By preventing the reuptake of dopamine by the dopamine transporter, cocaine increases synaptic dopamine (Ritz, Lamb, Goldberg, & Kuhar, 1987). In addition, chronic abuse of cocaine causes an overall decrease in dopaminergic function, a condition called hypodopaminergia (Dackis & O'Brien, 2001; Pilotte, Sharpe, Rountree, & Kuhar, 1996). By decreasing dopamine terminal density in the striatum (J. Lee, Parish, Tomas, & Horne, 2011), dopamine transporter synthesis (Cerruti, Pilotte, Uhl, & Kuhar, 1994) and dopamine transporter sensitivity (Pilotte, Sharpe, & Kuhar, 1994), cocaine use overtime significantly down regulates the dopaminergic signal. Evidence for this hypodopaminergic effect has been observed in human cocaine abusers (Hitri, Casanova, Kleinman, & Wyatt, 1994; Martinez et al., 2009; Volkow et al., 1990), rodents (Sharpe, Pilotte, Mitchell, & De Souza, 1991; Xia, Goebel, Kapatos, & Bannon, 1992) and primates (Kirkland Henry, Davis, & Howell, 2009). In addition to cocaine's effect on dopamine, drugs that increase dopaminergic transmission also increase acoustic startle amplitudes while antagonists of D1 and D2 receptors diminish the acoustic startle

response (Davis, 1985; Davis, Svensson, & Aghajanian, 1975; Meloni & Davis, 1999; Schwarzkopf, et al., 1993). Hence, the diminished acoustic startle amplitude in cocaine addicts could result from cocaine-induced hypodopaminergia.

The Correlative Relationship Between Acoustic Startle and Cocaine Use

The effect of cocaine on subsequent acoustic startle has been investigated previously. In rats, 7 days of cocaine treatments that were either response contingent (up to 170 mg/kg/day) or experimenter delivered (20 mg/kg/day, IP) produced no changes in acoustic startle amplitude compared to vehicle treated control animals in the first 24 hours after cocaine treatment ended (Mansbach, Markou, & Patrick, 1994). However, when the effect of seven once daily injections of cocaine (20 mg/kg/day, IP) were measured on acoustic startle 7 days after the last cocaine treatment, acoustic startle amplitude was significantly reduced in response to a 115-dB stimulus but not to a 95 or 105-dB one (Gordon & Rosen, 1999). The limited length of cocaine exposure in both of these experiments limits the interpretations that can be made about the impact of cocaine on acoustic startle amplitudes.

One study that did look at the effects of longer cocaine exposure in rats compared the effects of 2 and 8 weeks of injections (30 mg/kg/day, IP) on subsequent acoustic startle (Adams, Efferen, Duncan, & Rotrosen, 2001 2001). Although two weeks of daily cocaine injections did not reduce acoustic startle amplitudes compared to vehicle treated rats 1, 3, 14 or 28 days after the end of the cocaine treatment 8 weeks cocaine did at 1, 3 and 14 but not at 28 days after cocaine cessation suggesting that if acoustic startle is diminished by cocaine treatment, the effect may be transient. In the one primate study

looking at cocaine treatment and acoustic startle, rhesus macaques allowed to self-administer cocaine for 10 weeks had acoustic startle responses comparable to drug naïve rhesus macaques (Kirkland Henry, et al., 2009). Furthermore, there was no change in the ability of cocaine to increase startle amplitude in cocaine experienced macaques when compared to prior cocaine self-administration. These two studies which did lengthen the times-course of the cocaine treatment showed either no effect on acoustic startle or only transient effects on acoustic startle. In summary, the literature available does not provide convincing evidence that acoustic startle is dramatically diminished by previous cocaine exposure as was observed in the Efferen (2000) study. Moreover, in a pilot study we found that there was no decrease in overall acoustic startle amplitude after 14 to 28 days of cocaine self-administration compared to a startle test prior to cocaine intake. In addition, the excitatory effects of d-amphetamine, which releases dopamine and blocks reuptake also did not decrease after cocaine self-administration (supplementary data).

The second, more exciting possibility is that low startle amplitude is actually predictive of cocaine abuse. That is, low dopaminergic tone could predict both low startle amplitude and a greater reinforcing effect of cocaine via a greater increase in the signal-to-noise ratio when a given dose of cocaine is taken. The high prevalence of cocaine abuse emphasizes the need to identify individual factors that can be used to predict vulnerability to abuse cocaine, identify at risk populations and to institute preventative measures that reduce exposure. This could also be relevant to pre-clinical models of cocaine abuse. By identifying animals more likely to develop habitual cocaine seeking behaviors similar to those expressed by humans, a more clinically relevant model of cocaine abuse can be developed.

While there is currently no evidence of the possible predictive value of acoustic startle, none of the research that has been done can discount this possibility. Interestingly, acoustic startle is a highly heritable trait (Anokhin, et al., 2003; Cadenhead, Swerdlow, et al., 2000) that can be stable over time within an individual (Cocoran et al., 2011). It is conceivable, therefore, to imagine that the small magnitude of the acoustic startle response in cocaine addicted men (Cocoran et al., 2011; Efferen et al., 2000) preceded their cocaine use. Hence, the purpose of the dissertation was to evaluate whether low startle might be predictive of cocaine seeking.

Animal Models

Cocaine associated behaviors that could differ between individuals with high or low acoustic startle amplitudes include differences in sensitivity to cocaine, motivation to obtain cocaine, persistence in cocaine seeking, and likelihood to reinstate extinguished cocaine seeking (Belin, Berson, Balado, Piazza, & Deroche-Gamonet, 2011; Deroche-Gamonet, Belin, & Piazza, 2004). Increases in motivation, persistence of cocaine seeking, and repeated relapse to cocaine use following periods of abstinence are also criteria outlined for the diagnosis of substance abuse in the DSM-IV (*Diagnostic and Statistical Manual of Mental Disorders*, 2000).

If low startle individuals are more vulnerable to developing habitual patterns of cocaine use, they should display greater amounts of these behaviors. Lower functional dopaminergic transmission could mean that the magnitude of the reinforcing dopaminergic signal produced by cocaine in these people would be greater than in individuals with higher rates of dopaminergic transmission. If true, then the increased

magnitude of the reinforcing signal of cocaine in low startle individuals might make them more sensitive to smaller concentrations of cocaine. If cocaine is more reinforcing, than these individuals also would be more highly motivated to acquire cocaine, persistent in their cocaine seeking activities, and make it more difficult to abstain from cocaine use. The behavioral phenotype of novelty preference also has been shown to be predictive of cocaine abuse potential in rats (Belin, et al., 2011), predicting that low startle rats should also have a preference for novelty compared to high startle rats.

The studies described herein were carried out using a rat model of cocaine intravenous self-administration. The cocaine intravenous self-administration model is an operant task in which the delivery of cocaine is contingent upon the animal pressing an active lever (Weeks, 1962). A second inactive lever is also available but is not linked to the administration of cocaine. The two levers are used to assess cocaine specific versus non-specific lever presses. In the self-administration model acquisition is the period of time when the animal learns to obtain cocaine. A maintenance phase is defined as the period of time when the animal maintains a consistent level of cocaine self-administration across several days. Once the animal is maintaining responding it is possible to look at sensitivity to cocaine using a dose-response curve and well as other cocaine related behaviors such as motivation to obtain cocaine, persistence in cocaine seeking, and the reinstatement of extinguished cocaine seeking.

Sensitivity to cocaine is measured by evaluating the amount of cocaine or the number of cocaine injections a rat will self-administer at a given dose. If the concentration of the cocaine solution is below what the rat can detect, responding on the active lever should decrease to the rate observed on the inactive lever. The reinforcing

value of cocaine is inferred from the rate at which a selected cocaine dose is self-administered. Motivation to obtain cocaine is measured using a progressive ratio schedule of reinforcement. On this schedule the rat is required to make more and more presses on the active lever to obtain cocaine. The highest number of presses the rat makes to obtain a single dose of cocaine, beyond which the rat no longer presses the active level (breakpoint), is used as a measure of motivation. Persistence in cocaine seeking is defined by the number of bar presses on the active lever the rat makes during cued no drug, or time-out, intervals. This number typically increases as the reinforcing value of the cocaine concentration increases and is considered a measure of persistence in cocaine seeking because the unavailability of cocaine is clearly signaled to the animal (Belin, et al., 2011). When bar presses on the active lever no longer deliver cocaine, extinction occurs. Extinction is often defined as no significant difference in responses on the active and inactive levers or, as a limited number of responses on the active lever, typically less than 20% of the responses made during maintenance (Epstein, Preston, Stewart, & Shaham, 2006b 2006). After extinction, relapse or reinstatement of cocaine seeking can be measured (Epstein, et al., 2006b 2006). The animal can be presented with a drug, or a cue previously associated with access to cocaine, or some sort of stress. This results in a greater number of presses on the active lever than that seen after extinction.

The self-administration model is based on the assumption that both humans and animals can display cocaine craving based either on the desire for the reinforcing effects of cocaine or to avoid the adversity such as stress or withdrawal (Kalivas & McFarland, 2003; Pahwal, Hyman, & Sinha, 2008; Self & Nestler, 1998). The model differs from the

human condition because cocaine is readily available in the rat model and abstinence in humans is sometimes elected rather than being actively extinguished. Although the rat cocaine self-administration model has considerable face validity and is the preferred model of cocaine abuse (Ahmed, 2010), it is important to acknowledge the limitations of the model and the scope of what it can be used for to describe the human situation.

A paradigm that has been used to evaluate the prefernce for a novel environment, a trait shown to predict vulnerability to develop habitual cocaine self-administration in rats (Belin, et al., 2011), is the place preference paradigm. The place preference apparatus is a chamber consisting of two separate and physically distinct compartments that can be separated from each other by a door. By confining the rat to one chamber for a predetermined length of time, that chamber becomes familiar. When the rat is then allowed to freely explore both compartments, a novelty preference can be determined based upon the amount of time spent in the familiar compartment and the novel compartment.

Summary

Clinical data show that men with extensive histories of cocaine abuse have markedly diminished acoustic startle responses two weeks and up to one year after cocaine use has ended (Corcoran et al., 2011; Efferen et al., 2000). Currently it is not known if low acoustic startle amplitude precedes cocaine abuse, or is a consequence of it. However, the literature available does not provide convincing evidence that acoustic startle is substantially diminished by previous cocaine exposure and when it is it is only transient. Dopamine is central to the reinforcing effects of cocaine and can also exert

modulatory control over acoustic startle amplitude. Low drug naïve acoustic startle amplitudes, as well as an enhanced potential to abuse cocaine, could be accounted for by a low level of dopaminergic transmission by increasing the reinforcing signal of cocaine and inhibiting acoustic startle amplitude. The purpose of this dissertation was to evaluate whether drug naïve low acoustic startle predicts cocaine use based on an increased sensitivity to self-administer cocaine, greater motivation to obtain cocaine, greater persistence in cocaine seeking, a greater likelihood to reinstate extinguished cocaine seeking and higher novelty preference compared to high startle rats.

References

Adams, J. U., Efferen, T. R., Duncan, E. J., & Rotrosen, J. (2001). Prepulse inhibition of the acoustic startle response in cocaine-withdrawn rats. *Pharmacol Biochem Behav, 68*(4), 753-759. doi: S0091-3057(01)00478-6 [pii]

Ahmed, S. H. (2010). Validation crisis in animal models of drug addiction: Beyond non-disordered drug use toward drug addiction. *Neuroscience and Biobehavioral Reviews, 35*(2), 172-184. doi: DOI 10.1016/j.neubiorev.2010.04.005

Anokhin, A. P., Heath, A. C., Myers, E., Ralano, A., & Wood, S. (2003). Genetic influences on prepulse inhibition of startle reflex in humans. *Neuroscience Letters, 353*(1), 45-48. doi: DOI 10.1016/j.neulet.2003.09.014

Belin, D., Berson, N., Balado, E., Piazza, P. V., & Deroche-Gamonet, V. (2011). High-novelty-preference rats are predisposed to compulsive cocaine self-administration. *Neuropsychopharmacology, 36*(3), 569-579. doi: npp2010188 [pii]

10.1038/npp.2010.188

Cadenhead, K. S., Swerdlow, N. R., Shafer, K. M., Diaz, M., & Braff, D. L. (2000). Modulation of the startle response and startle laterality in relatives of schizophrenic patients and in subjects with schizotypal personality disorder: evidence of inhibitory deficits. *Am J Psychiatry, 157*(10), 1660-1668.

Cerruti, C., Pilotte, N. S., Uhl, G., & Kuhar, M. J. (1994). Reduction in dopamine transporter mRNA after cessation of repeated cocaine administration. *Brain Res Mol Brain Res, 22*(1-4), 132-138.

Corcoran, S., Norrholm, S. D., Cuthbert, B., Sternberg, M., Hollis, J., & Duncan, E. (2011). Acoustic startle reduction in cocaine dependence persists for 1 year of

abstinence. *Psychopharmacology, 215*(1), 93-103. doi: DOI 10.1007/s00213-010-2114-2

Dackis, C. A., & O'Brien, C. P. (2001). Cocaine dependence: a disease of the brain's reward centers. *J Subst Abuse Treat, 21*(3), 111-117. doi: S0740-5472(01)00192-1 [pii]

Davis, M. (1980). Neurochemical modulation of sensory-motor reactivity: acoustic and tactile startle reflexes. *Neurosci Biobehav Rev, 4*(2), 241-263.

Davis, M. (1985). Cocaine: excitatory effects on sensorimotor reactivity measured with acoustic startle. *Psychopharmacology (Berl), 86*(1-2), 31-36.

Davis, M., & Aghajanian, G. K. (1976). Effects of apomorphine and haloperidol on the acoustic startle response in rats. *Psychopharmacology (Berl), 47*(3), 217-223.

Davis, M., Gendelman, D. S., Tischler, M. D., & Gendelman, P. M. (1982). A primary acoustic startle circuit: lesion and stimulation studies. *J Neurosci, 2*(6), 791-805.

Davis, M., & Gendelman, P. M. (1977). Plasticity of the acoustic startle response in the acutely decerebrate rat. *J Comp Physiol Psychol, 91*(3), 549-563.

Davis, M., Svensson, T. H., & Aghajanian, G. K. (1975). Effects of d- and l-amphetamine on habituation and sensitization of the acoustic startle response in rats. *Psychopharmacologia, 43*(1), 1-11.

Deroche-Gamonet, V., Belin, D., & Piazza, P. V. (2004). Evidence for addiction-like behavior in the rat. *Science, 305*(5686), 1014-1017. doi: 10.1126/science.1099020305/5686/1014 [pii]

Diagnostic and Statistical Manual of Mental Disorders. (2000). (4 ed.). Washington, DC: American Psychiatric Association.

Efferen, T. R., Duncan, E. J., Szilagyi, S., Chakravorty, S., Adams, J. U., Gonzenbach, S., . . . Rotrosen, J. (2000). Diminished acoustic startle in chronic cocaine users. *Neuropsychopharmacology, 22*(1), 89-96. doi: S0893-133X(99)00089-5 [pii] 10.1016/S0893-133X(99)00089-5

Epstein, D. H., Preston, K. L., Stewart, J., & Shaham, Y. (2006). Toward a model of drug relapse: an assessment of the validity of the reinstatement procedure. *Psychopharmacology, 189*(1), 1-16. doi: DOI 10.1007/s00213-006-0529-6

Gordon, M. K., & Rosen, J. B. (1999). Lasting effect of repeated cocaine administration on acoustic and fear-potentiated startle in rats. *Psychopharmacology (Berl), 144*(1), 1-7.

Grillon, C. (2002). Startle reactivity and anxiety disorders: aversive conditioning, context, and neurobiology. *Biol Psychiatry, 52*(10), 958-975. doi: S0006322302016657 [pii]

Hitri, A., Casanova, M. F., Kleinman, J. E., & Wyatt, R. J. (1994). Fewer dopamine transporter receptors in the prefrontal cortex of cocaine users. *Am J Psychiatry, 151*(7), 1074-1076.

Kalivas, P. W., & McFarland, K. (2003). Brain circuitry and the reinstatement of cocaine-seeking behavior. *Psychopharmacology, 168*(1-2), 44-56. doi: DOI 10.1007/s00213-003-1393-2

Kehne, J. H., & Sorenson, C. A. (1978). The effects of pimozide and phenoxybenzamine pretreatments on amphetamine and apomorphine potentiation of the acoustic startle response in rats. *Psychopharmacology (Berl), 58*(2), 137-144.

Kirkland Henry, P., Davis, M., & Howell, L. L. (2009). Effects of cocaine self-administration history under limited and extended access conditions on in vivo striatal dopamine neurochemistry and acoustic startle in rhesus monkeys. . *Psychopharmacology (Berl), 205*(2), 237-247.

Koch, M., & Schnitzler, H. U. (1997). The acoustic startle response in rats--circuits mediating evocation, inhibition and potentiation. *Behavioural Brain Research, 89*(1-2), 35-49.

Lee, Lopez, D. E., Meloni, E. G., & Davis, M. (1996). A primary acoustic startle pathway: obligatory role of cochlear root neurons and the nucleus reticularis pontis caudalis. *J Neurosci, 16*(11), 3775-3789.

Lee, J., Parish, C.L., Tomas, D., & Horne, M.K. (2011). Chronic cocaine administration reduces striatal dopamine terminal density and striatal dopamine release which leads to drug-seeking behaviour. *Neuroscience, 174*, 143-150.

Mansbach, R. S., Markou, A., & Patrick, G. A. (1994). Lack of altered startle responding in rats following termination of self-administered or noncontingently infused cocaine. *Pharmacol Biochem Behav, 48*(2), 453-458. doi: 0091-3057(94)90552-5 [pii]

Martinez, D., Greene, K., Broft, A., Kumar, D., Liu, F., Narendran, R., . . . Kleber, H. D. (2009). Lower level of endogenous dopamine in patients with cocaine dependence: findings from PET imaging of D(2)/D(3) receptors following acute dopamine depletion. *Am J Psychiatry, 166*(10), 1170-1177. doi: appi.ajp.2009.08121801 [pii]

10.1176/appi.ajp.2009.08121801

Meloni, E. G., & Davis, M. (1999). Enhancement of the acoustic startle response in rats by the dopamine D1 receptor agonist SKF 82958. *Psychopharmacology (Berl), 144*(4), 373-380.

Pahwal, P., Hyman, S. M., & Sinha, R. (2008). Craving predicts time to cocaine relapse: Further validation of the Now and Brief versions of the cocaine craving questionnaire. *Drug and Alcohol Dependence, 93*(3), 252-259. doi: DOI 10.1016/j.drugalcdep.2007.10.002

Pilotte, N. S., Sharpe, L. G., & Kuhar, M. J. (1994). Withdrawal of repeated intravenous infusions of cocaine persistently reduces binding to dopamine transporters in the nucleus accumbens of Lewis rats. *J Pharmacol Exp Ther, 269*(3), 963-969.

Pilotte, N. S., Sharpe, L. G., Rountree, S. D., & Kuhar, M. J. (1996). Cocaine withdrawal reduces dopamine transporter binding in the shell of the nucleus accumbens. *Synapse, 22*(1), 87-92. doi: 10.1002/(SICI)1098-2396(199601)22:1<87::AID-SYN10>3.0.CO;2-X [pii]

10.1002/(SICI)1098-2396(199601)22:1<87::AID-SYN10>3.0.CO;2-X

Ritz, M. C., Lamb, R. J., Goldberg, S. R., & Kuhar, M. J. (1987). Cocaine receptors on dopamine transporters are related to self-administration of cocaine. *Science, 237*(4819), 1219-1223.

Roberts, D. C., Corcoran, M. E., & Fibiger, H. C. (1977). On the role of ascending catecholaminergic systems in intravenous self-administration of cocaine. *Pharmacol Biochem Behav, 6*(6), 615-620.

Roberts, D. C., & Koob, G.F. (1982). Disruption of cocaine self-administration following 6-hydroxydopamine lesions of the ventral tegmental area in rats. *Pharmacol Biochem Behav, 17*(5), 901-904.

Schwarzkopf, S.B., Bruno, J.P., & Mitra, T. (1993). Effects of haloperidol and SCH 23390 on acoustic startle and prepulse inhibition under basal and stimulated conditions. *Prog Neuropsychopharmacol Biol Psychiatry, 17*(6), 1023-1036.

Self, D. W., & Nestler, E. J. (1998). Relapse to drug-seeking: neural and molecular mechanisms. *Drug and Alcohol Dependence, 51*(1-2), 49-60.

Sharpe, L. G., Pilotte, N. S., Mitchell, W. M., & De Souza, E. B. (1991). Withdrawal of repeated cocaine decreases autoradiographic [3H]mazindol-labelling of dopamine transporter in rat nucleus accumbens. *Eur J Pharmacol, 203*(1), 141-144.

Volkow, N. D., Fowler, J. S., Wolf, A. P., Schlyer, D., Shiue, C. Y., Alpert, R., . . . et al. (1990). Effects of chronic cocaine abuse on postsynaptic dopamine receptors. *Am J Psychiatry, 147*(6), 719-724.

Weeks, J. R. (1962). Experimental Morphine Addiction - Method for Automatic Intravenous Injections in Unrestrained Rats. *Science, 138*(3537), 143-&.

Wise, R. A. (1984). Neural mechanisms of the reinforcing action of cocaine. *NIDA Res Monogr, 50*, 15-33.

Xia, Y., Goebel, D. J., Kapatos, G., & Bannon, M. J. (1992). Quantitation of rat dopamine transporter mRNA: effects of cocaine treatment and withdrawal. *J Neurochem, 59*(3), 1179-1182.

Chapter 2

Sensitivity to Cocaine

Abstract

Men with a history of cocaine abuse but who have stopped using cocaine exhibit markedly diminished acoustic startle responses (Corcoran, et al., 2011; Efferen, et al., 2000). It is unclear if the diminished acoustic startle amplitudes are a consequence of cocaine use or preceded cocaine use. If lower acoustic startle amplitudes preceded use, it would suggest that acoustic startle has predictive value in determining vulnerability to abuse cocaine. The aim of the current study was to investigate the predictive value of acoustic startle in determining sensitivity to cocaine. Using a rat intravenous self-administration of cocaine model, the ability of drug naïve high or low acoustic startle amplitudes to predict sensitivity to self-administered 0.0625, 0.125, 0.25, 0.5 and 1.0 mg/kg cocaine was assessed. Results suggest that low startle rats self-administered more cocaine than high startle rats and that at the 0.0625 mg/kg concentration only low startle rats continued to self-administer cocaine. This suggests that acoustic startle may have predictive value in determining sensitivity to cocaine.

Cocaine addicted men, not using cocaine at the time of testing, show markedly diminished acoustic startle responses compared to non-cocaine using control participants (Corcoran, et al., 2011; Efferen, et al., 2000). It is unclear from these clinical studies whether the low acoustic startle amplitude preceded the cocaine use or, if reduced acoustic startle was caused by chronic abuse of cocaine. However, the later hypothesis has not been strongly supported by rodent or primate data and when cocaine-induced decreases in startle have been seen the effects are only transient (Adams, et al., 2001; Gordon & Rosen, 1999; Kirkland Henry, et al., 2009; Mansbach, et al., 1994). In contrast, low startle in cocaine addicted men persists for at least a year following cocaine discontinuation (Corcoran, et al., 2011). If the diminished startle observed in these men predated cocaine use, it would suggest that acoustic startle had predictive value. If acoustic startle were a predictive factor it would have value for both clinical and pre-clinical models of cocaine abuse. There is currently no clear evidence regarding the direction of the relationship between acoustic startle and cocaine use and therefore the aim of the current study was to assess the potential value of acoustic startle in determining sensitivity to cocaine.

The acoustic startle response and the reinforcing effects of cocaine are both modulated by the neurotransmitter dopamine. Mesolimbic dopamine is important for the reinforcing effects of cocaine (D. C. Roberts, et al., 1977; D. C. Roberts & Koob, 1982). Cocaine blocks the reuptake of dopamine by antagonizing the dopamine reuptake transporter increasing the amount of synaptic dopamine available (Wise, 1984). By indirectly increasing dopaminergic transmission, cocaine exerts its main reinforcing effects (Ritz, et al.). Dopamine can also modulate the acoustic startle response. Agonists

of the dopaminergic D1 and D2 receptors enhance acoustic startle amplitudes and antagonists diminish it (Davis, 1985; Davis & Aghajanian, 1976; Davis, et al., 1975; Meloni & Davis, 1999; Schwarzkopf, et al., 1993). Hence, it is possible that low startle might be accounted for by low levels of basal dopamine transmission.

Lower basal levels of dopaminergic transmission also might increase the magnitude of cocaine's reinforcing signal. That is, the same amount of cocaine would have a higher signal-to-noise ratio in people with low compared to high tonic dopamine transmission. Differences in sensitivity to cocaine will detect differences in the magnitude of the reinforcing signal produced by cocaine. If the magnitude of the reinforcing dopaminergic signal is greater because of a lower tonic level of dopamine, then a smaller amount of cocaine should be able to produce a detectable reinforcing signal in these animals.

First developed and reported in 1962 (Weeks, 1962) the rat model of drug self-administration was designed as a paradigm in which drug seeking behaviors could be measured in freely moving rats and for which the delivery of drug was response contingent. This model has become the most widely used paradigm to model human drug abuse (Ahmed, 2010). In the current form of the self-administration paradigm, rats are placed in an operant chamber containing two levers and presses on a pre-selected active lever result in the administration of drug to the rat. Responses on the other inactive lever produce no scheduled events. The presence of the two levers allows the experimenter to more appropriately determine if responses on the active lever are prompted by the reinforcing effects of the drug rather than indiscriminate responding that is not goal directed.

Using this model, sensitivity to cocaine is measured as the change in volume of cocaine, or number of injections, that is self-administered when the concentration of the cocaine solution is increased or decreased (Steketee, 2003). The purpose of the current study was to measure cocaine sensitivity in rats that were preselected for having either high or low drug naïve acoustic startle amplitudes. If the magnitude of the reinforcing dopaminergic signal produced by cocaine is greater in animals with low drug naïve acoustic startle amplitudes, low startle animals should continue to self-administer cocaine at concentrations lower than those detectable by high startle rats.

The aim of this study was to use this test to determine if preexisting differences in rats acoustic startle amplitudes can predict sensitivity to cocaine. Specifically, will rats with low acoustic startle amplitudes have a greater sensitivity to cocaine, marked by self-administration of lower concentrations compared to high startle rats?

Methods

Animals

Group housed adult male Sprague-Dawley rats weighing between 250 and 300 grams upon arrival were used for the self-administration study. All rats had ad libitum access to food and water unless otherwise specified, and were kept on a 12 hour light/dark cycle with lights on at 800 hours. All procedures were approved by the Yerkes Institutional Animal Care and Use Committee prior to commencement.

Acoustic Startle Testing

A single acoustic startle chamber composed of a Plexiglas cage measuring 8 x 15 x 15 cm with a floor of 4 15 cm long stainless steel bars was used to complete all startle testing. The cage was positioned between 4 compression springs above the cage and a rubber stopper below. Between the cage and the rubber stopper was an accelerometer, movement of the Plexiglas cage displaced the accelerometer (PCM Peizotronics, Depew, NY) causing an electronic signal to be produced. The signal was then integrated, amplified and digitized by InstruNET (Model 100B; GW instruments, Sommerville, MA) and interfaced with a Macintosh G3 computer. Acoustic startle amplitude was defined as the maximal peak to peak voltage that occurred within the first 200 msecs of the onset of the acoustic stimulus (Cassella & Davis, 1986). A small speaker (Radio Shack Super Tweeters, range 5-40 kHz), positioned 5 cm in front of the Plexiglas cage generated a constant background white noise of 60 dB using a General Radio noise generator (ACO Pacific Inc., Belmont, CA). The startle stimuli (95, 105, and 115 dB) was 50-msec white noise bursts (5 msecs rise-decay) generated by the Macintosh G3 and produced by the same speaker which provided the background noise. The startle apparatus was enclosed in a metal, sound and light attenuating outer chamber (60cm x 80cm x 60cm).

To determine low and high startle amplitude groups, 20 rats were given three test sessions 20 min in length. A white noise of 60 Hz played for the duration of the session. Beginning 5 min into the session 30 auditory stimuli were presented at 30-sec intervals. Of the 30 auditory stimuli, there were 10 presentations each of 95, 105, and 115-dB stimuli. Once startle amplitudes from all three sessions were collected baseline acoustic startle amplitude was determined by measuring the mean acoustic startle amplitude

collapsed across sessions and stimulus intensity. High and low startle rats were designated as those with baseline acoustic startle amplitudes in the highest (n=5) and lowest quartiles (n=5). Rats with startle amplitudes in the two middle quartiles were not included in further analyses.

Jugular Catheterization

Jugular catheters were composed of 11 mm 22 gauge stainless steel guide cannula (Plastics One) with an L bend 5 mm from the bottom of the guide cannula. Cannula were overlaid with 12 cm of Silastic tubing inside diameter (ID) 0.03 cm, outside diameter (OD) 0.0635 cm (Fischer) and a second layer of 4 cm length of Silastic tubing ID 0.0635 cm, OD 0.119 cm protecting the junction between the cannula and tubing. The cannula with the tubing was then encased in a cranioplastic cement protective covering and a base of Polypro Mesh 500 Micron (Small Parts, Miami, FL) affixed to the bottom of the cranioplastic encasement. The exposed external port of the catheter was protected by a custom aluminum catheter head screw (Behavioral Pharmacology Inc.). All catheters were assembled in the Davis laboratory using a protocol provided by the Kyle Franz Lab (Georgia State University).

Jugular catheters were implanted after drug naïve baseline acoustic startle amplitudes were measured. All rats were anaesthetized with ketamine (intraperitoneally - IP, 75 mg/kg) and dexodomitor (IP, 0.5 mg/kg). Jugular catheters were inserted through an incision between the shoulder blades with the 10-cm Silastic tubing catheter extending subduraly over the right should to the right jugular vein. The catheter was inserted 4 cm into the jugular vein and secured in place with silk sutures. The secured catheter was

then flushed with 0.5 ml of 0.9% heparinized sodium chloride once daily to prevent clot formation. The external port of the catheter was protected from damage by an aluminum dust cap. At the end of the surgery, all rats were given a 0.5 mg/kg concentrated dose of meloxicam for pain management. Twenty-four hours after surgery, all incision sites were inspected and rats given additional meloxicam if necessary.

Patency of the jugular catheters was determined during daily catheter flushes with 0.9% heparinized sodium chloride. If the solution did not easily push through the catheter it was determined that the catheter was clogged and that animals data would not be included in the final data analyses. Furthermore, any rat determined to have an infection or illness was not included in the final analyses.

Self-Administration

Four identical rat self-administration operant chambers (Med Associates, St. Albans, VT) were used to conduct all self-administration testing. The self-administration chambers were constructed of two metal side panels and Plexiglas front, back and ceiling panels (30 x 24.1 x 21 cm) and a stainless steel rod floor. Two automated retractable levers (4.8 x 1.9 cm) were fitted to the right side metal panel 2.1 cm above the floor. Above each lever a white a cue light was affixed. Between the two levers, a pellet receptacle (7.6 x 8.3 cm) was positioned 2.1 cm above the floor. Connected to the food receptacle was a food hopper capable of dispensing 45 mg food pellets. An automated infusion pump (Med. Associates, St. Albans, VT) mounted with a 10 ml syringe rested on top of the self-administration chamber. The syringe delivered cocaine to the rat by way of Silastic tubing which entered the chamber through a protective metal leash and

connected to the cannula. Responses on the levers could be scheduled using MED-PC software to produce food or cocaine reinforcement. The rat self-administration chambers were housed inside sound attenuating outer chambers equipped with an exhaust fan and a camera connected to an external monitor for behavioral observation.

Before beginning cocaine self-administration, all rats were trained to operantly respond on a fixed ratio 1 schedule for a food reinforcer. During food training, rats were food restricted to maintain 90% of their free fed weight and fed a diet of Purina rat chow after the completion of the food self-administration testing for each of the 3 days. During once daily 2-hr sessions, rats were placed into the self-administration chambers. One of the levers in each chamber was designated the active lever and delivered 45 mg food pellets on a fixed ratio 1 schedule. Responses made on the inactive lever were recorded but produced no scheduled events. The active and inactive lever assignments were counter balanced across chambers and rats but remained the same for each rat for food and cocaine self-administration. To reach criteria for the food training task, rats were required to obtain 100 45 mg-food pellets in a single 2-hr session. Once this was achieved food training for that animal ended. If, by the end of the third session this criterion had not been met, the rat was excluded from further testing. At the end of food training all rats were returned to ad libitum access to food.

All cocaine self-administration testing occurred during once daily 2-hr sessions. At the beginning of each session, rats were connected to the cocaine infusion pump via the tubing and leash system. The session began with a 4-sec priming injection of cocaine followed immediately by the presentation of the active and inactive levers. The delivery of cocaine was set to a fixed ratio 1 schedule with a 20-sec time-out period following

each injection of cocaine. Responses on the active lever resulted in a 4-sec 0.1-ml cocaine injection and 20-sec presentation of the cue light above the active lever signaling the time-out. Responses on the inactive lever were recorded but produced no scheduled event. The session terminated at the end of 2 hr and the catheters were flushed with 0.5 ml of heparinized 0.9% sterile saline.

Once self-administration of the 0.5-mg/kg concentration of cocaine was acquired and had become stabilized, determined by 3 consecutive days of a consistent number of reinforced responses on the active lever with less than 10% variability around the mean, the concentration of cocaine was varied so that each rat, in a random and counterbalanced order, self-administered 1.0, 0.25, 0.125 and 0.0625 mg/kg cocaine concentrations. Each new concentration of cocaine was presented on consecutive days until the number of reinforced responses was stabilized with less than 10 % variability across the mean. The 3-day mean of stabilized responding for each cocaine concentration was used to determine the dose response curve for high and low startle animals.

Statistics

A repeated measures analysis of variance was used to analyze drug naïve baseline acoustic startle in high and low startle rats followed by t-tests on individual days. Pearson correlations were computed for days 1 and 2, 1 and 3, and 2 and 3 of baseline startle testing. Differences in the rate of reinforced responding on the active lever between low and high startle rats across cocaine concentrations was evaluated using a multivariate analysis of variance, using startle level as a between-subjects factor and dose as a within-subjects factor. Percent change scores were calculated for responding on

active versus inactive levers to compare preference for the active lever in high and low startle rats and analyzed with a repeated measures analysis of variance. Cohen D was also calculated for all analyses to assess effect size.

Results

The drug naïve acoustic startle amplitudes of low (n = 5) and high (n = 5) startle rats used for the sensitivity to self-administered cocaine experiment were significantly different [$F(1,8) = 67.314$, $p. = .000$ $d = 9.121$]. Independent samples t-tests showed significant differences in startle on days 1 [$t(8) = 2.709$, $p = .027$, $d = 3.810$], 2 [$t(8) = 3.378$, $p = .010$, $d = 4.778$] and 3 [$t(8) = 6.847$, $p < .000$, $d = 9.000$] see *Figure 1*. Startle amplitude across the 10 rats used to determine low and high startle groups was consistent across the three test sessions, with the following correlations: Day 1-Day 2 [$r(10) = .876$, $p = .001$] Day 2-Day -Day 3 [$r(10) = .760$, $p = .011$] and Day 1- Day 3 [$r(10) = .764$, $p = .010$]. High and low startle rats did not differ in the number of days necessary to acquire operant responding for a food reinfocer [$t(4)=.535$ $p=.621$ $d = .730$] or self-administration of cocaine [$t(4)=.886$, $p=.426$, $d = .670$] see *Figure 2*.

Low startle rats self-administered more cocaine as measured by the number of reinforced responses on the active lever at each concentration of cocaine and this effect was significant for the 1.0 mg/kg [$F(1,8)=7.281$, $p.=.27$, $d = 3.816$], 0.5 mg/kg [$F(1,8)=40.947$, $p <.000$, $d = 9.052$], and 0.0625mg/kg [$F(1,8)=9.082$, $p=.017$, $d = 4.262$] concentration, see *Figure 3*. Although an obvious trend towards a greater rate of cocaine self-administration was observed for the 0.25 and 0.125 mg/kg concentrations, this effect did not reach significance [0.25mg/kg, $F(1,8)= .207$, $p=.661$, $d = .648$; 0.125 mg/kg

F(1,8)=4.312, *p*=.072, *d* = 2.937]. Low startle rats responded more on the active than inactive lever compared to high startle rats when the concentration of cocaine was 0.0625 mg/kg (F(1,10) = 17.557, *p* = .003, *d* = 5.498], 0.5 mg/kg [F(1,10) = 15.896, *p* = .001, *d* = 5.043] or 1.0 mg/kg cocaine concentration [F(1,10) = 7.225, *p* = .028, *d* = 3.400] but not at the .0125 m/kg or 0.25 mg/kg concentrations [0.125 F(1,10) = 4.064, *p* = .079, *d* = 2.550; 0.25 F(1,10) = .177, *p* = .685, *d* = 3.400] *Figure 4*.

Discussion

Low startle rats self-administered more cocaine than high startle rats. More importantly, as indicated by the continued selective responding on the cocaine paired lever, low but not high startle rats were able to detect the lowest concentration of cocaine. The increased number of active lever presses at the 0.0625 mg/kg cocaine concentration suggests higher sensitivity to cocaine in the low startle rats.

These differences in sensitivity to cocaine may be related to differences in the functional transmission of dopamine in high and low startle rats. In squirrel monkeys, antagonism of D2 but not D1 receptors has been shown to increase sensitivity to cocaine (Howell & Byrd, 1992). Furthermore, an increase in D2 receptor availability is negatively correlated with the reinforcing effects of cocaine as measured by self-administration in rhesus macaques (Morgan et al., 2002). Therefore, it is possible that the enhanced sensitivity to cocaine observed in the low startle rats is due to a diminished basal rate of functional dopaminergic transmission that could also account for the diminished acoustic startle response in these animals.

Alternatively, a decrease in dopaminergic transmission could potentially increase post-synaptic receptor density as a compensatory measure. Co-administration of a D1 antagonist given with cocaine blocks the acquisitions of cocaine conditioned place preference (Shippenberg & Heidbreder, 1995) suggesting that D1 receptor density is positively correlated with sensitivity to cocaine. If low synaptic dopamine in low startle rats increased post-synaptic D1 receptor density it might be expected that unpotentiated acoustic startle amplitudes would be reduced because of lower synaptic release of dopamine but that sensitivity to cocaine, which increases synaptic dopamine, would be increased because of the greater D1 receptor density.

An increase in sensitivity to cocaine does not necessarily correlate with increased motivation to obtain cocaine or vulnerability to abuse cocaine (Ranaldi, Bauco, McCormick, Cools, & Wise, 2001). Differences in sensitivity to cocaine could however be an important factor in determining which individuals are presented with the opportunity to abuse cocaine. If cocaine is more reinforcing in a subset of the population due to increased sensitivity, this subset of the population may subsequently continue to seek out new opportunities to experience cocaine. By increasing their exposure these individuals may be more likely to develop problematic cocaine use compared to the rest of the population.

The current study did not address underlying physiological causes for the behavioral difference in cocaine sensitivity observed between high and low startle rats. It is our prediction that this difference in cocaine sensitivity is mediated by differences in functional dopaminergic transmission. Cocaine's primary reinforcing effect is via dopamine (D. C. Roberts, et al., 1977; D. C. Roberts & Koob, 1982). If low acoustic

startle rats have lower basal levels of dopamine it is possible that the magnitude of the reinforcing dopaminergic signal produced by cocaine would be greater than in rats with higher basal rates of dopaminergic transmission. This increase in sensitivity may be mediated by a compensatory change in post-synaptic dopamine receptor density. Dopamine can also modulate acoustic startle. Agonists of dopamine enhance acoustic startle and dopaminergic antagonists can diminish acoustic startle (Davis, 1985; Davis & Aghajanian, 1976; Davis, et al., 1975; Meloni & Davis, 1999; Schwarzkopf, et al., 1993). Given the role of dopamine in sensitivity to cocaine, it seems likely that dopamine plays an important role in determining the differences between high and low acoustic startle rats observed in this study.

The evidence provided here suggests that the differences in acoustic startle responses of cocaine addicted men and healthy controls (Corcoran, et al., 2011; Efferen, et al., 2000) may have preceded their cocaine use. However, a difference in sensitivity to cocaine, though promising, is not sufficient evidence to indicate a difference in vulnerability. It is important that future studies investigate differences between high and low startle animals on behavioral measures that are central traits of cocaine addiction in humans. These include, differences in motivation, drug seeking, and relapse. Differences in dopaminergic transmission should also be investigated. Differences in cocaine related behaviors are therefore the subject of the following chapters.

References

Adams, J. U., Efferen, T. R., Duncan, E. J., & Rotrosen, J. (2001). Prepulse inhibition of the acoustic startle response in cocaine-withdrawn rats. *Pharmacol Biochem Behav, 68*(4), 753-759. doi: S0091-3057(01)00478-6 [pii]

Ahmed, S. H. (2010). Validation crisis in animal models of drug addiction: Beyond non-disordered drug use toward drug addiction. *Neuroscience and Biobehavioral Reviews, 35*(2), 172-184. doi: DOI 10.1016/j.neubiorev.2010.04.005

Cassella, J. V., & Davis, M. (1986). Habituation, Prepulse Inhibition, Fear Conditioning, and Drug Modulation of the Acoustically Elicited Pinna Reflex in Rats. *Behavioral Neuroscience, 100*(1), 39-44.

Corcoran, S., Norrholm, S. D., Cuthbert, B., Sternberg, M., Hollis, J., & Duncan, E. (2011). Acoustic startle reduction in cocaine dependence persists for 1 year of abstinence. *Psychopharmacology, 215*(1), 93-103. doi: DOI 10.1007/s00213-010-2114-2

Davis, M. (1985). Cocaine: excitatory effects on sensorimotor reactivity measured with acoustic startle. *Psychopharmacology (Berl), 86*(1-2), 31-36.

Davis, M., & Aghajanian, G. K. (1976). Effects of apomorphine and haloperidol on the acoustic startle response in rats. *Psychopharmacology (Berl), 47*(3), 217-223.

Davis, M., Svensson, T. H., & Aghajanian, G. K. (1975). Effects of d- and l-amphetamine on habituation and sensitization of the acoustic startle response in rats. *Psychopharmacologia, 43*(1), 1-11.

Efferen, T. R., Duncan, E. J., Szilagyi, S., Chakravorty, S., Adams, J. U., Gonzenbach, S., . . . Rotrosen, J. (2000). Diminished acoustic startle in chronic cocaine users. *Neuropsychopharmacology, 22*(1), 89-96. doi: S0893-133X(99)00089-5 [pii]

10.1016/S0893-133X(99)00089-5

Gordon, M. K., & Rosen, J. B. (1999). Lasting effect of repeated cocaine administration on acoustic and fear-potentiated startle in rats. *Psychopharmacology (Berl), 144*(1), 1-7.

Howell, L. L., & Byrd, L. D. (1992). Enhanced sensitivity to the behavioral effects of cocaine after chronic administration of D2-selective dopamine antagonists in the squirrel monkey. *J Pharmacol Exp Ther, 262*(3), 907-915.

Kirkland Henry, P., Davis, M., & Howell, L. L. (2009). Effects of cocaine self-administration history under limited and extended access conditions on in vivo striatal dopamine neurochemistry and acoustic startle in rhesus monkeys.

. *Psychopharmacology (Berl), 205*(2), 237-247.

Mansbach, R. S., Markou, A., & Patrick, G. A. (1994). Lack of altered startle responding in rats following termination of self-administered or noncontingently infused cocaine. *Pharmacol Biochem Behav, 48*(2), 453-458. doi: 0091-3057(94)90552-5 [pii]

Meloni, E. G., & Davis, M. (1999). Enhancement of the acoustic startle response in rats by the dopamine D1 receptor agonist SKF 82958. *Psychopharmacology (Berl), 144*(4), 373-380.

Morgan, D., Grant, K. A., Gage, H. D., Mach, R. H., Kaplan, J. R., Prioleau, O., . . . Nader, M. A. (2002). Social dominance in monkeys: dopamine D-2 receptors and cocaine self-administration. *Nature Neuroscience, 5*(2), 169-174. doi: Doi 10.1038/Nn798

Ranaldi, R., Bauco, P., McCormick, S., Cools, A. R., & Wise, R. A. (2001). Equal sensitivity to cocaine reward in addiction-prone and addiction-resistant rat genotypes. *Behavioural Pharmacology, 12*(6-7), 527-534.

Ritz, M. C., Lamb, R. J., Goldberg, S. R., & Kuhar, M. J. (1987). Cocaine receptors on dopamine transporters are related to self-administration of cocaine. *Science, 237*(4819), 1219-1223.

Roberts, D. C., Corcoran, M. E., & Fibiger, H. C. (1977). On the role of ascending catecholaminergic systems in intravenous self-administration of cocaine. *Pharmacol Biochem Behav, 6*(6), 615-620.

Roberts, D. C., & Koob, G.F. (1982). Disruption of cocaine self-administration following 6-hydroxydopamine lesions of the ventral tegmental area in rats. *Pharmacol Biochem Behav, 17*(5), 901-904.

Schwarzkopf, S.B., Bruno, J.P., & Mitra, T. (1993). Effects of haloperidol and SCH 23390 on acoustic startle and prepulse inhibition under basal and stimulated conditions. *Prog Neuropsychopharmacol Biol Psychiatry, 17*(6), 1023-1036.

Shippenberg, T. S., & Heidbreder, C. (1995). Sensitization to the conditioned rewarding effects of cocaine: pharmacological and temporal characteristics. *J Pharmacol Exp Ther, 273*(2), 808-815.

Steketee, J. D. (2003). Neurotransmitter systems of the medial prefrontal cortex: potential role in sensitization to psychostimulants. *Brain Research Reviews, 41*(2-3), 203-228. doi: Doi 10.1016/S0165-0173(02)00233-3

Weeks, J. R. (1962). Experimental Morphine Addiction - Method for Automatic Intravenous Injections in Unrestrained Rats. *Science, 138*(3537), 143-&.

Wise, R. A. (1984). Neural mechanisms of the reinforcing action of cocaine. *NIDA Res Monogr, 50*, 15-33.

Figure Captions

Figure One. **Baseline acoustic startle amplitudes in high and low startle rats: Sensitivity to Cocaine.** Mean startle amplitudes for high (n=5) and low (n=5) startle rats that subsequently self-administered cocaine. There was a significant difference in startle amplitudes for each group, * $p < .05$.

Figure Two. **Acquisition of operant responding.** A) Low and high startle rats acquire operant responding for a food reinfocer at the same rate. B) Number of days necessary to acquire cocaine self-administration is comparable in high and low acoustic startle rats.

Figure Three. **Dose Response Curve.** Low acoustic startle rats achieved more cocaine reinforced responses on the active lever when compared to high startle rats and this difference was significant at 1.0, 0.5, and 0.0625 mg/kg cocaine concentrations, * $p < .05$.

Figure Four. **Percent change in responses on the active versus inactive lever.** Preferential responding on the active versus inactive lever was significantly greater in low startle rats when the concentration of cocaine was 0.0625 mg/kg, 0.5, mg/kg or 1.0 mg/kg * $p < .05$.

Figure 1.

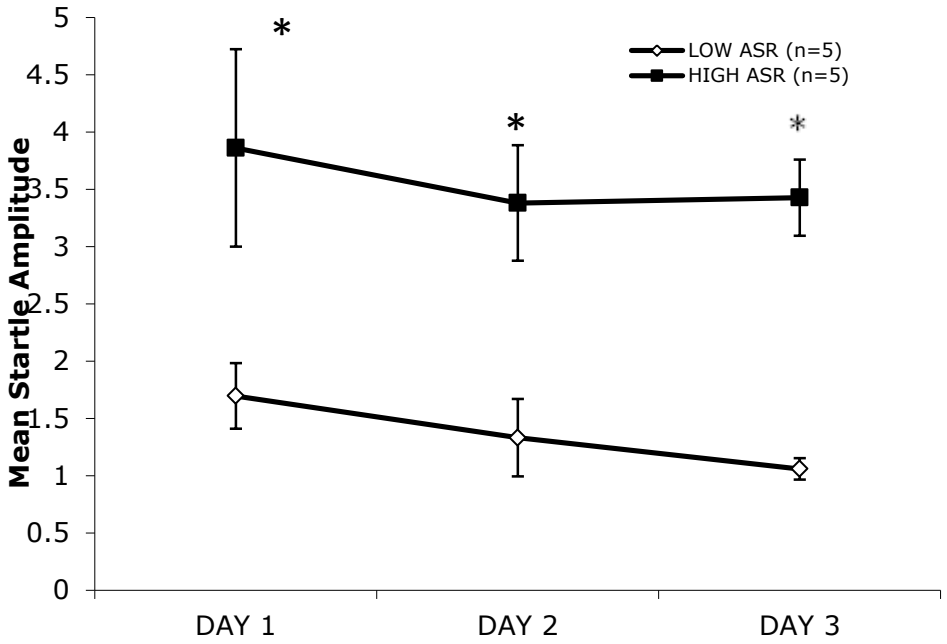
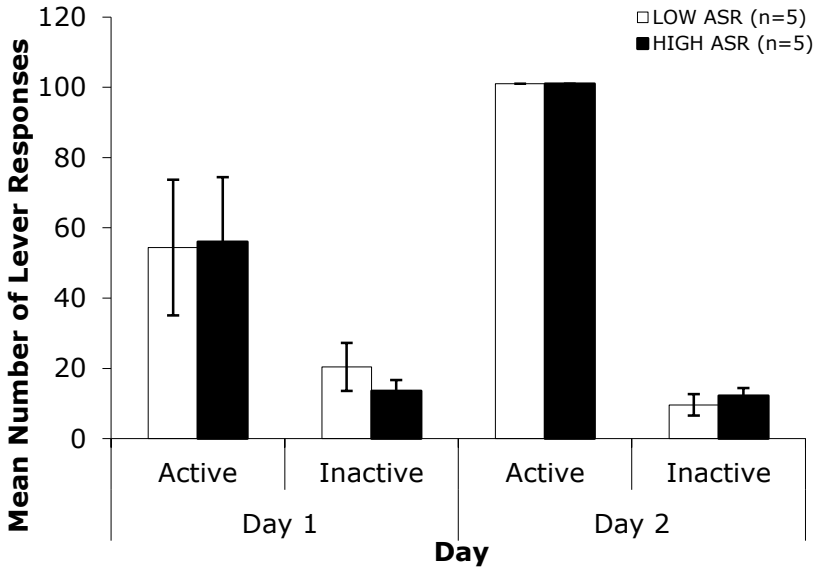
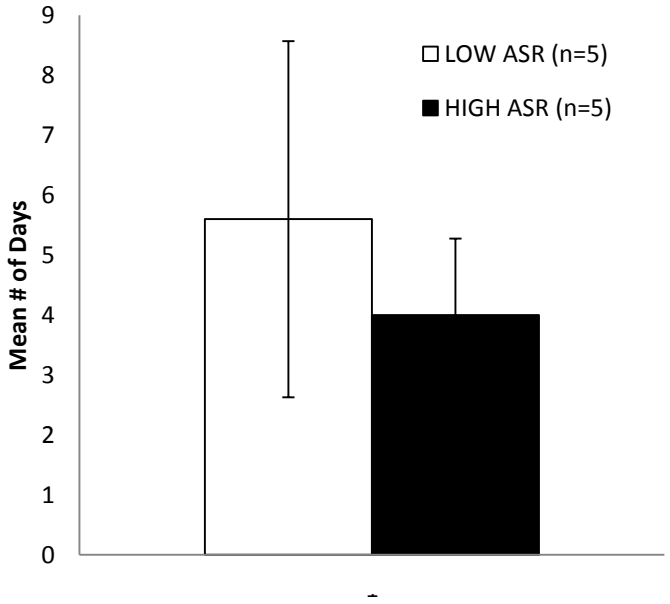

Mean Startle Amplitude
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
*
*
*
LOW ASR (n=5)
HIGH ASR (n=5)
DAY 1
DAY 2
DAY 3

Figure 2.


LOW ASR (n=5)
HIGH ASR (n=5)
Mean Number of Lever Responses
120
100
80
60
40
20
0
Active
Inactive
Active
Inactive
Day 1
Day 2
Day

A


LOW ASR (n=5)
HIGH ASR (n=5)
Mean # of Days
9
8
7
6
5
4
3
2
1
0

B

Figure 3.

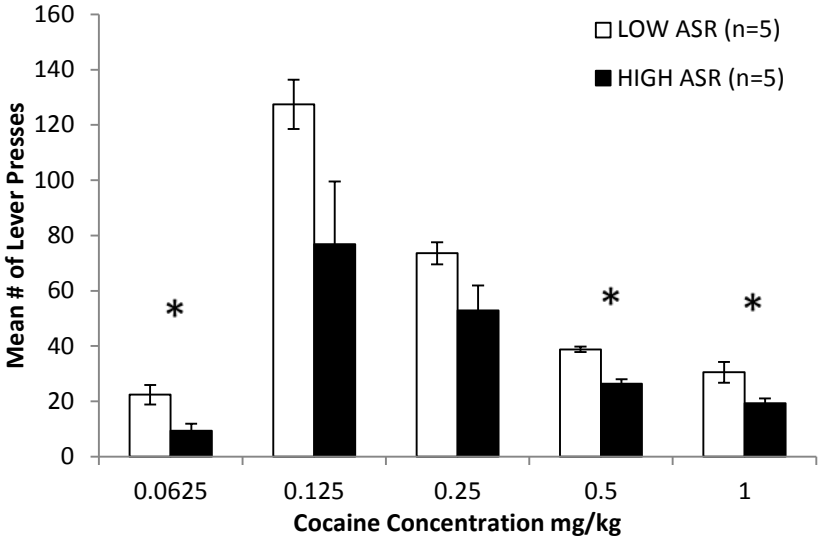
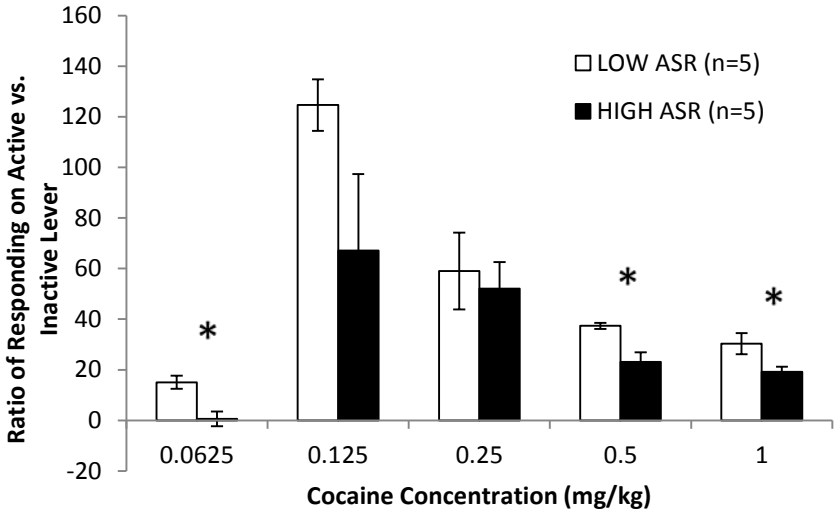

160
140
120
100
80
60
40
20
0
Mean # of Lever Presses
LOW ASR (n=5)
HIGH ASR (n=5)
*
*
*
0.0625
0.125
0.25
0.5
1
Cocaine Concentration mg/kg

Figure 4.


160
140
120
100
80
60
40
20
0
-20
Ratio of Responding on Active vs. Inactive Lever
LOW ASR (n=5)
HIGH ASR (n=5)
*
*
*
0.0625
0.125
0.25
0.5
1
Cocaine Concentration (mg/kg)

Chapter 3

Motivation and Reinstatement.

Abstract

Rats with drug naïve low acoustic startle amplitudes are more sensitive to cocaine than rats with drug naïve high acoustic startle amplitudes. The purpose of the current study was to determine if this difference extended to motivation to obtain and likelihood to reinstate cocaine use. Using a cocaine self-administration model, motivation to obtain cocaine was measured in rats with low or high acoustic startle amplitudes using a progressive ratio schedule. Breakpoints under the progressive ratio schedule were measured in response to a 0.125 mg/kg concentration of cocaine. Reinstatement of cocaine seeking following a priming dose of 10 mg/kg cocaine and a cocaine associated cue were also measured. Results show that drug naïve low acoustic startle rats reach higher breakpoints on a progressive ratio schedule and reinstated cocaine seeking at a greater rate in response to a cocaine prime or cocaine associated cue when compared to high startle rats.

Motivation to obtain cocaine is one of the criteria for the diagnosis of substance abuse in the DSM IV (*Diagnostic and Statistical Manual of Mental Disorders*, 2000). In rat models of drug self-administration, changes in drug-seeking motivation are measured using a progressive ratio schedule of reinforcement (Hodos, 1961). Motivation as measured on a progressive ratio schedule is particularly well suited to detecting the reinforcing properties of drugs of abuse and is readily manipulated by the co-administration of drugs that elevate or reduce that drugs reinforcing value (Arnold & Roberts, 1997).

Perhaps the most important criteria for cocaine abuse is relapse which is defined as the inability to maintain abstinence despite a desire to do so (Kirshenbaum, Olsen, & Bickel, 2009; Shaham, Shalev, Lu, De Wit, & Stewart, 2003). In animal models of cocaine abuse, cocaine relapse is measured as the reinstatement of cocaine seeking following extinction of this behavior (Aguilar, Rodriguez-Arias, & Minarro, 2009; Epstein, Preston, Stewart, & Shaham, 2006a). Cocaine relapse in humans and the reinstatement of cocaine seeking in rats is initiated by what are called relapse primes, these are; re-exposure to cocaine, the presentation of a cocaine associated cue, or the application of a stressor (Crombag & Shaham, 2002; de Wit & Stewart, 1981; Kalivas & McFarland, 2003). These primes increase cocaine craving by increasing the desire for the rewarding effects the drug and by decreasing the aversive effects caused by abstinence such as withdrawal or stress (Self & Nestler, 1998). It is the increase in cocaine craving brought about by these processes that is thought to be the motivator for subsequent cocaine abuse (Kalivas & McFarland, 2003; Koob, 2009; Self & Nestler, 1998).

In rodents performance on a progressive ratio schedule, a measure of motivation, can be predictive of an increased propensity to reinstate extinguished cocaine seeking (Anker & Carroll, 2011; Belin, et al., 2011; Deroche-Gamonet, et al., 2004; Hodos, 1961; Saunders & Robinson, 2011). Given that relapse is the most defining aspect of addiction and the predictive value of increased motivation in determining the probability of relapse, motivation is an important factor in determining differences in vulnerability to abuse cocaine.

As shown in Chapter 1, rats with low acoustic startle responses are more sensitive to cocaine when compared to rats with drug naïve high acoustic startle amplitudes. This is interesting because men with a history of cocaine abuse have significantly lower acoustic startle amplitudes when compared to non-cocaine using controls (Corcoran, et al.; Efferen, et al., 2000). Although an association between acoustic startle amplitude and cocaine sensitivity in rats provides some evidence that differences in startle amplitudes might precede cocaine use, this is not sufficient evidence of a vulnerability to abuse cocaine (Ranaldi, et al., 2001). Hence, further investigation of the relationship between acoustic startle and vulnerability to abuse cocaine is necessary. The aim of the present study was to investigate differences in motivation to obtain cocaine as expressed by high and low startle rats. Differences in motivation are important as they predict reinstatement potential of extinguished cocaine seeking behaviors in rats. The second aim of this study therefore was to investigate whether or not low startle rats would also be more likely to reinstate extinguished cocaine seeking behavior. This was done using both a cocaine prime, because of the prior evidence for differences in sensitivity to cocaine in high and low startle rats and a cue prime, given the clinical relevance of cued relapse in humans.

Methods

Animals

Male Sprague-Dawley rats were group housed and kept on a 12 hour light/dark cycle with lights on at 800 hours. Food and water were available ad libitum for the duration of the experiment with the exception of a 3 day period of food training on an operant procedure during which time animals were weight restricted to 90% of their free fed weight. All procedures were approved by the Institutional Animal Care and Use Committee.

Acoustic Startle Testing

Equipment and procedures for acoustic startle testing were described in detail in the methods section of chapter 1 and are available for review there. Briefly, acoustic startle was measured using a single acoustic startle chamber. To determine low and high startle amplitude groups, rats were given three 20-min startle sessions occurring once a day for 3 days between 800 and 1200 hours. Startle sessions consisted of 30 presentations of a 95, 105 and 115-dB auditory stimulus and the mean startle response for each session was taken as an average of the startle amplitude response to all three stimuli. Only rats with mean startle amplitudes in the lowest (n=7) and highest (n=6) quartiles were used for the completion of the self-administration study.

Intravenous Self-Administration and Progressive Ratio Schedule

Procedures for making and implanting jugular catheters were previously described and are available for review in chapter 1. High and low startle rats were tested

for differences in the ability to acquire operant responding using a food reinforcer with procedures also described in chapter 1. Rats were trained to self-administer a 0.5-mg/kg concentration of cocaine under a fixed ratio 1 schedule during once daily 120-min sessions until adequate maintenance of cocaine self-administration was observed, as described in Chapter 1. Once maintenance of cocaine self-administration was reached, low and high startle rats were tested for motivation to obtain cocaine using a progressive ratio schedule. The response requirements for the progressive ratio were determined using the response ratio = (5 x (0.2 x infusion number)-5 equation (D. C. Roberts & Bennett, 1993; D. C. S. Roberts, Loh, & Vickers, 1989). Breakpoints were calculated as the highest response requirement that was achieved and reinforced before a time interval of 60 min elapsed in which no further reinforcement was attained. Session lengths were a maximum of 5 hrs but were terminated earlier if the break point was achieved prior to the end of the 5-hr session. Break points were measured when the concentration of cocaine 0.125 mg/kg. This concentration was selected based on previous evidence showing the 0.125 mg/kg concentration to be the lowest detectable by both low and high startle rats. All rats underwent one progressive ratio session.

Extinction and Reinstatement of Cocaine Seeking

Following a minimum of 14 cocaine self-administration sessions extinction training began. During extinction training, rats were placed into the self-administration chambers for once daily 2-hr sessions with both the active and inactive levers extended into the chamber. Responses on both levers were recorded but active lever responses no longer produced cocaine reinforcement and the light above the active lever was deactivated. A minimum of 7 extinction training sessions occurred before reinstatement

of cocaine seeking was measured. Reinstatement of cocaine seeking was measured using both a cocaine prime (10 mg/kg IP) and a cue prime. During drug primed reinstatement of cocaine seeking, a priming injection of cocaine was administered immediately prior to the beginning 2-hr sessions in which responses on the active and inactive lever were recorded, although they resulted in no cocaine injections or cue light illumination when pressed. During cue primed reinstatement of cocaine seeking, the cue light above the previously active lever was illuminated for 4 sec to signal the beginning of the session along with activation of the automated pump which is audible to the rats but delivered no cocaine. Responses on the active lever resulted in the illumination of the cue light above the previously active lever and activation of the automated pump but no cocaine was administered. The two reinstatement tests were separated by a minimum of 7 days of extinction training and the order in which each primed reinstatement test occurred for each rat was randomly assigned.

Statistics

A repeated measures analysis of variance was used to analyze drug naïve baseline acoustic startle in high and low startle rats with independent t-tests to confirm differences in startle amplitudes on specific days. A Pearson correlation was also performed to assess the degree of correlation between separate days of startle amplitude testing across the 13 rats used to obtain low and high startle groups. Overall differences in performance on the progressive ratio schedule were measured using a one tailed independent t-test. A repeated measures analysis of variance was used to assess differences in the reinstatement of cocaine seeking between high and low startle rats with reinstatement prime as the

with-in subjects variable and startle group as the between subjects variable. Effect sizes for all statistics were calculated using Cohen D.

Results

Startle amplitude across the 13 rats used to determine low and high startle groups was consistent across the three test sessions, with the following correlations: Day 1-Day 2 [$r(16) = .489$, $p = .055$] Day 2-Day -Day 3 [$r(16) = .689$, $p = .003$] and Day 1- Day 3 [$r(16) = .609$, $p = .012$]. Drug naïve low (n = 7) and high (n = 6) acoustic startle rats significantly differed in acoustic startle amplitudes [$F(1,11) = 379.389$, $p = .000$, $d = 9.519$]. Independent samples t-tests showed significant differences in startle on days 1[$t(11) = 3.086$, $p = .01$, $d = 4.798$], 2 [$t(11) = 3.835$, $p = .003$, $d = 4.607$], and 3 [$t(11) = 6.886$, $p < .000$, $d = 8.711$], please refer to *Figure 1*.

High and low startle rats did not differ in the number of days necessary to acquire operant responding for a food reinfocer [$t(11) = .746$, $p = .471$, $d = .960$] *Figure 2A*. Low startle rats did acquire cocaine self-administration in fewer days then high startle rats [$t(11) = 2.594$, $p = 0.025$, $d = 3.103$] see *Figure 2B.*

Low acoustic startle rats reach higher breakpoints on a progressive ratio schedule when compared to high acoustic startle rats [$t(11)$ 1.781, $p = .051$, $d = 2.356$] *Figure 3*. Reinstatement of extinguished cocaine showed an overall group difference between high and low startle rats [$F(1,4) = 73.789$, $p = .001$, $d = 6.418$] with no significant difference in the effect of type of prime used [$F(1,4) = .022$, $p = .889$, $d = .116$] or significant interaction [$F(1,4) = .554$, $p = .498$] *Figure 4*.

Discussion

Low startle rats reached higher breakpoints on a progressive ratio schedule when compared to high startle rats. This suggests that not only are low startle rats more sensitive to cocaine than high startle rats (Chapter 1), but they may also be more motivated to obtain cocaine. This observation is interesting given the value of motivation to obtain cocaine in determining likelihood to reinstate extinguished cocaine seeking.

The difference in motivation to obtain cocaine between high and low startle rats could be related to a difference in dopaminergic function between the two phenotypes, although the literature is inconsistent. A different basal rate of dopaminergic transmission between high and low startle rats could alter the reinforcing value of cocaine. Clozapine is an atypical antipsychotic that works by blocking agonists of both dopamine and serotonin receptors (Gobbi & Janiri, 1999). A 10 mg/kg dose of clozapine administered before cocaine self-administration increases breakpoints reached by rats on a progressive ratio schedule (Loh, Fitch, Vickers, & Roberts, 1992) suggesting that reduced dopamine transmission increases motivation to obtain cocaine. On the other hand, decreasing dopaminergic transmission by blocking post-synaptic receptors decreases consumption of a food reinforcer and the amount of effort (climbing over obstructions in their path to reach the food) an animal would exert to obtain a food reinforcer (Aberman & Salamone, 1999; Kruzich & See, 2001; Salamone, Cousins, & Bucher, 1994; See, Kruzich, & Grimm, 2001). However, the several differences between these two studies and the different methods to decrease dopamine transmission make it unclear how to resolve these apparently opposite conclusions.

Another behavioral phenotype may also point to differences in dopaminergic function and account for differences in motivation to obtain cocaine. Recent studies have identified two distinct patterns of behavior in operant paradigms. These two behaviors are goal tracking and sign tracking. Goal trackers focus on the reinforcer while sign trackers spend more time focused on the cue associated with the goal (Flagel, Watson, Robinson, & Akil, 2007). These differences in approach behavior are attributed to differences in dopamine function. Sign trackers have larger amounts of D1 receptor mRNA compared to goal trackers; a finding that is consistent with other evidence showing that an increase in D1 correlates with an increased ability to learn operantly conditioned associations (Flagel, et al., 2007). Sign trackers are also more likely to develop sensitization to cocaine and to attribute incentive salience to reinforcer associated cues (Flagel, Watson, Akil, & Robinson, 2008 2009). Sign trackers also shower greater motivation to obtain cocaine (Saunders & Robinson, 2011) and to reinstate food- or cocaine seeking (Saunders & Robinson, 2011; Yager & Robinson, 2010). These data suggest that dopaminergic variability that drives differences in cocaine induced plasticity, operant learning, motivation to obtain cocaine, and vulnerability to reinstate cocaine seeking are likely due to complex differences in dopaminergic transmission that may include differences in post-synaptic D1 receptor density.

Similar to sign trackers, low startle rats may also be more likely to reinstate cocaine seeking than high startle rats. The literature on sign trackers suggests that this increased propensity to reinstate cocaine seeking, particularly cue induced cocaine seeking, is dependent upon an increased ability to learn operantly conditioned associations (Flagel, et al., 2007). Although the current study did not assess differences in

D1 receptor density of high and low startle rats, the differences in the rate of cue induced reinstatement suggest a difference in cocaine-cue associative learning. An important future study would therefore be to analyze differences in D1 receptor density of high and low startle rats.

In preclinical studies, motivation to obtain cocaine can be highly predictive of the rate at which cocaine seeking can be reinstated in an animal (Anker & Carroll, 2011; Belin, et al., 2011 Piazza, & Deroche-Gamonet, 2011; Deroche-Gamonet, et al., 2004 2004; Hodos, 1961; Saunders & Robinson, 2011). In the present study, reinstatement of extinguished cocaine seeking was measured in response to a cocaine prime and a cue prime. Low and high startle rats significantly differed in the reinstatement of extinguished cocaine seeking. If the low startle rats were more adept at acquiring the cued association between lever responding and cocaine delivery, than this could account for the heightened rate of reinstated cocaine seeking. In addition to this, low startle rats appear to be more sensitive to the reinforcing properties of cocaine and this increase could facilitate retention of operant learning and thus make reinstatement of the extinguished response more easily triggered.

In summary, low startle rats, in addition to being more sensitive to cocaine, also appear to be more motivated to obtain cocaine and to reinstate extinguished cocaine seeking. Interestingly these differences between low and high startle rats may in part be due to an increased ability of low startle rats to learn cocaine-cue associations. If this is the case it could explain both the increase in motivation to obtain cocaine as well as the increase in cocaine seeking reinstatement. In motivation, the lever and the cue light are cocaine associated cues. If the low startle rats made the association between lever and

cocaine more than high startle rats, and subsequently some of the hedonic value of the cocaine reinforcement was transferred to the lever, than low startle rats may continue to respond longer than high startle rats under a progressive ratio schedule. Additionally, because cocaine associated cues hold more hedonic value and have made stronger associative connections to cocaine in low startle rats, it should also be expected that these rats would also reinstate cocaine seeking at a greater rate than high startle rats.

These findings could have significant clinical value given that an increase in motivation to obtain cocaine is a significant predictor of cocaine addiction vulnerability and reinstatement of cocaine seeking which models cocaine relapse, a defining characteristic of the disorder. The current studies provide further evidence that the low startle amplitudes of cocaine addicted men first observed in clinical studies (Corcoran, et al., 2011; Efferen, et al., 2000) may have preceded the cocaine abuse. The behavioral phenotype of low acoustic startle thus may be predictive of cocaine abuse potential in rats. Chapter 3 will continue on this theme to determine if low startle rats have higher novelty seeking, another predictor of greater motivation to obtain cocaine.

References

Aberman, J. E., & Salamone, J. D. (1999). Nucleus accumbens dopamine depletions make rats more sensitive to high ratio requirements but do not impair primary food reinforcement. *Neuroscience, 92*(2), 545-552. doi: S0306-4522(99)00004-4 [pii]

Aguilar, M. A., Rodriguez-Arias, M., & Minarro, J. (2009). Neurobiological mechanisms of the reinstatement of drug-conditioned place preference. *Brain Res Rev, 59*(2), 253-277. doi: S0165-0173(08)00101-X [pii]

10.1016/j.brainresrev.2008.08.002

Anker, J. J., & Carroll, M. E. (2011). Adolescent nicotine exposure sensitizes cue-induced reinstatement of cocaine seeking in rats bred for high and low saccharin intake. *Drug Alcohol Depend*. doi: S0376-8716(11)00116-5 [pii]

10.1016/j.drugalcdep.2011.02.016

Arnold, J. M., & Roberts, D. C. (1997). A critique of fixed and progressive ratio schedules used to examine the neural substrates of drug reinforcement. *Pharmacol Biochem Behav, 57*(3), 441-447. doi: S0091305796004455 [pii]

Belin, D., Berson, N., Balado, E., Piazza, P. V., & Deroche-Gamonet, V. (2011). High-novelty-preference rats are predisposed to compulsive cocaine self-administration. *Neuropsychopharmacology, 36*(3), 569-579. doi: npp2010188 [pii]

10.1038/npp.2010.188

Corcoran, S., Norrholm, S. D., Cuthbert, B., Sternberg, M., Hollis, J., & Duncan, E. (2011). Acoustic startle reduction in cocaine dependence persists for 1 year of

abstinence. *Psychopharmacology, 215*(1), 93-103. doi: DOI 10.1007/s00213-010-2114-2

Crombag, H. S., & Shaham, Y. (2002). Renewal of drug seeking by contextual cues after prolonged extinction in rats. *Behav Neurosci, 116*(1), 169-173.

de Wit, H., & Stewart, J. (1981). Reinstatement of cocaine-reinforced responding in the rat. *Psychopharmacology (Berl), 75*(2), 134-143.

Deroche-Gamonet, V., Belin, D., & Piazza, P. V. (2004). Evidence for addiction-like behavior in the rat. *Science, 305*(5686), 1014-1017. doi: 10.1126/science.1099020 305/5686/1014 [pii]

Diagnostic and Statistical Manual of Mental Disorders. (2000). (4 ed.). Washington, DC: American Psychiatric Association.

Efferen, T. R., Duncan, E. J., Szilagyi, S., Chakravorty, S., Adams, J. U., Gonzenbach, S., . . . Rotrosen, J. (2000). Diminished acoustic startle in chronic cocaine users. *Neuropsychopharmacology, 22*(1), 89-96. doi: S0893-133X(99)00089-5 [pii] 10.1016/S0893-133X(99)00089-5

Epstein, D. H., Preston, K. L., Stewart, J., & Shaham, Y. (2006). Toward a model of drug relapse: an assessment of the validity of the reinstatement procedure. *Psychopharmacology (Berl), 189*(1), 1-16. doi: 10.1007/s00213-006-0529-6

Flagel, S. B., Watson, S. J., Akil, H., & Robinson, T. E. (2008). Individual differences in the attribution of incentive salience to a reward-related cue: influence on cocaine sensitization. *Behavioural Brain Research, 186*(1), 48-56. doi: S0166-4328(07)00373-7 [pii]

10.1016/j.bbr.2007.07.022

Flagel, S. B., Watson, S. J., Robinson, T. E., & Akil, H. (2007). Individual differences in the propensity to approach signals vs goals promote different adaptations in the dopamine system of rats. *Psychopharmacology, 191*(3), 599-607. doi: DOI 10.1007/s00213-006-0535-8

Gobbi, G., & Janiri, L. (1999). Clozapine blocks dopamine, 5-HT2 and 5-HT3 responses in the medial prefrontal cortex: an in vivo microiontophoretic study. *Eur Neuropsychopharmacol, 10*(1), 43-49. doi: S0924-977X(99)00055-3 [pii]

Hodos, W. (1961). Progressive ratio as a measure of reward strength. *Science, 134*, 943-944.

Kalivas, P. W., & McFarland, K. (2003). Brain circuitry and the reinstatement of cocaine-seeking behavior. *Psychopharmacology, 168*(1-2), 44-56. doi: DOI 10.1007/s00213-003-1393-2

Kirshenbaum, A. P., Olsen, D. M., & Bickel, W. K. (2009). A quantitative review of the ubiquitous relapse curve. *J Subst Abuse Treat, 36*(1), 8-17. doi: S0740-5472(08)00057-3 [pii]

10.1016/j.jsat.2008.04.001

Koob, G. F. (2009). Brain stress systems in the amygdala and addiction. *Brain Res, 1293*, 61-75. doi: S0006-8993(09)00602-7 [pii]

10.1016/j.brainres.2009.03.038

Kruzich, P. J., & See, R. E. (2001). Differential contributions of the basolateral and central amygdala in the acquisition and expression of conditioned relapse to cocaine-seeking behavior. *J Neurosci, 21*(14), RC155. doi: 20015433 [pii]

Loh, E. A., Fitch, T., Vickers, G., & Roberts, D. C. (1992). Clozapine increases breaking points on a progressive-ratio schedule reinforced by intravenous cocaine. *Pharmacol Biochem Behav, 42*(3), 559-562.

Ranaldi, R., Bauco, P., McCormick, S., Cools, A. R., & Wise, R. A. (2001). Equal sensitivity to cocaine reward in addiction-prone and addiction-resistant rat genotypes. *Behavioural Pharmacology, 12*(6-7), 527-534.

Roberts, D. C., & Bennett, S. A. (1993). Heroin self-administration in rats under a progressive ratio schedule of reinforcement. *Psychopharmacology (Berl), 111*(2), 215-218.

Roberts, D. C. S., Loh, E. A., & Vickers, G. (1989). Self-Administration of Cocaine on a Progressive Ratio Schedule in Rats - Dose-Response Relationship and Effect of Haloperidol Pretreatment. *Psychopharmacology, 97*(4), 535-538.

Salamone, J. D., Cousins, M. S., & Bucher, S. (1994). Anhedonia or anergia? Effects of haloperidol and nucleus accumbens dopamine depletion on instrumental response selection in a T-maze cost/benefit procedure. *Behavioural Brain Research, 65*(2), 221-229.

Saunders, B. T., & Robinson, T. E. (2011). Individual Variation in the Motivational Properties of Cocaine. *Neuropsychopharmacology, 36*(8), 1668-1676. doi: Doi 10.1038/Npp.2011.48

See, R. E., Kruzich, P. J., & Grimm, J. W. (2001). Dopamine, but not glutamate, receptor blockade in the basolateral amygdala attenuates conditioned reward in a rat model of relapse to cocaine-seeking behavior. *Psychopharmacology (Berl), 154*(3), 301-310.

Self, D. W., & Nestler, E. J. (1998). Relapse to drug-seeking: neural and molecular mechanisms. *Drug and Alcohol Dependence, 51*(1-2), 49-60.

Shaham, Y., Shalev, U., Lu, L., De Wit, H., & Stewart, J. (2003). The reinstatement model of drug relapse: history, methodology and major findings. *Psychopharmacology (Berl), 168*(1-2), 3-20. doi: 10.1007/s00213-002-1224-x

Yager, L. M., & Robinson, T. E. (2010). Cue-induced reinstatement of food seeking in rats that differ in their propensity to attribute incentive salience to food cues. *Behavioural Brain Research, 214*(1), 30-34. doi: DOI 10.1016/j.bbr.2010.04.021

Figure Captions

Figure One. **Baseline acoustic startle in high and low startle rats.** Startle amplitudes for high (n = 6) and low (n = 7) startle rats across 3 days of baseline acoustic startle testing. High and low startle rats significantly differ in baseline acoustic startle amplitudes, * $p < .05$.

Figure Two. **Acquisition of operant responding for a food or cocaine reinforcer.** A) High and low startle rats did not significantly in the number of day required to reach criterion on a operant food training task. B). Low startle rats acquired a steady state of cocaine self-administration in fewer days than high startle rats, * $p < .05$.

Figure Three. **Progressive Ratio Schedule.** Low startle rats reach significantly higher breakpoints when compared to high startle rats, * $p < .05$.

Figure Four. **Drug and cue reinstatement of cocaine seeking.** A) Following extinction of cocaine self-administration, low startle rats reinstate extinguished cocaine seeking at a significantly greater rate that high startle rats.

Figure 1.

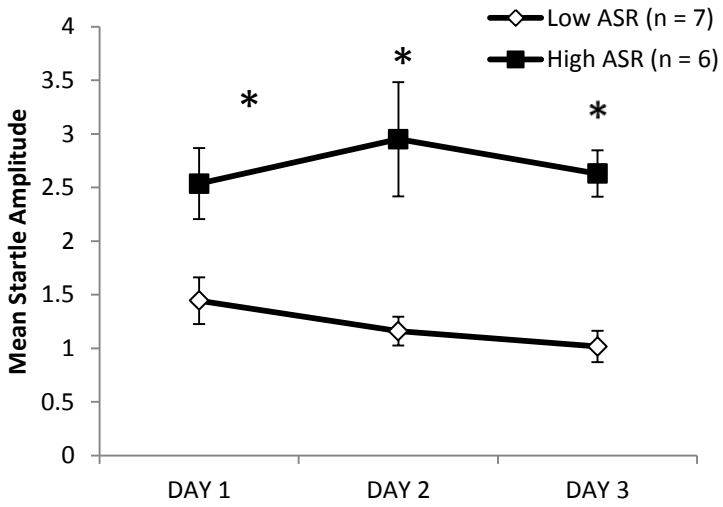
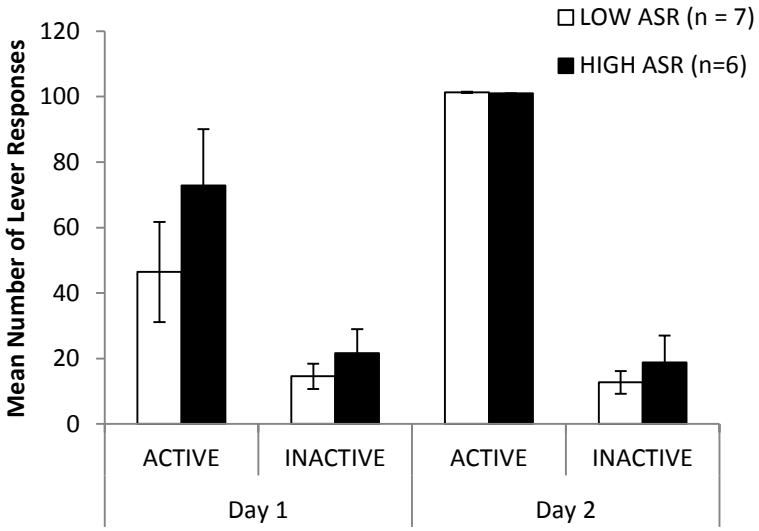
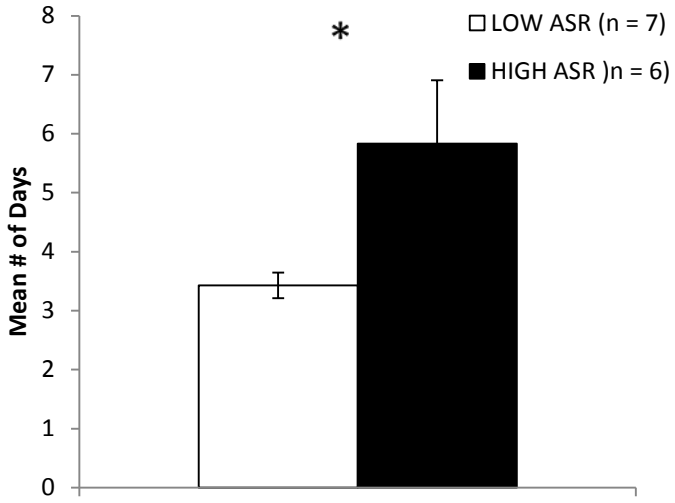

Low ASR (n = 7)
High ASR (n = 6)
Mean Startle Amplitude
4
3.5
3
2.5
2
1.5
1
0.5
0
*
*
*
DAY 1
DAY 2
DAY 3

Figure 2.


LOW ASR (n = 7)
HIGH ASR (n=6)
Mean Number of Lever Responses
120
100
80
60
40
20
0
ACTIVE
INACTIVE
ACTIVE
INACTIVE
Day 1
Day 2

A


*
LOW ASR (n = 7)
HIGH ASR)n = 6)
Mean # of Days
8
7
6
5
4
3
2
1
0

B

Figure 3.

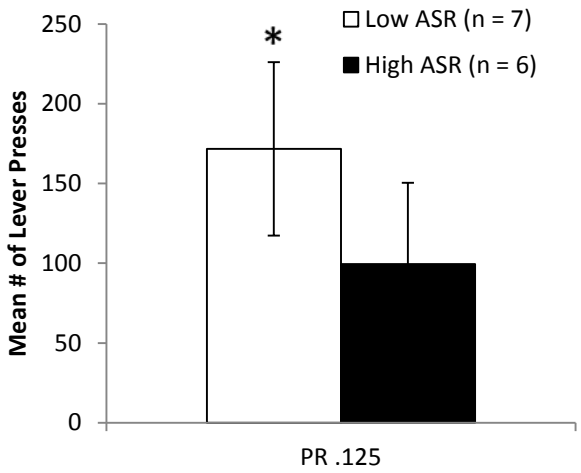
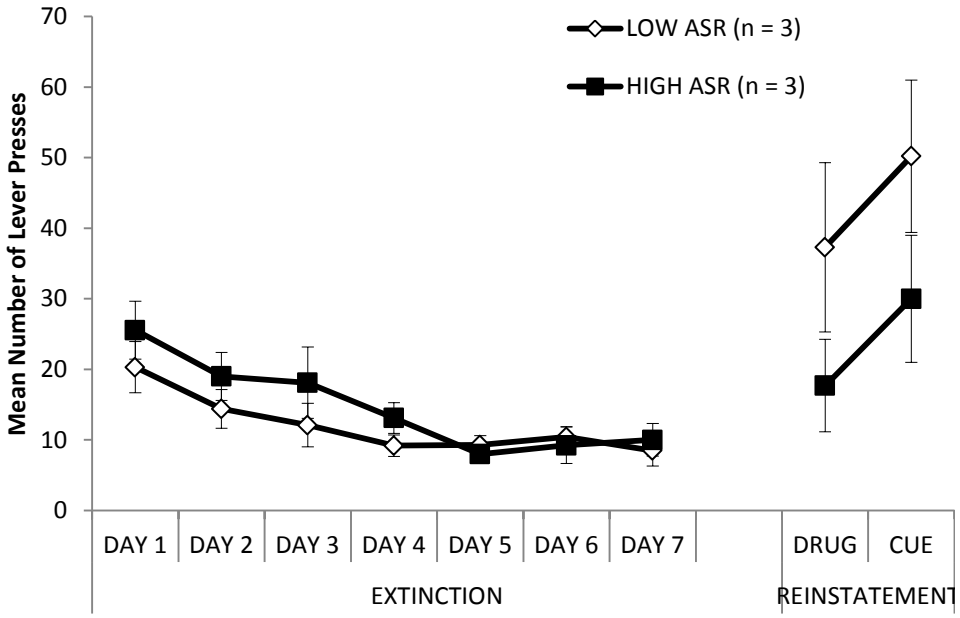

Low ASR (n = 7)
High ASR (n = 6)
*
Mean # of Lever Presses
250
200
150
100
50
0
PR .125

Figure 4.


LOW ASR (n = 3)
HIGH ASR (n = 3)
Mean Number of Lever Presses
70
60
50
40
30
20
10
0
DAY 1
DAY 2
DAY 3
DAY 4
DAY 5
DAY 6
DAY 7
DRUG
CUE
EXTINCTION
REINSTATEMENT

Chapter 4

Acoustic Startle, Novelty Preference and Persistence in Cocaine Seeking

Abstract

Preference for a novel environment positively correlates with greater motivation to obtain cocaine, persistence in cocaine seeking, and reinstatement of extinguished cocaine seeking. Interestingly, rats with drug naïve low acoustic startle amplitudes have greater cocaine sensitivity, motivation to obtain cocaine and reinstatement of extinguished cocaine seeking. The purpose of the present study was to determine if rats with drug naïve low acoustic startle would also have a greater novelty preference and if low startle rats would be more persistent in cocaine seeking when compared to high startle rats. Results indicate that low startle rats have a greater preference for a novel environment when compared to high startle rats and a greater persistence in cocaine seeking behavior. These data further indicate that drug naïve acoustic startle amplitudes are predictive of a vulnerability to abuse cocaine.

Cocaine abuse is a disorder of persistent cocaine seeking despite the negative consequences of doing so and repeated relapse regardless of a desire to abstain (Kirshenbaum, et al., 2009; Shaham, et al., 2003). Key components of this definition are increased motivation, persistence in cocaine seeking, and resistance to punishment. These criteria are also criteria for the diagnosis of substance abuse in the DSM-IV (*Diagnostic and Statistical Manual of Mental Disorders*). In animal models of cocaine abuse, those animals that display high degrees of these three behaviors are more likely to display compulsive cocaine self-administration behaviors such as increased reinstatement of extinguished cocaine seeking (Deroche-Gamonet, et al., 2004).

The Deroche-Gamonet model of cocaine abuse (Deroche-Gamonet, et al., 2004) captures the complexity of the disorder by modeling motivation, persistence in cocaine seeking, and resistance to punishment in a rat cocaine self-administration paradigm. This model focuses on multiple rather than individual behaviors to determine an animal's abuse potential, defined as propensity to reinstate extinguished cocaine seeking. In the Deroche-Gamonet model, rats that display these three behaviors are more likely to reinstate cocaine seeking after responding for cocaine has been extinguished. This finding is key, given that relapse to cocaine use is arguably the defining and most debilitating characteristic of cocaine addiction (Kirshenbaum, et al., 2009; Shaham, et al., 2003)

Preference for a novel environment in rats has subsequently been shown to predict performance in the Deroche-Gamonet model of cocaine abuse (Belin, et al., 2011). Rats with a preference for a novel environment reach higher break points on a progressive ratio, perform significantly more drug seeking behaviors and are more resistant to

punishment that is yoked to cocaine administration when compared to rats with a low preference for a novel environment (Belin, et al., 2011). Therefore, preference for a novel environment is a simple behavioral task that can be used to predict which rats will develop addiction-like patterns of behavior.

Acoustic startle is another individual factor that has been shown to correlate with and predict cocaine related behaviors. In humans, acoustic startle amplitudes of cocaine addicted men are significantly lower than those expressed by healthy controls (Corcoran, et al., 2011; Efferen, et al., 2000). As has been demonstrated in Chapters 1 and 2, acoustic startle amplitudes in rats carry predictive value in determining sensitivity to cocaine, motivation to obtain cocaine and reinstatement of extinguished cocaine seeking. Rats with drug naïve low acoustic startle amplitudes are more sensitive to cocaine and reach high break points on a progressive ratio schedule, indicative of greater motivation to obtain cocaine. Low startle rats are also reinstate extinguished cocaine seeking at a greater rate than high startle rats in response to a cocaine-related cue or cocaine injection. These data suggest the potential value of using acoustic startle to determine vulnerability to abuse cocaine.

Acoustic startle and novelty preference both seem to have predictive value in identifying cocaine associated behaviors. Given the reliability of the Deroche-Gamonet model in determining potential to abuse cocaine and the evidence for the predictive value of novelty preference in this paradigm, low startle rats which display many of the same behaviors may also exhibit a greater novelty preference. The current study will measure novelty preference and persistence in cocaine seeking in rats with drug naïve high and low acoustic startle amplitudes.

Methods

Animals

Adult male Sprague-Dawley rats were group housed in a climate controlled vivarium on a 12 hour light dark cycle with lights on at 800 hours. All rats were given free access to food and water unless otherwise stipulated. All procedures were approved by the animals care and use committee.

Acoustic Startle Testing

Equipment and procedures for acoustic startle testing were described in detail in chapter 1. To determine low and high startle amplitude groups for novelty seeking, 56 rats were given three 20-min tests sessions as described in Chapter 1. Rats with acoustic startle amplitudes in the highest (n=14) and lowest quartiles (n=14) were selected to represent high and low startle groups, the other rats were excluded from further use.

Novelty Preference

Procedures for determining novelty preference were adapted from Belin and colleagues (2011). A wooden conditioning chamber (65 cm x 35 cm x 35 cm) composed of two outer compartments (25 cm x 35 cm x 35 cm) separated by a central hallway (15 cm x 35 cm x 35 cm) and removable Plexiglas guillotine doors was used to conduct the novelty preference test. The outer compartments were made distinguishable from each other by the use of vertical or horizontal black and white stripes on the walls of opposing chambers as well as the use of a wire mesh floor in one compartment and a floor of steel

rods in the opposite compartment. The neutral hallway had white walls and a black Plexiglas floor.

Novelty preference tests were conducted between 800 and 1200 hours and composed of a single preference session. On the day of testing individual rats were placed in the central hallway with access to either compartment blocked by the guillotine doors. After 5 min, rats were removed from the central hallway and confined to either compartment A or B for 25 mins, counterbalanced across rats. At the end of the 25-min confinement rats were placed back into the central hallway and the guillotine doors were raised so that the rat was allowed to freely explore the familiar compartment, the central hallway, and the unfamiliar compartment for 15 min. Preference scores were determined as follows: time in unfamiliar compartment/(time in familiar + time in unfamiliar) * 100.

Cocaine Seeking Behavior

Cocaine seeking behavior was obtained from the low and high startle rats that completed the dose-response study in Chapter 1. Cocaine self-administration procedures have been previously described in Chapter 1. On a fixed ratio 1 schedule, rats were required to press the active cocaine paired lever to get intravenous injections of cocaine delivered through a jugular catheter. Although a fixed ratio 1 schedule was used, a 20-sec time out period followed each cocaine delivery during which no cocaine could be obtained; this time-out period was indicated by illumination of a cue light above the active lever for the 20-sec time out. Following stabilization on the maintenance phase, cocaine seeking behavior was defined as any response made on the cocaine associated lever during the cued time out periods when no cocaine was obtained.

Statistics

A repeated measure analysis of variance was used to evaluate differences in drug naïve acoustic startle amplitudes of high and low startle rats. Independent t-tests were used to assess differences in high and low startle on days 1, 2 and 3 of baseline startle testing. Pearson correlations were calculated to determine degree of correlation between startle on days 1 and 2, 2 and 3, and 1 and 3 for all 28 rats. An independent t-test was used to determine differences in novelty preference for high and low startle rats. A repeated measures analysis of variance was done on the number of time out bar presses using Group (low versus high startle) as a between-subjects factor and cocaine concentration as a within-subjects factor. Effect sizes were measured for all findings using Cohen's D.

Results

Low startle (n=14) and high startle (n=14) rats significantly differed in drug naïve acoustic startle [$F(1,26)=671.452$, $p = .000$, $d = 22.316$] please refer to *Figure 1*. Acoustic startle amplitudes of high and low startle rats significantly differed on days 1 [$t(26) = 6.346$, $p = .000$, $d = 27.023$], 2 [$t(26) = 6.452$, $p = .000$, $d = 7.889$] and 3 [$t(26) = 9.762$, $p = .000$, $d = 13.208$] of baseline startle testing. Pearson correlations showed a high degree of correlation between startle amplitudes on Day 1-Day 2 [$r(28) = .618$, $p = .000$], Day 2-Day -Day 3 [$r(16) = .800$, $p = .000$], and Day 1- Day 3 [$r(16) = .706$, $p = .000$]. Most important, novelty preference was significantly greater in low startle rats when compared to high startle rats [$F(1,27)=12.599$ $p = .001$, $d = 5.019$] please refer to *Figure 2*.

Low startle (n=5) and high startle (n=5) rats that completed a dose-response curve in Chapter 1 were used to assess time-out lever presses (Figure 1). A log10 transformation was performed on time-out lever press data to account for variability. Analysis of time-out lever presses showed a significant group effect between high and low startle rats [$F(1,8) = 23.104$, $p = .001$, $d = 6.654$] and dose [$F(1,8) = 13.828$, $p = .000$], with no significant interaction [$F(1,8) = .575$, $p = .683$] *Figure 4*.

Discussion

Acoustic startle amplitudes predicted performance on a novelty preference task. Low startle rats expressed significantly greater preferences for novel environments when compared to high startle rats. In addition, low startle rats were more persistent in cocaine seeking in comparison with high startle rats as measured by time-out lever presses. This effect was also varied by the concentration of cocaine.

Preference for a novel environment predicts the strength of amphetamine conditioned place preference conditioning, motivation to obtain cocaine, cocaine seeking, resistance to punishment, and the strength of reinstatement of previously extinguished cocaine seeking behaviors in rats (Bardo, Donohew, & Harrington, 1996; Belin, et al., 2011). The novelty preference phenotype therefore has significant predictive power in determining which rats will exhibit cocaine related behaviors that can be compared to cocaine addiction in humans. It is interesting therefore to note that low acoustic startle rats, which are more motivated to obtain cocaine, spend more time seeking cocaine during cued time-outs, and reinstate extinguished cocaine seeking at a greater rate than high startle rats, also display a significant preference for a novel environment.

Preference for a novel environment is dependent upon dopamine D1 receptors within the nucleus accumbens (Bardo, Lacy, & Mattingly, 1990; Bevins et al., 2002; Pierce, Crawford, Nonneman, Mattingly, & Bardo). Another behavioral phenotype dependent upon D1 receptors is sign tracking. Sign trackers are those animals for which the cue associated with cocaine comes to possess hedonic value and express greater motivation to obtain cocaine or food, and reinstate extinguished reinforcer seeking at a greater rate (Flagel, et al., 2008; Saunders & Robinson, 2011; Yager & Robinson, 2010 2007). Animals that express the sign tracking phenotype also have higher levels of D1 mRNA (Flagel, et al., 2007). These data suggest the importance of D1 receptors in the expression of behaviors associated with habitual cocaine use and suggest that low startle rats may also express larger amounts of the D1 receptor. Future studies should investigate this possibility.

It has been suggested that the increase in D1 mRNA in sign trackers and possibly in high novelty preferring rats is important for the acquisition of reinforcer-cue associations. The basolateral amygdala is important for the acquisition of cue-associations (Kruzich & See, 2001; See, McLaughlin, & Fuchs, 2003). Within the basolateral amygdala antagonism of D1 receptors blocks the acquisition of cocaine-cue associations and subsequently attenuates cue induced reinstatement of cocaine seeking (Berglind, Case, Parker, Fuchs, & See, 2006). Given the increased number of D1 receptors in the nucleus accumbens of sign trackers and rats with a preference for a novel environment, this increase could extend to the basolateral amygdala and would support the prediction that animals that express these phenotypes have better acquisition of cued associations. This may also be the case for low startle rats.

If low startle rats form stronger cocaine cue associations than high startle rats and consequently attribute greater hedonic value to the cocaine associated cue this may explain the increase in time-out lever presses. That is, low startle rats continue to lever press during intervals in which cocaine is not available because the lever, which is associated with the reinforcing value of cocaine, has acquired greater hedonic value in these rats.

This study cannot discount an alternative explanation, namely a difference in response inhibition. If low startle rats had less response inhibition than high startle rats it would be expected they would press the bars more often, regardless of whether or not cocaine was available. However, responding was selective to the cocaine associated lever, please refere to chapter 1, suggesting that the behavior was specific to a cue-associated stimulus and not a general increase in non-directed responding.

In conclusion, low acoustic startle rats also express the behavioral phenotype of novelty preference and greater cocaine seeking during cued time-outs when compared to high startle rats. These differences may be attributable to an increased expression of D1 receptors in both the nucleus accumbens and basolateral amygdala in low startle rats and future studies should further investigate this by looking directly at the acquisition of cued associations in high and low startle rats, and also measuring the density of D1 receptors in the nucleus accumbens and basolateral amygdala of low and high startle rats.

References

Bardo, M. T., Donohew, R. L., & Harrington, N. G. (1996). Psychobiology of novelty seeking and drug seeking behavior. *Behavioural Brain Research, 77*(1-2), 23-43.

Bardo, M. T., Lacy, M., & Mattingly, B. A. (1990). Effects of apomorphine on novelty-induced place preference behavior in rats. *Pharmacol Biochem Behav, 37*(1), 89-93. doi: 0091-3057(90)90046-K [pii]

Belin, D., Berson, N., Balado, E., Piazza, P. V., & Deroche-Gamonet, V. (2011). High-novelty-preference rats are predisposed to compulsive cocaine self-administration. *Neuropsychopharmacology, 36*(3), 569-579. doi: npp2010188 [pii]

10.1038/npp.2010.188

Berglind, W. J., Case, J. M., Parker, M. P., Fuchs, R. A., & See, R. E. (2006). Dopamine D1 or D2 receptor antagonism within the basolateral amygdala differentially alters the acquisition of cocaine-cue associations necessary for cue-induced reinstatement of cocaine-seeking. *Neuroscience, 137*(2), 699-706. doi: S0306-4522(05)00990-5 [pii]

10.1016/j.neuroscience.2005.08.064

Bevins, R. A., Besheer, J., Palmatier, M. I., Jensen, H. C., Pickett, K. S., & Eurek, S. (2002). Novel-object place conditioning: behavioral and dopaminergic processes in expression of novelty reward. *Behavioural Brain Research, 129*(1-2), 41-50. doi: S0166432801003266 [pii]

Corcoran, S., Norrholm, S. D., Cuthbert, B., Sternberg, M., Hollis, J., & Duncan, E. (2011). Acoustic startle reduction in cocaine dependence persists for 1 year of

abstinence. *Psychopharmacology, 215*(1), 93-103. doi: DOI 10.1007/s00213-010-2114-2

Deroche-Gamonet, V., Belin, D., & Piazza, P. V. (2004). Evidence for addiction-like behavior in the rat. *Science, 305*(5686), 1014-1017. doi: 10.1126/science.1099020305/5686/1014 [pii]

Diagnostic and Statistical Manual of Mental Disorders. (2000). (4 ed.). Washington, DC: American Psychiatric Association.

Efferen, T. R., Duncan, E. J., Szilagyi, S., Chakravorty, S., Adams, J. U., Gonzenbach, S., . . . Rotrosen, J. (2000). Diminished acoustic startle in chronic cocaine users. *Neuropsychopharmacology, 22*(1), 89-96. doi: S0893-133X(99)00089-5 [pii]10.1016/S0893-133X(99)00089-5

Flagel, S. B., Watson, S. J., Akil, H., & Robinson, T. E. (2008). Individual differences in the attribution of incentive salience to a reward-related cue: influence on cocaine sensitization. *Behavioural Brain Research, 186*(1), 48-56. doi: S0166-4328(07)00373-7 [pii]10.1016/j.bbr.2007.07.022

Flagel, S. B., Watson, S. J., Robinson, T. E., & Akil, H. (2007). Individual differences in the propensity to approach signals vs goals promote different adaptations in the dopamine system of rats. *Psychopharmacology, 191*(3), 599-607. doi: DOI 10.1007/s00213-006-0535-8

Kirshenbaum, A. P., Olsen, D. M., & Bickel, W. K. (2009). A quantitative review of the ubiquitous relapse curve. *J Subst Abuse Treat, 36*(1), 8-17. doi: S0740-5472(08)00057-3 [pii]

10.1016/j.jsat.2008.04.001

Kruzich, P. J., & See, R. E. (2001). Differential contributions of the basolateral and central amygdala in the acquisition and expression of conditioned relapse to cocaine-seeking behavior. *J Neurosci, 21*(14), RC155. doi: 20015433 [pii]

Pierce, R. C., Crawford, C. A., Nonneman, A. J., Mattingly, B. A., & Bardo, M. T. (1990). Effect of forebrain dopamine depletion on novelty-induced place preference behavior in rats. *Pharmacol Biochem Behav, 36*(2), 321-325.

Saunders, B. T., & Robinson, T. E. (2011). Individual Variation in the Motivational Properties of Cocaine. *Neuropsychopharmacology, 36*(8), 1668-1676. doi: Doi 10.1038/Npp.2011.48

See, R. E., McLaughlin, J., & Fuchs, R. A. (2003). Muscarinic receptor antagonism in the basolateral amygdala blocks acquisition of cocaine-stimulus association in a model of relapse to cocaine-seeking behavior in rats. *Neuroscience, 117*(2), 477-483. doi: S0306452202006656 [pii]

Shaham, Y., Shalev, U., Lu, L., De Wit, H., & Stewart, J. (2003). The reinstatement model of drug relapse: history, methodology and major findings. *Psychopharmacology (Berl), 168*(1-2), 3-20. doi: 10.1007/s00213-002-1224-x

Yager, L. M., & Robinson, T. E. (2010). Cue-induced reinstatement of food seeking in rats that differ in their propensity to attribute incentive salience to food cues. *Behavioural Brain Research, 214*(1), 30-34. doi: DOI 10.1016/j.bbr.2010.04.021

Figure Captions

Figure One. **Acoustic startle in low and high startle rats: Novelty Preference.** Mean startle amplitudes for high (n = 14) and low (n = 14) startle rats included in the novelty preference test. Low and high acoustic startle rats significantly differ in drug naïve baseline acoustic startle,* $p < .05$.

Figure Two. **Novelty preference.** Low startle rats display a greater level of novelty preference when compared to high startle rats, * $p < .05$.

Figure Three. **Acoustic startle in low and high startle rats: Cocaine Seeking.** Mean startle amplitudes for high (n = 5) and low (n = 5) startle rats included in the persistence in cocaine seeking analysis significantly differed, * $p < .05$.

Figure Four. **Persistence in cocaine seeking.** A) Low and high startle rats significantly differed in the rate of responding on the active lever during cued time-out periods of the cocaine self-administration session B) Log10 transformation of time-out lever presses used for analysis.

Figure 1.

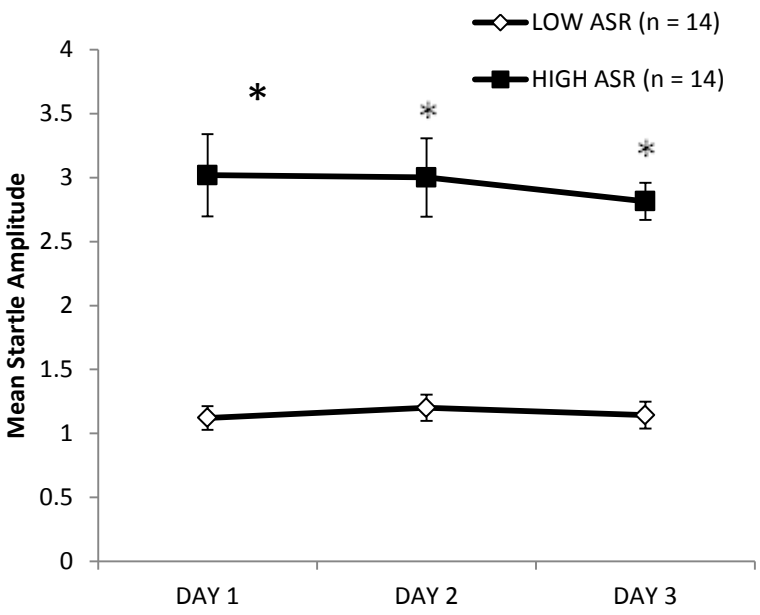

LOW ASR (n = 14)
HIGH ASR (n = 14)
Mean Startle Amplitude
4
3.5
3
2.5
2
1.5
1
0.5
0
*
*
*
DAY 1
DAY 2
DAY 3

Figure 2.

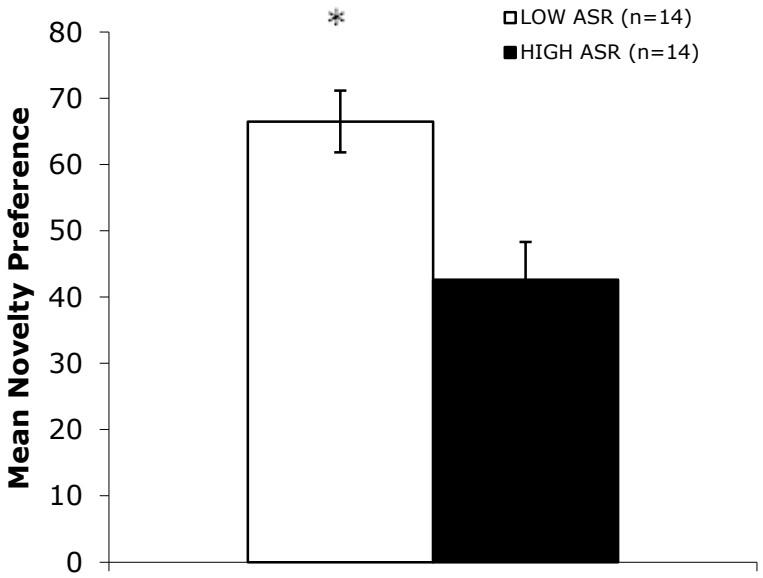

*
LOW ASR (n=14)
HIGH ASR (n=14)
Mean Novelty Preference
80
70
60
50
40
30
20
10
0

Figure 3.

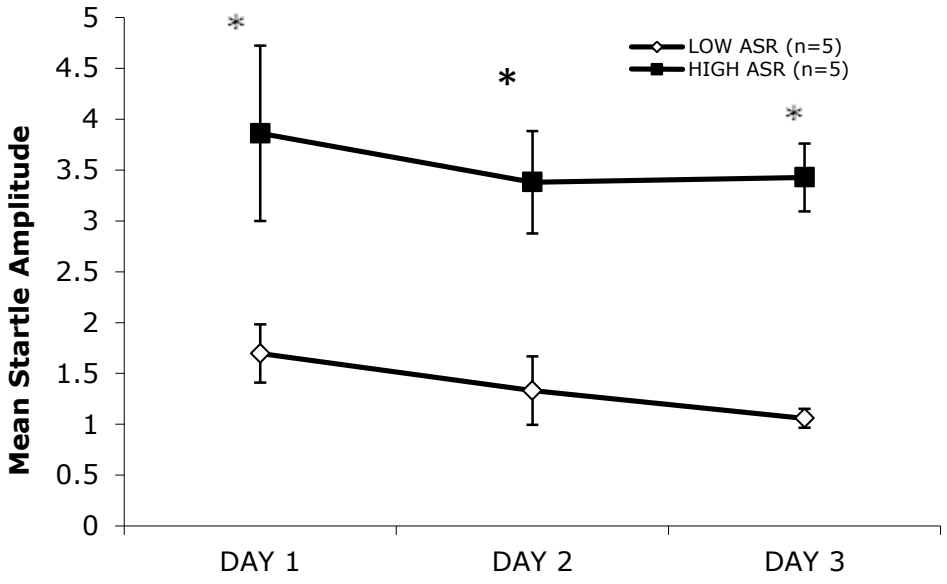
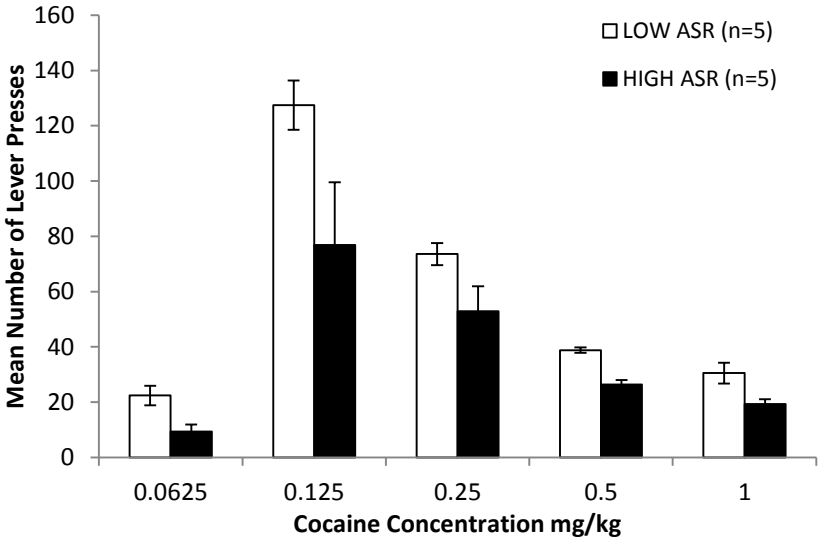
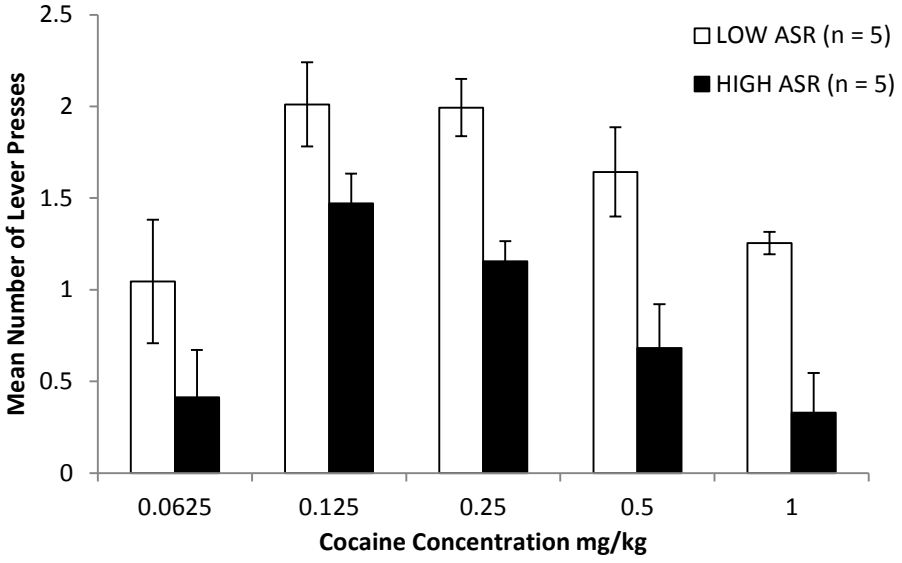

Mean Startle Amplitude
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
DAY 1
DAY 2
DAY 3
LOW ASR (n=5)
HIGH ASR (n=5)
*
*
*

Figure 4.

A


160
140
120
100
80
60
40
20
0
Mean Number of Lever Presses
LOW ASR (n=5)
HIGH ASR (n=5)
0.0625
0.125
0.25
0.5
1
Cocaine Concentration mg/kg

B


2.5
2
1.5
1
0.5
0
Mean Number of Lever Presses
LOW ASR (n = 5)
HIGH ASR (n = 5)
0.0625
0.125
0.25
0.5
1
Cocaine Concentration mg/kg

Chapter 5

General Discussion

The aim of this dissertation was to investigate the direction of the relationship between acoustic startle and cocaine use first identified in clinical samples of cocaine addicted men (Corcoran, et al., 2011; Efferen, et al., 2000). From these original studies it was unclear if cocaine use diminished acoustic startle, or if low startle preceded cocaine use. Evidence from pre-clinical studies and exploratory data (supplement A) suggest that cocaine treatment which is either response contingent or experimenter delivered has at best only limited and transient effects on acoustic startle (Adams, et al., 2001; Gordon & Rosen, 1999; Kirkland Henry, et al., 2009; Mansbach, et al., 1994). There is no evidence for or against the alternative hypothesis that low acoustic startle precedes and therefore predicts vulnerability to abuse cocaine. However, given that acoustic startle is a highly heritable trait (Anokhin, et al., 2003; Cadenhead, Light, Geyer, & Braff, 2000) and potentially stable over time it could be predictive.

Dopamine is a neurotransmitter through which cocaine produces it's reinforcing effect and has modulatory influence over acoustic startle (Davis, 1985; Davis & Aghajanian, 1976; Davis, et al., 1975; Meloni & Davis, 1999; Ritz, et al., 1987). Low dopaminergic transmission and potential changes in post-synaptic dopamine receptor density could account for changes in acoustic startle and response to cocaine. Investigating the potential predictive value of low acoustic startle in determining vulnerability to use cocaine in rats was therefore the focus of this dissertation.

The studies described here were aimed at identifying the predictive value of acoustic startle in measuring differences between high and low startle rats in sensitivity to cocaine, motivation to obtain cocaine, persistence in cocaine seeking and reinstatement of extinguished cocaine seeking. These behavioral measures are several of the criteria for

the diagnosis of substance abuse in humans and have been found to be highly predictive of the development of habitual cocaine use in rodent models of abuse (Belin, et al., 2011; de Wit & Stewart, 1981; Deroche-Gamonet, et al., 2004; *Diagnostic and Statistical Manual of Mental Disorders*, 2000). In addition to these cocaine associated behaviors, differences in novelty preference, which has previously been shown to predict cocaine abuse potential, was also measured in high and low startle rats (Belin, et al., 2011).

Low startle rats had heightened sensitivity to cocaine, increased motivation to obtain cocaine, and were more likely to reinstate extinguished cocaine seeking at a greater rate in response to a cocaine prime or cue when compared to high startle rats. Low startle rats also had a greater novelty preference when compared to high startle rats and greater persistence in cocaine seeking when compared to high startle rats. These data indicate that low acoustic startle is predictive of a vulnerability to abuse cocaine.

Clinically these findings support the use of individual factors which can help to identify at risk individuals who may benefit from a more intensive rehabilitation program. Given that acoustic startle amplitudes are highly heritable this may also be a tool for identifying non-cocaine using relatives who share a common genetic anomaly that may be contributing to increased vulnerability for cocaine abuse. In pre-clinical models of cocaine abuse, these data suggest that acoustic startle is a beneficial tool for identifying animals that express an increased vulnerability to develop habitual cocaine self-administration that is comparable to the addicted behaviors of humans.

These data may suggest that not only are low startle rats more sensitive to the reinforcing effects of cocaine, but that they may also be more adept at acquiring cocaine-

cue associations. Evidence from studies investigating the behavioral phenotype of sign-tracking versus goal tracking may elucidate this difference. Sign-trackers pay attention to the cue associated with the delivery of the reinforcer and goal trackers pay attention to the reinforcer (Flagel, et al., 2008; Flagel, et al., 2007). Rats that express sign tracking reach higher breakpoints on a progressive ratio and reinstate extinguished responding for a food or cocaine reinforcer at greater rates than goal trackers (Saunders & Robinson, 2011; Yager & Robinson, 2010). These differences are thought to be related to increased strength of the reinforcer-cue associations acquired by the sign-trackers. Furthermore, because of the strength of the associations to the reinforcer associated cues, sign-trackers assign hedonic value to the cues. Continued responding at those cues may therefore not only be motivated by a desire for the actual reinforcer but also be due to the fact that the cue has acquired reinforcement value of its own.

Differences in the acquisition of associative learning in response to a cocaine associated cue is at least in part modulated by D1 receptors in the basolateral amygdala (Berglind, et al., 2006). Though not measured in the amygdala, sign trackers do have more D1 mRNA in the nucleus accumbens compared to goal trackers (Flagel, et al., 2007). Therefore low startle rats could reach higher breakpoints on a progressive ratio schedule and have a greater number of time-out lever presses than high startle rats because of a stronger cocaine-cue association with the lever. If the lever subsequently acquired hedonic value for the low startle rats, this increase in reinforcement could facilitate an increased rate of responding on a progressive ratio schedule and an increased rate of cue primed reinstatement of cocaine seeking.

Other evidence that rats with a greater potential to abuse cocaine might have greater D1 receptor density is found in the behavioral phenotype of novelty preference. Rats that express a greater novelty preference are more motivated to obtain cocaine, persistent in cocaine seeking, and reinstate extinguished cocaine seeking at greater rates than rats with low novelty preferences (Belin, et al., 2011). Rats with greater novelty preferences have also been found to have greater amounts of D1 mRNA (Bardo, et al., 1990; Bevins, et al., 2002; Pierce, et al., 1990). Given that low startle rats express a greater novelty preference than high startle rats and many of the same behavioral traits as novelty preferring rats including greater motivation to obtain cocaine and likelihood to reinstate extinguished cocaine seeking, this evidence suggests that low startle rats may have greater D1 receptor density.

Alternatively dopamine may not be the driving force between the correlation between acoustic startle and cocaine abuse. In other psychiatric disorders such as schizophrenia in which there is an increase in dopaminergic transmission there is no difference in baseline acoustic startle amplitudes (Bird, Spokes, & Iversen, 1979; Curtis, Lebow, Lake, Katsanis, & Iacono, 1999; Lieberman, Kane, & Alvir, 1987; Owen et al., 1978). In rats bread for a sensitivity or insensitivity to the effects of apomorphine, a dopaminergic agonist, acoustic startle amplitudes are comparably affected by pretreatment with amphetamine (van der Elst, Wunderink, Ellenbroek, & Cools, 2007). Therefore, further investigation into the role of dopaminergic transmission between high and low startle rats is warranted. Given the potential role of D1 receptor in the cocaine associated behaviors and the possible increase in dopamine receptor density in

amphetamine potentiated acoustic startle this would be a primary target for future investigation.

In conclusion, the data presented here support the hypothesis that differences in acoustic startle amplitude precede cocaine abuse. Low startle rats were more sensitive to cocaine, more motivated to obtain it, more persistent in cocaine seeking and showed greater reinstatement of extinguished cocaine seeking in response to a cocaine or cue prime. Low startle rats also had a greater novelty preference than high startle rats and this behavioral phenotype has been previously shown to be predictive of cocaine abuse potential (Belin, et al., 2011). Exploratory data in our lab suggests that acoustic startle amplitudes in high and low startle rats were not diminished by the self-administration of cocaine and this finding is supported by the literature which does not show a significant or lasting impact of cocaine treatment on acoustic startle (Adams, et al., 2001; Gordon & Rosen, 1999; Kirkland Henry, et al., 2009; Mansbach, et al., 1994). Low dopaminergic transmission combined with an increase in post-synaptic receptor density could account for differences in acoustic startle and differences in cocaine sensitivity, motivation, seeking and reinstatement and well as the difference in novelty preference.

References

Adams, J. U., Efferen, T. R., Duncan, E. J., & Rotrosen, J. (2001). Prepulse inhibition of the acoustic startle response in cocaine-withdrawn rats. *Pharmacol Biochem Behav, 68*(4), 753-759. doi: S0091-3057(01)00478-6 [pii]

Anokhin, A. P., Heath, A. C., Myers, E., Ralano, A., & Wood, S. (2003). Genetic influences on prepulse inhibition of startle reflex in humans. *Neuroscience Letters, 353*(1), 45-48. doi: DOI 10.1016/j.neulet.2003.09.014

Bardo, M. T., Lacy, M., & Mattingly, B. A. (1990). Effects of apomorphine on novelty-induced place preference behavior in rats. *Pharmacol Biochem Behav, 37*(1), 89-93. doi: 0091-3057(90)90046-K [pii]

Belin, D., Berson, N., Balado, E., Piazza, P. V., & Deroche-Gamonet, V. (2011). High-novelty-preference rats are predisposed to compulsive cocaine self-administration. *Neuropsychopharmacology, 36*(3), 569-579. doi: npp2010188 [pii]

10.1038/npp.2010.188

Berglind, W. J., Case, J. M., Parker, M. P., Fuchs, R. A., & See, R. E. (2006). Dopamine D1 or D2 receptor antagonism within the basolateral amygdala differentially alters the acquisition of cocaine-cue associations necessary for cue-induced reinstatement of cocaine-seeking. *Neuroscience, 137*(2), 699-706. doi: S0306-4522(05)00990-5 [pii]

10.1016/j.neuroscience.2005.08.064

Bevins, R. A., Besheer, J., Palmatier, M. I., Jensen, H. C., Pickett, K. S., & Eurek, S. (2002). Novel-object place conditioning: behavioral and dopaminergic processes

in expression of novelty reward. *Behavioural Brain Research, 129*(1-2), 41-50. doi: S0166432801003266 [pii]

Bird, E. D., Spokes, E. G., & Iversen, L. L. (1979). Increased dopamine concentration in limbic areas of brain from patients dying with schizophrenia. *Brain, 102*(2), 347-360.

Cadenhead, K. S., Light, G. A., Geyer, M. A., & Braff, D. L. (2000). Sensory gating deficits assessed by the P50 event-related potential in subjects with schizotypal personality disorder. *Am J Psychiatry, 157*(1), 55-59.

Corcoran, S., Norrholm, S. D., Cuthbert, B., Sternberg, M., Hollis, J., & Duncan, E. (2011). Acoustic startle reduction in cocaine dependence persists for 1 year of abstinence. *Psychopharmacology, 215*(1), 93-103. doi: DOI 10.1007/s00213-010-2114-2

Curtis, C. E., Lebow, B., Lake, D. S., Katsanis, J., & Iacono, W. G. (1999). Acoustic startle reflex in schizophrenia patients and their first-degree relatives: evidence of normal emotional modulation. *Psychophysiology, 36*(4), 469-475.

Davis, M. (1985). Cocaine: excitatory effects on sensorimotor reactivity measured with acoustic startle. *Psychopharmacology (Berl), 86*(1-2), 31-36.

Davis, M., & Aghajanian, G. K. (1976). Effects of apomorphine and haloperidol on the acoustic startle response in rats. *Psychopharmacology (Berl), 47*(3), 217-223.

Davis, M., Svensson, T. H., & Aghajanian, G. K. (1975). Effects of d- and l-amphetamine on habituation and sensitization of the acoustic startle response in rats. *Psychopharmacologia, 43*(1), 1-11.

de Wit, H., & Stewart, J. (1981). Reinstatement of cocaine-reinforced responding in the rat. *Psychopharmacology (Berl), 75*(2), 134-143.

Deroche-Gamonet, V., Belin, D., & Piazza, P. V. (2004). Evidence for addiction-like behavior in the rat. *Science, 305*(5686), 1014-1017. doi: 10.1126/science.1099020 305/5686/1014 [pii]

Diagnostic and Statistical Manual of Mental Disorders. (2000). (4 ed.). Washington, DC: American Psychiatric Association.

Efferen, T. R., Duncan, E. J., Szilagyi, S., Chakravorty, S., Adams, J. U., Gonzenbach, S., . . . Rotrosen, J. (2000). Diminished acoustic startle in chronic cocaine users. *Neuropsychopharmacology, 22*(1), 89-96. doi: S0893-133X(99)00089-5 [pii] 10.1016/S0893-133X(99)00089-5

Flagel, S. B., Watson, S. J., Akil, H., & Robinson, T. E. (2008). Individual differences in the attribution of incentive salience to a reward-related cue: influence on cocaine sensitization. *Behavioural Brain Research, 186*(1), 48-56. doi: S0166-4328(07)00373-7 [pii] 10.1016/j.bbr.2007.07.022

Flagel, S. B., Watson, S. J., Robinson, T. E., & Akil, H. (2007). Individual differences in the propensity to approach signals vs goals promote different adaptations in the dopamine system of rats. *Psychopharmacology, 191*(3), 599-607. doi: DOI 10.1007/s00213-006-0535-8

Gordon, M. K., & Rosen, J. B. (1999). Lasting effect of repeated cocaine administration on acoustic and fear-potentiated startle in rats. *Psychopharmacology (Berl), 144*(1), 1-7.

Kirkland Henry, P., Davis, M., & Howell, L. L. (2009). Effects of cocaine self-administration history under limited and extended access conditions on in vivo striatal dopamine neurochemistry and acoustic startle in rhesus monkeys. . *Psychopharmacology (Berl), 205*(2), 237-247.

Lieberman, J. A., Kane, J. M., & Alvir, J. (1987). Provocative tests with psychostimulant drugs in schizophrenia. *Psychopharmacology (Berl), 91*(4), 415-433.

Mansbach, R. S., Markou, A., & Patrick, G. A. (1994). Lack of altered startle responding in rats following termination of self-administered or noncontingently infused cocaine. *Pharmacol Biochem Behav, 48*(2), 453-458. doi: 0091-3057(94)90552-5 [pii]

Meloni, E. G., & Davis, M. (1999). Enhancement of the acoustic startle response in rats by the dopamine D1 receptor agonist SKF 82958. *Psychopharmacology (Berl), 144*(4), 373-380.

Owen, F., Cross, A. J., Crow, T. J., Longden, A., Poulter, M., & Riley, G. J. (1978). Increased dopamine-receptor sensitivity in schizophrenia. *Lancet, 2*(8083), 223-226. doi: S0140-6736(78)91740-3 [pii]

Pierce, R. C., Crawford, C. A., Nonneman, A. J., Mattingly, B. A., & Bardo, M. T. (1990). Effect of forebrain dopamine depletion on novelty-induced place preference behavior in rats. *Pharmacol Biochem Behav, 36*(2), 321-325.

Ritz, M. C., Lamb, R. J., Goldberg, S. R., & Kuhar, M. J. (1987). Cocaine receptors on dopamine transporters are related to self-administration of cocaine. *Science, 237*(4819), 1219-1223.

Saunders, B. T., & Robinson, T. E. (2011). Individual Variation in the Motivational Properties of Cocaine. *Neuropsychopharmacology, 36*(8), 1668-1676. doi: Doi 10.1038/Npp.2011.48

van der Elst, M. C., Wunderink, Y. S., Ellenbroek, B. A., & Cools, A. R. (2007). Differences in the cellular mechanism underlying the effects of amphetamine on prepulse inhibition in apomorphine-susceptible and apomorphine-unsusceptible rats. *Psychopharmacology (Berl), 190*(1), 93-102. doi: 10.1007/s00213-006-0587-9

Yager, L. M., & Robinson, T. E. (2010). Cue-induced reinstatement of food seeking in rats that differ in their propensity to attribute incentive salience to food cues. *Behavioural Brain Research, 214*(1), 30-34. doi: DOI 10.1016/j.bbr.2010.04.021

Supplement A

Acoustic Startle Amplitudes Pre- and Post-Cocaine Self-Administration

While cocaine's reinforcing effect is significantly determined by its ability to enhance dopaminergic transmission, chronic abuse of cocaine can cause a hypodopaminergic state (Pilotte, et al., 1994; Pilotte, et al., 1996; Ritz, et al., 1987). In rats, cocaine self-administration decreases synaptic dopamine in the nucleus accumbens, the ability of cocaine to block the reuptake of dopamine, and consequently cocaine's ability to increase synaptic dopamine (Cerruti, et al., 1994; Dackis & O'Brien, 2001; J. Lee, et al., 2011; Mateo, Lack, Morgan, Roberts, & Jones, 2005; Pilotte, et al., 1994; Pilotte, et al., 1996). In addition to its central role in reinforcing and motivating cocaine use, dopamine also modulates acoustic startle. In rats, dopaminergic agonists increase acoustic startle amplitudes while dopaminergic antagonists diminish them (Davis, 1980; Davis, et al., 1975; Schwarzkopf, et al., 1993). Changes in dopaminergic transmission caused by chronic abuse of cocaine might therefore impact acoustic startle.

In rats neither 7 days of experimenter delivered cocaine (20 mg/kg IP) or self-administered cocaine (170 mg/kg/day) significantly altered acoustic startle in the first 24 hours or 1 week (7 once daily injections, 20 mg/kg, IP) post cocaine treatment (Gordon & Rosen, 1999; Mansbach, et al., 1994). In both of these studies, exposure to cocaine was considerably short, lasting only 7 days. The lack of an effect is potentially due to differences in length of cocaine exposure. However, 8 weeks of once daily injections of cocaine (30 mg/kg, IP) in rats diminishes acoustic startle amplitudes although this effect was longer detectable 28 days after the last cocaine exposure (Adams, et al., 2001). In rhesus macaques, although 10 weeks of cocaine self-administration did reduce dopaminergic transmission, it did not alter acoustic startle amplitudes in comparison with

drug naïve animals, nor did it effect the ability of acutely administered cocaine to enhance acoustic startle (Kirkland Henry, et al., 2009).

The literature therefore suggests that the impact of cocaine exposure on acoustic startle is at best minimal and transient. Alternatively, preexisting differences in dopaminergic function could account for both an increased vulnerability to abuse cocaine and reduced acoustic startle amplitudes. Lower levels of dopaminergic transmission could diminish startle amplitudes and increase the reinforcing change in the dopamine noise-to-signal ratio caused by cocaine. If this were the case, low functional rates of dopaminergic transmission would cause low startle and potentially increase vulnerability to abuse cocaine.

The purpose of this exploratory study was to determine in rats the role of dopamine in modulating differences in acoustic startle before and after cocaine exposure. If cocaine induced hypodopaminergia decreases acoustic startle amplitudes, startle amplitudes in high startle rats should be significantly reduced following cocaine self-administration.

Methods

Animals

Male Sprague-Dawley rats were group housed and kept on a 12 hour light/dark cycle with lights on at 800 hours. Food and water were available ad libitum for the duration of the experiment with the exception of a 3 day period of food training on an operant procedure during which time animals were weight restricted to 90% of their free fed weight. All procedures were approved by the Institutional Animal Care and Use Committee.

Cocaine Self-administration

Rats were initially trained to press respond on an operant task using a food reinforcer and then implanted with jugular catheters using techniques described in chapter 1.Using methods detailed in chapters 1 and 2, rats were trained to self-administer cocaine using a 0.5 mg/kg concentration of cocaine and then completed either a dose response curve or a progressive ratio schedule of reinforcement.

Acoustic Startle Testing

The equipment and procedures for acoustic startle testing have been described in chapter 1. Specific to the current set of experiments, acoustic startle was measured in drug naïve animals during 3 20 minute acoustic startle sessions using 30 presentations each of 95, 105 and 115 dB stimuli. Drug naïve acoustic startle was defined as the mean startle amplitude for each animal across the 3 days of baseline testing. Rats with acoustic startle responses in the highest and lowest quartiles were selected as high and low startle rats.

Amphetamine Challenge

Cocaine naive low and high startle rats underwent a 2 day amphetamine challenge of acoustic startle test. Using a cross-over within subjects design, all rats were injected one day with a 0.4 mg/kg (IP) concentration of amphetamine and one day with a vehicle injection. Immediately after injection rats were placed into the startle chamber and begun a single 20 minute acoustic startle session with 30 presentations each of the 95, 105 and 115 dB stimuli. The amphetamine challenge was repeated again a week after the end of cocaine self-administration.

Statistics

Differences in acoustic startle amplitudes were analyzed using a repeated measures analysis of variance. Differences in startle amplitudes on specific days were measured using independent t-tests. Pearson's correlations were calculated to compare acoustic startle data on days 1 and 2, 1 and 3, and 2 and 3. Repeated measures analysis of variance with drug and time as the within subjects variable and startle group as the between subjects variable were analyzed to compare acoustic startle amplitudes in response to either vehicle or 0.4 mg/kg amphetamine pre- and post-cocaine self-administration. Percent change scores were calculated to measure amphetamine potentiated startle. A repeated measures analysis of variance was used to measure differences between high and low startle rats in amphetamine potentiated startle with difference scores pre- and post-cocaine self-administration as the with-in subjects variable and high or low startle as the between subjects variable. Cohen D was calculated for all statistics to determine effect sizes.

Results

Acoustic startle amplitudes of high and low startle rats significantly differed [F(1, 21) = 233.736, p = .000, d = 8.450]. Differences in startle amplitudes were significant on days 1 [t(21) = 3.828, p = .001, d = 5.035], 2 [t(21) = 2.667, p = .014, d = 3.617] and 3 [t(21) = -3.978, p = .001 d = 5.469] please refer to *Figure 1*. Pearson correlations for days 1 and 2 [r(21) = .650, p = .001], 1 and 3 [r(21) = .710, p = .000] and 2 and 3 [r(1,21) = .800 p = .000] were significantly correlated. For the amphetamine challenge, startle amplitudes for high and low startle groups were significantly different [F(1,21) = 31.044, p = .000, d = 8.224], amphetamine significantly enhanced acoustic startle [F(1,21) = 49.946, p = .000, d = 5.949], and acoustic startle amplitudes were significantly increased following cocaine self-administration [F(1,21) = 25.791, p = .000, d = 4.338]. There was no significant drug by group [F(1,21) = .046, p = .832], time by group [F(1,21) = 3.116, p = .092], drug by time [F(1,21) = 3.182, p = .089] or drug by group by time [F(1,21) = 2.495, p = .057] interaction. Percent change scores for amphetamine potentiated startle showed a significant group effect [F(1,21)=5.752, p =.026] but no main effect of time [F(1,21)=.514, p = .481] or interaction of time X group [F(1,21)=3.702, p = .068] *Figure 3*.

Discussion

Comparing potentiated acoustic startle in high and low startle rats showed that amphetamine significantly enhanced startle and this effect was not altered in high or low startle rats by the self-administration of cocaine. This finding is supported by studies which find either no effect of cocaine on subsequent acoustic startle amplitudes (Kirkland

Henry, et al., 2009; Mansbach, et al., 1994) or only minimal or transient effects (Adams, et al., 2001; Gordon & Rosen, 1999).

In conclusion, amphetamine potentiated acoustic startle is significantly different in low and high startle rats and this is likely due to the difference in initial baseline startle. Cocaine self-administration did not significantly reduce acoustic startle amplitudes in high or low startle rats. This does not support the hypothesis that cocaine induced hypodopaminergia accounts for the low startle amplitudes observed in cocaine addicted men. Rather, these data suggest that low acoustic startle may have predictive value in determining vulnerability to abuse cocaine and that this is dependent upon differences in dopaminergic transmission.

References

Aberman, J. E., & Salamone, J. D. (1999). Nucleus accumbens dopamine depletions make rats more sensitive to high ratio requirements but do not impair primary food reinforcement. *Neuroscience, 92*(2), 545-552. doi: S0306-4522(99)00004-4 [pii]

Adams, J. U., Efferen, T. R., Duncan, E. J., & Rotrosen, J. (2001). Prepulse inhibition of the acoustic startle response in cocaine-withdrawn rats. *Pharmacol Biochem Behav, 68*(4), 753-759. doi: S0091-3057(01)00478-6 [pii]

Aguilar, M. A., Rodriguez-Arias, M., & Minarro, J. (2009). Neurobiological mechanisms of the reinstatement of drug-conditioned place preference. *Brain Res Rev, 59*(2), 253-277. doi: S0165-0173(08)00101-X [pii]

10.1016/j.brainresrev.2008.08.002

Ahmed, S. H. (2010). Validation crisis in animal models of drug addiction: Beyond non-disordered drug use toward drug addiction. *Neuroscience and Biobehavioral Reviews, 35*(2), 172-184. doi: DOI 10.1016/j.neubiorev.2010.04.005

Anker, J. J., & Carroll, M. E. (2011). Adolescent nicotine exposure sensitizes cue-induced reinstatement of cocaine seeking in rats bred for high and low saccharin intake. *Drug Alcohol Depend*. doi: S0376-8716(11)00116-5 [pii]

10.1016/j.drugalcdep.2011.02.016

Anokhin, A. P., Heath, A. C., Myers, E., Ralano, A., & Wood, S. (2003). Genetic influences on prepulse inhibition of startle reflex in humans. *Neuroscience Letters, 353*(1), 45-48. doi: DOI 10.1016/j.neulet.2003.09.014

Arnold, J. M., & Roberts, D. C. (1997). A critique of fixed and progressive ratio schedules used to examine the neural substrates of drug reinforcement. *Pharmacol Biochem Behav, 57*(3), 441-447. doi: S0091305796004455 [pii]

Bardo, M. T., Donohew, R. L., & Harrington, N. G. (1996). Psychobiology of novelty seeking and drug seeking behavior. *Behavioural Brain Research, 77*(1-2), 23-43.

Bardo, M. T., Lacy, M., & Mattingly, B. A. (1990). Effects of apomorphine on novelty-induced place preference behavior in rats. *Pharmacol Biochem Behav, 37*(1), 89-93. doi: 0091-3057(90)90046-K [pii]

Belin, D., Berson, N., Balado, E., Piazza, P. V., & Deroche-Gamonet, V. (2011). High-novelty-preference rats are predisposed to compulsive cocaine self-administration. *Neuropsychopharmacology, 36*(3), 569-579. doi: npp2010188 [pii]

10.1038/npp.2010.188

Berglind, W. J., Case, J. M., Parker, M. P., Fuchs, R. A., & See, R. E. (2006). Dopamine D1 or D2 receptor antagonism within the basolateral amygdala differentially alters the acquisition of cocaine-cue associations necessary for cue-induced reinstatement of cocaine-seeking. *Neuroscience, 137*(2), 699-706. doi: S0306-4522(05)00990-5 [pii]

10.1016/j.neuroscience.2005.08.064

Bevins, R. A., Besheer, J., Palmatier, M. I., Jensen, H. C., Pickett, K. S., & Eurek, S. (2002). Novel-object place conditioning: behavioral and dopaminergic processes in expression of novelty reward. *Behavioural Brain Research, 129*(1-2), 41-50. doi: S0166432801003266 [pii]

Bird, E. D., Spokes, E. G., & Iversen, L. L. (1979). Increased dopamine concentration in limbic areas of brain from patients dying with schizophrenia. *Brain, 102*(2), 347-360.

Cadenhead, K. S., Light, G. A., Geyer, M. A., & Braff, D. L. (2000). Sensory gating deficits assessed by the P50 event-related potential in subjects with schizotypal personality disorder. *Am J Psychiatry, 157*(1), 55-59.

Cadenhead, K. S., Swerdlow, N. R., Shafer, K. M., Diaz, M., & Braff, D. L. (2000). Modulation of the startle response and startle laterality in relatives of schizophrenic patients and in subjects with schizotypal personality disorder: evidence of inhibitory deficits. *Am J Psychiatry, 157*(10), 1660-1668.

Cassella, J. V., & Davis, M. (1986). Habituation, Prepulse Inhibition, Fear Conditioning, and Drug Modulation of the Acoustically Elicited Pinna Reflex in Rats. *Behavioral Neuroscience, 100*(1), 39-44.

Cerruti, C., Pilotte, N. S., Uhl, G., & Kuhar, M. J. (1994). Reduction in dopamine transporter mRNA after cessation of repeated cocaine administration. *Brain Res Mol Brain Res, 22*(1-4), 132-138.

Corcoran, S., Norrholm, S. D., Cuthbert, B., Sternberg, M., Hollis, J., & Duncan, E. (2011). Acoustic startle reduction in cocaine dependence persists for 1 year of abstinence. *Psychopharmacology, 215*(1), 93-103. doi: DOI 10.1007/s00213-010-2114-2

Crombag, H. S., & Shaham, Y. (2002). Renewal of drug seeking by contextual cues after prolonged extinction in rats. *Behav Neurosci, 116*(1), 169-173.

Curtis, C. E., Lebow, B., Lake, D. S., Katsanis, J., & Iacono, W. G. (1999). Acoustic startle reflex in schizophrenia patients and their first-degree relatives: evidence of normal emotional modulation. *Psychophysiology, 36*(4), 469-475.

Dackis, C. A., & O'Brien, C. P. (2001). Cocaine dependence: a disease of the brain's reward centers. *J Subst Abuse Treat, 21*(3), 111-117. doi: S0740-5472(01)00192-1 [pii]

Davis, M. (1980). Neurochemical modulation of sensory-motor reactivity: acoustic and tactile startle reflexes. *Neurosci Biobehav Rev, 4*(2), 241-263.

Davis, M. (1985). Cocaine: excitatory effects on sensorimotor reactivity measured with acoustic startle. *Psychopharmacology (Berl), 86*(1-2), 31-36.

Davis, M., & Aghajanian, G. K. (1976). Effects of apomorphine and haloperidol on the acoustic startle response in rats. *Psychopharmacology (Berl), 47*(3), 217-223.

Davis, M., Gendelman, D. S., Tischler, M. D., & Gendelman, P. M. (1982). A primary acoustic startle circuit: lesion and stimulation studies. *J Neurosci, 2*(6), 791-805.

Davis, M., & Gendelman, P. M. (1977). Plasticity of the acoustic startle response in the acutely decerebrate rat. *J Comp Physiol Psychol, 91*(3), 549-563.

Davis, M., Svensson, T. H., & Aghajanian, G. K. (1975). Effects of d- and l-amphetamine on habituation and sensitization of the acoustic startle response in rats. *Psychopharmacologia, 43*(1), 1-11.

de Wit, H., & Stewart, J. (1981). Reinstatement of cocaine-reinforced responding in the rat. *Psychopharmacology (Berl), 75*(2), 134-143.

Deroche-Gamonet, V., Belin, D., & Piazza, P. V. (2004). Evidence for addiction-like behavior in the rat. *Science, 305*(5686), 1014-1017. doi: 10.1126/science.1099020

305/5686/1014 [pii]

Diagnostic and Statistical Manual of Mental Disorders. (2000). (4 ed.). Washington, DC: American Psychiatric Association.

Efferen, T. R., Duncan, E. J., Szilagyi, S., Chakravorty, S., Adams, J. U., Gonzenbach, S., . . . Rotrosen, J. (2000). Diminished acoustic startle in chronic cocaine users. *Neuropsychopharmacology, 22*(1), 89-96. doi: S0893-133X(99)00089-5 [pii]

10.1016/S0893-133X(99)00089-5

Epstein, D. H., Preston, K. L., Stewart, J., & Shaham, Y. (2006a). Toward a model of drug relapse: an assessment of the validity of the reinstatement procedure. *Psychopharmacology (Berl), 189*(1), 1-16. doi: 10.1007/s00213-006-0529-6

Epstein, D. H., Preston, K. L., Stewart, J., & Shaham, Y. (2006b). Toward a model of drug relapse: an assessment of the validity of the reinstatement procedure. *Psychopharmacology, 189*(1), 1-16. doi: DOI 10.1007/s00213-006-0529-6

Flagel, S. B., Watson, S. J., Akil, H., & Robinson, T. E. (2008). Individual differences in the attribution of incentive salience to a reward-related cue: influence on cocaine sensitization. *Behavioural Brain Research, 186*(1), 48-56. doi: S0166-4328(07)00373-7 [pii]

10.1016/j.bbr.2007.07.022

Flagel, S. B., Watson, S. J., Robinson, T. E., & Akil, H. (2007). Individual differences in the propensity to approach signals vs goals promote different adaptations in the dopamine system of rats. *Psychopharmacology, 191*(3), 599-607. doi: DOI 10.1007/s00213-006-0535-8

Gobbi, G., & Janiri, L. (1999). Clozapine blocks dopamine, 5-HT2 and 5-HT3 responses in the medial prefrontal cortex: an in vivo microiontophoretic study. *Eur Neuropsychopharmacol, 10*(1), 43-49. doi: S0924-977X(99)00055-3 [pii]

Gordon, M. K., & Rosen, J. B. (1999). Lasting effect of repeated cocaine administration on acoustic and fear-potentiated startle in rats. *Psychopharmacology (Berl), 144*(1), 1-7.

Grillon, C. (2002). Startle reactivity and anxiety disorders: aversive conditioning, context, and neurobiology. *Biol Psychiatry, 52*(10), 958-975. doi: S0006322302016657 [pii]

Hitri, A., Casanova, M. F., Kleinman, J. E., & Wyatt, R. J. (1994). Fewer dopamine transporter receptors in the prefrontal cortex of cocaine users. *Am J Psychiatry, 151*(7), 1074-1076.

Hodos, W. (1961). Progressive ratio as a measure of reward strength. *Science, 134*, 943-944.

Howell, L. L., & Byrd, L. D. (1992). Enhanced sensitivity to the behavioral effects of cocaine after chronic administration of D2-selective dopamine antagonists in the squirrel monkey. *J Pharmacol Exp Ther, 262*(3), 907-915.

Kalivas, P. W., & McFarland, K. (2003). Brain circuitry and the reinstatement of cocaine-seeking behavior. *Psychopharmacology, 168*(1-2), 44-56. doi: DOI 10.1007/s00213-003-1393-2

Kehne, J. H., & Sorenson, C. A. (1978). The effects of pimozide and phenoxybenzamine pretreatments on amphetamine and apomorphine potentiation of the acoustic startle response in rats. *Psychopharmacology (Berl), 58*(2), 137-144.

Kirkland Henry, P., Davis, M., & Howell, L. L. (2009). Effects of cocaine self-administration history under limited and extended access conditions on in vivo striatal dopamine neurochemistry and acoustic startle in rhesus monkeys. . *Psychopharmacology (Berl), 205*(2), 237-247.

Kirshenbaum, A. P., Olsen, D. M., & Bickel, W. K. (2009). A quantitative review of the ubiquitous relapse curve. *J Subst Abuse Treat, 36*(1), 8-17. doi: S0740-5472(08)00057-3 [pii]

10.1016/j.jsat.2008.04.001

Koch, M., & Schnitzler, H. U. (1997). The acoustic startle response in rats--circuits mediating evocation, inhibition and potentiation. *Behavioural Brain Research, 89*(1-2), 35-49.

Koob, G. F. (2009). Brain stress systems in the amygdala and addiction. *Brain Res, 1293*, 61-75. doi: S0006-8993(09)00602-7 [pii]

10.1016/j.brainres.2009.03.038

Kruzich, P. J., & See, R. E. (2001). Differential contributions of the basolateral and central amygdala in the acquisition and expression of conditioned relapse to cocaine-seeking behavior. *J Neurosci, 21*(14), RC155. doi: 20015433 [pii]

Lee, Lopez, D. E., Meloni, E. G., & Davis, M. (1996). A primary acoustic startle pathway: obligatory role of cochlear root neurons and the nucleus reticularis pontis caudalis. *J Neurosci, 16*(11), 3775-3789.

Lee, J., Parish, C.L., Tomas, D., & Horne, M.K. (2011). Chronic cocaine administration reduces striatal dopamine terminal density and striatal dopamine release which leads to drug-seeking behaviour. *Neuroscience, 174*, 143-150.

Lieberman, J. A., Kane, J. M., & Alvir, J. (1987). Provocative tests with psychostimulant drugs in schizophrenia. *Psychopharmacology (Berl), 91*(4), 415-433.

Loh, E. A., Fitch, T., Vickers, G., & Roberts, D. C. (1992). Clozapine increases breaking points on a progressive-ratio schedule reinforced by intravenous cocaine. *Pharmacol Biochem Behav, 42*(3), 559-562.

Mansbach, R. S., Markou, A., & Patrick, G. A. (1994). Lack of altered startle responding in rats following termination of self-administered or noncontingently infused cocaine. *Pharmacol Biochem Behav, 48*(2), 453-458. doi: 0091-3057(94)90552-5 [pii]

Martinez, D., Greene, K., Broft, A., Kumar, D., Liu, F., Narendran, R., . . . Kleber, H. D. (2009). Lower level of endogenous dopamine in patients with cocaine dependence: findings from PET imaging of D(2)/D(3) receptors following acute dopamine depletion. *Am J Psychiatry, 166*(10), 1170-1177. doi: appi.ajp.2009.08121801 [pii]

10.1176/appi.ajp.2009.08121801

Mateo, Y., Lack, C. M., Morgan, D., Roberts, D. C. S., & Jones, S. R. (2005). Reduced dopamine terminal function and insensitivity to cocaine following cocaine binge self-administration and deprivation. *Neuropsychopharmacology, 30*(8), 1455-1463. doi: DOI 10.1038/sj.npp.1300687

Meloni, E. G., & Davis, M. (1999). Enhancement of the acoustic startle response in rats by the dopamine D1 receptor agonist SKF 82958. *Psychopharmacology (Berl), 144*(4), 373-380.

Morgan, D., Grant, K. A., Gage, H. D., Mach, R. H., Kaplan, J. R., Prioleau, O., . . . Nader, M. A. (2002). Social dominance in monkeys: dopamine D-2 receptors and cocaine self-administration. *Nature Neuroscience, 5*(2), 169-174. doi: Doi 10.1038/Nn798

Owen, F., Cross, A. J., Crow, T. J., Longden, A., Poulter, M., & Riley, G. J. (1978). Increased dopamine-receptor sensitivity in schizophrenia. *Lancet, 2*(8083), 223-226. doi: S0140-6736(78)91740-3 [pii]

Pahwal, P., Hyman, S. M., & Sinha, R. (2008). Craving predicts time to cocaine relapse: Further validation of the Now and Brief versions of the cocaine craving questionnaire. *Drug and Alcohol Dependence, 93*(3), 252-259. doi: DOI 10.1016/j.drugalcdep.2007.10.002

Pierce, R. C., Crawford, C. A., Nonneman, A. J., Mattingly, B. A., & Bardo, M. T. (1990). Effect of forebrain dopamine depletion on novelty-induced place preference behavior in rats. *Pharmacol Biochem Behav, 36*(2), 321-325.

Pilotte, N. S., Sharpe, L. G., & Kuhar, M. J. (1994). Withdrawal of repeated intravenous infusions of cocaine persistently reduces binding to dopamine transporters in the nucleus accumbens of Lewis rats. *J Pharmacol Exp Ther, 269*(3), 963-969.

Pilotte, N. S., Sharpe, L. G., Rountree, S. D., & Kuhar, M. J. (1996). Cocaine withdrawal reduces dopamine transporter binding in the shell of the nucleus accumbens. *Synapse, 22*(1), 87-92. doi: 10.1002/(SICI)1098-2396(199601)22:1<87::AID-SYN10>3.0.CO;2-X [pii]

10.1002/(SICI)1098-2396(199601)22:1<87::AID-SYN10>3.0.CO;2-X

Ranaldi, R., Bauco, P., McCormick, S., Cools, A. R., & Wise, R. A. (2001). Equal sensitivity to cocaine reward in addiction-prone and addiction-resistant rat genotypes. *Behavioural Pharmacology, 12*(6-7), 527-534.

Ritz, M. C., Lamb, R. J., Goldberg, S. R., & Kuhar, M. J. (1987). Cocaine receptors on dopamine transporters are related to self-administration of cocaine. *Science, 237*(4819), 1219-1223.

Roberts, D. C., & Bennett, S. A. (1993). Heroin self-administration in rats under a progressive ratio schedule of reinforcement. *Psychopharmacology (Berl), 111*(2), 215-218.

Roberts, D. C., Corcoran, M. E., & Fibiger, H. C. (1977). On the role of ascending catecholaminergic systems in intravenous self-administration of cocaine. *Pharmacol Biochem Behav, 6*(6), 615-620.

Roberts, D. C., & Koob, G.F. (1982). Disruption of cocaine self-administration following 6-hydroxydopamine lesions of the ventral tegmental area in rats. *Pharmacol Biochem Behav, 17*(5), 901-904.

Roberts, D. C. S., Loh, E. A., & Vickers, G. (1989). Self-Administration of Cocaine on a Progressive Ratio Schedule in Rats - Dose-Response Relationship and Effect of Haloperidol Pretreatment. *Psychopharmacology, 97*(4), 535-538.

Salamone, J. D., Cousins, M. S., & Bucher, S. (1994). Anhedonia or anergia? Effects of haloperidol and nucleus accumbens dopamine depletion on instrumental response selection in a T-maze cost/benefit procedure. *Behavioural Brain Research, 65*(2), 221-229.

Saunders, B. T., & Robinson, T. E. (2011). Individual Variation in the Motivational Properties of Cocaine. *Neuropsychopharmacology, 36*(8), 1668-1676. doi: Doi 10.1038/Npp.2011.48

Schwarzkopf, S.B., Bruno, J.P., & Mitra, T. (1993). Effects of haloperidol and SCH 23390 on acoustic startle and prepulse inhibition under basal and stimulated conditions. *Prog Neuropsychopharmacol Biol Psychiatry, 17*(6), 1023-1036.

See, R. E., Kruzich, P. J., & Grimm, J. W. (2001). Dopamine, but not glutamate, receptor blockade in the basolateral amygdala attenuates conditioned reward in a rat model of relapse to cocaine-seeking behavior. *Psychopharmacology (Berl), 154*(3), 301-310.

See, R. E., McLaughlin, J., & Fuchs, R. A. (2003). Muscarinic receptor antagonism in the basolateral amygdala blocks acquisition of cocaine-stimulus association in a model of relapse to cocaine-seeking behavior in rats. *Neuroscience, 117*(2), 477-483. doi: S0306452202006656 [pii]

Self, D. W., & Nestler, E. J. (1998). Relapse to drug-seeking: neural and molecular mechanisms. *Drug and Alcohol Dependence, 51*(1-2), 49-60.

Shaham, Y., Shalev, U., Lu, L., De Wit, H., & Stewart, J. (2003). The reinstatement model of drug relapse: history, methodology and major findings. *Psychopharmacology (Berl), 168*(1-2), 3-20. doi: 10.1007/s00213-002-1224-x

Sharpe, L. G., Pilotte, N. S., Mitchell, W. M., & De Souza, E. B. (1991). Withdrawal of repeated cocaine decreases autoradiographic [3H]mazindol-labelling of dopamine transporter in rat nucleus accumbens. *Eur J Pharmacol, 203*(1), 141-144.

Shippenberg, T. S., & Heidbreder, C. (1995). Sensitization to the conditioned rewarding effects of cocaine: pharmacological and temporal characteristics. *J Pharmacol Exp Ther, 273*(2), 808-815.

Steketee, J. D. (2003). Neurotransmitter systems of the medial prefrontal cortex: potential role in sensitization to psychostimulants. *Brain Research Reviews, 41*(2-3), 203-228. doi: Doi 10.1016/S0165-0173(02)00233-3

van der Elst, M. C., Wunderink, Y. S., Ellenbroek, B. A., & Cools, A. R. (2007). Differences in the cellular mechanism underlying the effects of amphetamine on prepulse inhibition in apomorphine-susceptible and apomorphine-unsusceptible rats. *Psychopharmacology (Berl), 190*(1), 93-102. doi: 10.1007/s00213-006-0587-9

Volkow, N. D., Fowler, J. S., Wolf, A. P., Schlyer, D., Shiue, C. Y., Alpert, R., . . . et al. (1990). Effects of chronic cocaine abuse on postsynaptic dopamine receptors. *Am J Psychiatry, 147*(6), 719-724.

Weeks, J. R. (1962). Experimental Morphine Addiction - Method for Automatic Intravenous Injections in Unrestrained Rats. *Science, 138*(3537), 143-&.

Wise, R. A. (1984). Neural mechanisms of the reinforcing action of cocaine. *NIDA Res Monogr, 50*, 15-33.

Xia, Y., Goebel, D. J., Kapatos, G., & Bannon, M. J. (1992). Quantitation of rat dopamine transporter mRNA: effects of cocaine treatment and withdrawal. *J Neurochem, 59*(3), 1179-1182.

Yager, L. M., & Robinson, T. E. (2010). Cue-induced reinstatement of food seeking in rats that differ in their propensity to attribute incentive salience to food cues. *Behavioural Brain Research, 214*(1), 30-34. doi: DOI 10.1016/j.bbr.2010.04.021

Figure Captions

Figure 1. **Acoustic startle in low and high startle rats: Amphetamine Challenge.** Drug naïve acoustic startle amplitudes for high and low startle rats were significantly different across 3 days of baseline testing, * $p < .05$.

Figure 2. **Amphetamine potentiated acoustic startle amplitudes pre- and post-cocaine self-administration.** Acoustic startle amplitudes of high and low startle rats were significantly different and amphetamine compared to saline significantly enhanced acoustic startle. Acoustic startle amplitudes were elevated post cocaine self-administration when compared to the drug naïve, pre-cocaine self-administration condition, * $p < .05$.

Figure 3. **Amphetamine potentiated % change in startle amplitudes.** Amphetamine potentiated acoustic startle amplitudes in low startle rats significantly more than high startle rats before exposure to cocaine but this difference was not significant after cocaine self-administration

Figure 1.

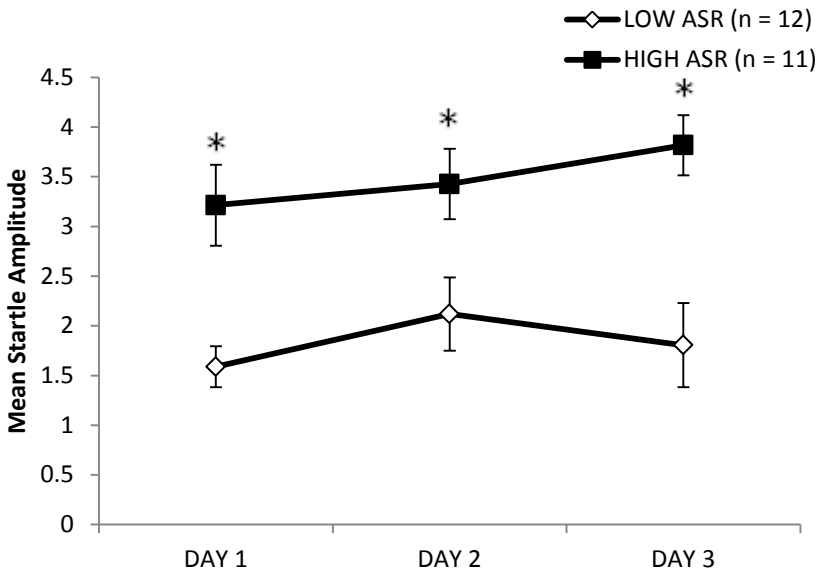

LOW ASR (n = 12)
HIGH ASR (n = 11)
Mean Startle Amplitude
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
DAY 1
DAY 2
DAY 3
*
*
*

Figure 2.

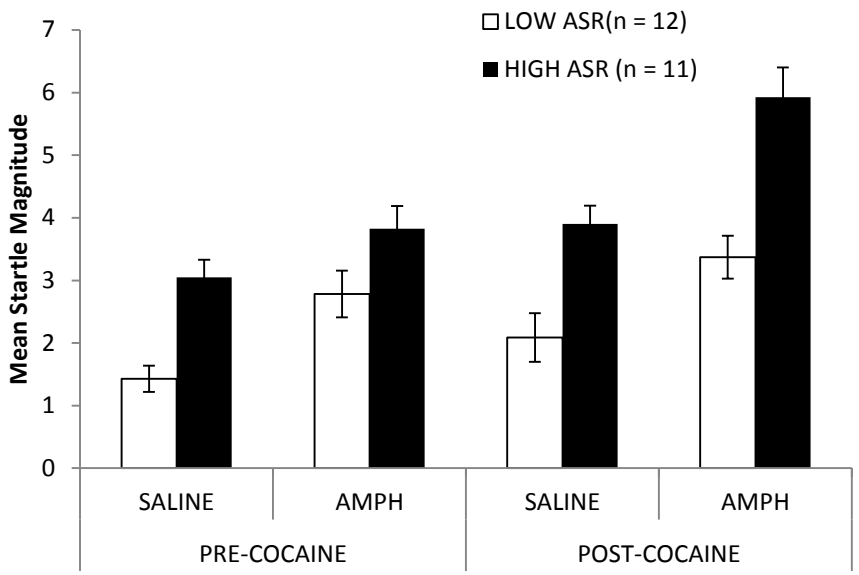
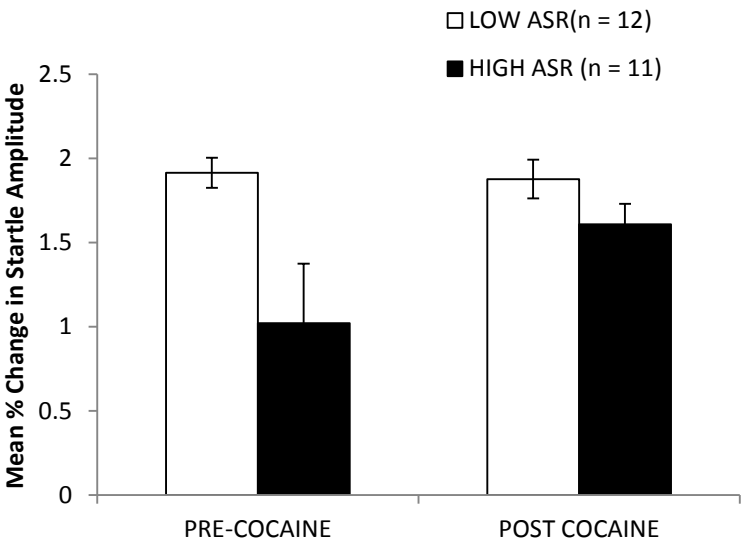

LOW ASR(n = 12)
HIGH ASR (n = 11)
Mean Startle Magnitude
0
1
2
3
4
5
6
7
SALINE
AMPH
SALINE
AMPH
PRE-COCAINE
POST-COCAINE

Figure 3.


LOW ASR(n = 12)
HIGH ASR (n = 11)
Mean % Change in Startle Amplitude
2.5
2
1.5
1
0.5
0
PRE-COCAINE
POST COCAINE