ASSESSING INDIVIDUAL DIFFERENCES: NOVELTY AND ULTRASONIC VOCALIZATIONS PREDICT ACUTE AND CHRONIC D-AMPHETAMINE RESPONSE IN RATS

by

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Abstract

Novelty-seeking and sensation-seeking are traits implicated in initial drug experimentation and relapse in human populations. To research the neurobiological substrates that are implicated in novelty/sensation-seeking that predispose an individual to drug use, a rodent model was used. Recently, 50 kHz ultrasonic vocalizations (USV) have been identified as indices of affective state and are evoked by several drugs of abuse, specifically when these drugs of abuse have their pharmacological effects in the mesolimbic dopamine path. Secondly, genetic breeding of high and low vocalizers suggests not only are they different in the calling frequency, but also to drug sensitivity, suggesting ultrasonic vocalizations may be a behavioral marker for individual differences in the mesolimbic dopamine circuit. Two sensation/novelty seeking screens and an ultrasonic vocalization screen were used in rats to predict the locomotor and 50 kHz USV response to a low (.3 mg/kg) and high dose (1.0 mg/kg) of amphetamine. Correlation analysis revealed none of the novelty screens were correlated. Simultaneous regression analyses indicated amphetamine dose-dependently increased locomotor activity acutely and chronically but did not increase 50 kHz USV. The USV assessment predicted USV response to amphetamine acutely and chronically but was not dose dependent. No interactions among any predictors were observed. Previous research has dichotomized the novelty/sensation-seeking trait and found significant differences between high and low novelty responders. The current research provides evidence for maintaining continuous individual difference variables, and suggests each screen measures a different trait implicated in addiction.

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CHAPTER 1 – Introduction

Rationale

Several models of drug abuse suggest the transfer from drug use to dependence is a learned process, dependent on Pavlovian conditioning. Through repeated pairings of environmental cues and drug administration the user *learns* to associate those cues with drug taking (Siegel, Baptisa, Kim, McDonald, & Weise-Kelly, 2000). The Opponent Process theory of motivation attempts to understand the physiological processes of motivation and its application to affect, aversion, and drug abuse (Solomon & Corbit, 1974). This model predicts that the user will be motivated to seek out and use drugs because of two different processes. The "A" process is initially evoked by a stimulus, for example hedonic feeling after opiate administration. The second process, the "B" process occurs in response to the A process and is in opposition of the A process. Through repeated pairings and associated contextual stimuli the B process becomes increasingly stronger and can even precede the A process. In this example, the B process can evoke withdrawal symptoms. The user, through negative reinforcement, is motivated to pursue the opiate to alleviate the B process and return to the A-like state. The Opponent Process model has been revolutionary in attempting to explain tolerance, negative reinforcement, and context-induced relapse (Siegel, Hinson, Krank, & McCully, 1982; Crombag, Bossert, Koya, & Shaham, 2008)

Similarly, the Proponent Process model acknowledges elements of Pavlovian conditioning, but further explains drug seeking is motivated by incentive sensitization (Robinson & Berridge, 1993). Incentive salience is attributing the rewarding value of a pleasurable stimulus to a non-rewarding stimulus. Through repeated pairings the non-rewarding stimuli come to activate the same neural networks the drug of abuse once did, and the user becomes motivated to

seek previously administered drugs of abuse. However, these models have their shortcomings. First, the Opponent Process model does not account for relapse after long periods of sobriety because, according to the model (Solomon & Corbit, 1974), without stimulation the B process would return to baseline and motivation should no longer be a concern. Second, not all drug users escalate to drug dependent after continued use of the drug (Anthony, Warner, & Kessler, 1994) suggesting there are substantial differences among individuals that may predispose them to compulsive drug taking. The current study investigated the individual differences associated with drug use and attempted to predict which traits are important to making the transfer from drug use to drug dependence using an animal model of addiction.

Assessing Individual Differences

The first attempts to understand the interaction of personality type and drug abuse did not yield many promising theories in drug dependence. However, focusing on specific personality traits such as novelty and sensation seeking has been more promising (Zuckerman, Bone, Neary, Mangelsdorff, & Brustman, 1972; Pihl & Spiers, 1978). According to Zuckerman et al. (1972), sensation seeking is a need for novel or complex experience and a motivation to take physical or social risks to obtain these experiences. Sensation seeking has been measured in human populations using the Sensation Seeking Scale (SSS) developed by Zuckerman, Kolin, Price, & Zoob (1964). The SSS is a forced-choice personality assessment designed to define sensation seeking. Similarly, novelty seeking has been assessed in human populations using the TPQ Novelty Seeking Scale (NSS) (Cloninger, 1987). The NSS questionnaire attempts to assess the tendency for novel stimuli to produce excitation leading to exploratory behavior and avoidance of experienced stimuli (Helmus, Downey, Arfken, Henderson, & Schuster, 2001).

One major contribution of the SSS and NSS has been identification of personality traits that may contribute to drug seeking behavior. Zuckerman et al. (1972) found that the SSS was related to experiences with risky choices with sex, alcohol and drugs. In their 1972 study, participants were given the SSS questionnaire in addition to an *Experiences* questionnaire (unpublished). Results from the study suggest SSS scores and experiences with sex, alcohol, and drugs were positively correlated. Interestingly, the "Thrill and adventure seeking" subscales from the SSS questionnaire had significant correlations with drug use. Galizio and Stein (1983) extended this finding in their research. Together they found not only was sensation seeking correlated with drug use, but higher scores on the SSS scales were also correlated with a *polydrug user*, participants who reported using stimulants, hallucinogens or a variety of drugs. Further, *depressant* drug users didn't score as high on the SSS scale relative to the *polydrug users*. In addition to drug taking behavior, novelty may influence efficacy of treatment programs and put novelty seekers more at risk for relapse. Helmus et al. (2001) administered NSS questionnaires to heroin dependent participants in treatment programs and found high novelty seekers were more likely to drop-out of treatment before the end of program, when compared to low novelty seekers. However, high novelty seekers had higher retention rates at the start of the treatment program. Taken together, this may suggest when treatment protocols are novel they may provide enough stimulation to motivate the user, but when treatment protocols become redundant the stimulation is no longer novel and may influence the user to seek more novel stimulation or possibly relapse (Helmus et al., 2001). As stated, sensation/novelty seeking influences risk taking, seeking out drugs, and the potential for relapse. It also predicts which drugs are abused, and the willingness to maintain a treatment program in human populations.

Animal Models of Individual Differences

Given the relationship between novelty seeking and drug use, animal models have been developed to understand the neurobiological substrates and how they are related to each other. Novelty seeking in animals has been assessed using an inescapable novelty test (Beckmann, Marusich, Gipson, & Bardo, 2011; Cain, Saucier, & Bardo, 2005; Belin, Berson, Balado, Piazza, & Deroche-Gamonet, 2011), where animals are placed in a novel open arena and locomotor activity is measured via total distance traveled (Cain et al., 2005; Beckman et al., 2011) or total beam breaks (Belin et al., 2011). After the test session, animals are divided into two groups via a median split on the locomotor dependent variable (total distance or beam breaks), yielding high and low responders. It is thought animals displaying more locomotor activity achieve more arousal from the novel environment and consequently display more locomotion. The novelty place preference is an additional novelty assessment frequently used in animal models (Belin et al., 2011; Cain et al., 2005; Vidal-Infer, Arenas, Daza-Losada, Aguilaar, Minarro, & Rodriguez-Arias, 2012). In the novelty place preference assessment animals are confined to one contextually distinct side of the place preference chamber for an allotted period of time, called *habituation*. Following habituation, the animal is presented concurrently with a novel contextually distinct environment and the habituated environment, and the animal can pass freely between the two contextually different environments. After the test session, animals are divided into two groups via a median split on the percentage of time spent in the novel side, yielding high and low novelty seekers. Although these tests are measuring novelty in rodents, the two tests are not correlated (Beckman et al., 2011) and may be measuring different aspects of novelty, regardless of whether median split group assignment is used (Cain, Smith, & Bardo, 2004) or when novelty remains a continuous variable and regression analyses are used (Cain et al., 2005).

Inescapable Novelty Screen

High responder and low responder rodents not only differ in their response to a novel environment, but they also differ in their response to low doses of amphetamine, (Cain et al., 2004; Cain et al., 2005; Piazza, Deminiere, Maccari, Mormede, Moal, & Simon, 1990) and cocaine (Beckman et al., 2011). In the Cain et al. (2004) study, rats were identified as either high or low responders based on the locomotor activity in the inescapable novelty test. Animals identified as high responders showed a decrease in self-administration of amphetamine when a novel stimulus was presented. However, the effect was only transient relative to the low responders. The effects from the novel stimuli decreased responding for the initial two time blocks in the high responders, while the effect persisted for the initial four time blocks in the low responders. Rats classified as high and low responders based on the *novelty place preference* were not significantly different in responding for amphetamine when a novel stimulus was presented. The second major finding from Cain et al. (2004) was that the effects of a novel stimulus to decrease self-administration of amphetamine appear to affect only responding for low doses of amphetamine. High and low responders (classified via inescapable novelty test) differed in responding only at the lowest dose of amphetamine (0.003 mg/kg/infusion), with the high responding rats responding significantly less than the low responding rats. At 0.01 mg/kg, novel stimuli reduced responding for both responders and at the doses above 0.03 mg/kg, neither high or low responders reduced responding for amphetamine. Animals classified as high and low responders base on *novelty place preference* test did not differ in responding when novel stimuli were presented during the self-administration of amphetamine.

Novelty Place Preference Screen

Although the novelty place preference screen has not proven beneficial in predicting acquisition of self-administration (Cain et al., 2004; Klebaur, Bevins, Segar & Bardo, 2001) it has been used to predict conditioned rewarding effects of cocaine (Vidal-Infer et al., 2012). In this study, mice classified as high novelty seekers in a novelty place preference assessment spent more time in a drug-paired context when administered a low dose of cocaine (1.0 mg/kg/infusion) when compared to their low novelty seeking counterparts. These results were not sex dependent and occurred after just one pairing of cocaine, suggesting the high novelty seekers are more sensitive to the rewarding effects of the low doses of cocaine.

Interestingly, novelty place preference, predicts which animals will demonstrate addiction-like criteria such as: persistence in drug seeking, motivation for the drug, and resistance to punishment (Belin et al., 2011). These same criteria are not predicted by the inescapable novelty screen. More specifically, rats were first screened using the inescapable novelty and novelty place preference assessments, followed by self-administration of cocaine (0.8 mg/kg). Following 60 days of self-administration, animals were tested for addiction-like criteria: persistence in drug seeking, motivation for the drug, and resistance to punishment. Persistence in drug seeking was measured as responding on the active response during no drug periods. Motivation for the drug was measured with a progressive ratio schedule of reinforcement. Finally, resistance to punishment was measured by the number of cocaine infusions earned in the presence of a footshock. High and low responding rats as categorized by the inescapable novelty assessment did not differ in any addiction criteria or for the animals' addiction score. However, high responders in the novelty place preference showed a significantly higher addiction score than low responding animals. Further, high novelty preference rats also demonstrated more persistence to drug seek than their low novelty preference comparison group.

Similarly, resistance to punishment was also predicted by the high responding novelty place preference animals, and not low responding novelty place preference responders, nor the inescapable novelty assessment. These criteria have also been implicated in impulsive rodents as well (Belin, Mar, Dalley, Robbins, & Everitt, 2008). These results suggest that indeed, the two novelty screens are uncorrelated and are measuring two different aspects of drug dependence.

Differences Between Novelty Screens

The differences in the novelty screen may be due to the fact the inescapable novelty screen has been shown to elevate corticosterone, a hormone implicated in the stress response (Piazza, Maccari, Deminiere, Le Moal, Mormede, & Simon, 1991). In Piazza et al. (1991) study, animals identified as high responders in an inescapable novelty assessment showed increased levels of corticosterone after 120 minutes of exposure to a novel environment, while the low responders returned to basal levels. In agreement with works cited above, high responders, but not low responders acquired self-administration of amphetamine. However, low responders did acquire amphetamine self-administration when they were pretreated with an experimenter-administered acute dose of corticosterone. In addition, when corticosterone and amphetamine were self-administered concurrently the high and low responders did not differ in the number of infusions earned. Taken together, the data suggest higher locomotor responses in an inescapable novelty assessment, and self-administration of amphetamine are related to increased levels of corticosterone. Furthermore, it may suggest corticosterone contributes to the reinforcing effects of amphetamine and high responding animals may satiate in regards to the reinforcing effects of the drug more quickly.

Corticosterone may moderate dopaminergic transmission in the mesolimbic dopamine circuit. Rouge-Pont, Piazza, Kharouby, Le Moal, and Simon (1993) monitored dopaminergic

activity in the nucleus accumbens in the response to novelty and concluded it can be used as a predictive tool to estimate dopaminergic transmission in response to stress. Similarly, nucleus accumbens dopaminergic activity during novelty can be used to predict dopaminergic influx resulting from a non-selective dopamine agonist, apomorphine (Piazza, Barrot, Rouge-Pont, Marinelli, Maccari, Abrous, Simon, & Le Moal, 1996). These results provide strong support for the hypothesis that corticosterone plays an integral role in the nucleus accumbens and increases the psychomotor effects of amphetamine through modulation of mesolimbic dopamine levels. This is particularly important because dopaminergic transmission in the nucleus accumbens has been identified as crucial neuroanatomical structure in reward processing and reward seeking behavior (Beyene, Carelli, & Wightman, 2010). Barrot, Marinelli, Abrous, Rouge-Pont, Le Moal, and Piazza, (2000) concluded the 'hyper-responsiveness' of the nucleus accumbens shell but not the core was directly modulated by corticosterone. Following an adrenalectomy, dopamine levels in the shell of the nucleus accumbens were reduced during mild stress, a manipulation to elevate corticosterone concentrations. Alternately, dopamine levels in the core remained unchanged following the adrenalectomy, suggesting that corticosterone preferentially affects nucleus accumbens shell dopamine levels.

Novelty Behavior is Dopamine Dependent

While differences in the stress response system may contribute to individual differences in response to novelty, corticosterone does not maintain a conditioned place preference (Dietz, Wang, & Kabbaj, 2007), suggesting that by itself it not rewarding and may act as a moderator of dopaminergic activity. Furthermore, the inescapable novelty assessment is largely dopamine dependent, as novelty-induced locomotor activity has been linked to the mesoaccumbens-pallidal circuit (Mogenson, & Nielsen, 1984). Within this circuit, projections from the ventral tegmental

area to the nucleus accumbens are largely dopaminergic. Following microinjections of fluphenazine (a dopamine antagonist) into the nucleus accumbens, novelty-induced locomotor activity was reduced without affecting basal locomotor activity in non-novel trials. These results indicate that novelty-induced locomotor activity is largely dopamine dependent and tied to the nucleus accumbens, and can be surmounted with dopamine antagonists. Just as novelty-induced locomotor activity can be reduced with dopamine antagonists, amphetamine-induced conditioned place preference is largely dopamine dependent and reduced by dopamine antagonism (Spyraki, Fibiger, & Phillips, 1982). The effects of amphetamine-induced place conditioning were assessed across three doses of amphetamine (0.5, 1.0 and 5.0 mg/kg) and amphetamine produced a robust place preference across all doses (Spyraki et al., 1982). This effect was abolished across all doses of amphetamine with the administration of two doses of haloperidol (0.15 and 1.0 mg/kg), and with neurotoxic lesions to the nucleus accumbens. Amphetamine induces dopaminergic transmission in the nucleus accumbens that is rewarding, and that can be abolished by dopamine antagonist haloperidol, further implicating the mesolimbic dopamine system in the rewarding effect of amphetamine.

Similarly, the novelty place preference is dopamine dependent. Dopamine D_1 and D_2 receptors have been implicated in novelty place preference (Bardo, Bowling, Roninet, Rowlett, Lacy, & Mattingly, 1993). The D_1 receptor agonist (SKF 38393) dose-dependently impeded novelty-maintained place preference, with lower doses (1 and 3 mg/kg) failing to eliminate the place preference. The D_1 receptor antagonist (SCH 23390) robustly eliminated novelty place preference across all doses (0.3–3.0 mg/kg). Similar to the D_1 receptor agonist, the D_2 receptor agonist (Quinpirole) abolished novelty-maintained place preference, but this effect was not dose dependent. Finally, D_2 receptor antagonist (Eticlopride) did not affect novelty-maintained place

preference at any dose tested. Taken together, these results indicate D_1 and D_2 receptors may influence novelty place preference behavior through two different processes. It is likely that the dopamine agonists made the experienced and novel sides rewarding, while the antagonists blocked dopamine transmission from the novel environment. However, D_1 dopamine receptors seem to be more involved with the incentive properties of novelty (Bardo et al., 1993).

Recent evidence implicates dopamine located within the nucleus accumbens that contributes to the novelty response. Mogenson and Nielsen, (1984) first observed a role of the nucleus accumbens in exploratory locomotion. Intracranial microinjections of glutamate antagonist L-glutamic-acid diethyl ester HCL (GDEE) dose-dependently (20µg/1µl) decreased exploratory locomotion. In addition, novel environments induce dopaminergic transmission in the shell but not the core (Rebec, Christensen, Guerra, & Bardo, 1997). Using fast-scan cyclic voltammetry, Rebec et al. (1997) recorded dopaminergic transmission in awake and behaving animals in real time in the nucleus accumbens shell, core and bisection area so called the 'shore'. Upon the animals' first entry into the novel environment, dopaminergic activity spiked in the shell and 'shore' but not in the core. This provides strong support that entry into a novel environment from a previously experienced environment evokes a dopamine response in the shell of the nucleus accumbens and further supports the mesolimbic dopamine system involvement in the novel response.

An Additional Individual Difference Measure

The inescapable novelty and novelty place preference assessments have been valuable tools for understanding different components of drug dependence in animal models. However, the two assessments are uncorrelated and seem to measure different aspects of novelty and drug dependence despite being dependent on dopaminergic activity. Second, the predictive validity of the two assessments is largely dose dependent, with the most robust findings occurring at lower

doses. It may be advantageous to employ an additional measure to examine additional aspects of novelty, or possibly create a synergistic relationship with one of the previous assessments. This additional measure should be: (i) a behavioral assessment, (ii) measured as a continuous variable, and (iii) dependent on the mesolimbic dopamine pathway.

Anderson (1953) was one of the first scientists to recognize that rodents emit ultrasonic sounds. Anderson (1953) did not draw any type of inference from these sounds but later research on ultrasonic vocalizations has attempted to understand the motivations from the rodents emitting these sounds (Barfield, & Geyer, 1972; Blanchard, Flannelly, & Blanchard, 1986; Burgdorf, Knutson, & Pankseep, 2000, 2001; Panksepp, & Burgdorf, 2000; Brudzynski, 2007). Recently, ultrasonic vocalizations have been used as a dependent measure to help understand drug abuse. Rat ultrasonic vocalizations have been dichotomized into two separate call types, 22 kHz and 50 kHz calls (Panksepp & Burgdorf, 2000; Brudzynski, 2007; Schwarting, Jegan, & Wohr, 2007; Burgdorf & Panksepp, 2001). Twenty-two kHz ultrasonic vocalizations are primarily evoked by aversive stimuli such as: predatory odor (Blanchard , Blanchard, & Griebel, 2005), aggressive rat behavior (Thomas, Takahashi, & Barfield, 1983), sudden acoustic noise or air puff (Brudzynski & Holland, 2005), and during post ejaculatory refractory periods (Barfield & Geyer, 1972). Alternatively, 50 kHz ultrasonic vocalizations are commonly evoked by appetitive stimuli such as: anticipation of play (Knutson, Burgdorf, & Panksepp, 1998), anticipation of rewarding brain stimulation (Burgdorf et al., 2000), tickling, termed heterospecific play (Burgdorf & Panksepp, 2001; Panksepp & Burgdorf, 2000), and drugs of abuse including psychomotor stimulants: cocaine and amphetamine (Meyer, Ma, & Robinson, 2012; Ahrens, Ma, Maier, Duvauchelle, & Schallert, 2009; Browning, Browning, Maxwell, Dong, Jansen, Panksepp, & Sorg, 2011; Barker, Root, Ma, Jha, Megehee, Pawlak, & West, 2010;

Sadananda, Natusch, Karrenbauer, & Schwarting, 2012), caffeine (Simola, Ma, Schallert, 2010), and methylphenidate (Simola, Fenu, Costa, Pinna, Plumitallo, & Morelli, 2012). Further, conditioned stimuli predicting rewarding stimuli also evoke 50 kHz ultrasonic vocalizations (Knutson et al., 1998; Burgdorf et al., 2000). Interestingly, withdrawal from alcohol, opiates, and cocaine evoke 22 kHz ultrasonic vocalizations and cease with reinstatement of the drug (Knapp, Duncan, Crews, & Breese, 1998; Mutschler & Miczek, 1998; Vivian & Miczek, 1991).

Reinforcing Stimuli Evoke 50 kHz Ultrasonic Vocalizations

To understand the foundation of ultrasonic vocalizations research in drug addiction, one must understand the behavior in multiple contexts. Ultrasonic vocalizations as a measure in neuroscience began by studying aversive and appetitive stimuli. Fifty kHz ultrasonic vocalizations are understood to reflect a positive affective state because they have been correlated with reinforcing stimuli. In an attempt to elucidate the meaning of 50 kHz ultrasonic vocalizations Burgdorf et al. (2000) implanted electrodes into the ventral tegmental area, an area of the brain that supports self-stimulation. In this paradigm animals were first given experimenter-administered brain stimulation and all types of vocalizations were observed. Animals initially vocalized in the 50 kHz range in response to the brain stimulation, but because the interval was predictable animals were temporally conditioned and the 50 kHz ultrasonic vocalizations began to happen before the stimulation, suggesting 50 kHz ultrasonic vocalizations can be evoked in anticipation of rewarding stimuli. However, these results do not suggest the animal finds the stimulation rewarding. One method to elucidate if the animal's vocalization at 50 kHz represents positive affect is to examine vocalizations in a self-stimulation paradigm. Again, when an animal self-administered electric brain stimulation, 50 kHz vocalizations

increased. These results suggest the brain stimulation is rewarding and that the 50 kHz vocalizations are indices of positive affect.

Conditioned stimuli previously paired with reward have also had the capacity to evoke 50 kHz and 22 kHz ultrasonic vocalizations further relating these call types in affective state. When deprived, food can become a powerful reinforcer and Burgdorf et al. (2000) examined the effects of food as a reward. When food deprived rats were given access to food after presentation of a conditioned stimulus, 50 kHz ultrasonic vocalizations increased during the stimulus. When rats were presented with the conditioned stimulus without food, 50 kHz ultrasonic vocalizations declined and 22 kHz ultrasonic vocalizations increased, indicating a negative affective state. Conditioned place preference has also been used to understand these affective call types. Ultrasonic vocalizations have even been used to evaluate the reinforcing properties of amphetamine and morphine (Knutson, Burgdorf, & Panksepp, 1999). Using a conditioned place preference paradigm, rats emitted more 50 kHz ultrasonic vocalizations in the sides paired with amphetamine or morphine.

Another behavioral task that induces 50 kHz ultrasonic vocalizations is heterospecific play termed "tickling". In this procedure animals are given tactile stimulation with fast finger movements around the head, nape of the neck and ventral sides for 15 sec followed by 15 sec of no tactile stimulation and this procedure is cycled for 2 min (Panksepp et al., 2000). Data on heterospecific play indicate it is not generic tactile stimulation but intentional tickling that is responsible for evoking 50 kHz ultrasonic vocalizations. Animals learn to approach the experimenter's hand and will even self-administer heterospecific play versus generic tactile stimulation. Furthermore, a conditioned place preference can be established when a rat is tickled in a contextual specific arena. In this experiment, animals spent more time with the

experimenter's hand despite not receiving tickle stimulation. Moreover, Burgdorf, Kroes, Moskal, Pfaus, Brudzynski, and Pansepp (2008) extended the work on heterospecific play and 50 kHz ultrasonic vocalizations were grouped into subtypes of flat and frequency modulated (FM) call types and concluded the FM call types were more implicated in reward. Animals in this experiment also had the ability to self-administer recordings of 22 kHz USVs, flat and FM 50 kHz ultrasonic vocalizations and white noise. Animals self-administered significantly more FM 50 kHz ultrasonic vocalizations than any other recording type, suggesting these specific call types were more rewarding than the other call types tested, and these calls have also been implicated in inducing approach behavior (Wohr & Schwarting, 2007). Taken, together with Burgdorf et al. (2008), 50 kHz calling is rewarding and has the potential to induce appetitive behaviors.

Breeding For 50 kHz Ultrasonic Vocalizations

There are vast behavioral differences between animals characterized as high and low vocalizers. The effects from breeding for 50 kHz USVs has primarily been researched in animal emotion (Burgdorf, Panksepp, Brudzynski, Kroes, & Moskal, 2005; Burgdorf, Panksepp, Brudzynski, Beinfeld, Cromwell, Kroes, & Moskal, 2009). Animals were dichotomized into high and low vocalizers, and bred within their respective groups. To identify animals as high and low vocalizers, animals underwent four heterospecific play sessions. The fourth session was recorded and scored. Such experiments (Burgdorf et al., 2005; 2009) bred rats for high and low 50 kHz ultrasonic vocalizations. Breeding was completed for 5 or 14 generations and the effects of selective breeding were observed on differential rates of ultrasonic vocalization calling, anxiogenic-like behavior, depressive behavior, sensitivity for sucrose and social contact. After 4 generations, high and low vocalizing animals displayed differential rates of 50 kHz ultrasonic

vocalizations calling, and high 50 kHz vocalizing animals displayed lower levels of the 22 kHz ultrasonic vocalizations (Burgdorf et al., 2005). Furthermore, animals bred for higher levels of 50 kHz ultrasonic vocalizations after 14 generations show pronounced differences in many behaviors. These animals show more 50 kHz ultrasonic vocalizations in response to tickling, as well as alterations in the forced swim test, (Burgdorf et al., 2009; Mallo, Matrov, Herm, Koiv, Eller, Rinken, & Harro, 2007). Furthermore, rats bred to be 50 kHz or 22 kHz vocalizers differ in their response to systemic amphetamine. Animals bred to be high 50 kHz callers displayed increased locomotor activity relative to the low 50 kHz callers after an acute dose of amphetamine (1.5 mg/kg), (Brudzynski, Gibson, Silkstone, Burgdorf, Kroes, Moskal, & Panksepp, 2011a). Differences observed in call rate in response to amphetamine indicate differences in reward sensitivity to amphetamine. Higher 50 kHz ultrasonic vocalizations callers are more sensitive to the reinforcing effects of stimulant drugs and can possibly be used to understand the individual differences associated with reward magnitude.

Psychomotor Stimulant-induced 50 kHz Ultrasonic Vocalizations

Ultrasonic vocalizations have primarily been researched with cocaine and amphetamine because these drugs have their pharmacological effect in the mesolimbic dopamine pathway. Other psychomotor stimulants such as caffeine (Simola, 2010) and nicotine (Garcia, unpublished) do not seem to influence or may even reduce ultrasonic vocalizations. However, the number of studies researching these compounds has been few. Ultrasonic vocalizations have been a useful tool for understanding the sensitization and tolerance effects observed with cocaine and amphetamine, and have been unequivocal behavioral measures for understanding the affective development of stimulant addiction: from acquisition to withdrawal. During acquisition of cocaine self-administration, 50 kHz ultrasonic vocalizations are negatively correlated with

how quickly the animal acquired self-administration (Browning et al., 2011) indicating initial affective response to drugs is important for the rate of acquisition, with a more rewarding response producing a more rapid rate of cocaine acquisition. Ultrasonic vocalizations have been particularly sensitive to modifications of dose (Barker, Root, Ma, Jha, Megehee, Pawlak, & West, 2010). Results from the Barker et al. study revealed ultrasonic vocalizations are sensitive to the cocaine dose during a self-administration paradigm. At the lower dose (0.355 mg/kg i.v.), significantly more short 22 kHz ultrasonic vocalizations were observed than at the higher dose (0.71 mg/kg i.v.), while animals in the higher dose had significantly more FM 50 kHz ultrasonic vocalizations. Similar to 50 kHz ultrasonic vocalizations, 22 kHz vocalizations have multiple call categories, but less is known about the differences between them. Short 22 kHz ultrasonic vocalizations in comparison to their longer 22 kHz counterparts, short ultrasonic vocalizations may be indicative of an aversive stimulus (Brudzynski, Bihari, Ociepa, & Fu, 1993) or possibly cholinergic activity in the mesolimbic circuit as Bihari, Hrycyshyn, and Brudzynski (2003) found that 22 kHz ultrasonic vocalizations were evoked by acetylcholine transmission. The differences observed in ultrasonic vocalizations between these two doses of intravenous cocaine could be representative two motivational states. The animals in the lower dose group may be motivated through negative reinforcement, while animals in the higher group are motivated through positive reinforcement and are more able to control blood concentrations of cocaine. However, 22 kHz ultrasonic vocalizations following cocaine administration are not typical and could possibly be explained by motivational states of the animal. Evidence has surfaced implicating motivation and ultrasonic vocalizations (Coffey, Barker, Ma, Root, Martinez, Horvitz, & West, 2013). Results from this Pavlovian study suggest that expected value can influence ultrasonic vocalizations of both call types, such that when the expected value of a reinforcer (sucrose) is

high and the actual reward is low 22 kHz calling increases, suggesting ultrasonic vocalizations may be subject to the expected value of the reinforcer. Increases in 22 kHz ultrasonic vocalizations likely indicate a negative affective state by the animal. Interestingly this affective relationship remains intact with psychomotor stimulants. When rats are allowed to self-administer stimulants and stable responding is met across several days, 50 and 22 kHz ultrasonic vocalizations change if the rat is restricted in the amount of drug allowed to administer and when they are given a loading dose to elevate them above satiety levels (Barker, Simmons, Servillo, Bercovicz, Ma, Root, Pawlak, & West, 2013). This result suggests that ultrasonic vocalizations are sensitive to reward value and affective states change as a result of reward value changes.

Amphetamine has also been shown to increase 50 kHz ultrasonic vocalizations, and these vocalizations have been stable across time among individual rats, with differences in ultrasonic vocalizations primarily existing between rats (Taracha, Hamed, Krzascik, Lehner, Skorzewska, Plaznik, & Chrapusta, 2012). Surprisingly, although vocalizations remained stable across time, FM 50 kHz calling after the animals' first exposure did not predict sensitization of FM 50 kHz ultrasonic vocalizations after repeated exposure to a high dose of amphetamine (2.0 mg/kg). However, these analyses were conducted using a median split method and may not have been powerful enough to detect differences between animals clumped around the median (Cain et al., 2005) and the dose used may have been too high to detect individual differences between animals. Amphetamine has also been shown to elevate FM 50 kHz ultrasonic vocalizations after repeated intravenous exposure (Ahrens et al., 2009). In this study animals' initial ultrasonic vocalizations and locomotor response to amphetamine was observed as well as after repeated exposure. After just two trials, FM 50 kHz ultrasonic vocalizations had significantly increased from the previous day while locomotor activity did not become significant until the third day.

This suggest FM 50 kHz ultrasonic vocalizations may be a more sensitive measure of behavioral sensitization and can be used in conjunction with locomotor activity to measure behavioral sensitization of psychomotor stimulants.

50 kHz Ultrasonic Vocalizations Dependent on Mesolimbic Dopamine

The results from the aforementioned studies on 50 kHz ultrasonic vocalizations and drugs of abuse suggest 50 kHz ultrasonic vocalizations are likely dependent on mesolimbic dopamine. Neurobiological evidence has emerged to provide strong support for this hypothesis. Burgdorf, Wood, Kroes, Moskal, and Panksepp (2007) used electric brain stimulation and pharmacological manipulations to attempt to elucidate the neural underpinnings of 50 kHz ultrasonic vocalizations. Animals had cannula implanted in the ventral tegmental area, and experimenters delivered electric brain stimulation and measured the induced 50 kHz ultrasonic vocalizations. Animals were then shaped to self-stimulate the electric stimulation and 50 kHz ultrasonic vocalizations were recorded. When electrical brain stimulation was ON animals displayed increased bar pressing and increased 50 kHz ultrasonic vocalizations than when the electrical brain stimulation was OFF. However, this effect was only observed in animals that vocalized during the experimenter-administered electric brain stimulation. Animals failing to vocalize during the experimenter-administered electric brain stimulation also failed to acquire self-stimulation, possibly suggesting the importance of individual differences in 50 kHz call rate, reward sensitivity, and mesolimbic dopamine function. Finally, animals were microinjected directly into the ventral tegmental area with tri-D-Ala-Gly-N-methyl-Phe-Gly-ol (DAMGO) a μ-opiate agonist and conditioned place preference and 50 kHz ultrasonic vocalizations were observed. Animals that showed an increase in 50 kHz ultrasonic vocalizations in response to

DAMGO also demonstrated a conditioned place preference, while animals not showing an increase in vocalization rate failed to show a condition place preference.

Neurotoxic and electrolytic lesions have also suggested a role for the mesolimbic dopamine system in the generation of 50 kHz ultrasonic vocalizations (Burgdorf et al., 2007). Animals had been habituated to heterospecific play and they had demonstrated 'high levels' of 50 kHz ultrasonic vocalizations. Following electrolytic and neurotoxic lesions (8 µg of 6-OHDA bilaterally) to the ventral tegmental area, FM 50 kHz ultrasonic vocalizations were significantly reduced during subsequent heterospecific play sessions. However, flat 50 kHz and 22 kHz ultrasonic vocalizations were not affected. Further tissue analysis revealed a significant reduction in dopamine concentrations in the nucleus accumbens. These results suggest that FM 50 kHz ultrasonic vocalizations are dopamine dependent and FM 50 kHz ultrasonic vocalizations may be implicated in reward processing in the mesolimbic dopamine circuit.

Although DAMGO had been used to help elucidate the neural correlates of 50 kHz ultrasonic vocalizations it was an indirect measure of dopaminergic activity. To understand the role of dopamine receptors in 50 kHz ultrasonic vocalizations, quinpirole (dopamine D_2/D_3 receptor agonist), has recently been used to provide a more direct manipulation of dopamine receptors. Quinpirole dose dependently increased 50 kHz ultrasonic vocalizations when injected directly into the nucleus accumbens shell (Brudzynski, Komadoski, & St. Pierre, 2012). This relationship depended on a number of factors: First, at lower (0.25 µg) and higher doses (3, 6 and 10 µg) quinpirole increased 50 kHz calling. Second, moderate doses (.5 and 1 µg) and the highest dose (20 µg) of the dopamine agonist failed to elevate 50 kHz ultrasonic vocalizations. Specific antagonism to the D_2 and D_3 receptor revealed that at the lower doses quinpirole seemed to be preferentially working on the D_3 receptor, while at the higher doses the drug is

nonspecifically binding to both D_2 and D_3 receptors. This provides strong evidence that dopamine D_3 receptors in the nucleus accumbens are implicated in drug-induced 50 kHz ultrasonic vocalizations calling. Similarly, Brudzynski, Silkstone, Komadoski, Scullion, Duffus, Burgdorf, Kroes, Moskal, and Panksepp (2011b) microinjected amphetamine into the nucleus accumbens shell and observed 50 kHz ultrasonic vocalizations in 3 lines of selectively bred animals (similar to Brudzynski et al., 2011a). The results suggested the number of amphetamine-induced 50 kHz ultrasonic vocalizations depended on which genetic line the animal came from, with the high and random lines vocalizing more than the low line of animals. Interestingly, all three lines were significantly different from each other, suggesting the high vocalizing animals have increased dopaminergic sensitivity resulting from alterations in the nucleus accumbens shell. This alteration in the nucleus accumbens shell may make these animals more sensitive to drugs of abuse specifically targeting the mesolimbic dopamine circuit. Thus, this increased sensitivity would likely create a hyper-responsive animal to the reinforcing properties of drugs and possibly influence the animal to be a compulsive drug seeker.

Prior models have attempted to understand addiction through learned processes and have been quite successful in explaining many aspects of addiction, yet how these models account for individual differences in compulsive drug taking is not as well understood. Individual difference variables such as novelty/sensation seeking have been correlated with individuals who are more likely to experiment with drugs. To further understand the contribution of these personality traits animals model have been developed. Specifically, the inescapable novelty and novelty place preference have been developed and have been reliable predictors of self-administration and reward sensitivity. Surprisingly, although these screens are measuring novelty and are both understood to be dopamine dependent, they seem to be measuring different aspects of novelty.

These two novelty screens have been paramount in individual differences animal research, but could benefit from an additional individual difference measure. Ultrasonic vocalizations have been reliable measures of affective states in rodents and demonstrate the potential to measure individual differences. Ultrasonic vocalizations have been used previously to study psychomotor stimulants and have been shown to sensitize with repeated exposures, suggesting they could be used in conjunction with locomotor activity to track stimulant behavioral sensitization. Furthermore, 50 kHz ultrasonic vocalizations are largely dopamine dependent, further promoting the idea that ultrasonic vocalizations could be correlated with one of the novelty screens and predict more variance in animal models of addiction. If it is these same individuals (rats) who are high in novelty seeking and ultrasonic vocalizations that are more prone or sensitive to the psychomotor effects of amphetamine, could they be the same individuals more liable to become compulsive drug seekers and abusers?

Individual Difference Screen Hypotheses

$H_{1:}$ The inescapable novelty screen and novelty place preference screen will not be correlated, such that there will not be a positive or negative relationship between the screens.

$H_{2:}$ Ultrasonic vocalizations observed during the heterospecific manipulation (H-USV) will be correlated with the inescapable novelty screen, but not the novelty place preference screen.

Amphetamine Hypotheses

$H_{1:}$ Amphetamine will dose dependently increase locomotor activity, such that both the low dose (0.3 mg/kg) and high dose (1.0 mg/kg) will increase locomotor activity relative to the saline group, but high dose (1.0 mg/kg) will result in greater locomotor activity

when compared to the low (0.3 mg/kg) dose. Furthermore, repeated treatments of amphetamine will progressively increase locomotor activity, such that on the challenge day animals with previous amphetamine exposure will have increased locomotor activity when compared to the saline treated animals. Repeated treatments of the high dose (1.0 mg/kg) will result in greater locomotor activity than repeated treatments of the low dose (0.3 mg/kg) amphetamine dose.

$H_{2:}$ Amphetamine will dose dependently increase 50 kHz ultrasonic vocalizations, such that the low dose (0.3 mg/kg) will increase 50 kHz ultrasonic vocalizations relative to the saline group, and the high dose (1.0 mg/kg) will increase 50 kHz ultrasonic vocalizations when compared the low dose (0.3 mg/kg). Amphetamine will also progressively increase 50 kHz ultrasonic vocalizations with repeated treatments of amphetamine, such that on the challenge day animals with previous amphetamine treatments will have increased 50 kHz ultrasonic vocalizations in response to amphetamine when compared to the saline treated animals. Repeated treatments of the high dose (1.0 mg/kg) will result in more 50 kHz ultrasonic vocalizations when compared to the animals with repeated low dose (0.3 mg/kg) treatments.

$H_{3:}$ The inescapable novelty screen will have a dose dependent relationship with locomotor activity, such that there will be a unique positive relationship at the low dose (0.3 mg/kg) and no relationship at the high dose (1.0 mg/kg). The relationship between the inescapable novelty screen and locomotor activity is expected to be dose dependent because differences between lower and higher responders will be reduced at the high dose (1.0 mg/kg).

H_4: The inescapable novelty screen will have a dose dependent relationship with amphetamine-induced 50 kHz ultrasonic vocalizations, such that there will be a unique positive relationship at the low dose (0.3 mg/kg) and no relationship at the high dose (1.0 mg/kg). This is predicted because the high dose is likely to increase 50 kHz ultrasonic vocalizations regardless of whether the animal was a higher or lower responder in the inescapable novelty screen.

H_5: The novelty place preference will not have a relationship with amphetamine-induced locomotor activity. This non-significant relationship will remain throughout the course of chronic treatment and during the challenge day.

H_6: The novelty place preference will not have a relationship with amphetamine-induced 50 kHz ultrasonic vocalizations. It is expected that the non-significant relationship will remain throughout repeated amphetamine exposure and the challenge day.

H_7: The H-USV screen will have a dose dependent relationship with amphetamine-induced locomotor activity. The H-USV screen will have a positive relationship, such that the highest ultrasonic callers will have greater locomotor activity in response to a low dose (0.3 mg /kg) amphetamine. However, this relationship will diminish at the high dose (1.0 mg/kg) of amphetamine because the high dose will increase locomotion regardless of higher or lower vocalizers in the H-USV screen.

H_8: The H-USV screen will have a dose dependent relationship with amphetamine-induced 50-kHz ultrasonic vocalizations, such that the highest vocalizers will show the greatest ultrasonic vocalization response to the low dose (0.3 mg/kg) of amphetamine and this relationship will diminish at the high dose (1.0 mg/kg) of amphetamine, because the

high dose (1.0 mg/kg) will increase 50 kHz ultrasonic vocalizations regardless of higher or lower vocalizers in the H-USV screen.

CHAPTER 2 – General Methods

Animals and Housing Environment

Thirty adolescent male Sprague-Dawley rats were purchased from Charles River Laboratories. The animals were approximately 30 days old on arrival. Animals were housed individually in a temperature and humidity controlled room. Animals were housed individually because social housing conditions have been known to affect ultrasonic vocalization. Animals were housed in standard transparent polyurethane cages with CAREfresh bedding with free access to food and water for the complete duration of the experiment. Animals were handled daily for 1 minute each day to habituate them to experimentation. Experimentation began 10 days after arrival. The colony room was maintained on 12:12 light dark cycle, with lights on at 7:00-19:00. All behavioral testing was conducted during 11:00-15:00 hours. All procedures were approved by the Kansas State University Institutional Animal Care and Use Committee and the Guide for the Care and Use of Animals (National Research Council, 2011).

Drugs and Solutions

Sodium chloride (Sigma, St. Louis, MO) was dissolved in distilled water to yield a concentration of 0.9% saline. d-amphetamine sulfate (Sigma, St. Louis, MO) was dissolved in 0.9% saline and was administered at doses of either 0.3 mg/kg or 1.0 mg/kg. Each drug or vehicle treatment was administered in a volume of 1 ml/kg intraperitoneally (i.p.). Doses of 0.3 and 1.0 mg/kg were chosen because these doses are known to produce locomotor activity without producing stereotyped behaviors (Pijnenburg, Honig, & Van Rossum, 1975).

Furthermore, the inescapable novelty and novelty place preference assessments are most robust at lower doses (Cain et al., 2004; 2005), and are likely measuring different aspects of drug abuse.

General Apparatus

Inescapable novelty

Six locomotor activity chambers measuring 46.6 X 46.6 X 46.6 cm were used to measure locomotor activity. Each locomotor chamber was constructed of transparent plexiglass walls and plastic flooring that was covered with pine shavings bedding. A photobeam sensor surrounded the locomotor chamber and was approximately 2.54 cm above the plastic flooring. The photobeam was comprised of a 16 x 16 (x-axis) photocell array. Each photocell was spaced 2.54 cm apart (Coulbourn Instruments, TruScan 2.01) and measured the amount of horizontal movement in centimeters. The amount of horizontal movement (cm) was also recorded in 5-minute blocks of time for each session. A white noise generator (70 dB) was used to create background noise to mask sounds from other chambers and from experimenter generated sounds.

Novelty Place Preference

The apparatus consisted of three compartments constructed of plexiglass. The end compartments of the apparatus are 29x23x45 (L x W x H) cm. The walls of one compartment were white and the floor of this compartment is made of wire mesh (13x13) mm. Below the white-walled compartment pine shavings bedding were contained in a litter tray. The walls of the other compartment were black and the floor of this compartment was made of 15 metal rods (6 mm in diameter) spaced 2 cm apart center-to-center. Compressed pellet bedding was contained in the litter tray below black-walled compartment. The smaller center compartment is 19x23x45 (L x W x H) cm. Its walls and floor are painted grey and constructed entirely of plexiglass. The solid walls that separate the three compartments were replaced on the preference test day with

similarly painted walls that had 10.5x10.5 cm opening in the bottom center to allow access to all compartments of the apparatus.

Ultrasonic Arena

All ultrasonic recording was conducted in a separate room, and each animal was tested individually. During the 'tickling' screen, the ultrasonic arena was comprised of a standard transparent shoebox cage. The cage floor was covered with Carefresh® litter bedding. On amphetamine training sessions, the ultrasonic arena was comprised of a standard opaque shoebox cage. The cage floor was covered with compressed pellet bedding to provide an additional contextual cue.

Ultrasonic Data Collection and Classification

Ultrasonic vocalizations were recorded using an ultrasonic microphone (Ultramic 200K) purchased from dodotronic.com and SEAwave recording software. Both the microphone and software were connected to a separate computer that automatically saved each sound file. Analysis of each sound file was done using AviSoft SASLab Pro Bioacoustics sound analysis software. Ultrasonic vocalizations were counted automatically and scored similar to Brudzynski (2005) and Wright, Gourdon, and Clarke (2010). Briefly, after the recording sessions a spectrogram was created for each animal that displayed all sounds recorded in the possible 1-100 kHz sound range. SASLab Pro automatically marked all potential ultrasonic vocalizations, and parameter estimates were measured. A trained researcher then identified the shape of the ultrasonic vocalization. Ultrasonic vocalization shapes were identified as either: flat, harmonic or FM. During this step, all ultrasonic vocalizations were verified using the playback function in SASLab Pro. The playback function reduced the speed of the playback and allows the ultrasonic vocalizations to become audible. Parameter estimates were then copied in Microsoft Excel and,

using Excel's functions, ultrasonic vocalizations were categorized into different call types. Twenty-two kHz ultrasonic vocalizations were operationally defined as occurring in the frequency of 20-28 kHz. In addition they were required to be flat in shape with no visual fluctuations and occurring for more than 100 milliseconds. Fifty kHz ultrasonic vocalizations were operationally defined as occurring in a range of 35-90 kHz and lasting longer than 100 milliseconds. Flat 50 kHz ultrasonic vocalizations were flat in shape with no visual fluctuations. Harmonic 50 kHz ultrasonic vocalizations were operationally defined as have 1 or 2 fluctuations, a change in frequency, and/or complex shape that was not flat or frequency modulated (FM). Frequency modulated (FM) 50 kHz ultrasonic vocalizations were operationally defined as having 3 or more fluctuations in frequency and occurring for at least 100 milliseconds.

Locomotor Data Collection

The same locomotor chambers used for the inescapable novelty screen were used to record locomotor activity during the acute amphetamine, repeated amphetamine, and sensitization tests.

Procedure

Individual Difference Screens

All animals received all novelty screens in the same order: inescapable novelty, novelty place preference, heterospecific play. The order was determined on level of intrusiveness, with the least intrusive test completed first. For the inescapable novelty screen, animals were weighed then subsequently placed inside of the activity chamber for 30 minutes. Distance traveled was recorded every 5 minutes and summed at the end of the session to yield a total distance traveled (cm). The following day animals were screened using the novelty place preference screen. Each

animal was placed in either the white or the black side of the place preference chamber for 30 minutes for habituation. During this habituation period animals were restricted to the one side of the chamber. After two days of habituation the partition between the compartments was removed, and animals were placed into the grey center and allowed access to both sides of the box for 15 minutes. Time spent on each the habituated and novel side was recorded. Also, latency to enter any side, and latency to enter the novel side was recorded. A preference ratio (time spent in novel compartment / total time in both the familiar and novel compartments) was calculated for each animal. An animal was considered to be in a compartment when both front paws were in the compartment. The following day the animals were transported to a holding room. From the holding room, animals were moved into the ultrasonic recording room individually. Animals were wrestled and 'tickled' using gentle, fast-finger movements along the back and neck, similar to conspecific play. 'Tickling' occurred for 15 seconds, followed by 15 seconds of no stimulation. This cycle repeated for 2 minutes. Each animal experienced 3 days of 'tickling' to habituate the animals to the procedure. On the fourth day animals underwent the same procedure, but the ultrasonic microphone recorded potential ultrasonic vocalizations of any call type.

Amphetamine Training Sessions

After all screens were complete, animals were divided into two squads, A and B, such that responders were equally distributed in each squad. Animals were block randomized to a drug condition, such that higher and lower novelty responders and ultrasonic callers were equally distributed between the saline, low (0.3 mg/kg) and high (1.0 mg/kg) drug groups. To start an amphetamine training session, all animals in either squad A or B were weighed in the colony room, followed by transport to a holding room. While in the holding room, animals individually

received an injection of its respective drug and were immediately placed back into its homecage. After 15 minutes, the injected animal was transported to the ultrasonic room and recorded for 2 minutes. When the recording was finished, the animal was immediately transported to an activity chamber and locomotor activity was recorded for 60 minutes. Each animal in the squad underwent this procedure for 7 training sessions. Training sessions alternated between Squad A and Squad B for a total of 14 days. Ultrasonic recording occurred on training Days 1 and 7. Days 2-6 the microphone was on but did not record potential ultrasonic sounds. After the seventh training session, Squad A and B did not receive any drug treatment for 14 days and were only handled to change bedding or cages.

Amphetamine Challenge

After the 14 day rest period, all animals were given a challenge dose of amphetamine. Saline animals (n=10) were divided into two groups (n=5), one group received the low dose (0.3 mg/kg) and the other group received the high dose (1.0 mg.kg). As a result, all animals received either a low (0.3 mg/kg) or high (1.0 mg/kg) dose of d-amphetamine. Briefly, animals were weighed and transported to the holding room, where they all received an i.p. administered dose of amphetamine. After 15 minutes, the animal was transported to the ultrasonic room and recorded for 2 minutes and subsequently taken to the activity chambers and locomotor activity was recorded for 60 minutes.

CHAPTER 3 – Results

Relationships Between The Screens

Previous research has shown the inescapable novelty and novelty place preference are uncorrelated, but neither screen has been correlated with ultrasonic vocalizations. Correlation analyses were completed to understand the relationship between the different novelty/sensation

seeking screens and ultrasonic vocalizations. Correlation analyses revealed the inescapable novelty screen was not correlated with the novelty place preference screen ($r(28) = .03$, $p > .05$) (Figure 1). The inescapable novelty screen was also not correlated with 50 kHz USV observed during the H-USV screen ($r(28) = -17$, $p > .05$) (Figure 2). The novelty place preference was not correlated to 50 kHz ultrasonic vocalizations observed during the H-USV screen ($r(28) = .08$, $p > .05$) (Figure 3). Taken together, these results suggest the novelty/sensation seeking screens and the H-USV screen are not related and are measuring different individual difference traits. In addition, this suggests they will predict different aspects of amphetamine-induced locomotor activity and amphetamine-induced 50 kHz ultrasonic vocalizations.

Amphetamine Session Analyses

To understand how novelty/sensation seeking, H-USV, and amphetamine predict locomotor and ultrasonic vocalization response to amphetamine, separate regression analyses were used. This type of analysis was used to maintain the continuous nature of each novelty/sensation-seeking and H-USV assessment. Treatment group was entered as a categorical variable and had 3 levels (Saline 0.0 mg/kg, Low 0.3 mg/kg, and High 1.0 mg/kg). In summary, the inescapable novelty, novelty place preference, and H-USV screens and amphetamine treatment were used to predict locomotor and ultrasonic vocalization response to amphetamine on Days 1 and 7. On the Challenge day, all animals received an amphetamine dose and thus there were 4 levels of this categorical variable: acute low (0.3 mg/kg), repeated low (0.3 mg/kg), acute high (1.0 mg/kg) and repeated high (1.0 mg/kg). The acute animals previously had 7 treatments with saline. The repeated animals previously had 7 treatments with their respective dose of amphetamine.

Before any linear regression analyses were completed all dependent measures were plotted using a histogram to determine if the distributions of scores were approximately normal. None of the dependent measures appeared to be skewed and none were transformed. The homogeneity of variance assumption was also met by examining the residual plots. The residuals appeared to be evenly dispersed without any concerns. To reduce the potential for multicolinearity the predictor variables were centered prior to testing any interactions.

Day 1 Locomotor Response to Amphetamine

The regression analysis revealed that the overall model predicted locomotor response to amphetamine on Day 1 ($R^2 = .77$, $p < .001$). However, the inescapable novelty screen, novelty place preference screen, and H-USV screen did not predict the locomotor response to amphetamine (see Table 1 for summary). Both the low ($\beta = .49$, $p < .001$) and high ($\beta = .87$, $p < .001$) doses of amphetamine showed significant positive relationships with locomotor response on Day 1 (Figure 4). These relationships indicate that amphetamine increased locomotor response relative to the saline group. Furthermore, the high (1.0 mg/kg) dose increased locomotor activity above and beyond the low dose (0.3 mg/kg). Treatment group was the best predictor of the animals' locomotor responses on Day 1 and that the animals' response to novelty or response to heterospecific play were not useful in predicting locomotor response to the initial exposure to amphetamine. All possible interactions were tested independently using a hierarchical regression technique, such that one interaction was tested in the model, then removed and another interaction term entered. Thus, interactions were assessed 1 at a time. Interaction terms were added to the model and a significant change in R^2 was used as a criterion to determine potential interactions. There were no interactions observed when trying to predict locomotor response to treatment on the animals first treatment.

Day 1 50 kHz USV Response to Amphetamine

The regression analysis revealed that the overall model predicted the 50 kHz ultrasonic vocalization response to acute amphetamine on Day 1 (R^2 = .46, p = .008). Importantly, neither the high (1.0 mg/kg) nor the low dose (0.3 mg/kg) showed any difference from the saline animals, suggesting amphetamine did not influence 50 kHz ultrasonic vocalizations with acute treatment. The inescapable novelty and novelty place preference screens did not show any relationship with 50 kHz ultrasonic vocalizations in response to treatment on Day 1 (see Table 2 for summary). However, the H-USV screen did show a significant unique positive relationship with the 50 kHz ultrasonic vocalizations on Day 1 (β = .67, p < .001), such that the highest 50 kHz vocalizing animals during the H-USV screen were the highest 50 kHz vocalizing animals on Day 1 regardless of drug treatment condition (Figure 5). Amphetamine at either dose did not change 50 kHz ultrasonic vocalizations, these results suggest that 50 kHz ultrasonic vocalizations remained stable from the H-USV screen to the animals' first exposure to amphetamine or saline. All possible interactions were tested independently (described above) using a hierarchical regression technique and did not result in a significant change in R^2.

Day 7 Locomotor Response to Amphetamine

The regression analysis revealed that the overall model predicted the locomotor response to repeated amphetamine on Day 7 (R^2 = .75, p < .001). Both the low dose (β =.50, p < .001) and the high dose (β =1.02, p < .001) showed a positive relationship with locomotor response to repeated amphetamine treatment, revealing the low (0.3 mg/kg) and high (1.0 mg/kg) doses increased locomotor activity relative to the saline group (Figure 6). Repeated administrations of the high dose (1.0 mg/kg) induced greater locomotor activity when compared to repeated administrations of the low dose (0.3 mg/kg). The inescapable novelty screen, the novelty place

preference screen, and the H-USV screen did not predict locomotor response to repeated amphetamine or saline (see Table 3 for summary). All possible interactions were tested independently using hierarchical regression analysis (described above) and did not result in a significant change in R^2.

Day 7 50 kHz USV Response to Amphetamine

The regression analysis revealed the overall model did not predict 50 kHz ultrasonic vocalizations response to repeated amphetamine or saline on Day 7 (R^2 = .33, p = .07). Neither dose of amphetamine changed A50 kHz ultrasonic vocalizations indicating call rates were not different between the saline, low (0.3 mg/kg) and high (1.0 mg/kg) doses. Although amphetamine did not increase ultrasonic vocalization, the H-USV screen showed a positive relationship with 50 kHz ultrasonic vocalizations in response to repeated amphetamine (β =.47, p = .01), with the highest 50 kHz vocalizing animals in the H-USV screen also vocalizing the most following 7 treatments of amphetamine or saline (Figure 7). This shows 50 kHz ultrasonic vocalizations remain stable across 7 administrations of two different doses of amphetamine. The inescapable novelty and novelty place preference screens did not show any relationship with 50 kHz ultrasonic vocalization responses to repeated amphetamine or saline (see Table 4 for summary). All possible interactions were tested independently using hierarchical regression (described above) and did not result in a significant change in R^2.

Challenge Day Locomotor Response to Amphetamine

The regression analysis revealed the overall model predicted the locomotor response to amphetamine on the Challenge day (R^2 = .49, p < .01). The inescapable novelty, novelty place preference, and the H-USV screens did not show a significant relationship with locomotor response on the Challenge day (see Table 5. for summary). Amphetamine-induced locomotor

response was predicted by the dose and number of exposures of amphetamine (Figure 8). Animals administered a repeated low dose of amphetamine showed a non-significant positive relationship with locomotor response, suggesting that the animals that received the repeated low dose (0.3 mg/kg) were not different in locomotor response to amphetamine from animals that received the acute low dose of amphetamine. The acute and repeated high dose (1.0 mg/kg) showed a significant positive relationship with locomotor response (β = .61, p = .005), demonstrating these animals showed greater locomotor response than the animals that were administered the acute low dose (0.3 mg/kg) of amphetamine.. The animals' locomotor response to the acute high dose and the repeated high dose were not different. All possible interactions were tested independently using hierarchical regression (described above and did not result in a significant change in R^2.

Challenge Day 50 kHz USV Response to Amphetamine

The simultaneous regression analysis revealed that the overall model did not predict the 50 kHz ultrasonic vocalizations response to amphetamine on the Challenge day (R^2 = .35, p = .10). The novelty place preference showed a non-significant positive relationship with 50 kHz ultrasonic vocalizations. The inescapable novelty assessment showed a significant negative relationship with 50 kHz ultrasonic vocalizations on the Challenge day (β = -.37, p = .04), indicating that the higher responding animals in the inescapable novelty screen vocalized less at 50 kHz during the Challenge day of the experiment (Figure 10). The H-USV screen did not show a significant relationship with 50 kHz ultrasonic vocalizations observed on the challenge day (β = .22, p = .23), suggesting that the 50 kHz ultrasonic vocalization mechanism change during drug withdrawal (Figure 9). None of the amphetamine groups showed a change in amphetamine-induced 50 kHz ultrasonic vocalizations when compared to the acute low dose (0.3 mg/kg)

animals. The repeated low dose of amphetamine showed a non-significant positive relationship with amphetamine-induced 50 kHz ultrasonic vocalizations. The acute high dose showed a non-significant positive relationship with amphetamine-induced 50 kHz ultrasonic vocalizations. The repeated high dose of amphetamine showed a non-significant positive relationship with amphetamine-induced 50 kHz ultrasonic vocalizations. These results lend support that 50 kHz ultrasonic vocalizations are not affected by low or high doses of amphetamine and they are not influenced by acute or repeated amphetamine (see Table 6. for summary). All possible interactions were tested using hierarchical regression (described above) and did not result in a significant change in R^2.

CHAPTER 4 - Discussion

The first purpose of the present study was to examine the relationship between the inescapable novelty, novelty place preference, and H-USV screens. Previous research has shown that the inescapable novelty and novelty place preference screens are not correlated. The results from the current study replicate previous literature (Beckman et al., 2011; Cain, et al., 2005), and provide evidence that rodent models of novelty are indeed measuring different aspects of novelty. To date, ultrasonic vocalizations have not been correlated with the inescapable novelty screen or the novelty place preference screen. The current hypothesis that 50 kHz ultrasonic vocalizations observed during the H-USV screen would be correlated with the inescapable novelty screen was not supported. The H-USV screen was also not correlated with the novelty place preference screen. Given that the novelty screens and H-USV screen were not related it is likely that they are measuring different aspects of novelty or different individual difference traits.

The second purpose of this study was to examine if the separate novelty screens and H-USV screen could be used to predict the locomotor response to acute and repeated amphetamine.

Locomotor response was not predicted from any of the novelty screens, which is in contrast to previous literature. It was hypothesized that the inescapable novelty screen would dose dependently predict locomotor response to amphetamine, because the inescapable novelty screen has been shown to predict locomotor response to low unit doses of amphetamine (Hooks, Jones, Smith, Neill, & Justice, 1991; Bevins, Klebaur, & Bardo, 1997). The current study did not replicate this previous relationship, but the results are in support of Cain et al. (2005) showing when regression analyses are used the inescapable novelty screen is no longer a significant predictor.

The third purpose of this study was to examine if the separate novelty screens and the H-USV screen could be used to predict the ultrasonic vocalization response to acute and repeated amphetamine. Also, in contrast to previous research and the proposed hypotheses, acute and chronic amphetamine did not increase 50 kHz ultrasonic vocalizations at either dose. Interestingly, the H-USV screen showed a significant positive relationship with 50 kHz ultrasonic vocalizations on Day 1 and Day 7 during the amphetamine training phase of the experiment, which supported the current hypothesis. Surprisingly, the inescapable novelty and novelty place preference screens did not show any relationship with 50 kHz ultrasonic vocalizations during the amphetamine training phase during the experiment. However, on the challenge day, the inescapable novelty screen showed a negative relationship with the observed 50 kHz ultrasonic vocalizations. This result demonstrates for the first time a novelty measure can be used to predict 50 kHz ultrasonic vocalization after a 14-day drug withdrawal.

Predicting Amphetamine-induced 50 kHz USV

Surprisingly, neither the high (1.0 mg/kg) nor the low dose (0.3 mg/kg) of amphetamine used in the present experiment increased 50 kHz ultrasonic vocalizations. While this is in

opposition to the majority of the previously published literature, recent research has failed to show any relationship between amphetamine-induced locomotor activity and 50 kHz ultrasonic vocalizations or any difference between rats dichotomized into high and low callers in their locomotor response to acute or repeated amphetamine (Taracha, Kaniuga, Chrapusta, Maciejak, Sliwa, Hamed, & Krzascik, 2014). However, in general 50 kHz ultrasonic vocalizations are evoked by a range doses of amphetamine doses, including those as low as 0.25 mg/kg (Wright et al., 2010). It is unlikely that our doses of amphetamine were not effective because each dose of amphetamine increased locomotor activity relative to the animals that were treated with saline, and demonstrate amphetamine-induced 50 kHz ultrasonic vocalizations and locomotor activity are neurologically distinct. Previous research using genetic breeding for high 50 kHz ultrasonic vocalization callers lead to increased locomotor activity when administered a high dose of amphetamine (1.5 mg/kg) when compared to both the low and random genetic lines, suggesting the brain areas are at least related (Brudzynski et al., 2011). As mentioned earlier, 50 kHz ultrasonic vocalizations are evoked by increased dopaminergic function specifically in the shell of the nucleus accumbens, while Towell, Willner, and Muscat (1987) documented that amphetamine-induced locomotor activity was specifically related to the core of the nucleus accumbens. The current results support that 50 kHz ultrasonic calling and locomotor activity are controlled by distinct neurological areas, because 50 kHz ultrasonic vocalizations did not have any relationship with amphetamine-induced locomotor activity.

Therefore, the current study replicated previous research suggesting amphetamine-induced locomotor activity and 50 kHz ultrasonic vocalizations are not correlated, but did not replicate previous research showing 50 kHz ultrasonic vocalizations are sensitive to amphetamine. One possible explanation as to why amphetamine did not increase 50 kHz

ultrasonic vocalizations is that the animals could have conditioned to the tickle arena apparatus. Previous research has shown ultrasonic vocalizations are evoked to non-pharmacological conditioned stimuli (Panksepp et al., 2000). In Panksepp et al.'s (2000) experiment after three conditioning sessions, animals associated a tickle box with the rewarding tickling procedure and vocalized in response to the conditioned tickle box and not in response to a non-tickle box. Given that the animals in Panksepp et al.'s (2000) and the current study were approximately the same age, it is likely that the animals in the current study conditioned to the tickle arena and vocalized in response to being placed inside the tickle arena, and not in response to their respective treatment conditions. This would account for the similar frequencies in the number of 50 kHz ultrasonic vocalization observed across all treatment conditions, and explain why neither dose of amphetamine increased 50 kHz ultrasonic calling when compared to the saline treated animals.

Our H-USV screen training protocol involved tickling the animals for 3 days to habituate them to the procedure and then measured vocalizations on the fourth day. Although precautions were utilized to make the tickle arena and the amphetamine training environments contextually distinct, it is possible the context failed to be distinct, and the ultrasonic vocalizations during the amphetamine training procedures were evoked in anticipation of heterospecific play. This explanation would explain why the saline, low dose (0.3 mg/kg) and high dose (1.0 mg/kg) were ineffective in altering 50 kHz ultrasonic vocalizations. Previous research has shown that saline groups typically vocalize approximately 5-15 calls per minute (Brudzynski et al. 2011; Wright, Dobosiewicz, & Clarke, 2013). In the current experiment, during the two minute recording session saline animals had an average of 254.60 and 283.90, 50 kHz ultrasonic vocalizations on Day 1 and Day 7 respectively. These values are well above previously published research and

lend support that the saline animals were vocalizing in response to a conditioned environment and not the intended control manipulation. Thus, the saline animals had an elevated response and resulted in an increased difficulty to observe the effect that either dose of amphetamine could have had on 50 kHz ultrasonic vocalizations.

The animals treated with saline demonstrated that the conditioned response to the tickling arena is long-lasting and does not deteriorate under extinction conditions. In the current study rats were not tickled or extensively handled after the H-USV screen was completed. If the amphetamine training context failed to be distinct from the tickle arena then each amphetamine training day served as an extinction trial. It would be expected that the animal would learn the context no longer predicts tickle stimulation and instead, predicts no stimulation. Thus, it would be expected that the 50 kHz ultrasonic vocalization responses would decrease after several trials. However, there was no decrease in ultrasonic responding from Day 1 to Day 7, suggesting the conditioned 50 kHz ultrasonic vocalization response is long-lasting and may be resistant to a short extinction phase. It is not clear how long the conditioned response would remain intact, but it persisted for 7 extinction trials over 14 days. This suggests that the reward induced by the tickle stimulation could have long-lasting receptor changes in the hedonic mesolimbic structures, such as the nucleus accumbens, but these hypotheses are yet to be examined. An alternative explanation for amphetamine's failure to increase 50 kHz ultrasonic vocalizations is that, at the doses tested (0.3 and 1.0 mg/kg), the drug failed to add rewarding value above and beyond the conditioned effect of the tickle arena. Therefore, the current results suggest the conditioned stimulus of the tickle arena is a strong stimulus capable of evoking 50 kHz ultrasonic vocalizations and the conditioned stimulus is more rewarding than the doses of amphetamine tested.

Although amphetamine did not change ultrasonic vocalizations, the H-USV screen was successful in predicting ultrasonic vocalization response during acute (Day 1) and chronic (Day 7) amphetamine treatment. Interestingly, the animals that vocalized the most during the H-USV screen remained the highest vocalizers during amphetamine training. Recent research suggests that ultrasonic vocalizations are individually stable across time (Mallo et al., 2007; Wright, Dobosiewicz, & Clarke, 2013), suggesting ultrasonic vocalization response is trait-like. While other research has shown bedding affects ultrasonic vocalizations (Schwarting et al., 2007). The present study showed ultrasonic vocalizations remained stable and did not change when different bedding types were used and lends support to the hypothesis that ultrasonic vocalizations are trait-like.

The current results provide additional support that the ultrasonic vocalization response is trait-like and individually stable across time (Mällo et al., 2007; Taracha et al., 2012). Amphetamine can change 50 kHz ultrasonic vocalizations by increasing the absolute number of 50 kHz ultrasonic vocalizations or by shifting from flat and harmonic vocalizations to FM ultrasonic vocalizations. It is these FM vocalizations that are sensitive to repeated exposures to amphetamine (Ahrens, Ma, Maier, Duvauchelle, & Schallert, 2009). Given that the low dose (0.3 mg/kg) and high dose (1.0 mg/kg) did not increase 50 kHz ultrasonic vocalizations and neither dose affected FM 50 kHz ultrasonic vocalizations when compared to the saline treated animals, these results suggest the emotional and motivational state remained consistent across all animals and across acute and chronic treatments, providing strong support that these are trait-like responses that remain stable over time.

On the Challenge day, all animals received either the low (0.3mg/kg) or the high dose (1.0 mg/kg) of amphetamine after a 14-day break. The H-USV screen did not predict

amphetamine-induced 50 kHz ultrasonic vocalizations, however, there was a unique negative relationship between the inescapable novelty screen and amphetamine-induced 50 kHz ultrasonic vocalizations. This result suggests the highest responding animals in the inescapable novelty screen vocalized less in response to the challenge doses of amphetamine, suggesting the rewarding effect of amphetamine is blunted in those higher responding animals. This blunted effect is intriguing because it could explain why the higher responding animals in the inescapable novelty screen self-administer more low-unit doses of amphetamine (Cain et al., 2004; Piazza et al., 1989). Conditioned place preference and conditioned taste aversion are paradigms used to evaluate the rewarding value of a drug. Interestingly, high and low (assigned via median split) responding animals in the inescapable novelty screen do not show differences in conditioned place preference but do in a conditioned taste aversion paradigm. Low responding animals are more sensitive to the aversive effects of amphetamine when compared to high responding animals, suggesting the high responding animals are less sensitive to the rewarding value of amphetamine (Towell, Willner, & Muscat, 1987). Fifty kHz ultrasonic vocalizations in response to amphetamine after a 14-day withdrawal are in support of the conditioned aversion theory, and suggest that high responding animals are less sensitive to the rewarding value of amphetamine when compared to the low responding animals. Furthermore, 50 kHz ultrasonic vocalizations are negatively correlated with self-administration acquisition, such that higher 50 kHz callers acquire self-administration in in a shorter amount of time, suggesting the rewarding effect of drug is important for acquisition of self-administration. Taken together, these results lend support that ultrasonic vocalizations could help reveal differences between higher and lower novelty seeking animals in reward processing of amphetamine after a withdrawal period and suggest there is a

negative relationship between high responding animals in the inescapable novelty screen and 50 kHz ultrasonic vocalizations.

However, there is another explanation for this negative relationship. Corticosterone alters dopaminergic function in the nucleus accumbens (Barrot et al., 2000). High responding rats are hypothesized to have a dysfunctional stress response that contributes to their increased vulnerability to drug use. High responding animals in the inescapable novelty screen have an elevated corticosterone concentration that persists for a longer duration when compared to the low responding animals when placed in a novel environment (Piazza et al., 1991). The novelty place preference screen does not elevate corticosterone concentrations and high novelty preferring animals in the novelty place preference screen are not different from low novelty preferring animals in corticosterone concentrations after exposure to the novelty place preference screen. The elevation of the corticiosterone is hypothesized to account for the differences between the inescapable novelty screen and novelty place preference screen and could explain why the screens are uncorrelated. The blunted ultrasonic vocalization response observed in the higher responder could be the result of a greater corticosterone concentration induced during the withdrawal period. Fifty and 22 kHz ultrasonic vocalizations do decrease and increase respectively under withdrawal conditions (Mutschler, & Miczek, 1998), and may suggest corticosterone may alter ultrasonic vocalizations, especially because corticosterone moderates dopaminergic transmission in the nucleus accumbens shell (Rouge-Pont et al., 1993).

Fifty kHz ultrasonic vocalizations have been shown to be dopamine dependent at the D2 receptor (Brudzynski et al., 2011) and specifically evoked by amphetamine microinjections into both the nucleus accumbens core and shell, with a greater response rate evoked by microinjections into the shell (Brudzynski et al., 2011Burgdorf et al., 2001). Recent evidence has

implicated diverging roles for the shell and core with repeated amphetamine treatment. Following brief (1-3 day) and longer (10-14 day) abstinence periods shell neuronal firing excitability decreases in the shell, while the core shows an increase in excitability (Kourrich, & Thomas, 2011). If 50 kHz ultrasonic vocalizations are dependent on nucleus accumbens shell, more so than nucleus accumbens core excitability, then a decrease in excitability observed by Kourrich and Thomas (2011) could explain why the H-USV screen did not predict vocalization response after a prolonged abstinence period. According to Kourrich and Thomas (2011), the nucleus accumbens shell becomes down regulated during the withdrawal period, suggesting the excitability is intact during repeated amphetamine exposures. Thus, the current results suggest the H-USV screen can predict prolonged repeated amphetamine exposures until a withdrawal period. Based on the current results, it may be possible to predict these hypothesized adaptations to the nucleus accumbens shell from the inescapable novelty screen.

Predicting Amphetamine-induced Locomotor Activity

The H-USV screen did not show any relationship with amphetamine-induced locomotor activity on Day 1, Day 7 or the Challenge day. This result did not support the hypothesis and suggests 50 kHz ultrasonic vocalizations observed in response to rewarding tickle stimulation have no relationship with amphetamine-induced locomotor activity. Furthermore, it indicates the brain area associated with 50 kHz ultrasonic vocalizations and amphetamine-induced locomotor activity are likely different or that the behaviors are measuring different constructs within addictive behavior.

Previous research has indicates there is a common neural mechanism implicated in the response to a novel environment and response to amphetamine (Hooks et al., 1991). The nucleus accumbens has been identified as a possible area in the rat brain that contributes to exploratory

locomotor activity (Mogenson et al., 1984). Therefore, animals with a greater exploratory locomotor response to a novel environment (inescapable novelty screen) may have an increased dopaminergic response to the novel environment and to amphetamine. Interestingly, high responding animals have demonstrated dose-dependent differences in locomotor sensitization to repeated amphetamine exposure (Hooks et al., 1991). At higher doses (1.5 mg/kg), the locomotor activity measured is increased to a maximal level, making it difficult to see any further increases. This result offers an explanation as to why the current experiment did not observe differences between the repeated (1.0 mg/kg) and acute (1.0 mg/kg) groups on the challenge day. It is possible that at the high dose (1.0 mg/kg) animals were maximally activated, making it difficult to establish a difference between animals with acute amphetamine exposure and animals with repeated amphetamine exposure on the challenge day.

While previous research has demonstrated that high novelty seeking animals in the inescapable novelty screen show an increased response to non-contingent amphetamine, the current study did not replicate that effect. This result was not surprising at the high dose (1.0 mg/kg) of amphetamine tested, because the differences between higher and lower responding animals diminish with increasing doses of amphetamine. The inescapable novelty screen's prediction of locomotor activity in response to non-contingent amphetamine likely diminishes because higher doses of amphetamine induce a greater locomotor response regardless of whether the animal was a higher or lower responder in the inescapable novelty screen. In contrast, lower doses parse out differences in amphetamine sensitivity between high and low responders in the inescapable novelty screen (Bevins et al., 1997; Hooks et al., 1991; Mathews et al., 2010). However, when regression analyses are utilized the inescapable novelty screen no longer predicts acquisition of self-administration of amphetamine, even when large samples are used (Cain et al.,

2005). Previous research has successfully used the inescapable novelty screen as a dichotomous variable to predict locomotor response to amphetamine (Hooks et al., 1991; Bevins et al.,1997; Matthews et al., 2010), and self-administration of amphetamine (Cain et al., 2004; Piazza et al., 1989). Importantly, regression analyses and more advanced mixed effect models have not determined a relationship between the inescapable novelty screen and self-administration of amphetamine (Cain et al., 2005; Marusich, Darna, Charnigo, Dwoskin, & Bardo, 2011). The current study has produced similar findings to the self-administration literature in that, when regression analyses are used the inescapable novelty screen does not predict locomotor response to amphetamine, and suggests that when the inescapable novelty screen is treated as a continuous variable it may not predict or show any relationship with amphetamine-induced locomotor activity.

The novelty place preference screen did not show any relationship with amphetamine-induced 50 kHz ultrasonic vocalizations and it did not show a relationship with amphetamine-induced locomotor activity. This result is in support of previous research and determines there is no added predictive value by including the novelty place preference screen when predicting amphetamine-induced locomotor activity. The novelty place preference screen was included in the current study because the novelty place preference screen had not previously been used to understand amphetamine-induced 50 kHz ultrasonic vocalizations. It is now clearer that the novelty place preference is not related to 50 kHz ultrasonic vocalizations in response to a low dose (0.3 mg/kg) or high dose (1.0 mg/kg) and furthermore is not related to 50 kHz ultrasonic vocalizations observed in the H-USV screen. Interestingly, the novelty place preference screen has a unique relationship with self-administration models when regression statistical techniques are used (Cain et al., 2005) and predicts compulsive drug taking (Belin et al., 2011). Given that

the novelty place preference screen has been a useful predictor in several self-administration paradigms and has not predicted amphetamine-induced locomotor activity it can be concluded that the addiction paradigms are measuring two distinct addiction behaviors. Possibly, the novelty place preference screen could be measuring the animals' motivation to self-administer especially because the novelty screen predicts compulsive drug taking (Belin, et al., 2011).

Importance of Regression Analyses

Traditionally, median split techniques have been applied to the inescapable novelty and novelty place preference screens to understand how high and low responders are different in drug response, self-administration acquisition, and the transition to compulsive drug taking. Similarly, differences between high and low ultrasonic vocalization callers have also been explored and revealed differences in amphetamine-induced 50 kHz ultrasonic vocalization, and amphetamine-induced locomotion.

Importantly, the distribution of the novelty responses in the inescapable novelty and novelty place preference screens are approximately normal (Cain et al, 2005). Thus, less optimal approaches to data analysis such as median split are not appropriate for creating high and low responding groups. The inescapable and novelty place preference distribution of scores were approximately normal in the current experiment and provide a rationale for not completing a median split technique to create groups. Instead, a more appropriate and powerful technique is to maintain the measurement precision in our novelty screens. To maintain the precision, each of the predictors remained continuous, as opposed to creating categorical 'high' and 'low' groups (i.e. median split or dichotomizing). The negative effects of dichotomizing continuous predictors are well known and as Irwin and McClelland (2003) suggest dichotomizing predictor variables results in a reduction of the R^2, because of the increase in variability.

More concerning is the fact that artificial categorization of continuous variables can result in spurious effects, especially when more than one continuous predictor is dichotomized (Maxwell & Delaney, 1993). Traditionally in psychology, drug dose is treated as a categorical variable. When combined with the categorization of the inescapable novelty screen or novelty place preference screen that would yield two variables with artificial categorization, which by Maxwell and Delaney's (1993) observations suggest could create spurious effects in the analyses. Therefore, future research should continue to use regression statistical approaches to understand the relationships between novelty and amphetamine-induced locomotor activity, and other animal models of drug addiction.

While the current study did not replicate previous research with the inescapable novelty screen predicting amphetamine-induced locomotor activity, it does offer strong support that the inescapable novelty screen does not predict locomotor activity in response to a low (0.3 mg/kg) or high (1.0 mg/kg) dose of amphetamine. It is certainly possible the differences between previous research and this one is the maintenance of continuous predictors and regression analyses. In Cain et al. (2005) the inescapable novelty screen did not predict self-administration behavior when regression analyses were used. Although that study was examining self-administration behavior, there is a commonality between the two experiments. When regression analyses are used the inescapable novelty screen no longer predicts self-administration or amphetamine-induced locomotor activity. Furthermore, the current study replicated that the inescapable novelty screen loses its predictive validity when it is combined with other individual difference measures even when large samples of animals are used (Cain et al., 2005; Marusich, Darna, Charnigo, Dwoskin, & Bardo, 2011).

Limiting Factors

This study was, to my knowledge the first to use ultrasonic vocalizations, inescapable novelty, and novelty place preference as continuous predictors to understand amphetamine-induced locomotor activity and ultrasonic vocalizations. While previous research has dichotomized novelty responses into high and low responders, which is problematic for the reasons discussed above, they have used analyses that account for the dependence of observations, such as repeated measures ANOVA. A simultaneous regression analysis was used to predict locomotor activity and 50 kHz USV on Day 1, Day 7 and the Challenge day. Essentially, each regression analysis is treating the data as if each observation is independent, when in fact they were not. Without accounting for the dependence of observations from Day 1 to Day 7 and Day 7 to the Challenge day it is possible that relationship estimations could be overestimated, because the correlations between each day are not accounted for in the simultaneous regression model (Sainani, 2010). The comparisons could lead to an increased Type I error rate. Given that inescapable novelty, novelty place preference, and H-USV screens did not predict locomotor activity acutely or chronically, it is likely an inflated Type I error rate was not observed. However, in predicting 50 kHz ultrasonic vocalizations the H-USV screen and inescapable novelty screen were predictive. Violating the independence of observations assumptions could have influenced the β coefficients and overestimated the relationships observed. Although, the relationships between the respective screens and treatment-induced ultrasonic vocalizations may be overestimated for Day 7 and the Challenge day, the independence of observations assumption was satisfied for Day 1. Given that amphetamine did not increase ultrasonic vocalizations, it is possible the β coefficients associated with the Day 7 and the Challenge day are most likely accurate.

Furthermore, violating the independence of observations assumption can also lead to *underestimating* β coefficients with within group comparisons. This suggests the β coefficients observed on Day 7 and the Challenge day may also be underestimated and deemed to be non-significant. Therefore, the β coefficients for Day 7 and the Challenge day could be stronger, and thus explain the non-significant findings that were observed with the novelty screen and H-USV screens.

In addition another limiting factor in the present study that could push the results out of significance could have been the lack of power to observe such an effect. Previous research maintaining the continuous precision of predictor variables used larger samples than what was used in the current study (Cain et al., 2005), the present study was in conjunction with the result that the inescapable novelty screen does not predict amphetamine-induced locomotor activity when regression analyses are used. More powerful statistical methods such as mixed effects modeling employed by Marusich et al. (2011) did not find a relationship between the inescapable novelty screen and amphetamine self-administration, suggesting the effect size may be overestimated. Thus, the current study may have been underpowered to observe an effect of any of the screens, especially when drug is treated as a categorical variable because it uses additional degrees of freedom. Babyak (2004) suggests that linear regression model should effectively have 10-15 observations for each predictor in the model. To illustrate the lack of power, the current study attempted to use 5-6 predictors (depending on the phase of the experiment) and 30 animals which only leave 5-6 observations per predictor. When sample sizes are small, the influence of each observation is more substantial because there are not as many observations to mitigate outlier values. Therefore, the β coefficients are more likely to fluctuate, sometimes resulting in significance and other times falling out of significance.

Thus, a remedy to establish stability in the β coefficient estimates is to increase the sample size or to decrease the number of predictors. Increasing the sample size to the suggested 60-90 animals would yield a more stable and accurate estimate of the β coefficients. This would enable future research to understand the true β coefficients and more accurately replicate data. In addition, understanding the true β coefficient will progress research to include and exclude necessary individual difference screens and yield more accurate predictions of animal drug behavior. In short, the model would be a better fit, without overfitting the data and generalize to future samples. Previous research has shown the inescapable novelty and novelty place preference are not correlated, which suggests it may benefit future predictive models to exclude one of the screens altogether, but that depends on a sensitization model (Hooks et al., 1991), acquisition of self-administration (Cain et al., 2004) or compulsive self-administration model (Belin et al., 2009). Given that these novelty screens are not correlated they offer a unique opportunity to understand novelty's contribution to addictive behavior. The inescapable novelty screen and novelty place preference are both measuring novelty, but they seem to be measuring different aspects of novelty, each with its own contribution to understand addictive behavior in the novelty and sensation seeker. Therefore, it is necessary to continue to research both novelty screens in separate addiction paradigms to truly understand each screen's relationship with addiction.

Another possible limitation may have been the age of the rats. The rats were approximately 45 days old during the H-USV screen, followed by 28 days of amphetamine training. At the Challenge day, the animals were 73 days old and the loss in prediction from the H-USV screen and the negative relationship with 50 kHz ultrasonic vocalization observed with the inescapable novelty screen could be attributed to the animals aging. Animals for the current

experiment were selected at the current age because adolescent male rats are most receptive to tickling at this age (Burgdorf & Panksepp, 2000). Ultrasonic vocalizations do remain stable across time, but some research suggests that 50 kHz ultrasonic vocalizations vary as a function of age with animals decreasing 50 kHz ultrasonic vocalization calling as early as 2 months of age (Mallo et al., 2007). At the final ultrasonic recording animals were approximately 13 days older than 2 months, suggesting that the age of the animal may have contributed to the non-significant prediction of the H-USV screen and the significant negative relationship with the inescapable novelty screen.

Conclusion

The inescapable novelty screen and the novelty place preference screen are thought to measuring novelty and sensation-seeking behavior in rodents. However, the current study is in support of previous research and suggests they are measuring two different aspects of novelty and sensation-seeking behavior. It also suggests that novelty and sensation-seeking are complex behaviors in the rodent and more research needs to be completed to understand which aspects of novelty these screens are measuring. Furthermore, these novelty screens are related to different animal models of drug dependence further implicating them as separate, but necessary components to understanding drug dependences. Ultrasonic vocalizations offer another behavioral measure to understand individual differences in rewarding stimuli and affective state of the animal. Importantly, novelty and sensation-seeking and ultrasonic vocalizations are complex animal behaviors that are not fully understood, but do offer an avenue to understand predispositions to develop compulsive drug taking.

Ultrasonic vocalizations in conjunction with the inescapable novelty screen offer a capability to predict neurological changes that are occurring in response to repeated

amphetamine exposure particularly after withdrawal periods, and suggest both novelty and affective state are implicated in drug response. Although we can infer these neurological changes as measured through behavior, more direct measures need to be utilized to understand under what circumstances these changes are occurring. Nonetheless, novelty and sensation seeking are traits identified in humans that predict experimentation with drugs, types of drugs, and treatment success. In addition, human affective state has been documented in treatment success and relapse vulnerability. While many people experiment with drugs of abuse only a fraction escalate to drug dependence or uncontrolled drug taking. It is important to understand how novelty and sensation-seeking contribute to drug experimentation, drug response, and self-administration and how affective state is changed during each drug phase and withdrawal. These data suggest rodents' novel behavior and affective state can successfully be used to predict amphetamine response, and provide strong support for researching novelty and affective state to understand addictive behavior.

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Figure 1. A scatterplot showing the total distance traveled (cm) in the inescapable novelty screen and the calculated ratios from the novelty place preference screen.

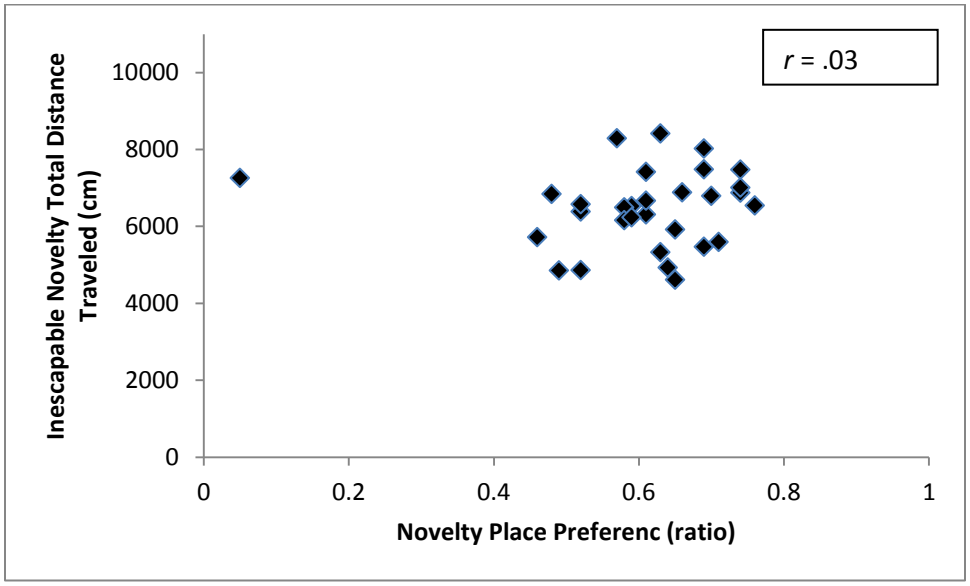

Figure 2. A scatterplot showing the total distance traveled (cm) in the inescapable novelty screen and the number of 50 kHz ultrasonic vocalizations in the heterospecific screen.

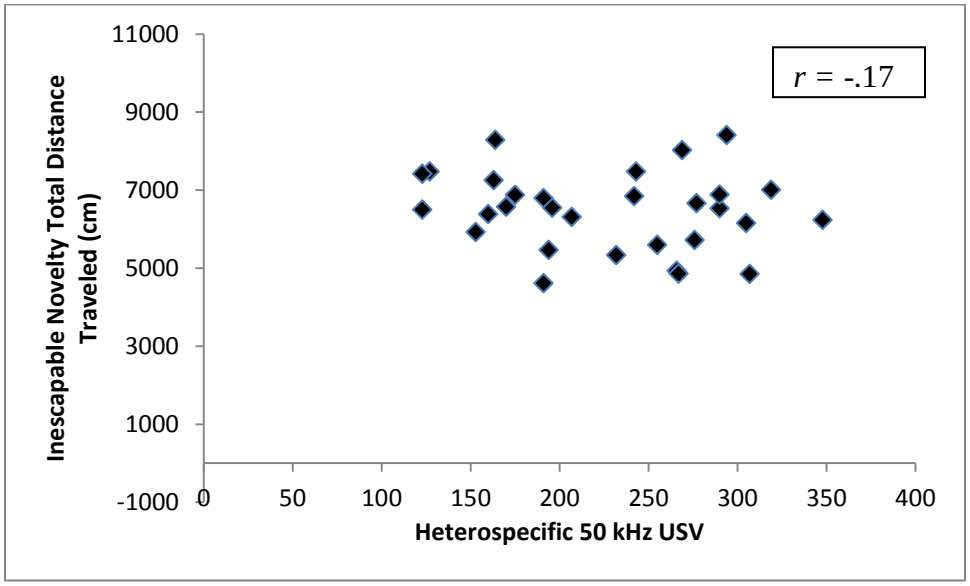

Figure 3. A scatterplot showing and the calculated ratios from the novelty place preference screen and the number of 50 kHz ultrasonic vocalizations in the heterospecific screen.

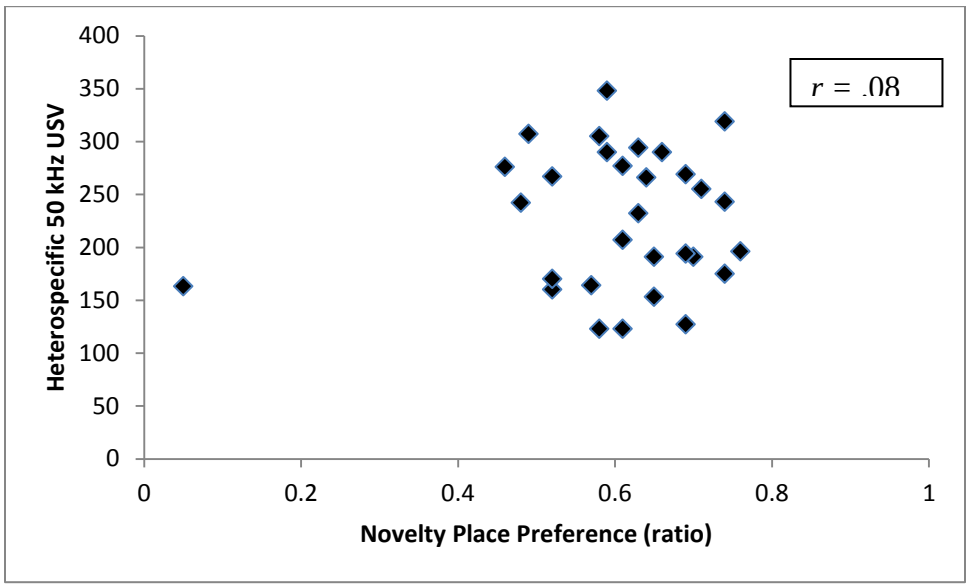

Figure 4. The predicted total distance traveled in response to the respective drug treatment plotted as a function of low, mean and high values of the inescapable novelty response for Day 1.

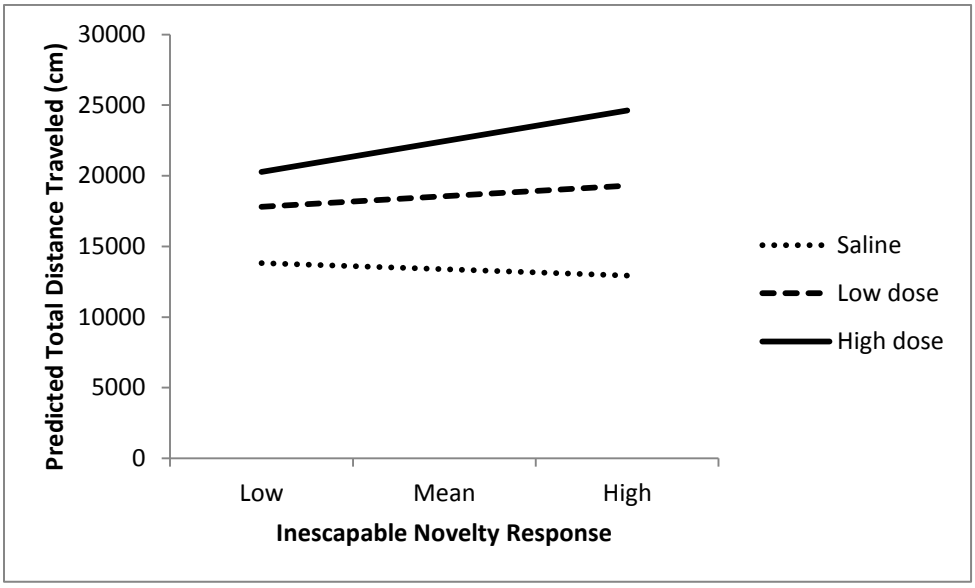

Figure 5. The predicted total 50 kHz ultrasonic vocalizations in response to the respective drug treatment plotted as a function of low, mean and high values of the heterospecific screen response for Day 1.

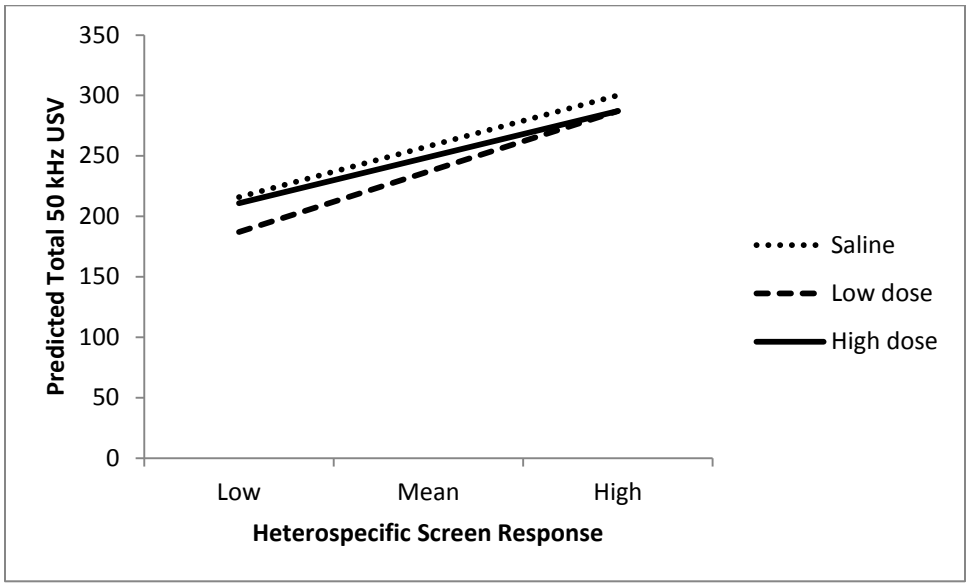

Figure 6. The predicted total distance traveled in response to the respective drug treatment plotted as a function of low, mean and high values of the inescapable novelty response for Day 7.

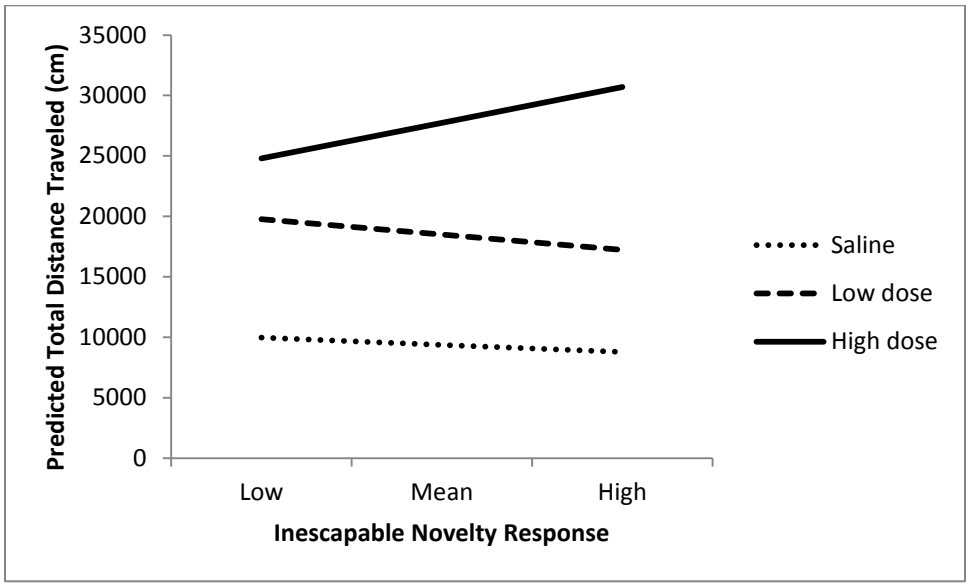

Figure 7. The predicted total 50 kHz ultrasonic vocalizations in response to the respective drug treatment plotted as a function of low, mean and high values of the heterospecific screen response for Day 7.

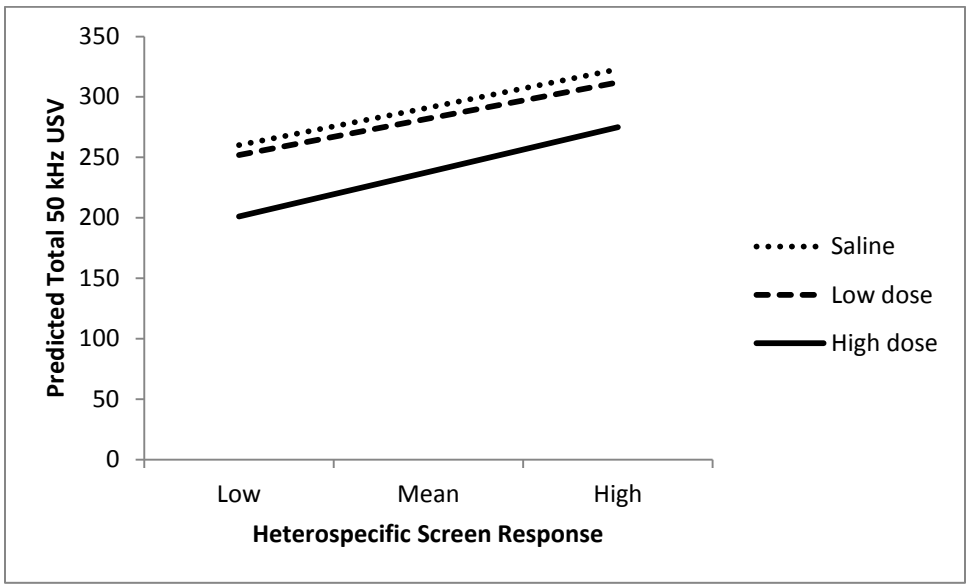

Figure 8. The predicted total distance traveled in response to the respective drug treatment plotted as a function of low, mean and high values of the inescapable novelty response for the Challenge day when all animals received a treatment of amphetamine.

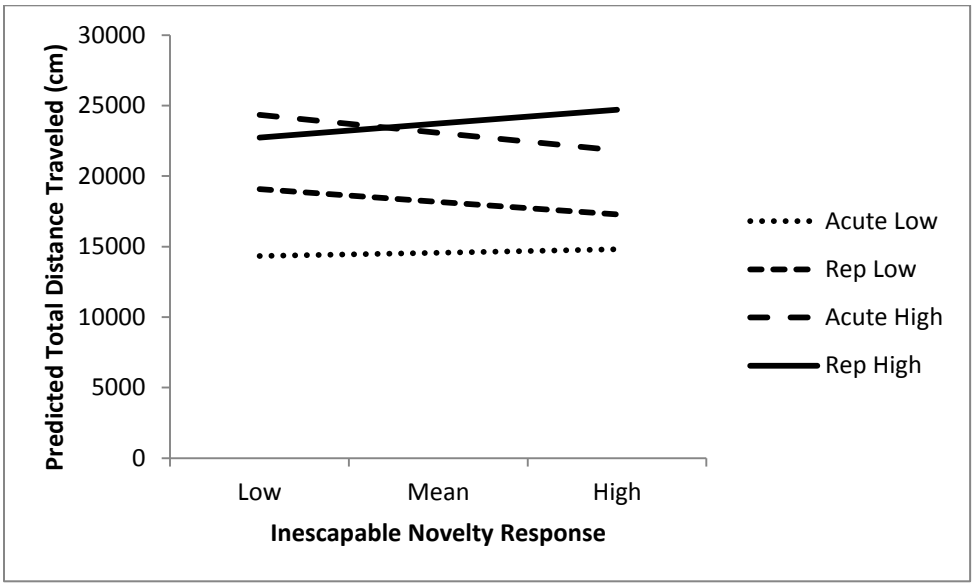

Figure 9. The predicted total 50 kHz ultrasonic vocalizations in response to the respective drug treatment plotted as a function of low, mean and high values of the heterospecific screen response for Challenge day when all animals received a treatment of amphetamine.

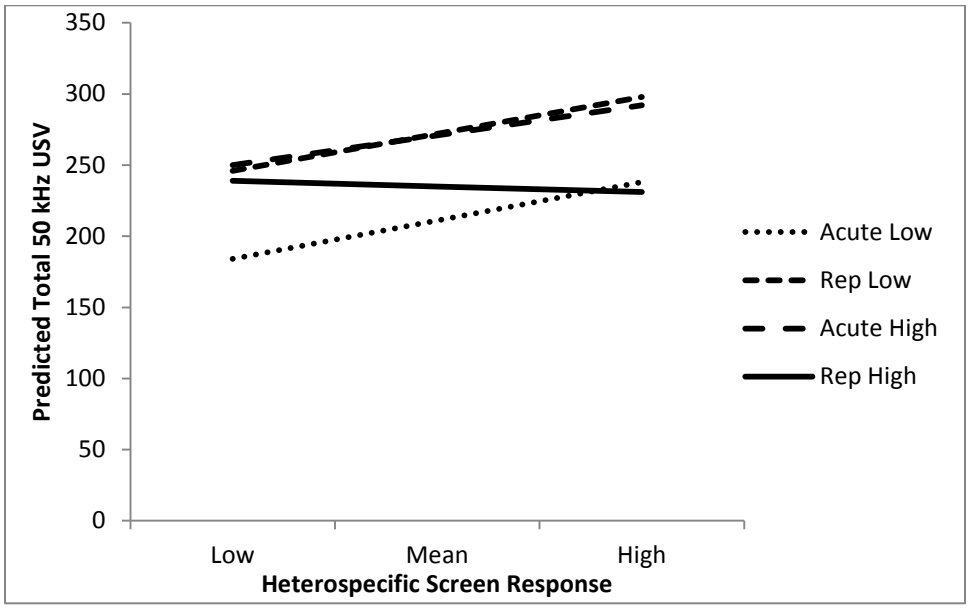

Figure 10. The predicted total 50 kHz ultrasonic vocalizations in response to the respective drug treatment plotted as a function of low, mean and high values of the inescapable novelty screen response for Challenge day when all animals received a treatment of amphetamine.

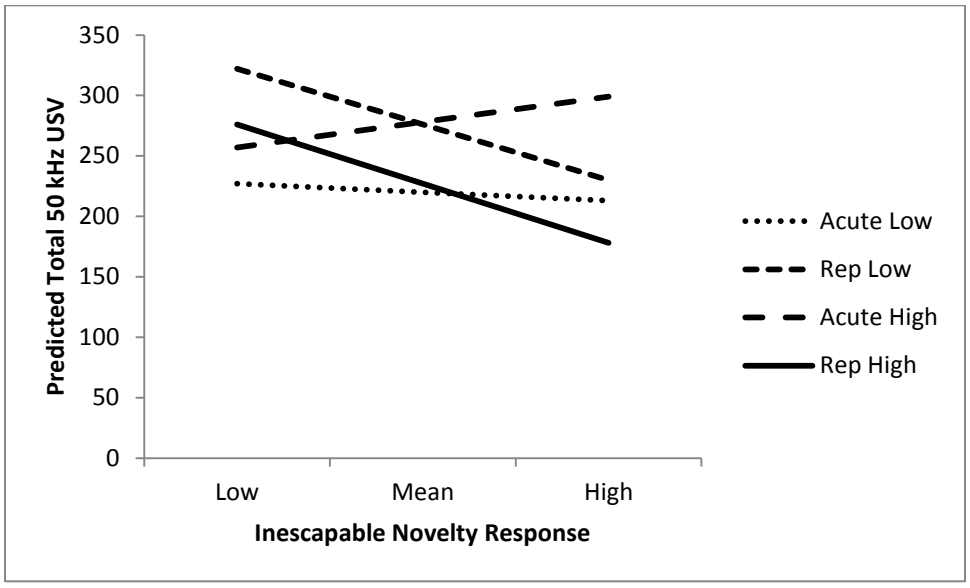

Table 1. A summary table of the predictors, standardized coefficients, t-values and significance for the regression model when predicting the locomotor response to amphetamine on Day 1. All interactions were tested independently.

Acute locomotor response (Day 1)		
Predictor	**Standardized β coefficient**	***t***
Intercept	0 [1]	2.71*
Inescapable Novelty	0.13	1.30
Novelty place preference	-0.21	-1.90
H-USV 50 kHz	0.12	1.17
Amphetamine 0.3 mg/kg	0.49	4.29***
Amphetamine 1.0 mg/kg	0.86	6.85***
Amp 0.3 X Inescapable	0.17	1.08
Amp 1.0 X Inescapable	0.23	1.89
Amp 0.3 X Novelty place	-0.19	-1.44
Amp 1.0 X Novelty place	-0.24	-0.93
Amp 0.3 X H-USV 50 kHz	-0.07	-0.38
Amp 1.0 X H-USV 50 kHz	-0.04	-0.25
Inescapable X Novelty place	-0.21	-1.69
Inescapable X H-USV 50 kHz	-0.04	-0.35
Novelty place X H-USV 50 kHz	0.09	0.64

*$p < .05$, ***$p < .001$

[1] Unstandardized intercept value 12,038

Table 2. A summary table of the predictors, standardized coefficients, t-values and significance for the regression model when predicting the 50 kHz USV response to amphetamine on Day 1. All interactions were tested independently.

Acute 50 kHz USV response (Day 1)		
Predictor	**Standardized β coefficient**	***t***
Intercept	0 [2]	1.19
Inescapable Novelty	-0.01	1.30
Novelty place preference	-0.01	-1.90
H-USV 50 kHz	0.67	4.31**
Amphetamine 0.3 mg/kg	-0.15	-0.88
Amphetamine 1.0 mg/kg	-0.09	-0.45
Amp 0.3 X Inescapable	0.24	0.94
Amp 1.0 X Inescapable	0.10	0.52
Amp 0.3 X Novelty place	-0.08	-0.38
Amp 1.0 X Novelty place	0.17	0.42
Amp 0.3 X H-USV 50 kHz	-0.08	0.29
Amp 1.0 X H-USV 50 kHz	-0.04	-0.13
Inescapable X Novelty place	-0.23	-1.18
Inescapable X H-USV 50 kHz	0.28	1.78
Novelty place X H-USV 50 kHz	0.31	1.56

$^{**}p < .01$

[2] Unstandardized intercept value 110

Table 3. A summary table of the predictors, standardized coefficients, t-values and significance for the regression model when predicting the locomotor response to amphetamine on Day 7. All interactions were tested independently.

	Chronic locomotor response (Day 7)	
Predictor	**Standardized β coefficient**	***t***
Intercept	0 [3]	0.74
Inescapable Novelty	-0.04	-0.35
Novelty place preference	0.09	0.74
H-USV 50 kHz	0.07	0.63
Amphetamine 0.3 mg/kg	0.50	4.18***
Amphetamine 1.0 mg/kg	1.02	7.74***
Amp 0.3 X Inescapable	-0.06	-0.34
Amp 1.0 X Inescapable	0.17	1.36
Amp 0.3 X Novelty place	-0.17	-1.21
Amp 1.0 X Novelty place	0.02	0.07
Amp 0.3 X H-USV 50 kHz	-0.07	0.41
Amp 1.0 X H-USV 50 kHz	-0.01	-0.07
Inescapable X Novelty place	-0.18	-1.34
Inescapable X H-USV 50 kHz	-0.19	-1.72
Novelty place X H-USV 50 kHz	0.02	0.12

***$p < .001$

[3] Unstandardized intercept value 6090

Table 4. A summary table of the predictors, standardized coefficients, t-values and significance for the regression model when predicting the 50 kHz USV response to amphetamine on Day 7. All interactions were tested independently.

Chronic 50 kHz USV response (Day 7)		
Predictor	**Standardized β coefficient**	***t***
Intercept	0 [4]	2.90**
Inescapable Novelty	-0.18	-1.02
Novelty place preference	-0.18	-0.98
H-USV 50 kHz	0.47	2.70*
Amphetamine 0.3 mg/kg	-0.06	-0.31
Amphetamine 1.0 mg/kg	-0.35	-1.63
Amp 0.3 X Inescapable	-0.14	-0.51
Amp 1.0 X Inescapable	-0.24	-1.10
Amp 0.3 X Novelty place	-0.30	-1.38
Amp 1.0 X Novelty place	0.17	0.40
Amp 0.3 X H-USV 50 kHz	-0.01	-0.05
Amp 1.0 X H-USV 50 kHz	-0.05	0.18
Inescapable X Novelty place	0.17	0.77
Inescapable X H-USV 50 kHz	0.07	0.38
Novelty place X H-USV 50 kHz	0.11	0.50

*$p < .05$ **$p < .01$

[4] Unstandardized intercept value 312

Table 5. A summary table of the predictors, standardized coefficients, t-values and significance for the regression model when predicting the locomotor response to amphetamine on Challenge day. All interactions were tested independently.

Challenge dose locomotor response (Challenge day)		
Predictor	**Standardized β coefficient**	***t***
Intercept	0 [5]	2.32*
Inescapable Novelty	-0.10	-0.64
Novelty place preference	-0.04	-0.24
H-USV 50 kHz	0.06	0.41
Repeated Amp 0.3 mg/kg	0.33	1.53
Acute Amp 1.0 mg/kg	0.61	3.13**
Repeated Amp 1.0 mg/kg	0.83	3.51**
Rep Amp 0.3 X Inescapable	-0.15	-0.39
Acute Amp 1.0 X Inescapable	-0.13	-0.46
Rep Amp 1.0 X Inescapable	0.06	0.22
Rep Amp 0.3 X Novelty place	-0.18	-0.59
Acute Amp 1.0 X Novelty place	-0.08	-0.22
Rep Amp 1.0 X Novelty place	-0.12	-0.14
Rep Amp 0.3 X H-USV 50 kHz	-0.04	-0.12
Acute Amp 1.0 X H-USV 50 kHz	-0.02	-0.07
Rep Amp 1.0 X H-USV 50 kHz	0.17	0.52
Inescapable X Novelty place	-0.21	-0.99
Inescapable X H-USV 50 kHz	-0.09	-0.55
Novelty place X H-USV 50 kHz	0.01	0.05

*$p < .05$ **$p < .01$

[5] Unstandardized intercept value 17,658

Table 6. A summary table of the predictors, standardized coefficients, t-values and significance for the regression model when predicting the 50 kHz USV response to amphetamine on Challenge day. All interactions were tested independently.

Challenge dose 50 kHz USV response (Challenge day)		
Predictor	**Standardized β coefficient**	***t***
Intercept	0 [6]	2.36*
Inescapable Novelty	-0.37	-2.16*
Novelty place preference	0.14	0.74
H-USV 50 kHz	0.22	1.25
Repeated Amp 0.3 mg/kg	0.38	1.55
Acute Amp 1.0 mg/kg	0.29	1.30
Repeated Amp 1.0 mg/kg	0.10	0.39
Rep Amp 0.3 X Inescapable	-0.36	-0.90
Acute Amp 1.0 X Inescapable	0.17	0.59
Rep Amp 1.0 X Inescapable	-0.23	-0.86
Rep Amp 0.3 X Novelty place	-0.35	-1.03
Acute Amp 1.0 X Novelty place	-0.43	-1.15
Rep Amp 1.0 X Novelty place	-1.31	-1.43
Rep Amp 0.3 X H-USV 50 kHz	-0.01	-0.02
Acute Amp 1.0 X H-USV 50 kHz	-0.03	-0.10
Rep Amp 1.0 X H-USV 50 kHz	-0.23	-0.64
Inescapable X Novelty place	0.14	0.59
Inescapable X H-USV 50 kHz	0.09	0.30
Novelty place X H-USV 50 kHz	0.25	1.10

**p < .05*

[6] Unstandardized intercept value 291